Effect of lead on growth and physiological responses of Hanjuang plant (*Cordyline fruticosa*)

L Herlina1*, B Widianarko 2 and H R Sunoko 3

1 Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Semarang-Indonesia
2 Faculty of Agricultural Technology, Soegijapranata Catholic University, Semarang -Indonesia
3 Faculty of Medicine, Diponegoro University, Semarang-Indonesia

Corresponding author: linaherlina@mail.unnes.ac.id

Abstract. The research aimed to analyze the growth and physiological response of *Cordyline fruticosa* plant. In this study, plants were grown on soil contaminated with lead at various concentrations. The parameters observed included leaf area, the leaves number, stem dry biomass, leaf and roots, photosynthetic pigment, and antioxidant enzyme activity (CAT and APX) which were analyzed after 60 days. The results were plants growth, dry biomass, and photosynthetic pigments decreased with the increasing of lead concentrations while the activity of ascorbate peroxidase (APX) and catalase (CAT) increased. This study signifies the potential of *C. fruticosa* for lead tolerance.

1. Introduction

Lead (Pb) is a heavy metal element that has potential to pollute the environment of water, soil, and air. Pb was the second most dangerous element after As [1] to animals, plants, animals, and humans [2]. The main sources of lead pollution in the environment are from transportation, industrial effluents, recycling, pesticides, and fertilizers. The accumulation of lead metal in the soil will affect plants especially in the root system [3]. Lead has low solubility and translocation from the roots to the organs on the ground, so a lot of lead accumulates in the roots [4]. The absorption process of lead metal from plant root to leaf forms complex compounds and so lead taken to plant tissue [5].

Plants would respond to the entry of lead into the tissues as environmental stress and provide changes as an adaptation response. Lead accumulation causes systemic damage that will affect the metabolic function of plants, such as inhibiting photosynthesis, respiration, germination, and crop production [6]. Lead has an impact on plants such as chlorosis, damages cell walls, and decreases chlorophyll biosynthesis. One of the physiological responses can be seen from the chlorophyll levels, while the enzymatic response can be seen from the activity of antioxidant enzymes [7].

Antioxidant enzymes an important as a protection system for plant oxidative stress was caused by the presence of heavy metals [8]. Antioxidants and enzymes protect plants from oxidative damage, the combined activity of SOD, POX, and CAT are needful for the toxic defense of Reactive Oxygen Species (ROS). ROS are toxic compounds that are produced from aerobic metabolisms during the electron transfer that takes place in mitochondria, chloroplasts and peroxisomes. The aim of this study was to determine the effect of Pb on growth and physiological response of the *C. fruticosa* plants, and
assess the sensitivity to the lead ions presence in the environment, which was the basis for selecting certain plant species that are tolerant to unprofitable of environmental conditions.

2. Methods

2.1. Materials
The soil was collected from the garden taken from a depth of 15-20 cm, air-dried, and sieved with 2 mm mesh size. The physical and chemical characteristics of the soil that will be used in this study were: clay soil texture, pH 6.61, 0.44 C-organic, P 137.93 mg / 100g, K 13.23 mg / 100g, CEC 16.6 cmol(+). Kg(-1), 1.22 cm / hour permeability, 11.25% air content, 1.18 g / cm3 soil volume, 2.15 g / cm3 specific gravity 45.12% porosity and 27.47 mg. kg(-1) lead [9].

2.2. Growth condition and plant analysis
The planting medium consisted of a mixture of 1500 g dry soil and 500 g vermicompost. Pb(NO3)2 was given as much 0, 250 mg.kg(-1) and 375 mg.kg(-1). Plants were harvested after eight weeks of lead exposure and data about plant growth recorded as the area of leaves, leaf number, and biomass. After the were harvested, the fresh weight of the stem, leaf, and root were measured, then the parts of the plant were oven-dried at 70°C for three days and the dry weights were measured.

2.3. Chlorophyll content and enzymatic antioxidants analysis
Chlorophylls contents from the extraction of fresh leaves, with 80% acetone, chlorophyll a and chlorophyll b content were measured using UV-VIS spectrophotometer (PerkinElmer Lambda 25) at 665 and 649 nm (Lichtenthaler, 1987).

APX was determined to the method by Nakano and Asada (1987). The reaction mixture was 3 ml, consisted of 100 µl ascorbate 5 mM, 2.8 ml potassium phosphate buffer 100 mM pH 7.0, 100 µl H2O2 0.3 mM, and enzyme extract. Ascorbate peroxidase (APX) enzyme activity measured using UV-VIS spectrophotometer at 290 nm wavelength. Catalase (CAT) enzyme activity measured according to the method by Aebi (1984). The reaction mixture 3 ml, consisted of 100 µl H2O2, 300 mM, 2.8 ml of phosphate buffer pH 7.0 and 100 µl enzyme extract. The catalase was measured using Uv-VIS spectrophotometer at 240 nm wavelength.

3. Results and Discussion
After two months exposed to Pb metal, there was no difference Leaf area and leaves number of C. fruticosa in control and treated group (Fig.1). Leaf area and leaves number were not significantly affected under metal Pb stressed while the stems, roots, leaves dry weight significantly decreased after two months exposed to Pb metal (Table 1).

| Table 1. Effects of various Pb concentration on root, stem, and leaf dry biomass of C. fruticosa. |
|---------------------------------|---------|---------|--------|
| Pb concentration (mg.kg(-1))    | Root    | Stem    | Leaf   |
| 0                              | 1778.67±166.17b | 4844.33±276.4 c | 4289.33±263.96 b |
| 250                            | 1497.0±167.29 a | 4040.0±199.21b | 3125.0±111.13 a |
| 375                            | 1114.0±257.73 a | 3375.67±115.52a | 3096.0±148.35 a |

* different letters indicate that values are significantly different at P<0.05

The LSD test results of root, and stem dry weight in all treatments were significantly different from the control. The dry weight of leaves exposed to Pb metal of 250 mg.kg(-1) was not significantly different from metal exposure of 375 mg.kg(-1). This study result was same as the research [10] which reported that treatment of 50 ppm of lead caused a decrease in root dry weight in alang-alang.
seedlings. Lead exposure causes inhibit root growth due to deficiency of nutrients and an imbalance in consequence of the presence of lead with lead accumulation and ion absorption [11]. The decrease in stem biomass exposed to lead, due to inhibition of plant growth, was further explained by [12] that stem dry biomass is also affected by plant diameter. The Plants exposed to lead cause a decrease in stem diameter and diameter of vascular bundles. The decrease in leaves biomass is a sign of plant toxicity. The form of plant adaptation to toxicity through detoxification by shedding leaves [13].

The effect of various concentrations of Pb treatment (250 and 375 mg.kg\(^{-1}\)) on chlorophyll content (mg/L) of \textit{C. fruicosa} was depicted in Figure 2. Chlorophyll contents decreased with increasing Pb levels in the growth medium. The reductions of chlorophyll a, chlorophyll b, and chlorophyll total contents were 8.42% a,13.49%; 7.97%,10.82%; 2.12%, 8.16% respectively in treated plants compared to the control. Pb metal exposure had no significant effect on chlorophyll content.

The inhibition of photosynthesis occurs because disruption of metal ions that bind photosynthetic enzymes and chloroplast membranes [14]. Chlorophyll biosynthesis was inhibited in the presence of lead by interfering with the absorption of crucial photosynthetic pigment elements, such as potassium, magnesium, calcium, potassium, and ions [15], inhibiting porphobilinogen deaminase and aminolevulinic acid (ALA) dehydrase enzymes needed in chlorophyll biosynthesis.
Figure 3. Effect of various Pb concentrations on ascorbate peroxidase (APX) and catalase (CAT) of *C. fruicosa*. Different letters indicate that values are significantly different at P<0.05

The results of the enzyme antioxidant activity showed that the activity of catalase and ascorbate peroxidase antioxidant enzymes was significantly increased at Pb concentration in *C. fruicosa*. The LSD test results of catalase and ascorbate peroxidase in all treatments were significantly different from the control. Plants will produce antioxidants in the form of catalase and ascorbate peroxidase in response to heavy metals. The first defense system against ROS is carried out by the SOD enzyme which catalyzes the reaction of superoxide radical release to H$_2$O$_2$ and O$_2$. APX is the main enzyme in the ascorbate cycle that plays a role in converting H$_2$O$_2$ to H$_2$O and O$_2$. Catalase enzyme plays a role in reducing excess ROS that occurs under stress conditions. A balanced condition between SOD activity and active APX or CAT inside the cell is critical to keep in order superoxide and H$_2$O$_2$ radicals are always in steady-state. Catalase and ascorbate peroxidase are key enzymes to defend cells against oxidative stress due to ROS such as H$_2$O$_2$. The degradation of H$_2$O$_2$ into H$_2$O and O$_2$ will be carried by catalase to the peroxisomes to the vacuole, cell wall, and cytosol, so it will not interfere with plant metabolism.

4. Conclusion
This study results showed that leaves number, leaf area, biomass, and chlorophyll content of *C. fruicosa* decreased with increasing Pb concentration, while the activity of antioxidant enzymes (CAT and APX) increased with increasing Pb concentrations. This study proved that *C. fruicosa* can accumulate and tolerate Pb stress by changing physiological character. The ability of this plant to accumulate and tolerate metal emphasize makes this species an excellent candidate to recover metal contaminated soil.

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