Effect of Organic-Based Humic Acid on Yield of Rice Grown in Heavy Metal Contaminated Soil

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ABSTRACT

Application of certain fertilizer during long-term food crops production, might increase the heavy metal (HM) level in soil. An inexpensive and easy method to decrease this contaminant uptake by plants is application of humic acid (HA) which has the capacity to bind toxic substances. Pot experiment had been conducted to observe the influence of organic-based HA on the growth and yield of rice grown in soil contaminated with Cadmium and Lead. The experiment laid in Randomized Block Design with four treatments and six replications. Rice was grown in Inceptisols and treated with HA extracted from various organic matter. Control plants did not receive any HA. All plants had not shown any HM toxicity symptoms. The plant height as well as straw dry weight in HA-treated soil was no different with control plant. Various HA decreased available Cadmium and Lead in soil and in unhusked grain but did not affect yield traits. However, the reduced content of HM was also caused by soil acidity that shift to neutral during puddling. This experiment suggested that HA have a role to control HM availability in paddy soil and hence reduced their uptake by rice plants.

Keywords: Cadmium, Grain Yield, Lead, Soil acidity

1. INTRODUCTION

Naturally, the level of heavy metal Cadmium (Cd) and Lead (Pb) in soil depends on the soil genesis since certain rock of soil parent material contain the metals. Igneous rock Granite and Basalt as well as metamorphic rocks such as Gneiss and Schists contained Cd up to 0.01-1.6 mg/kg and 0.05-1.87 mg/kg respectively (Alloway, 1995). The content of Pb in igneous and sediment rocks were as low as 1.9-8.3 mg/kg and 10-7.1 mg/kg respectively (Davies, 1995). Anthropogenic activities including phosphate fertilizer and organic matter application possibly increase their content. For growing lowland rice in Indonesia, organic matter (OM) amendment is needed to increase organic carbon (C), nitrogen (N) and phosphor (P) level in soil since tropical soil is usually low in OM (Ross, 1993; Craswell and Lefroy, 2001).

Various types of manure contained 28 mg/kg Cd, 9 mg/kg Hg, 60 mg/kg Ni and 48 mg/kg Pb in order of chicken > ostrich > sheep > cattle > goat (Irshad et al., 2013). Cadmium levels in phosphate fertilizers reached 1.94-113 mg/kg (Setyorini et al., 2003). Long term application of P fertilizer and organic matter increased the level of Cd and Pb soil (Adhikari et al., 2020). The Cd and Pb caused a decrease in seedling vigor and growth as a result of stress reactions which is indicated by the increase in toxicity indicator substances of amines – betaine and putrescine (Solanki and Dhankhar, 2011; Li et al., 2020).

Decontamination of heavy metals (HM) in agricultural soil is important to increase crop productivity and reduce the risk of HM accumulation in edible part of food crops. The availability of HM in soil highly depends on the acidity; soil acidification, enhance the Cd and Pb mobility in the order of Cd > Pb (Kicińska et al., 2022); this leads to HM accumulation by plants.

Reducing the mobility of heavy metals in the soil can be done by using humic acids (HA) which have a carboxyl group (-COOH) and a hydroxyl group (-OH). Humic acids are ubiquitous in natural environments and derived from the degradation of plant and microbial substances (Zhou et al., 2014). Humic acid (HA) is a soil organic component that determines soil quality by reducing soil density, forming aggregates and increasing air infiltration. A number of studies have proven the role of HA as a source of plant nutrients and microorganisms so that HA help to improve plants growth (Boguta and Sokołowska, 2013). Moreover, HA possibly benefit plant growth by two mechanisms: improving nutrient uptake and providing plant hormones (Nikbakht et al., 2008).

The negative charge of the acid will form coordination bonds with HM cations (Zhou et al., 2015). The HA-HM chelation reduces the availability of HM in the soil and limits its uptake by roots. Humic acid is usually obtained through coal extraction through an unsustainable process. However, HA fractionated from organic matter is reported effective in reducing Cd levels in waters (Paul and Jayakumar 2010).

Rice is staple food of majority of nation in East- as well as South East Asia including Indonesia. To maintain rice productivity, the remediation of contaminated soil where the rice is grown is prominent. The study about the role of humic acid is important in order to find sustainable method for HM decontamination in soil. This pot experiment had been carried out to verify the effect of organic-based humic acid on the growth and yield of rice grown in Cd- and Pb-contaminated soil.

2. MATERIALS AND METHODS

The pot experiment had been conducted in the Agricultural Environment Research Centre in Pati located in tropical area with the altitude of 7 m above sea level. The soil was Inceptisols with the acidity of 5.2. The soil texture was clay and contained 1.98% organic carbon, 0.78% total N. Before experiment the soil contained 0.08 mg/kg Cd and 0.59 mg/kg Pb respectively. The HA were prepared by extracting municipal organic waste, cow manure and local peat. After being fractionated, HA content in the three organic matters were 0.6%, 3% and 2% respectively.

2.1 Experimental Design

The experiment was arranged in Randomized Block Design with four HA treatments and six replications. The treatments were:

A : without humic acid (control)
B : 0.225 g of municipal waste humic acid
C : 1.125 g of cow manure humic acid
D : 0.750 g of peat humic acid.

The rice seedlings were grown in a black plastic pot that contain 7.5 kg soil of Inceptisols order. In order to obtain the need of humic acid for single pot, the doses were calculated based on the content of humic acid in each organic matter mention above; and recommended organic matter in rice cultivation, 10 t/ha. The said doses were applied for single pot.

2.2 Experimental Establishment

The seedlings of Rice cv Ciherang were used in this experiment. Before planting, the potted soils were irrigated to form 5 cm-height standing water. The potted soils were contaminated artificially by 8 mg/kg Cd, 250 mg/kg Pb at seven day after puddling. Solid HA were applied and mixed with potted soil evenly at day 14 prior to incubate for another seven days. Two 21-day old rice seedlings were transplanted in each potted-soil soon after soil incubation.

Rice plants were fertilized with 0.38 g Urea, 0.47 g SP-36, and 0.13 g KCI. This fertilizer amount was calculated according to recommended doses of N, P and K fertilizer for lowland rice; 100 kg/ha N, 125 kg/ha P_2O_5 and 33.33 kg/ha K_2O. Plants received Urea and KCI twice at planting time and 21 days after planting; while SP-36 was applied at the planting time. Irrigation was carried out by unsterilized HM-free distilled water. All plants were grown in the greenhouse until harvest time at 100 days according to the description of Rice cv Ciherang released by Indonesian Ministry of Agriculture.

2.3 Parameters and Statistical Analysis

At harvest time (100 days after transplanting), the level of available Cd and Pb in soil were analysis by Atomic Adsorption

Spectrophotometry after soil extraction with 1 M NaOH. The yield parameter included tassel number in a pot, productive (filled) grain number in a tassel, unhusked grain dry weight, and hay dry weight. All data were analyzed by Analysis of Variance (Anova) with $p \leq 0.05$), and the Duncan Multiple Range (DMR) Test at $p \leq 0.05$ when the treatment affect the parameters significantly based on ANOVA.

3. RESULTS AND DISCUSSION

3.1 Available Cadmium and Lead in Soil

The content of available Cd and Pb in soil after rice harvesting was significantly affected by HA treatments. Soil received no HA had less available both HM (Table 1). The result showed that HA application resulted in the Cd and Pb level in soil was about 5-8.7% and 7.9-17.7% respectively compared to the control. Soil was contaminated by 8 mg/kg Cd and 250 mg/kg Pb and before treatment soil contained 0.08 mg/kg Cd and 0.59 mg/kg Pb. Despite higher level of Pb, the end of experiment the available Pb level in soil was not exceed available Cd.

The humic acid in the soil is unstable and will undergo further humification and mineralization (Boguta and Sokołowska, 2013). Due to the limited study duration, it was clear that HA has not underwent any notable degradation, HA clearly demonstrated its ability to bind Cd and Pb. The decrease in the levels of both metals was caused by the complexation reaction between the HA chelate and metal cations (Zhou et al., 2015). In the HA-M complex, the solubility of the metal can be increased if the HM forms a complex with free HA; or decreased if the metal was scavenged by a humic film on the mineral surface (Calace et al., 2009).

The HAs applied in this experiment was free HA. Therefore, the immobilization and the decrease in the availability of HA were also controlled by increasing the pH from 5.2 became neutral during irrigation irrespective of HA type (Table 1). Neutral soil induces the Cd and Pb immobilization but 70% Cd in soils remain mobile at neutral or slightly alkaline pH (Kicińska et al., 2021).

Table 1 Effect of different humic acid on available Cd and Pb, and soil acidity of heavy metal-contaminated soil after rice harvesting

Treatments	Available HM (mg/kg)		Soil acidity
	Cd	Pb	
A: without HA (control)	4.20 a	2.65 a	6.87
B: 0.225 g of municipal waste HA	0.21 b	0.21 b	6.94
C: 1.125 g of cow manure HA	0.25 b	0.45 b	6.97
D: 0.750 g of peat HA	0.36 b	0.47 b	6.95

Numbers followed by the same letter are not significantly different based on DMR test at $p \leq 0.05$

3.2 Rice Growth

Humic acid treatment did not affected plant height at 28 and 56 days after transplanting (Figure 1) as well as dry weight of hay after harvest (Figure 2). Based on Alloway (1995) review, the minimum threshold of Cd and Pb contamination in soil is 5 mg/kg and 200 mg/kg. The results found that rice withstand slight Cd and Pb contamination in paddy soil partly because of neutral soil reaction. Low available Cd and Pb in soil did not cause HM visible toxicity syndrome in rice during vegetative stage.

During the experiment, there was no growth difference between rice treated with HA and control. This clearly verified that in this experiment HA did not induced plant growth. This result differed with the positive effect of HA to provide nutrition, mainly nitrogen, for rice and hence its growth as previously reported (Suhardjadinata et al., 2015; Suntari et al., 2015). The absence of a plant-growth-promoting effect of Has may be due to the low dose of HAs applied to the soil or by the quality of the HAs extracted from organic matter.

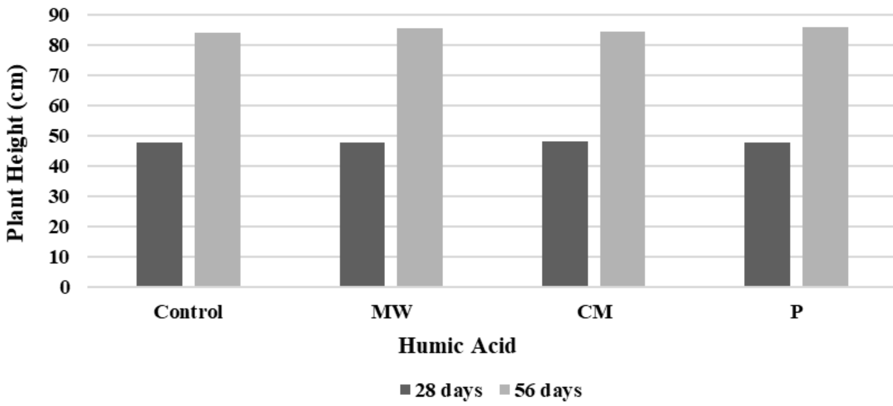

Figure 1 Effect of different humic acids on plant height of rice grown in contaminated soil at 28 and 56 days after planting. Humic acids were extracted from Municipal wastes (MW); Cow manure (CM); and Peat (P).

The hay dry weight did not determine by HA application (Figure 2). The increased of hay dry weight only demonstrated by rice treated with HA extracted from cow manure but the difference was not significant compared to the control. The data confirmed the resistance of lowland rice on low Cd and Pb concentration in soil. Despite most of Cd in soil might be still available for root uptake in neutral soil environment (Kicińska et al., 2021).

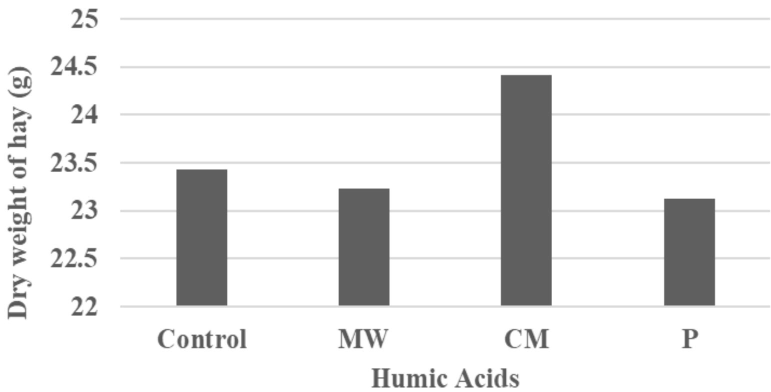

Figure 2 Effect of different humic acids on hay dry weight of rice grown in HM contaminated soil

3.3 Yield Parameters

The absence of negative effect of HM on the rice growth and biomass resulted in the non-significant effect of various HA on rice yield parameters. Clearly, the HA had no role to induce tiller number, tassel number, number of grains in a tassel and unhusked grain dry weight in a potted soil (Table 2). Tiller number measured at 56 days was not affected by HA application. In general rice cv Ciherang has 14-17 tiller number; Table 2 showed that tiller number per clump was 17-19.

Table 2 Effect of different humic acids on yield traits of rice grown in potted soil contaminated with Cd and Pb

Treatments	Tiller number	Tassel number	Grains in a tassel	Unhusked grain dry weight (g/pot)
A: without HA (control)	18	14.8 a	90.0 a	54.50 a
B: 0.225 g of municipal waste HA	17	14.8 a	97.8 a	53.71 a
C: 1.125 g of cow manure HA	19	14.6 a	98.6 a	53.51 a
D: 0.750 g of peat HA	17	13.5 a	95.5 a	53.11 a

Numbers followed by the same letter are not significantly different based on DMR test at $p \leq 0.05$

The unhusked grains contained small amount Cd and Pb mainly when rice grown in potted soil with various HA application (Table 3). The rice received any HA significantly had lower Cd and Pb compared to control plant. The HA reduced the availability of both HA in soil (Table 1) so that HM uptake by roots was limited. The maximal threshold of both HM in food crops was 0.003-1.0 mg/kg of Cd and 0.005-10.0 mg/kg of Pb based on Indonesian National Standard 7387: 2009. This greenhouse experiment verified that rice grown in potted soil slightly contaminated with Cd and Pb produce grain with low content of both HM particularly when HA was added.

Table 3 Cadmium and Pb content in unhusked grain of rice grown in contaminated soil following various humic acid application

Treatments	HM in unhusked grain (mg/kg)	
	Cd	Pb
A: without HA (control)	0.99 a	1.09 a
B: 0.225 g of municipal waste HA	0.09 b	0.22 b
C: 1.125 g of cow manure HA	0.04 b	0.11 b
D: 0.750 g of peat HA	0.10 b	0.11 b

Numbers followed by the same letter are not significantly different based on DMR test at $p \leq 0.05$

4. CONCLUSION

Contamination of Cd and Pb was carried out intentionally into the soil to increase the levels of both heavy metals to 8 mg/kg Cd and 250 mg/kg Pb. The experiment showed that rice cv Ciherang grown in potted soil contaminated with Cd and Pb did not cause growth decrease or any toxicity symptoms towards rice. Humic acids was extracted from various organic matter reduced the available Cd and Pb in the soil after rice harvest, as well as Cd and Pb level in rice straw and unhusked grains. However, all humic acid did not increase the growth and yield of rice. The absence of the effect of plant-growth-promotion may be due to the non-optimal dose of HA and the quality of HA which was not analyzed in this experiment.

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REFERENCES

Adhikari, T., R. C. Gowda, R. H. Wanjari and M. Singh. 2020. Impact of continuous fertilization on heavy metals content in soil and food grains under 25 years of long-term fertilizer experiment. Communications in Soil Science and Plant Analysis 52(2): 1-17.

Alloway, B.J. 1995. Cadmium. In Alloway, B. J. (Ed) Heavy Metals in Soil. Blackie Academic and Professional, Glasgow, United Kingdom.

Boguta, P. and Z Sokołowska. 2013. Interactions of Humic Acids with Metals. Instutut Agrofizyki im Bohdana Dobrańskiego PAN. Lublin. Poland.

Calace, N., D. Deriu, B. Petronio and M. Pietroletti, 2009. Adsorption isotherms and breakthrough curves to study how humic acids influence heavy metal–soil interactions. Water, Air, and Soil Pollution 204 (1-4): 373–383.

Craswell, E.T. and R.D.B. Lefroy. 2001. The role and function of organic matter in tropical soils. In Martius, C., Tiessen, H., Vlek, P.L.G. (Eds) Managing Organic Matter in Tropical Soils: Scope and Limitations. Developments in Plant and Soil Sciences, vol 93. Springer, Dordrecht, Germany.

Davies, B.E. 1995. Lead. In Alloway, B. J. (Ed) Heavy Metals in Soil. Blackie Academic and Professional, Glasgow, United Kingdom.

Kicińska, A, R. Pomykala and M Izquierdo-Diaz. 2021. Changes in soil pH and mobility of heavy metals in contaminated soils.

European Journal of Soil Science 73(1): e13203

Li. C., F. Yu, Y. Li, W. Niu, J. Li, J. Yang and K. Liu. 2020. Comparative analysis of the seed germination of pakchoi and its phytoremediation efficacy combined with chemical amendment in four polluted soils. International Journal of Phytoremediation. 22(11): 1-12.

Nikbakht, A., M. Kafi, M. Babalar, Y. P. Xia, A. Luo and N-A, Etemadi. 2008. Effect of humic acid on plant growth, nutrient uptake, and postharvest life of gerbera, Journal of Plant Nutrition 31(12): 2155-2167.

Paul, V.I. and P. Jayakumar. 2010. A comparative analytical study of cadmium and humic acids contents of two lentic water bodies in Tamil Nandu, India. Journal of Environmental Health Science & Engineering 7(2):137-144.

Ross, S.M. 1993. Organic matter in tropical soils: current conditions, concerns and prospects for conservation. Progress in Physical Geography: Earth and Environment. 17(3): 265-305.

Setyorini, D., E Soeparto and Sulaeman. 2003. Konsentrasi logam berat dalam pupuk. Prosiding Seminar Nasional Peningkatan Kualitas Lingkungan dan Produk Pertanian. Puslitbang Tanah dan Agroklimat. Bogor. hal 219-229

Solanki, R. and R. Dhankhar. 2011. Biochemical changes and adaptive strategies of plants under heavy metal stress. Biologia 66: 195-204.

Suhardjadinata, Y. Sunarya and T. Tedjaningsih. 2015. Increasing nitrogen fertilizer efficiency on wetland rice by using humic acid. Journal of Tropical Soils 20(3): 143-148.

Suntari, R., R. Retnowati, Soemarno and M. Munir. Determination of urea-humic acid dosage of vertisols on the growth and production of rice. Agrivita 37(2): 185-192.

Zhou, S., J. Xu, G. Yang and L. Zhuang. 2014. Methanogenesis affected by the co-occurrence of iron (III) oxides and humic substances. FEMS Microbiological Ecology 88 (1):107–120

Zhou, S., S. Chen, Y. Yuan and Q Lu. 2015. Influence of humic acid complexation with metal ions on extracellular electron transfer activity. Scientific Reports 5:17067.