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Accumulation of Pb in *Brassica juncea* in response to sludge paper waste application

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Abstract
Sludge is one of the wastes produced by the paper industry which has the potential to be used as an ameliorant material. However, the obstacle is that sludge is considered a toxic and dangerous material because of the presence of heavy metals in it, especially the heavy metal Pb. The aims of this study were to determine the number of heavy metals accumulated in cocopeat and biochar planting media combined with sludge paper waste, roots and leaves of the *Brassica juncea* plant as accumulator plants. The pot experimental was set up in a Single Factor Randomized Group Design consisting of 6 treatments and 3 replications. The treatment used was a combination of planting media (cocopeat and biochar) with sludge paper waste consisting of treatments A (cocopeat), B (biochar), C (cocopeat + paper sludge organic fertilizer 3:1), D (biochar + fertilizer paper sludge organic fertilizer 3:1), E (cocopeat + paper sludge organic fertilizer 1:1), F (biochar + paper sludge organic fertilizer 1:1). The results showed that biochar media had a higher accumulation of heavy metal Pb in plant roots, while cocopeat media translocated more to leaves. The study showed that biochar and cocopeat media are effective media capable of accumulating and translocating the heavy metal Pb, both in the planting medium and into plant tissue However, the value of the Translocation Factor in each treatment was included in the low category, namely TF<1.

Keywords
Brassica juncea, Paper waste, Pb, Heavy metal

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Introduction

Paper is one of the materials whose level of need is increasing every year throughout the world, both for school, printing and office needs. [1] in their research reported that paper consumption based on data from the World Wildlife Fund (WWF) was around 400 million tons per year with residues of around 5-16% of the amount of paper produced. Even in Europe, the Confederation of European Paper Industries (CEPI) [2] in 2012 suggested recycling waste paper into useful materials. It is estimated that one ton of paper production produces around 40-50 kg of sludge paper waste.

Paper processing produces waste from the Wastewater Treatment Plant (WWTP) in the form of solid waste called sludge. Indonesian Government Regulation No. 22 of 2021 states that sludge is included in Category 2 B3 waste (Hazardous and Toxic Materials), where its use must pass a TCLP (Toxicity Characteristic Leaching Procedure) test [3]. The use of sludge is also limited only as a material for making bricks or adobe bricks. Meanwhile, sludge from the WWTP for the paper industry is included as a soil ameliorant whose function is to improve soil properties [4]. Sludge is a very important soil conditioner for sustainable sustainability and can add to the organic matter content of the soil and provide macro and micro nutrients to the soil. The content of organic matter in sludge can improve the physical, chemical and biological properties of the soil. In addition, sludge also contains 4.89% Organic C, 0.96% N, 0.22% P_2O_5, 0.08% K_2O and 1.58% $CaCO_3$, so that sludge has the potential to be used as a source of nutrition, especially in sub- optimal.

Sludge has a high C/N of about 20 [5], if it is directly applied to the soil, it will cause nitrogen immobilization. Therefore, the provision of sludge as a nutrient additive must go through the composting stage first. If the C/N is too high, the activity of microorganisms will be inhibited. In addition, the content of heavy metals in organic sludge fertilizers must also be identified. The results of the analysis of organic fertilizers from sludge waste containing Pb, which are above the minimum organic technical requirements and soil repairers Decree of the Minister of Agriculture of Indonesia No. 261/KPTS/SR.310/M/4/2019, where the analysis results show Pb levels around 53.44 mg /kg, while based on technical requirements the maximum Pb value is around <50 mg/kg [6].

Pb is a heavy metal with a density of 11.34 g/cm3. Pb is one of the heavy metals that have the potential to be toxic if it is in excess concentration in the soil. Elemental Pb is a heavy metal that is not essential for plants and is currently considered a pollutant that can pollute the soil and the environment [7]. In addition, Pb compounds can enter through the breath and affect health. The forms of Pb are generally insoluble, but the most soluble forms are Pb acetate, Pb chloride and Pb nitrate. The content of heavy metal Pb contained in organic fertilizer sludge waste must be identified further to determine the level of pollution and absorption of heavy metal Pb in soil and plants.

Accumulator plants have the ability to accumulate metals and organic compounds, either in the roots or translocated to the leaves and stems of plants. Heavy metal content in plants is influenced by the period of time the plant is in contact with heavy metals, its levels and plant morphology and physiology [8]. Plant roots can directly absorb soluble heavy metals, especially in the form of free cations. Accumulator plants are able to absorb heavy metals exceeding the metal content in the soil and accumulate in plant parts. There are about 400 species of accumulator plants and 22 families that have been identified, one of which is the family Brassicaceae which has the most species of heavy metal accumulators.

Brassica species can accumulate significant amounts of Pb in roots [9]. Caisim (*Brassica juncea*) plants can accumulate Pb in the canopy about 34,500 g/kg [10]. Caisim plants can be used as Pb accumulator plants so that Pb uptake can be identified as normal or excessive thresholds for human consumption. In addition, to determine the amount of Pb contained in paper mill sludge that can accumulate in the growing media and caisim plants. The success of organic fertilizer derived from sludge waste is determined by the accumulation and level of pollution in both the soil and plants so that sludge waste fertilizer can be used, especially as an ameliorant in agriculture. Utilization of sludge waste into organic fertilizer is an agricultural challenge in the future to overcome the reduction of pollution of soil and water bodies from sludge waste produced by large industries. The aims of this study were to determine the number of heavy metals accumulated in cocopeat and biochar planting media combined with sludge paper waste, roots and leaves of the *Brassica juncea* plant as accumulator plants.

Methods

The research was conducted in the greenhouse of the Faculty of Agriculture, Universitas Singaperbangsa Karawang, Wst Java. from January to March 2021. TCLP Sludge paper waste analysis was carried out at Teknologi Mineral dan Batubara laboratory. Meanwhile, sludge chemical analysis was carried out at the Indonesian Center for Biodiversity and Biotechnology.

Experimental establishment

The pot experimental was set up in a Single Factor Randomized Group Design consisting of 6 treatments. The treatment used was a combination of planting media (cocopeat and biochar) with sludge paper waste consisting of treatments A (cocopeat), B (biochar), C (cocopeat + paper sludge organic fertilizer 3:1), D (biochar + fertilizer paper sludge organic fertilizer 3:1), E (cocopeat + paper sludge organic fertilizer 1:1), F (biochar + paper sludge organic fertilizer 1:1). All combination treatments were replicated three times. The ratio of planting media per polybag is adjusted to the specific gravity of each material in a polybag measuring 35 x 17.5 x 35 cm. After the media is mixed with sludge paper waste, the media is incubated for 2 weeks.

Cocopeat and biochar medium

Cocopeat and biochar have different specific gravity, so the treatment application uses a weight ratio between cocopeat and biochar with sludge paper waste. The results of the analysis of the specific gravity of cocopeat showed a value of 0.029 g/cm3, while the specific gravity of biochar was around 0.045 g/cm3. Analysis of Pb in Cocopeat and biochar used for planting media was carried out using the $HClO_4$ and HNO_3 extraction methods. The results of the analysis showed that the cocopeat and biochar used had a Pb concentration of 0 mg/kg.

Sludge paper waste

The sludge paper waste used is the result of composting with a mixture of cow dung, lime, bran, effective microorganisms and molasses for 28 days. The specific gravity of sludge paper waste after being composted is around 0.13 g/cm3. The results of sludge organic waste analysis showed that the C-organic content contained was around 17.73%, with a total N-content of 1.01% and a C/N ratio of 18. In addition, the analysis results also showed a total P_2O_5 content of approx. 0.67% and a total K_2O of about 3.22% with a pH level of 7.84. Meanwhile, the results of TCLP analysis using the EPA Test Method 1311 showed that paper sludge waste contained a Pb concentration of around 53.44 mg kg^{-1}. Based on the standard available Pb content has a limit of around >50 mg kg^{-1}. This shows that the heavy metal content of Pb in sludge waste is still relatively high, so it is necessary to test accumulator plants of *Brassica juncea* in order to find out how much heavy metal is absorbed by plants on cocopeat and biochar growing media.

Planting and chemical fertilizer

Planting is done using seeds that have been sown previously for 2 weeks. Each polybag is filled with 1 plant seed, the distance between polybags is 25 x 25 cm. The chemical fertilizer composed of 500 kg ha^{-1} of NPK 15:15:15 or the equivalent of 3,125 g per polybag were applied 7 days after planting. The fertilizer placed in a circular band lay 1 cm below the media surface at a distance of 2 cm from the stem. All plants were irrigated with water in the morning and afternoon.

Harvesting

Harvesting is done when the *Brassica juncea* are 30 days after planting. Harvesting is done by uprooting the entire plant and its roots.

Analysis of Pb content

Pb content analysis was carried out on the combination of planting media after harvest, plant roots *Brassica juncea* and *Brassica juncea* leaves using the $HClO_4$ and HNO_3 extraction methods.

Coefficient and Translocation Factor of Pb

Plant and planting media relationships were assessed for Pb accumulation in shoots and roots. According to [11]EPA (2000) the accumulation coefficient (AC), defined as the plant/planting media concentration quotient was calculated.

AC= (Croot or shoots /Csoil)

Where Croot or shoot = Concentration of heavy metal in root part (mg/kg) and Cplant medium = Concentration in plant medium (mg/kg). Moreover, according to Machiol et al. (2004) the translocation factor (TF) was calculated to estimate the transfer of heavy metals from roots to shoots of *Brassica juncea* **TF= (Cshoots /Croot).**

Statictical analysis

All data taken from the pot experiment were analyzed by one-way analysis of variance (ANOVA) to test the significance of the factor. If the results of the analysis of variance show a significant difference, then to find out which treatment gave the highest results, a further test was carried out with the Duncan Multiple Range Test (DMRT) at 5% level.

Results and Discussion

Based on ANOVA, the effect of the combination of planting media with sludge paper waste showed a significant effect on the Pb content in the leaves of the *Brassica juncea* plant (Figure 1).

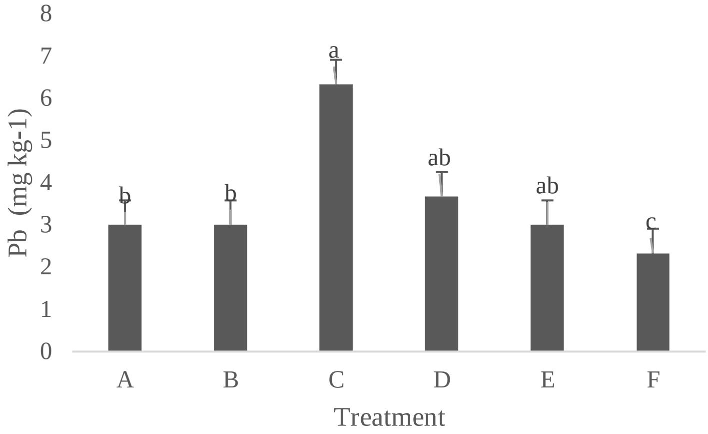

Figure 1. Pb Content in Leaf *Brassica juncea*

Figure 1 shows that there is a significant effect of the combination of planting media and sludge organic fertilizer on the Pb content in *Brassica juncea* leaves. The highest yield was obtained by treatment C (3:1), not significantly different from treatments D, E and F and significantly different from treatments A and B. The highest Pb content of around 6.33 mg kg^{-1} was found in treatment C (3:1). While the lowest Pb content was found in treatment F (1:1).). This shows that the ability of *Brassica juncea* to absorb the heavy metal Pb is quite large, both with media using cocopeat and biochar combined with sludge paper waste. *Brassica juncea* is a heavy metal accumulator plant that has the ability to absorb metal elements and is able to store them in its stems and leaves [12]. Pb mobility in *Brassica juncea* plants was higher by using cocopeat media, where cocopeat was able to provide more available Pb that could be absorbed and translocated to all parts of the plant compared to biochar media combined with sludge paper waste. Meanwhile, *Brassica juncea* plants grown with biochar media and sludge paper waste accumulated more in plant roots, rather than translocating them to the plant canopy.

Figure 2 shows that the accumulation of heavy metal Pb in plant roots originating from sludge paper waste was higher when using biochar media in treatment F (1:1) of 9.52 mg kg-1 and treatment D (3:1) of 6.25 mg kg-1 compared to cocopeat medium. This is inversely proportional to the accumulation of the heavy metal Pb in the canopy of the *Brassica juncea* plant. In cocopeat media, the heavy metal Pb was accumulated in the plant canopy more than in the roots. This is likely due to the presence of Pb bonds with organic matter, making it difficult for plants to translocate to parts of the plant canopy. In line with research conducted [13] that biochar has pores and is able to produce organic acids which help in binding minerals and heavy metals more strongly so that heavy metals are not translocated to parts of the plant canopy, but still accumulate in the roots *Brassica juncea* plant.

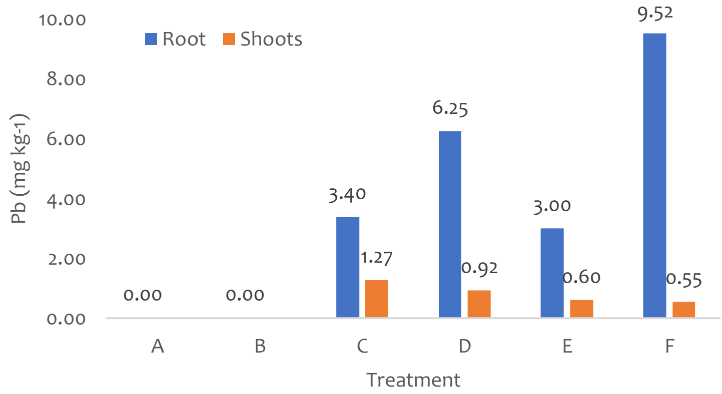

Figure 2. Accumulation coefficient Pb in root and shoots

Figure 3 show that the highest Pb content in the roots of *Brassica juncea* was found in treatment F (1:1), not significantly different from treatment D (3:1) and significantly different from other treatments. The highest Pb contained in the roots in the sludge organic fertilizer treatment was around 39.67 mg kg^{-1}, with biochar growing media. Meanwhile, the lowest was obtained by treatment A (Cocopeat) of around 6.67 mg kg^{-1} found in cocopeat growing media without the use of sludge organic fertilizer. In treatment C (3:1), the highest Pb content was around 17 mg kg^{-1}. This shows that the cocopeat growing media provides more available Pb which can be absorbed by the roots of the *Brassica juncea* plant, although it is possible that the Pb did not come from sludge waste organic fertilizer.

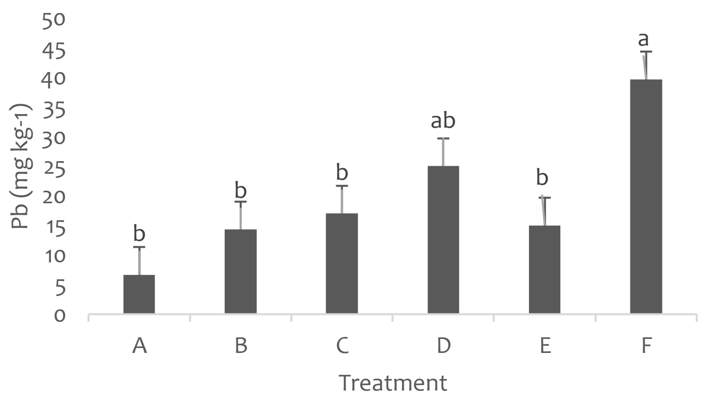

Figure 3. Pb Content in Root *Brassica juncea*

Biochar is a pyrolysis decomposition product, where biochar will provide more Pb than cocopeat media. Therefore, the Pb absorbed by plant roots with biochar media was more than the Pb absorbed by plant roots with cocopeat media. Cocopeat is an organic material containing lignin, cellulose, hemicellulose, several pectin and extractives (fats, alcohols, phenols, terpenes, steroids, resins, resins and waxes) which can increase the adsorption of metal ions [14], so that metal ions are absorbed by plant roots is lower than that of Pb in the media.

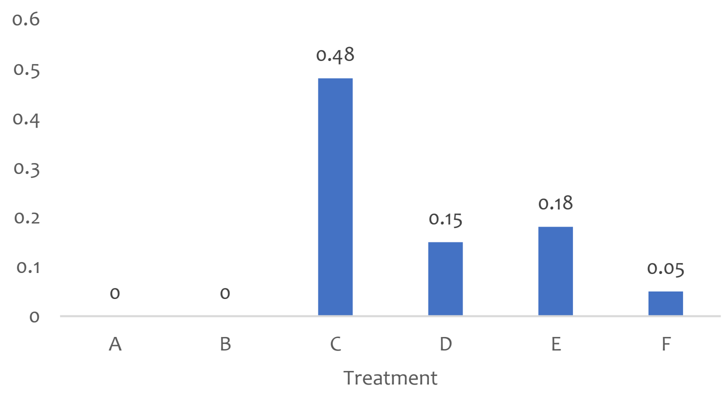

Figure 4. Translocation Factor root to shoots

The translocation of heavy metal factors is related to the chemical properties/metal elements to be extracted for translocation and distribution to plant parts [15]. The heavy metal Pb is an immobile metal, so it is usually distributed in the same pattern in plants, the concentration is in roots > shoots > leaves > fruit > seeds. Translocation factor is the ratio of metal concentrations in the shoots to the roots which indicates the ability to transfer metals from the roots to the shoots of the plant (Sharma et al 2016). *Brassica juncea* is a plant that belongs to the hyperaccumulator class of heavy metals. The results of the study (Figure 4) showed that treatment C (3:1) could translocate the heavy metal Pb higher than the other treatments. Nevertheless, the translocated metal compounds were low, namely around TF = 0.48. [16] The value of TF> 1 indicates that plants are very effective at translocating heavy metals from roots to plant parts. This is related to the amount of accumulated Pb metal that can be absorbed by plants using biochar or cocopeat media combined with sludge paper waste which is included in the safe category for consumption. Metal translocated with cocopeat or biochar media combined with sludge paper waste has TF <1.

Cocopeat and biochar are organic materials that can produce organic acids and form chelate bonds with metal compounds and form organometallic bonds. It is possible that this has caused Pb metal in the media and roots not to accumulate and not to be maximally translocated to the top of the plant. However, [17] the normal limit for Pb levels in plants is around 0.2 -20 mg kg^{-1}, so it is necessary to pay attention if the metal accumulates in food plants which have a relatively longer age compared to *Brassica juncea* plants.

Figure 5 shows that there is a significant effect on the combination of planting media and sludge organic fertilizer on the Pb content in the *Brassica juncea* planting medium. The highest yield was obtained by treatment C (3:1), not significantly different from treatment E and significantly different from treatments A, B, D, and F. Treatment C (3:1) contained Pb of approx. 5 mg kg^{-1}, but in treatment E (1:1) also contained about 5 mg kg^{-1} Pb. This shows that cocopeat growing media with sludge is more capable of absorbing large amounts of Pb than biochar growing media with sludge.

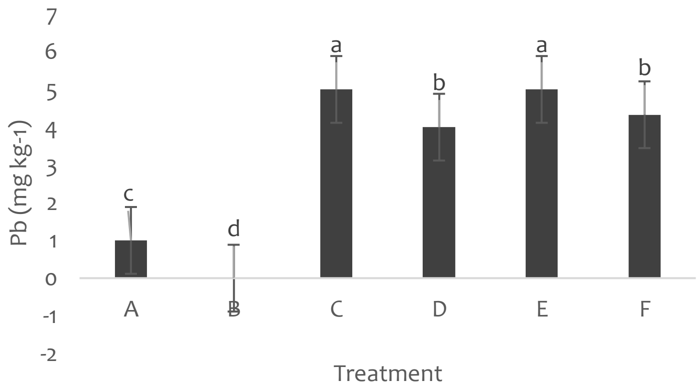

Figure 5. Pb content in growing media cocopeat, biochar and combination with sludge paper waste

The degree of variation in Pb concentration that is more accumulated by cocopeat can be attributed to the high amount of Pb entering the filter layer and the high affinity of Pb for the lignin contained in cocopeat, causing Pb ions to enter deeper [18]. Meanwhile, Biochar is also a potential bio-adsorbent for pollutants because it has a large surface area, porous structure, abundant surface functional groups, and high cation exchange capacity (CEC). However, the type of metal also affects differences in the uptake of these metals in plants. Metal absorption is dominated by complexation with functional groups, cation exchange, and precipitation [19][20]. The adsorption effect of various heavy metal ion adsorption capacities competitively affecting the biochar adsorption results, so that Pb was considered the most easily absorbed heavy metal.

Conclusion

Based on the research results, the heavy metal content of Pb in the roots, leaves and growing media of cocopeat and biochar combined with sludge paper waste is still relatively safe. The combination of cocopeat media and sludge paper waste provides more heavy metal Pb both in the media and in the *Brassica juncea* plants, compared to using Biochar. However, the value of the Translocation Factor in each treatment was included in the low category, namely TF <1.

Acknowledgments

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