doi: 10.11594/jtls.08.01.02
THE JOURNAL OF TROPICAL LIFE SCIENCE
OPEN ACCESS Freely available online
VOL. 8, NO. 1, pp. 6 – 10, January 2018
Submitted May 2016; Revised July 2017; Accepted November 2017

Cadmium (Cd) Absorption and Phenol Content in *Pogostemon* Exposed to Heavy Metals

Elly Proklamasiningsih *, Iman Budisantoso, Kamsinah

Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia

ABSTRACT

Patchouli (*Pogostemon cablin* Benth.) is an important plant used by industrial facilities to absorb cadmium (Cd) in polluted land. We performed an experiment using plant medium polluted with both Cd and lead (Pb) with added humic acid. The aims of this study were to 1) determine the effects of humic acid in growth medium contaminated with Cd and Pb on the absorption of Cd and phenol content in patchouli, and 2) determine the Cd tolerance level of the growth media. A completely randomized factorial design was used for the experiment with two factors. The heavy metals were a combination of pure $PbNO_3$ and Cd (PC) with a ratio 1 : 1, and included five concentrations: PC0 (without $PbNO_3$ and without Cd); PC1 (250 ppm $PbNO_3$ + 250 ppm Cd); PC2 (500 ppm $PbNO_3$ + 500 ppm Cd); PC3 (750 ppm $PbNO_3$ + 750 ppm Cd); PC4 (1,000 ppm $PbNO_3$ + 1,000 ppm Cd) and humic acid concentration (0; 6,000; 12,000; and 18,000 ppm). Each treatment was replicated three times. The parameters observed were plant biomass, Cd absorption, and phenol content. The application rate of humic acid to the plant medium containing heavy metals influenced the growth of patchouli, Cd absorption, and phenol content. An application rate of 12,000 ppm of humic acid reduced the toxicity of the heavy metals and increased the dry biomass and phenol content of patchouli.

Keywords: *Cadmium, humic acid, phenol, Pogostemon*

INTRODUCTION

Cultivated on contaminated metal land given this patchouli (*Pogostemon cablin* Benth.). Is an industrial plant as a producer of essential oil (patchouly oil). This plant is widely cultivated in Sumatra, specially in Aceh, North Sumatra and West Sumatra. *P. cablin* is known as Aceh patchouli which has high productivity. Until now there is no information about the influence of heavy metals on patchouli growth, therefore researchers conducted this experiment. Experiments to use heavy metals in patchouli plant is done because this plant is an industrial plant that is not consumed orally, so it is expected this plant can be plant has a good prospect as a producer of patchouli oil.

Soil heavy metal contamination can affect human health if the soil is planted with crops. Soil contamination caused by heavy metals cannot be avoided, especially near waste disposal sites, since contamination can originate from waste that contains dangerous heavy metals. In addition, in open dumping waste disposal systems, runoff water can contaminate the surrounding soil. The most common heavy metals found in contaminated soil are cadmium (Cd), lead (Pb), and chromium (Cr) [1]. Intensive care is required to amend soil contaminated with heavy metals.

Heavy metal contamination disturbs plant metabolic processes [2, 3]. Little physiological research, such as studies on absorbed particles, transportation inside the plant, and the extent of absorption through a carrier, has been reported. Plant species have different sensitivities and exhibit different abilities to accumulate heavy metals. One study found that Cd could obstruct glutamine synthesis and inhibited glutamine synthetase activity and stimulated glutamate dehydrogenase one in order to tolerate cadmium stress [4]. Another study observed the accumulation of cadmium in wheat shoots, roots and glumes could be inhibited by chlorimuron-ethyl to some extent after exposure to 1000 mg/kg Cd [5].

The toxicity of metals in soil can be reduced by

*Corresponding author:
Elly Proklamasiningsih
Faculty of Biology, Jenderal Soedirman University
Jalan Dr. Soperano No 63, Purwokerto, Indonesia 53122
E-mail: elly.proklamasi@gmail.com

How to cite:
Proklamasiningsih E, Budisantoso I, Kamsinah (2018) Cadmium (Cd) Absorption and Phenol Content in *Pogostemon* Exposed to Heavy Metals. J. Trop. Life. Science 8 (1): 6 – 10.

amending the soil with organic material [6, 7] such as humic acid [8, 9]. Humic acid is a polyelectrolytic macromolecule with –COOH and –OH functional groups; therefore, it can form complexes with metal ions [10], and is a final form of organic decomposition into acid-base-soluble fractions. Several studies [11, 12] found that application of humic acid to planting medium containing Pb, Cu, and Mn increased the metal content of corn.

Several plants can absorb and accumulate Cd in individual organs. For example, *Bolboschoenus maritimus* (L.) pepper accumulates the highest Cd concentration in its rhizomes, whereas *Salix* accumulates the highest Cd concentration in its leaves and stems [12]. One report showed that *Pistia stratiotes* could absorb Pb concentrations of up to 10 mg/kg [13].

The present study examined patchouli (*P cablin* Benth), which is an industrial plant with high economic value and high prospects development of Patchouli alcoho because it is a producer of an essential oil, known as patchouli oil [14]. Essential oils contain phenol compounds, most of which are derived from free eugenols, some eugenol acetates, and sesquiterpenes, as well as small amounts of esters, ketones, and alcohols. Patchouli oil is used widely in the cosmetics and perfume industry. The purpose of this research was to determine the effects of amending soil contaminated with Cd and Pb with humic acid on the Cd absorption and phenol content of patchouli, and to determine the highest metal concentration of the planting medium that can be tolerated by patchouli.

MATERIALS AND METHODS

Planting medium preparation

The planting medium used in this study was soil passed through a 2-mm sieve containing various humic acid and heavy metal concentrations.

The heavy metals were a combination of pure $PbNO_3$ and Cd (PC) with a ratio 1:1, and included five concentrations:

1. PC0 (without Pb NO3 and without Cd);
2. PC1 (250 ppm PbNO3 + 250 ppm Cd);
3. PC2 (500 ppm PbNO3 + 500 ppm Cd);
4. PC3 (750 ppm PbNO3 + 750 ppm Cd);
5. PC4 (1000 ppm PbNO3 + 1000 ppm Cd)

The humic acid treatment included four concentrations: 0 ppm (H0); 6,000 ppm (H1); 12,000 ppm (H2), and 18,000 ppm (H3). Distilled water was added to the planting medium until it reached field capacity, and was then wrapped in a polybag. Each polybag contained 5 kg of planting medium.

Patchouli culturing and effect of planting medium

Plant seedling cuttings originated from the first 30 cm of the stem and were directly planted in the medium after four weeks of treatment. The experiment was conducted in polybags that were 40 cm in height and 35 cm in diameter, with one plant per polybag. The parameters observed included plant biomass, Cd absorption in plant organs, and phenol content. The observations were performed when plants were 90 days old.

Determination of Cd distribution in plants

The Cd determination in plant was assayed according Hsu and Kao (2003) [15]. The plants were separated into two different parts, as roots and shoots. Both of them were dried at 80°C for 48 hours, and then ashed in Muffle furnace at 550°C for 20 hours. The ash residue was incubated with 31% HNO_3 and 17.5% H_2O_2 at 75°C for 2 hours, and then dissolved in distilled water. The Cd concentration in the digest was determined using an atomic absorption spectrophoto-meter (AA-6800, Shimadzu, Japan).

Determination of phenol content

The phenol content was determined using the Folin-Ciocalteau method [16]. The working principal of this extraction is that the measured compound (phenol) is dissolved in methanol. First, 2 g of patchouli leaves and stems were dipped into boiling alcohol for 3 min, and then pulverized. Next, 1 g of homogenate was taken for extraction in 2.5 mL of 70% methanol using a rotary shaker. This process was repeated to optimize the amount of phenol compounds while minimizing the amount of sediment. The phenol extract was formed, set aside for a few minutes until sediment formed, and then centrifuged at 5,000 rpm for 10 minutes.

The phenol content of the extract was determined at an extract concentration of 1 mg/mL. The reaction was made using 0.5% methanol-extracted compound, 2.5 mL 10% Folin-Ciocalteau reagent dissolved in water, and 2.5 mL of 7.5% $NaHCO_3$. The blank consisted of 0.5 mL methanol, 2.5 mL 10% Folin-Ciocalteau reagent in water, and 2.5 mL of 7.5% $NaHCO_3$. The sample was incubated in an incubator at 45°C for 45 minutes. Absorbance was determined with a spectrophotometer at λ max = 765 nm. Three samples were prepared for each treatment for the analysis and the average values were

determined. The same procedure was repeated using gallic acid standard and the calibration curve was interpreted. Based on the measured absorbance, the phenolic content (mg/mL) was determined from the calibration curve; therefore, the phenol concentration inside the extract was stated in terms of gallic acid equivalent (mg GAE/g extract).

Experimental design

This research uses experimental method with Completely Randomized Design (CRD) factorial pat-tern with two factors. Cd and $PbNO_3$ concentrations (PC0; PC1; PC2; PC3; and PC4) and humic acid concentration (H0; H1; H2; H3). Each combination of treatments was repeated three times.

Statistical analysis

Analysis of variance with Tukey's test was used for the statistical analysis in *SPSS* for *Windows* software.

RESULTS AND DISCUSSION

The results showed that the use of humic acid in planting medium exposed to heavy metals on nilam cuttings caused significant effect on root Cd concentration ($F = 1283.7$; $p < 0.00$), Cd rod ($F = 932.5$; $p < 0.00$), leaf Cd ($F = 773.1$, $P < 0.00$) absorbed by plant and plant biomass ($F = 8,895$; $p < 0.00$). Giving humic acid 12,000 ppm can reduce metal toxicity on exposed media Pb and Cd 500 ppm (H2PC2) concentration so that plant biomass is increased (Table 1).

The ability of patchouli to absorb Cd can be measured through the highest plant biomass and highest

Table 1. Plant biomass (g) and Cd concentration in patchouli root, stem, and leaf (mg/kg) for each treatment

No.	*Treatment*	*Mean (± SD)*			
		Biomass (g)	*Cd (root) (mg/kg)*	*Cd (stem) (mg/kg)*	*Cd (leaf) (mg/kg)*
1.	H0PC0	29.96 (± 0.63) fghi	0.03 (± 0.00) n	0.03 (± 0.00) n	0.02 ± 0.00 l
2.	H0PC1	28.06 (± 0.30) ghij	0.07 (± 0.00) j	0.04 (± 0.00) m	0.02 ± 0.00 j
3.	H0PC2	26.70 (± 0.10) hij	0.08 (± 0.00) h	0.05 (± 0.00) j	0.03 ± 0.00 gh
4.	H0PC3	24.20 (±0.06) ij	0.09 (± 0.00) g	0.06 (± 0.00) h	0.04 ± 0.00 e
5.	H0PC4	22.09 (± 0.08) j	0.09 (± 0.00) f	0.06 (± 0.00) h	0.05 ± 0.00 c
6.	H1PC0	38.11 (± 0.29) cde	0.03 (± 0.00) m	0.03 (± 0.00) n	0.02 ± 0.00 l
7.	H1PC1	37.42 (± 0.05) cde	0.06 (± 0.00) k	0.05 (± 0.00) l	0.03 ± 0.00 h
8.	H1PC2	35.92 (± 0.25) cdef	0.09 (± 0.00) g	0.05 (± 0.00) l	0.03 ± 0.00 f
9.	H1PC3	33.64 (± 0.16) defg	0.09 (± 0.00) f	0.05 (± 0.00) k	0.04 ± 0.00 d
10.	H1PC4	28.11 (± 7.87) ghij	0.10 (± 0.00) d	0.08 ±0.00 e	0.05 ± 0.00 a
11.	H2PC0	45.90 (± 0.58) a	0.03 (± 0.00) mn	0.03 ±0.00 n	0.02 ± 0.00 l
12.	H2PC1	35.97 (± 8.69) cdef	0.06 (± 0.00) l	0.05 ±0.00 jk	0.02 ± 0.00 jk
13.	H2PC2	39.96 (± 0.60) abcd	0.11 (± 0.00) c	0.08 ±0.00 c	0.05 ± 0.00 b
14.	H2PC3	38.68 (± 0.55) bcde	0.10 (± 0.00) e	0.06 ±0.00 i	0.05 ± 0.00 a
15.	H2PC4	38.42 (± 0.46) cde	0.11 ± 0.00 b	0.08 ±0.00 b	0.03 ± 0.00 i
16.	H3PC0	45.03 (± 0.45) ab	0.03 ± 0.00 m	0.03 ±0.00 n	0.02 ± 0.00 m
17.	H3PC1	41.28 ± 0.24 abc	0.08 ± 0.00 i	0.09 ±0.47 a	0.02 ± 0.00 k
18.	H3PC2	33.05 ± 9.66 efgh	0.09 ± 0.00 f	0.08 ±0.00d	0.03 ± 0.00 i
19.	H3PC3	36.42 ± 0.44 cde	0.10 ± 0.00 d	0.07 ± 0.00 g	0.03 ± 0.00 g
20.	H3PC4	28.90 ± 8.01 ghi	0.12 ± 0.00 a	0.07 ± 0.00 f	0.03 ± 0.00 gh

Note: - Means followed by the same letters are not as different for each treatment means ($p < 0.00$).

- H0: no humic acid; H1: 6,000 ppm humic acid; H2: 12,000 ppm humic acid; H3: 18,000 ppm humic acid; PC0: no $PbNO_3$ and without Cd; PC1: 250 ppm $PbNO_3$ + 250 ppm Cd; PC2: 500 ppm PbNO3 + 500 ppm Cd; PC3: 750 ppm PbNO3 + 750 ppm Cd; PC4: 1000 ppm Pb NO3 + 1000 ppm Cd

Table 2. Patchouli stem and leaf phenolic content with humic acid and heavy metal addition

No.	Treatment	Mean (± SD) (mg/mL)	
		Stem phenol concentration	Leaf phenol concentration
1.	H0PC0	0.10 (± 0.01) i	0.18 (± 0.01) g
2.	H0PC1	0.06 (± 0.01) k	0.11 (± 0.01)j
3.	H0PC2	0.05 (± 0.01) l	0.09 (± 0.01) k
4.	H0PC3	0.04 (± 0.01) lm	0.08 (± 0.01) k
5.	H0PC4	0.03 (± 0.01) m	0.08 (± 0.01) k
6.	H1PC0	0.18 (± 0.01) bc	0.26 (± 0.01) c
7.	H1PC1	0.14 (± 0.01) fg	0.17 (± 0.01) g
8.	H1PC2	0.12 (± 0.01) h	0.16 (± 0.01) h
9.	H1PC3	0.07 (± 0.01) k	0.14 (± 0.01) i
10.	H1PC4	0.06 (± 0.01) k	0.10 (± 0.01) j
11.	H2PC0	0.20 (± 0.01) a	0.30 (± 0.01) a
12.	H2PC1	0.17 (± 0.01) cd	0.30 (± 0.01) a
13.	H2PC2	0.16 (± 0.01) de	0.30 (± 0.01) a
14.	H2PC3	0.15 (± 0.01) ef	0.28 (± 0.01) b
15.	H2PC4	0.12 (± 0.01) h	0.25 (± 0.01) d
16.	H3PC0	0.18 (± 0.01) b	0.26 (± 0.01) c
17.	H3PC1	0.14 (± 0.01) fg	0.22 (± 0.01) e
18.	H3PC2	0.14 (± 0.01) g	0.20 (± 0.01) f
19.	H3PC3	0.11 (± 0.01) hi	0.14 (± 0.01) i
20.	H3PC4	0.09 (± 0.01) j	0.11 (± 0.01) j

Note: - Means followed by the same letters are not as different for each treatment means ($p < 0.00$).

- H0: no humic acid; H1: 6,000 ppm humic acid; H2: 12,000 ppm humic acid; H3: 18,000 ppm humic acid PC0: no $PbNO_3$ and without Cd; PC1: 250 ppm $PbNO_3$ + 250 ppm Cd; PC2: 500 ppm $PbNO_3$ + 500 ppm Cd; PC3: 750 ppm $PbNO_3$ + 750 ppm Cd; PC4: 1000 ppm $PbNO_3$ + 1000 ppm Cd

Cd concentration which can be translocated to leaf organ. In H2PC2 it is evident that during the Cd translocation from the root organ to the leaf organ inside the xylem does not cause damage to the molecules present in the xylem.

Cd accumulated on the root, and only some was translocated to the leaves and stems (Table 1). This indicated that Cd had complexed with humic acid, reducing its mobility. Cd on the plant increased with increasing humic acid and heavy metal concentrations, probably due to the formation of chelates from humic acid. Humic compounds can effectively bind to certain elements. Metal absorption by plant systems occurs along with nutrient uptake from soil water. Although high concentrations of metals can be toxic to plants, humic acid addition can bind to such toxic metals in soil water [10].

Plants exposed to Cd and Pb in soil without humic acid amendment had lower biomasses. Amendment with 1,200 ppm humic acid decreased the metal toxicity in the exposed medium to 1,000 ppm. Moreover, 1,200 ppm and 1,800 ppm humic acid increased the dry plant biomass and Cd concentration adsorbed on the root. It also decreased the metal toxicity threshold to 1000 ppm Cd/Pb. These results indicated that chelates formed in the presence of humic acid. Humic compounds have been found to be very effective in binding to metals via complexation [17].

Table 2 presents the stem and leaf phenolic contents in plants exposed to heavy metals and humic acid. Treatments that resulted in higher phenol concentrations also resulted in high dry biomasses. Phenol biosynthesis begins with malonic acid and shikimate acid, while these

compounds form from monosaccharides during photosynthesis. Therefore, high rates of photosynthesis result in high production of phenol as a secondary metabolite. Plant growth parameters can be used as response indicators for plants cultivated in contaminated planting medium. Application of 1,200 ppm humic acid can increase the leaf phenol concentrations of plants in medium contaminated with as much as 750 ppm Cd/Pb.

CONCLUSION

Applying humic acid to plant medium contaminated with heavy metals can affect Cd absorption, patchouli growth, and patchouli oil content. Application of 1,200 ppm humic acid can decrease heavy metal toxicity, thereby increasing the dry biomass and phenolic content of patchouli.

ACKNOWLEDGMENT

The authors would like to thank the LPPM Universitas Jenderal Soedirman, which funded this research through the Program Penelitian Fundamental in 2014.

REFERENCES

1. Li T, Cheng H, Oh K, Hosono S (2014) Effect of humic acid and bacterial manure on distribution of heavy metals in different organs of maize. International Journal of Environmental Science and Development 5 (4): 393 – 397. doi: 10.7763/IJESD.2014.V5.516.
2. Chen X, Wang J, Shi Y et al. (2011) Effects of cadmium on growth and photosynthetic activities in pakchoi and mustard. Botanical Studies 52 (1): 41 – 46.
3. Gong X, Yin L, Chen J, Guo C (2015) Overexpression of the iron transporter *NtPIC1* in tobacco mediates tolerance to cadmium. Plant Cell Reports 34 (11): 1963 – 1973. doi:10.1007/s00299-015-1843-4.
4. Afef NH, Houda G, Chiraz CH (2012) Response of *Arabidopsis thaliana*, seedlings to cadmium in relation to ammonium availability. Bulletin of Environmental Contamination and Toxicology 89 (6): 1175 – 1180. doi: 10.1007/s00128-012-0840-3.
5. Jin C, Zhou Q, Zhou Q, Fan J (2010) Effects of chlorimuron-ethyl and cadmium on biomass growth and cadmium accumulation of wheat in the phaiozem area, Northeast China. Bulletin of Environmental Contamination and Toxicology 84 (4): 395 – 400. doi: 10.1007/s00128-009-9635-6.
6. Saha JK, Panwar NR, Coumar MV (2013) Effect of methods of preparation on distribution of heavy metals in different size fractions of municipal solid waste composts. Environmental Monitoring and Assessment 185 (11): 8815 – 8821. doi: 10.1007/s10661-013-3214-3.
7. He XS, Xi BD, Li D et al. (2014) Influence of the composition and removal characteristics of organic matter on heavy metal distribution in compost leachates. Environmental Science and Pollution Research 21 (12): 7522 – 7529. doi: 10.1007/s11356-014-2674-5.
8. Sardashti A, Angtarash MH, Asadi A (2015) Defining the metal ions contaminants in the leaves and growing place soil of cultivated *A. triphylla* plant with addition of humic substances to root. International Journal of Agriculture and Crop Sciences 8 (2): 142 – 148.
9. Topcuoglu B (2013) Effects of humic acids on the phytoextraction efficiency of sludge applied soil. International of Chemical, Environmental and Biological Sciences 1 (1): 21 – 24.
10. Khaled H, Fawy HA (2011) Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. Soil and Water Research 6 (1): 21 – 29.
11. Arancon NQ, Edwards CA, Lee S, Byrne R (2006) Effects of humic acids from vermicomposts on plant growth. European Journal of Soil Biology 42 (Supplement 1): S65 – S69. doi: 10.1016/j.ejsobi.2006.06.004.
12. Ling T, Jun R, Fangke Y (2011) Effect of cadmium supply levels to cadmium accumulation by *Salix*. International Journal of Environmental Science and Technology 8 (3): 493 – 500. doi: 10.1007/BF03326235.
13. Proklamasiningsih E, Hernayanti H (2010) Rizofiltrasi logam Pb (Plumbum) pada beberapa jenis tumbuhan air. Biosfera 27 (1): 30 – 37.
14. Xu F, Yang Q, Wu L et al. (2017) Investigation of inclusion complex of patchouli alcohol with β-cyclodextrin. PloS One 12 (1): e0169578. doi: 10.1371/journal.pone.0169578
15. Hsu YT, Kao CH (2003) Role of abscisic acid in cadmium tolerance of rice (*Oryza sativa* L.) seedlings. Plant Cell Environmental 26 (6): 867 – 874. doi: 10.1046/j.1365-3040.2003.01018.x.
16. Stankovic MS (2011) Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. Kragujevac Journal of Science 33 (2011): 63 – 72.
17. Mastrángelo M, Afonso MDS, Ferrari L (2011) Cadmium toxicity in tadpoles of *Rhinella arenarum* in relation to calcium and humic acids. Ecotoxicology 20 (6): 1225 – 1232. doi: 10.1007/s10646-011-0667-4.