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 PbNO₃ and Cd (PC) with a ratio 1:1, and included five concentrations: PC0 (without PbNO₃ and without Cd); PC1 (250 ppm PbNO₃ + 250 ppm Cd); PC2 (500 ppm PbNO₃ + 500 ppm Cd); PC3 (750 ppm PbNO₃ + 750 ppm Cd); PC4 (1,000 ppm PbNO₃ + 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:
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 alcohol 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, 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 PbNO₃ 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); 6000 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 cultivation 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% HNO₃ and 17.5% H₂O₂ at 75°C for 2 hours, and then dissolved in distilled water. The Cd concentration in the digest was determined using an atomic absorption spectrophotometer (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% NaHCO₃. The blank consisted of 0.5 mL methanol, 2.5 mL 10% Folin-Ciocalteau reagent in water, and 2.5 mL of 7.5% NaHCO₃. 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>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Biomass (g)</th>
<th>Cd (root) (mg/kg)</th>
<th>Cd (stem) (mg/kg)</th>
<th>Cd (leaf) (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>H0PC0</td>
<td>29.96 ± 0.63</td>
<td>0.03 ± 0.00 n</td>
<td>0.03 ± 0.00 n</td>
<td>0.02 ± 0.00 l</td>
</tr>
<tr>
<td>2.</td>
<td>H0PC1</td>
<td>28.06 ± 0.30</td>
<td>0.07 ± 0.00 j</td>
<td>0.04 ± 0.00 m</td>
<td>0.02 ± 0.00 j</td>
</tr>
<tr>
<td>3.</td>
<td>H0PC2</td>
<td>26.70 ± 0.10</td>
<td>0.08 ± 0.00 h</td>
<td>0.05 ± 0.00 j</td>
<td>0.03 ± 0.00 gh</td>
</tr>
<tr>
<td>4.</td>
<td>H0PC3</td>
<td>24.20 ± 0.06</td>
<td>0.09 ± 0.00 g</td>
<td>0.06 ± 0.00 h</td>
<td>0.04 ± 0.00 e</td>
</tr>
<tr>
<td>5.</td>
<td>H0PC4</td>
<td>22.09 ± 0.08</td>
<td>0.09 ± 0.00 f</td>
<td>0.06 ± 0.00 h</td>
<td>0.05 ± 0.00 c</td>
</tr>
<tr>
<td>6.</td>
<td>H1PC0</td>
<td>38.11 ± 0.29</td>
<td>0.03 ± 0.00 m</td>
<td>0.03 ± 0.00 n</td>
<td>0.02 ± 0.00 l</td>
</tr>
<tr>
<td>7.</td>
<td>H1PC1</td>
<td>37.42 ± 0.05</td>
<td>0.06 ± 0.00 k</td>
<td>0.05 ± 0.00 l</td>
<td>0.03 ± 0.00 h</td>
</tr>
<tr>
<td>8.</td>
<td>H1PC2</td>
<td>35.92 ± 0.25</td>
<td>0.09 ± 0.00 g</td>
<td>0.05 ± 0.00 l</td>
<td>0.03 ± 0.00 f</td>
</tr>
<tr>
<td>9.</td>
<td>H1PC3</td>
<td>33.64 ± 0.16</td>
<td>0.09 ± 0.00 f</td>
<td>0.05 ± 0.00 k</td>
<td>0.04 ± 0.00 d</td>
</tr>
<tr>
<td>10.</td>
<td>H1PC4</td>
<td>28.11 ± 0.07</td>
<td>0.10 ± 0.00 d</td>
<td>0.08 ± 0.00 e</td>
<td>0.05 ± 0.00 a</td>
</tr>
<tr>
<td>11.</td>
<td>H2PC0</td>
<td>45.90 ± 0.58</td>
<td>0.03 ± 0.00 mn</td>
<td>0.03 ± 0.00 n</td>
<td>0.02 ± 0.00 l</td>
</tr>
<tr>
<td>12.</td>
<td>H2PC1</td>
<td>35.97 ± 0.89</td>
<td>0.06 ± 0.00 l</td>
<td>0.05 ± 0.00 jk</td>
<td>0.02 ± 0.00 jk</td>
</tr>
<tr>
<td>13.</td>
<td>H2PC2</td>
<td>39.96 ± 0.60</td>
<td>0.11 ± 0.00 c</td>
<td>0.08 ± 0.00 c</td>
<td>0.05 ± 0.00 b</td>
</tr>
<tr>
<td>14.</td>
<td>H2PC3</td>
<td>38.68 ± 0.55</td>
<td>0.10 ± 0.00 e</td>
<td>0.06 ± 0.00 i</td>
<td>0.05 ± 0.00 a</td>
</tr>
<tr>
<td>15.</td>
<td>H2PC4</td>
<td>38.42 ± 0.46</td>
<td>0.11 ± 0.00 b</td>
<td>0.08 ± 0.00 b</td>
<td>0.03 ± 0.00 i</td>
</tr>
<tr>
<td>16.</td>
<td>H3PC0</td>
<td>45.02 ± 0.45</td>
<td>0.03 ± 0.00 m</td>
<td>0.03 ± 0.00 n</td>
<td>0.02 ± 0.00 m</td>
</tr>
<tr>
<td>17.</td>
<td>H3PC1</td>
<td>41.28 ± 0.24</td>
<td>0.08 ± 0.00 i</td>
<td>0.09 ± 0.00 g</td>
<td>0.02 ± 0.00 k</td>
</tr>
<tr>
<td>18.</td>
<td>H3PC2</td>
<td>33.05 ± 9.66</td>
<td>0.09 ± 0.00 f</td>
<td>0.08 ± 0.00 d</td>
<td>0.03 ± 0.00 i</td>
</tr>
<tr>
<td>19.</td>
<td>H3PC3</td>
<td>36.42 ± 0.44</td>
<td>0.10 ± 0.00 d</td>
<td>0.07 ± 0.00 g</td>
<td>0.03 ± 0.00 g</td>
</tr>
<tr>
<td>20.</td>
<td>H3PC4</td>
<td>28.90 ± 8.01</td>
<td>0.12 ± 0.00 a</td>
<td>0.07 ± 0.00 f</td>
<td>0.03 ± 0.00 gh</td>
</tr>
</tbody>
</table>

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 Pb NO$_3$ + 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].

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