



EFFECT OF FUNGI AND MANURE ON CADMIUM CONTENT AND BIOMASS OF MAIZE GROWN IN CADMIUM CONTAMINATED TAILING FROM BANGKA INDONESIA

Nofalia Nurfitriani^{1†} --- Reginawanti Hindersah²

¹Student at Faculty of Agriculture, Universitas Padjadjaran, Bandung, Indonesia

²Lecturer at Departement of Soil Microbiology, Faculty of Agriculture, Universitas Padjadjaran, Bandung, Indonesia

ABSTRACT

Cadmium (Cd) contamination from tailings disposal that occur in tin mining in Bangka cause a serious soil health problem. One of the ways to reduce Cd concentration in soil is bioremediation. The research was conducted to determine the influence of fungi and cattle manure on dry weight and Cd content of maize as well as fungi population in the rhizosphere of maize grown in tailing collected from tin mining area in Bangka. Green house research was set up in Factorial Randomized Block Design. Maize hybrid P21 was grown for six weeks in tailing contaminated by $CdCl_2 \cdot 0,5H_2O$ up to 8 mg kg^{-1} and inoculated with *Humicola* sp. or *Fusarium* sp. without and with several dose of cattle manure. Interaction effect between fungi and cattle manure clearly affect the rhizosphere fungal population, however did not give any significant effect to dry weight and Cd content. Application of *Fusarium* sp. 10^7 spores mL^{-1} with manure of 22 g pot^{-1} lead to increased fungal population. There was an independent effect of cattle manure to decrease Cd content and increase dry weight of maize.

Keywords: Cadmium, Fungi, Cattle manure, Maize (*Zea mays* L.)

Contribution/ Originality

This study contibutes in developing the soil microbiology and soil bioremediation studies in Indonesia. This study also as a reference and an information about what kind of fungi and the right dose of cattle manure to improve soil quality and productivity in tailing at Bangka Island.

1. INTRODUCTION

Bangka is the biggest tin mining island in Indonesia. The tin mining activities cause changes in the characteristics of the chemical, physical and biological of soil. The decline in soil physical properties such as the destruction of soil structure, sensitive to erosion, and low water holding ability [1]. In mining site of Bangka, C-organic of both top soil and bulk soil was less than 1,78%

and the texture was sandy. Sandy soils are characterized by a lack of structure, low water traction, high water permeability and consequently low indigenous microbial population.

Pollution by heavy metals such as cadmium (Cd), manganese (Mn), lead (Pb), Zinc (Zn), Copper (Cu) and chromium (Cr) due to post mining activities [1] was massive in mining area. In Bangka post mining locations, Cd contaminated soil was already exceeds the threshold with a concentration range from 0,67 to 12,36 mg kg⁻¹. According to Alloway and Ayres [2], amount of Cd in uncontaminated soil is 0-1 mg kg⁻¹ of soil. Heavy metal has a conversely effect to soil, agricultural crops and threat food chain. In human being, heavy metal is one the causes of cancer incidence [3]. Heavy metal was considered as 10 priority pollutants by the US Environmental Protection Agency [4]. Indonesian National Standardization Agency (Standar Nasional Indonesia) [5] set a threshold of Cd in food is around 0,1 – 1 mg kg⁻¹. Bioremediation is one of the ways to reduce Cd concentration in soil. Microorganisms such as fungi is one of great bioremediator. Fungi has bigger potential for bioremediation because of its growth and its wide range hyphae in the soil [6].

Fungi are good in heavy metal retention through the accumulation of heavy metals such as Cd, Cu, Hg, Ti and Zn in both of miselium or spora of fungi. Fungi release its metabolite such as sulphide acid or oxalic acid and other extracell materials such as polysaccharide and melanin, which could reduce the heavy metal concentration in soil [7] and furthermore limited heavy metal uptake by plant roots. According to Environmental Research Institute of Agriculture [1] viability of *Fusarium* sp. and *Humicola* sp. in the liquid culture with Cd verified that those fungi are relatively resistant to Cd and potentially to be used as biological agents of bioremediation of heavy metal contaminated soil. Other scientist has been reported that there are some fungi survived in polluted soil with heavy metals such as (*Humicola grisea*, *Fusarium* sp., *Nannizzia* sp., *Curvularia* sp. dan *Helementrho sporium* [8].

Organic matter amendment is necessary to enhance bioremediation by using of heterotrophic microbes. The growth of fungi would be increased in the presence of organic matters as an energy source. Organic matter could improve soil condition, soil chemical and physical characteristics and also affect stability of soil structure [9], and subsequently would increase the availability of oxygen for its proliferation and activity. Long term effect of organic matter application are improve microbial population and influence availability of nutritions [10]. Alongside that, decomposed organic matters released some organic acids which could bind the heavy metals [11] so as the availability of heavy metal will be decreased [12]. Cattle manure application may reduce the availability of heavy metals, in addition, it is a right way to maintain soil quality and increase fungi population [13]. Organic manure provide C-organic and high nutrition to increase microbes activity. Research conducted by Vinhal-Freitas, et al. [14] resulted in increases in microbial activity after application of 20 g kg⁻¹ of compost. Application of cattle manure up to 30 t ha⁻¹ is able to improve dry weight of maize plant [15]. Application of 20 t ha⁻¹ organic manure and inoculation of arbuscular mycorrhiza fungi reveal that this combination

strongly support the growth of maize plant [16]. Under these conditions, the addition of organic material has an important role in increasing crop yields.

2. MATERIALS AND METHODS

The experiment was conducted in a green house at Faculty of Agriculture Universitas Padjadjaran, Bandung - Indonesia on April – Juli 2013. The tin tailing used in this experiment obtained from Bangka tin mining field in Indonesia. Composite soil samples (0 to 20 cm depth) were collected from the various sites in Air Hitam Village, Bangka Island. Prior to experiments, soil were sterilized and analyzed in laboratory. C-organic content of sample was 0,56%, N-total 0,056% and $19,5 \times 10^3$ cfu/g of fungi population.

2.1. Cd Spiking

Cd pollution was simulated through the application of $\text{CdCl}_2 \cdot 0,5\text{H}_2\text{O}$ to achieve a soil concentration of 8 mg kg^{-1} . This Cd spiked is aimed to reach the same condition with one in the native field (Bangka Island). The Cd-spiked treatments were left for estimated 2 weeks prior to transplanting of maize seed.

2.2. Metal-Resistant Fungi

Cadmium resistant fungi *Humicola* sp. and *Fusarium* sp. were isolated from the rhizosphere of native plants grown in tailing at Bangka tin mining. *Fusarium* sp. was from native Akasia (*Acacia auriculiformis*) and *Humicola* sp. was from native Harendong (*Melastoma*). At previous research, the resistance of both of species on Cd was determined.

2.3. Pot Experiment

The pot experiment was set up in a Factorial Randomized Block Design with two treatment factors and three replications using maize (*Zea mays* L) as a test plant. The first factor was fungi species i.e without and with non pathogenic *Humicola* sp. or *Fusarium* sp., and the second one was dose of cattle manure i.e without and with 11 or 22 g pot⁻¹.

Pots were filled with one kilogram of sterilized tailing contaminated with $\text{CdCl}_2 \cdot 0,5\text{H}_2\text{O}$ up to 8 mg kg^{-1} and incubated for two weeks. Then, different dose of manure were added and incubated for a week prior to sowing. After that, seeds of the commercial hybrid maize P21 were sown and grown for six weeks. Two days after sowing, 10 mL of fungi species containing 10^7 spora mL⁻¹ was pour around seedling. At the end of experiment, Cd content and dry weight of maize as well as rhizosphere fungi population were determined.

2.4. Fungi Population Count

For fungi population analysis, total plate count method (Johnson and Curl, 1972 in [17]) was followed using Potato Dextrose Agar (PDA) medium incubated at 30°C for one week. Soil samples were taken from maize rhizosphere. Colony forming units (CFU) were estimated by

counting the number of colonies. *Humicola* sp. has black cottony on its surface, while *Fusarium* sp. has red colour on its surface.

2.5. Dry Weight Quantification

The sample plants for biomass quantification were harvested after 6 weeks. Roots and shoots were dried at 70°C to constant weight [18] and then weighed separately.

2.6. Heavy Metal Assay

Cadmium concentrations were determined by using spectrophotometer at 228,8 nm. One mL of HClO₄ and 5 mL of HNO₃ was added to 2,5 g dried plant samples (0.5 mm) in digestion tube, and leave overnight. The samples were then heated in digestion block 100°C within 1,5 hours, then temperature spiked to 130°C within 1 hour, 150°C within 2 hours subsequently. After the yellow vapor runs out, digestion temperature increased to 200°C to form a white vapor. After cooling, the solution was diluted to 10 mL with deionized water and filtered through a Whatmann No.40 filter paper. The filtrates were analyzed for Cd by *Atomic Absorption Spectroscopy* (AAS).

All preparations and performances for soil analysis presented in this paper were undertaken within a standard methods described by *Soil Research Institute of Indonesia (Balai Penelitian Tanah)* [19].

2.7. Statistical Analysis

All data were statistically analyzed using the SPSS. Differences between treatments were determined by the least significant difference ($P < 0.05$) from the analysis of variance (ANOVA) [20].

3. RESULTS

3.1. Fungi Population in Rhizosphere of Maize

Result revealed that there was an interaction between fungi and cattle manure to fungi population in rhizosphere of maize was significant. Result of statistical analysis in Table 1 showed that application of fungi *Fusarium* sp. and cattle manure at 22 g generate the highest fungi population in rhizosphere, $6,91 \times 10^5$ cfu g⁻¹. Application of without fungi treatment and cattle manure at 22 g resulted as the lowest fungi population in maize rhizosphere, $0,64 \times 10^5$ cfu g⁻¹.

Before experiment, soil fungi population was $1,95 \times 10^4$ cfu g⁻¹. Following inoculation of *Fusarium* sp., total fungi population was increased at any dose of cattle manure. It showed that inoculation of 10 ml of *Fusarium* sp. at the same time of cattle manure at 22 g was the best treatment and it could increase fungi population in maize rhizosphere up to 10,7 fold from the lowest fungi population ($0,64 \times 10^5$ cfu g⁻¹) (Table 1).

Table-1. Effect of Fungi and Dose of Cattle Manure to Fungi Population in Maize Rhizosphere Grown in Cd-Contaminated Tailing

Cattle manure	Fungi Population (10^5 cfu g^{-1})		
	Without Fungi	<i>Humicola</i> sp.	<i>Fusarium</i> sp.
0 g	2,83 b A	2,67 b A	4,66 a B
11 g	0,98 a A	0,81 a A	5,07 a B
22 g	0,64 a A	0,70 a A	6,91 b B

Description: values followed by the same letter are not significantly different according to 5% Duncan's Multiple Range Test.

Cadmium has adversely affects to plant growth and decreases the diversity and biomass of microorganisms, however, there are quite a lot of microorganisms that resistant to heavy metals and play a role in detoxification mechanisms [21]. In this study, in pot with cattle manure, effect of *Fusarium* sp. to fungi population was better than that of *Humicola* sp. (Table 1). *Fusarium* sp. was more rapidly growing and its hypae was more spreading fast than *Humicola* sp. According to Sanyal, et al. [22], metals ion are not harmful to the *Fusarium oxysporium*, even *Fusarium oxysporium* could grow faster with the presence od heavy metals ion. The high population of *Fusarium* sp. was also influenced by root exudates. Fungi population are common in rhizosphere due to organic matter and exudate which is a source of additional nutrients for microorganism [23].

Organic matter affects to the availability of plant nutrients which is in turn could induce the presence of microbes [11]. Increased presence and activity of microbes due to its organic manure contains carbon which is used as a source of energy [24] thus fungi population is increasing. Besides that, with addition of organic manure such as cattle manure leads to higher Cd absorption. Cattle manure is able to bind Cd ion [25] and also could help the growth of fungi through the supply of nutrition, thus fungi and plant could grow well.

3.2. Plant Dry Weight

Research revealed that there was no interaction effect between fungi and dose of cattle manure on maize dry biomass. However, there was an independent effect of cattle manure on maize dry biomass. Table 2 describes that application of cattle manure at 22 g could afford highest maize dry biomass up to 1,116 g.

Plant dry weight (root and shoot) indicate efficient level of plant metabolism [26]. Plant dry weight is consider as plant growth indicator, due to it is an accumulation net result during the plant life [27]. Maize plant treated with Cd contamination causes a decrease on plant dry weight and yield, as well as reducing nitrogen content in the plant tissues also lowering protein content in its seed [28]. The lowest maize dry weight belongs to maize plant without cattle manure, 0,348. It caused by the lack of nutrient supply and the availability of Cd lead to stunted growth.

Table-2. Effect of Cattle manure to Plant Dry Weight of Maize Grown in Cd-contaminated Tailing

Treatments	Plant Dry Weight (g)
Fungi	
Without Fungi	0,643 a
<i>Humicola</i> sp.	0,794 a
<i>Fusarium</i> sp.	0,745 a
Cattle manure	
Without manure	0,348 a
Cattle manure (11 g)	0,717 b
Cattle manure (22 g)	1,116 c

Description : values followed by the same letter are not significantly different according to 5% Duncan's Multiple Range Test.

Application of cattle manure at 22 g in maize was able to provide sufficient nutrients in order to be able to photosynthesis properly thus the plant metabolism running out well. In addition, this treatment could improve plant dry weight up to 3,2 fold than without cattle manure treatment.

3.3. Cd Content in Maize

According to statistics analysis, there was no interaction effect between fungi and cattle manure, however, there was an independent effect of manure on lowering Cd content in maize biomass (Table 3). Cadmium content in plant decreased along with manure application at 11 g and 22 g. The application of organic matter caused heavy metal bound strongly to organic compound, thus heavy metal such as Cd was unavailable to the plant. The highest Cd content in plant (16,837 mg kg⁻¹) belongs to plant without manure treatment, this happened because of there was no compound which could bind heavy metal, and plant could not survive any longer in the condition with Cd. The more Cd uptake by plant, the more Cd content in plant, generally.

Table-3. Change in Cd Content in Maize Following Fungi Inoculation and Cattle Manure

Treatments	Plant Cd Content (mg kg ⁻¹)	Cd Uptake (mg/g)
Fungi		
Without Fungi	11,014 a	7,082
<i>Humicola</i> sp.	9,140 a	7,257
<i>Fusarium</i> sp.	8,087 a	6,025
Cattle manure		
Without manure	16,837 b	5,859
Cattle manure (11 g)	6,352 a	4,554
Cattle manure (22 g)	5,052 a	5,638

Description : values followed by the same letter are not significantly different according to 5% Duncan's Multiple Range Test.

Chelation processes between metal and organic acid usually occurs while the increase of organic acids causing ligand exchange between organic anions (such as humic acid and fulvic acid to the free -OH). Increased levels of organic matter followed by increased cation exchange capacity (CEC) and clay fraction. Soil containing organic matter generally contains clay colloid

which be able to bind cations in soil [27]. Humic acid takes a role in stimulates root and plant shoot growth, furthermore humic acid has high CEC which is could bind toxic heavy metal.

Humic acid released by organic manure leads to Cd cheating by its humic, thus Cd availability in soil will be decreased [12]. In this study, plants treated with manure typically have higher plant height due to nutrient intake from organic matter and Cd binding by organic acids released by decomposed organic matter. Statistic analysis described that there was no significant effect of fungi inoculation. Regardless on statistical analysis, Cd content of whole maize biomass decreased by fungal inoculation (Table 3) although it was not significant. However, *Humicola* sp. and *Fusarium* sp. were potentially reduce Cd uptake by plant through organic acids released by those fungi, one of organic acids was oxalic acid [29]. Oxalic acid could reduce Cd toxicity in plant and could improve root length, alongside that, oxalic acid could hinder Cd uptake by plant root [30]. Cadmium immobilization by fungi as well as manure also influenced Cd uptake by roots. Our research verified that phenomenon clearly. This evidence demonstrated that Cd resistant fungi might be used as bioremediation bioagents; and that efficacy of bioremediation could improve by manure addition.

3.4. Symptoms of Cd Toxicity in Plant

Impaired growth of maize in this study due to the poor condition of soil physical characteristics, the presence of heavy metals and limited space to grow and soil nutrients. In this study, maize faced obstacles in its growth this could happened due to the plants were suffered by heavy metals Cd poisoning. Maize could accumulate Cd [31], however with the presence of Cd even in low concentrations could decrease root growth.

Cadmium has adversely effect to photosynthesis process due to Cd could interfere chlorophyll synthesis [32] and photosynthetic electron transport by inhibit the located in photosystem II [33]. Presence of Cd hinder growth of lateral roots while main root turn to brown, stiff and circular [34]. Heavy metals are toxic to maize, plant shoot had stunted growth [28]. Nitrogen content in shoot and root would be decreased in plant which is exposed by metals. Cadmium could cause wilted, yellowing leaves and blackened roots. In this study, maize plant also had the same symptoms which seem to wilt and yellowing leaves (Picture 1) and stunted growth (Picture 2).



Picture-1. Symptoms of Cd toxicity, the leaves were yellowing and wilting.



Picture-2. Picture showed different plant height of treatments of a_0b_0 (left), a_0b_1 (centre) and a_0b_2 (right). Treatment of a_0b_0 has stunted growth due to Cd existence.

4. CONCLUSION

There was an interaction between fungi isolate with cattle manure to fungi population in the rhizosphere of maize. However there was no interaction between fungi and cattle manure to plant dry weight and Cd concentration of maize plant. There was an independent effect of cattle manure to plant dry weight and Cd concentration of maize plant.

Combination of the best treatment was inoculation of *Fusarium* sp. with addition of cattle manure at 22 g pot^{-1} to fungi population in the rhizosphere of maize plant. Application of heavy metals resistant indigenous fungi and cattle manure were quite able affected decreasing Cd

concentration in plant and also capable to increase plant dry weight and total fungi population which then affect to maize plant growth.

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