Conference Paper

The Growth and Antioxidative Responses of *Sonchus oleraceus* (Linnaeus, 1753) Under Cu(II), Pb(II), Cd(II) and Cr(VI) Stress Condition

Nur Hidayaturrohmah[1], Sri Kasmiyati[1], Sucahyo[1], and Zane Vincēviča-Gaile[2]

[1]Faculty of Biology, Satya Wacana Christian University, Jl. Diponegoro 52-60, Salatiga, Indonesia 50711
[2]Department of Environmental Science, University of Latvia, Raiņa Bulvāris 19, Riga, Latvia, LV-1586, European Union

Abstract

Plants growing in soil containing heavy metal polutan such as chromium (Cr), lead (Pb), cadmium (Cd) and copper (Cu) will be stunted, and increase production of Reactive Oxygen Species (ROS). In dealing with the excess amount of ROS, plants have an enzymatic defense system, using superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxide (APX). The aim of this study was to determine and analyze the *Sonchus oleraceus* (Linnaeus, 1753) plant response to heavy metals stress, seen from the growth and antioxidative defense enzymatically. Research carried out using a Completely Randomized Design (CRD) with four treatments and five replicates. The metal treatment was 10 mg · L^{-1}. The presence of heavy metals in the growing medium significantly decreased the plant height and leaf area, so the impact is on the weight of wet and dry weight. The metal treatments of Cr, Cd, Pb and Cu increase the activity of SOD and APX enzymes but decrease the activity of the CAT enzyme. Chromium is a metal that has a significant influence on the growth and activity of SOD, APX, and CAT enzymes in *S. oleraceus*.

Keywords: antioxidative responses; growth; heavy metal; *Sonchus oleraceus;* toxicity.

Corresponding Author:
Sri Kasmiyati
kas@staff.uksw.edu

Received: 11 February 2017
Accepted: 08 March 2017
Published: 26 March 2017

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the ICBS Conference Committee.

OPEN ACCESS

1. Introduction

Heavy metal pollution is one of the problems of the environment in which the presence of a metal in the soil, air and waters exceeds the threshold and being toxic. Heavy metal is an element that has a density of more than 5 g · cm^{-3} [1]. Heavy metals are naturally contained in the soil but can also come from agricultural and industrial waste as well as the burning of transportation. On soil, heavy metal pollution lead to various problems including the disruption of the growth of soil organisms and plants as well as the problems associated with aesthetics (discoloration and odor) [2].

Heavy metals can be essential for plants for example copper (Cu), but Cu is only needed in very small amounts, the exessive concentration can be toxic to plants. According to Rokhmah research, 0.3 mg · L^{-1} dissolved Cu concentrations disrupt the growth of rice plants [3]. Heavy metals that are non-essential for plants such as lead

(Pb), cadmium (Cd) and chromium (Cr). The metals are toxic to plants despite in the low amount. In plants, high concentrations of Pb can interfere the process of photosynthesis, chlorophyll synthesis and synthesis of antioxidant enzymes [4]. Cd is very reactive and can activate a lot of enzyme activity that needed by the cell [5]. Cd accumulates in potato plants inhibit the formation of chlorophyll thereby reducing biomass [6]. Cr accumulation can increase the activity of antioxidative enzymes and decrease the rate of growth in *Sorghum* plants [7, 8].

The plasma membrane is the first part that is in direct contact with heavy metals, so that it will disrupt the absorption of other essential elements. Along with the heavy metal absorption of nutrients and water uptake by plant roots, then the compound will accumulate to plant's parts such as roots, stems, and leaves [9]. The presence of heavy metals can disrupt metabolic processes and developmental germination [10]. Heavy metals cause the decrease of leaf area and plant dry weight, root plasma membrane damage, a decrease in photosynthetic activity and the growth of bean plants [11]. Heavy metal also affects the lowered spinach plant leaf area, causing the death of cells in the leaf tips and leaf growth rate slows [12].

Heavy metal toxicity in plants are also associated with the producer of ROS (Reactive Oxygen Species) which reached 30 times higher than normal conditions [13]. ROS are oxygen derivatives oxidizing compounds that are highly reactive. ROS can be H_2O_2 (hydrogen peroxide), O_2 (superoxide radicals), OH (hydroxyl radical), HO_2 (hydrogen oxide radicals), etc. [14]. Under normal conditions, ROS are produced in low quantities in the cell organelles, but when the amount exceeds the amount of ROS increases antioxidative defense, it can cause a condition called oxidative stress. ROS can damage the membrane and react with biomolecules, thereby disrupting normal cellular structure and function are indicated by abnormalities (symptoms) [15].

Antioxidant defenses needed to reduce oxidative damage from ROS. The defense can be enzymatic and non-enzymatic [16]. In enzymatically for example using enzymes superdioxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Balance the activity of SOD, CAT and APX are important in suppressing ROS in cells. SOD is an initial defense against ROS, followed by CAT and APX work [17]. SOD functions to change the O_2 into H_2O_2. CAT works to change the H_2O_2 into H_2O and O_2. APX function converts H_2O_2 into H_2O and MDHA (monodehydroascorbate) [18]. Oxidative defense becomes an important mechanism for plants tolerant to fight heavy metal stress, because the plant cannot move so susceptible to heavy metal stress in their habitat.

Sonchus oleraceus (Linnaeus, 1753) or known as *tempuyung* is a weed that is included in the annual plant that grows wild in the area with an altitude of 200 m to 2 200 m above sea level in areas exposed to sunlight and a little shade. This plant is used as food and medicine. *S. oleraceus* can be used as test plants as easily obtained and grown, its life cycle is relatively short at around 3 mo and widely used as a medicinal plant for diseases such as inflammation, kidney stones and blood purifier.

The research using *S. oleraceus* the treatment of heavy metal is still rare. Most of it only research on the content of *S. oleraceus* for use as a medicine. The aim of this

study was to determine and analyze the plant response to stress *S. oleraceus* heavy metals, in terms of the growth of and enzymatic antioxidative defense.

2. Materials and Methods

Research carried out experimentally using a Completely Randomized Design (CRD) with four treatments and five replications. The research carried out with four treatments and five replications. Treatment given is heavy metal stress Cr (VI), Cd (II), Cu (II) and Pb (II) with a concentration of 10 mg · L^{-1} were determined by a preliminary test. Data were analyzed using one way ANOVA at 5% significance level to determine the effect of treatment on each parameter. Turkey continued testing to determine the mean or average mutually significantly different. The program used was SPSS 17.0 software.

2.1. The planting of seeds of S. oleraceus and preparation of test plants

The germinated seeds in one pot using a mixture of soil and compost medium with a ratio of 1 : 1. After 30 d, the test plants were selected based on height and number of leaves uniform. Test plant was moved in polythene bags containing soil medium, one polybag one plant, then acclimatized for 7 d.

2.2. The treatment of heavy metal stress

Cr, Cd, Cu and Pb concentrations of 10 mg · L^{-1} are given at the beginning of treatment. Heavy metal compounds used are $K_2Cr_2O_7$, $CdCl_2$, $CuSO_4$ and Pb $(CH_3COO)_2$ obtained from the laboratory of biochemistry and Molecular Cell Biology, Faculty of Biology, Satya Wacana Christian University, Salatiga. The solution of heavy metals are provided on each polybag (500 g soil) as many as 50 mL. Treatment was given for 30 d. During the treatment period, each of the plants are watered using tap water 20 mL.

2.3. The measurement of plant growth

The growth measured by plant height, number of leaves, leaf area, dry weight and wet weight. Measurements of plant height and number of leaves is done during the treatment, while the leaf area, fresh weight and dry weight at the end of the treatment time.

2.4. The height of plant

Plant height measurements performed every 2 d. Plant measured from the base of the stem to the highest branches of plants.

2.5. Numbers of leaves and leaf area

Observation of the number of leaves every 2 d. Whole leaf is calculated including dried and withered, except the leaves are still buds. Leaf area was measured by gravimetric methods, principally through the leaf area estimated weight ratio. The measures undertaken which is draw on the fifth leaf buds on a piece of paper which produces replicas of leaves. Then cut leaf replica leaf area was estimated based on the following Equation ((1)) [19]:

$$LD = \frac{Wr}{Wt} \times LK \quad (1)$$

Description: LD = Broad Leaf, Wr = weight paper replicas of leaves, Wt = total weight of paper, LK = the total area of the paper.

2.6. Dry weight and wet weight

The fresh weight of plant was measured by means of a whole plant except the root is cleaned and weighed. Plants put in a petri dish and then dried in an oven at 70°C for 2 d to obtain a constant weight, then weighed to determine the plant dry weight.

2.7. Antioxidative enzyme activity measurements

2.7.1. Enzyme sample preparation

Extraction of antioxidative enzymes using methods Sunkar [20]. A total of 200 mg of wet second and third leaves from the shoots were mixed with 1.5 mL of extraction buffer, which is 0.2 M sodium phosphate (pH 7.8) containing 0.1 mM EDTA. Pulverized using a mortar and pestel, then centrifuged at 15.000 g for 20 min at 4°C. Supernatant was used for determination of the activity of SOD, CAT and APX.

2.7.2. Superoxide Dismutase (SOD)

A total of 20 μL enzyme extract and 20 μL of 1 mM riboflavin was added in 1.5 mL of the reaction mixture. The reaction mixture in the form of 50 mM sodium phosphate buffer (pH 7.8) containing NaH2PO4.2H2O and Na2HPO4.I2H2O; 0.2 mL EDTA 2mM; methionine 9.9 mM; Nitroblue tetrasolium (NBT) 55 μM and 0.025% Triton X100. The reaction begins when riboflavin is added to the mixture, then placed under fluorescent light 15 Watt at a distance of 20 cm for 10 min. The reaction was stopped by turning off the lights and closing the tube with less black plastic. Shells and controls used are the same reaction mixture, but the sample was replaced with phosphate buffer, and then placed in a dark place for the blank and the light to control. Absorbance was measured at a wavelength of 560 nm using a spectrophotometer (Shimadzu UV-vis mini 1240). SOD activity (2) is determined by the amount of enzyme that produces 50% inhibition of NBT reduction photo formazen formed by O_2^-. One unit of enzyme

activity is expressed by the amount of enzyme that reduces the absorbance of up to 50% of control. Enzyme activity is expressed in units per gram of wet weight (WW).

$$\begin{aligned}&\text{SOD activity (U/grams WW)}\\&= (\text{absorbance control} - \text{absorbance of the sample}) : \text{absorbance of control} : 50\%\\&\times \text{volume of mixture} : \text{concentration of the enzyme protein.}\end{aligned} \tag{2}$$

2.7.3. Catalase (CAT) and Ascorbate Peroxydase (APX)

The assay mixture (3 mL) contained 1.99 mL sodium phosphate buffer (pH 7.0), 10 μL enzyme extract and 1 mL H_2O_2 12.058 mM, for CAT measurement. Absorbance was measured at a wavelength of 240 nm using a spectrophotometer (Shimadzu UV-vis mini 1240). The extinction coefficient of H_2O_2 (40 mM^{-1} cm^{-1} at 240 nm) was used to calculate the enzyme activity that was expressed in terms of millimoles of H_2O_2 per minute per gram fresh weight.

APX activity was determined from the decrease in absorbance at 290 nm due to oxidation of ascorbate in the reaction. The 3 mL assay mixture contained 2.7 mL 50 mM potassium phosphate buffer (pH 7.0), 150 μL 10 mM ascorbate, and 30 μL enzyme extract. 150 μL H_2O_2 was added last to initiate the reaction, and the decrease in absorbance was recorded for 3 min. The extinction coefficient of H_2O_2(2,8 mM^{-1} cm^{-1} at 290 nm) for reduced ascorbate was used in calculating the enzyme activity that was expressed in terms of millimoles of ascorbate per minute per gram fresh weight.

CAT and APX enzyme activity (3) was determined using the following Equation (3).

$$\begin{aligned}&\text{Enzyme activity } (\mu\text{mol/mg protein})\\&= (\Delta \text{ absorbance} : \text{a}) \times (1 : \text{molar extinction coefficient})\\&\times (\text{total volume} : \text{volume of enzyme})\\&\times (\text{total volume of enzyme extract} : \text{WW sample})\\&\times \text{total protein} \times 1000\end{aligned} \tag{3}$$

3. Result and Discussion

S. oleraceus leaf morphology after exposure to heavy metals for 30 d showed different results (Figure 1). Visually, the leaves on the plant by exposure to Cu look normal and green, not much different from the metal leaves that are not given heavy metal (control). While the leaf is exposed to a yellowish-green colored metals Pb, Cd metal exposed leaves are pale green and wrinkled. In contrast to the leaves by exposure to Cr, green-purple color of the leaves and the leaves are dried.

The height growth of plants *S. oleraceus* with heavy metals Cu treatment was not significantly different to the control plants, while the plants are both significantly different from that given exposure to Pb, Cd and Cr (Figure 2).

Figure 1: The second leaf of *S. oleraceus* on heavy metal stress conditions.

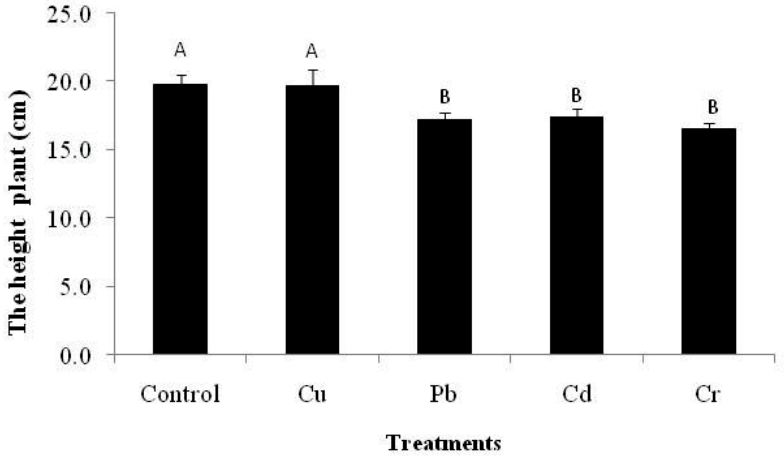

Figure 2: The height of *S. oleraceus* plants in heavy metal stress conditions. The notation a, b show a significant difference ($p \leq 0.05$) between treatments.

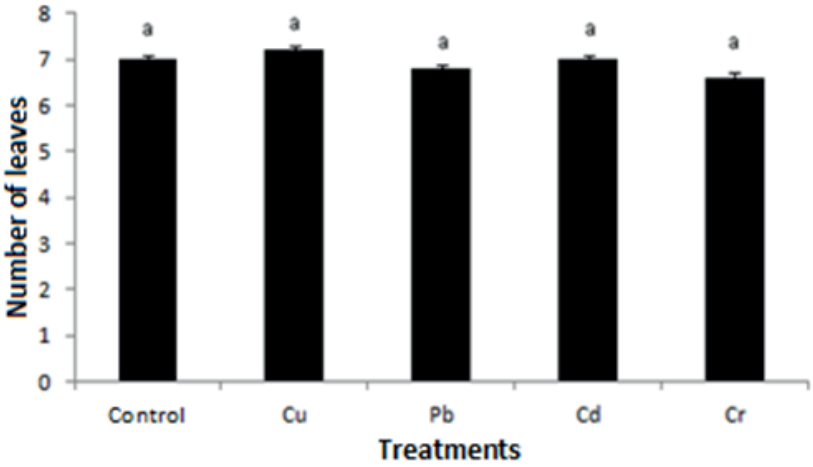

Figure 3: Number of leaves *S. oleraceus* on heavy metal stress conditions.

The provision of heavy metals Cu, Pb, Cd, and Cr in plants *S. oleraceus* not lead to a decrease in the number of leaves significantly compared to controls (Figure 3).

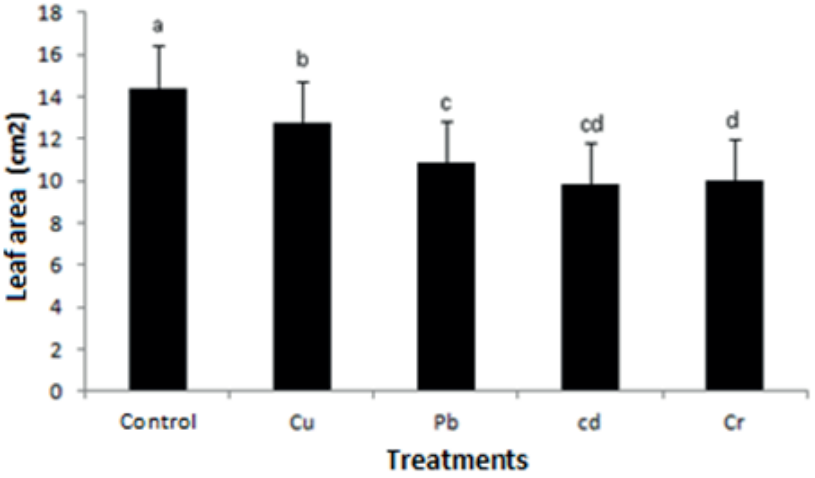

Figure 4: Leaf area *S. oleraceus* on heavy metal stress condition. The notation a, b, c, d show a significant difference ($p \leq 0.05$) between treatments.

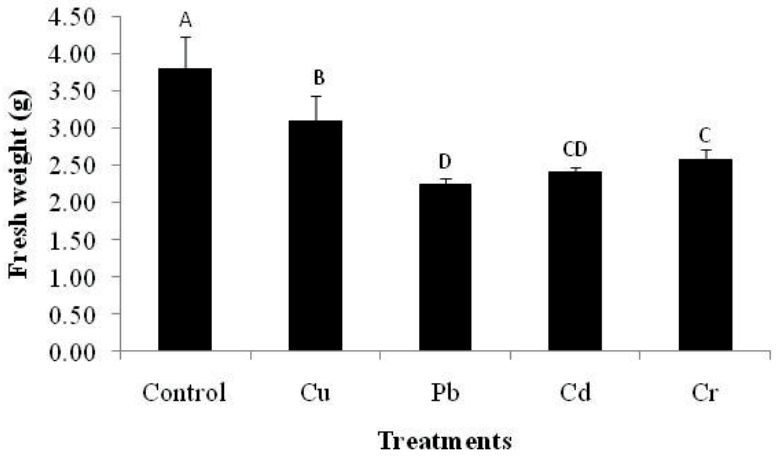

Figure 5: The weight of wet plant *Sonchus oleraceus* on heavy metal stress conditions. The notation a, b, c, d show a significant difference ($p \leq 0.05$) between treatments.

The treatments of heavy metals showed significantly different results in leaf area *S. oleraceus*. Control plants had an average of greatest leaf area followed by treated plant with Cu, Pb, Cr and Cd was smallest (Figure 4).

The fresh and dry weight of plants *S. oleraceus* were significantly different at each treatment of heavy metals, except control and Cu on a dry weight parameters (Figure 5 and 6). Both have the same downward trend. Cr exposure causes decreased *S. oleraceus* high, followed by exposure to Cd, Pb and Cu.

The treatment of heavy metals Cu, Pb, Cd and Cr overall have significant effects on the enzyme activity of SOD. SOD activity in plants treated with heavy metals all increased compared to control plants (Figure 7). The SOD activity is in *Sonchus oleraceus* with exposure to Cr increased 97.3%, followed by the plant with exposure to Pb, Cd and Cu with the percentage increase respectively by 27.2%, 13.4% and 6.8%.

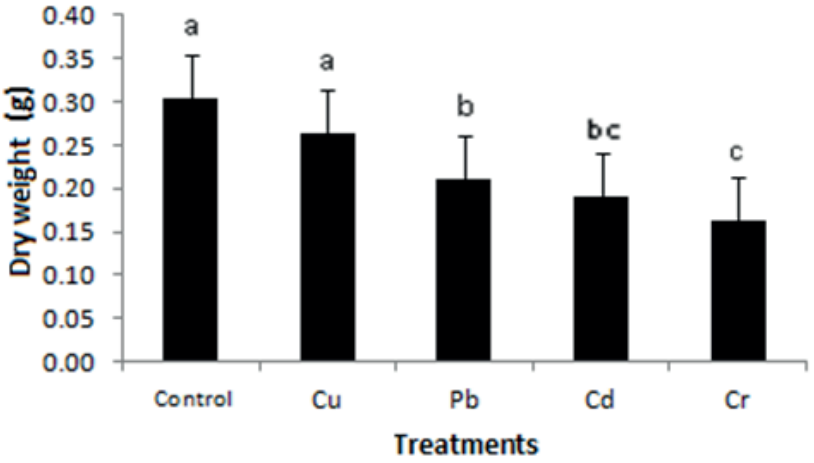

Figure 6: The dry weight of the plant *Sonchus oleraceus* on heavy metal stress conditions. The notation a, b, c, d show a significant difference ($p \leq 0.05$) between treatments.

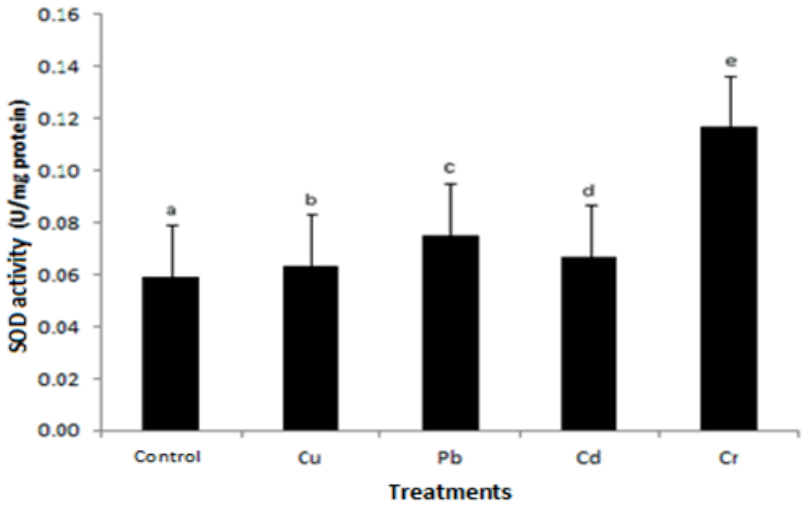

Figure 7: The SOD activity *S. oleraceus* plants in heavy metal stress conditions. The notation a, b, c, d, e shows a significant difference ($p \leq 0.05$) between treatments.

APX enzyme activity in plants *S. oleraceus* with exposure to heavy metals Cu, Pb, Cd and Cr significantly increased compared to control plants (Figure 8). Treatment of Cu and Pb give different results significant to the entire treatment with increased activity of APX of 19.8% and 62.3%, while plants exposed metal against metal Cd,Cr and the reverse of it are not significantly different, but both are significantly different from other treatments. Increased activity of APX in *Sonchus oleraceus* with exposure to Cd metal by 79.20%, while those exposed to Cr amounting to 78.90%.

Unlike the enzyme SOD and APX, CAT enzyme activity in plants by exposure to heavy metals had a significant reduction compared to the control. *Sonchus oleraceus* plants with exposure to Cr has the smallest CAT enzyme activity with a decrease of 85.9%. Followed by a decline in CAT activity in plants exposed to Pb, amounted to 38.8%, and Cd by 26.5% and amounted to 21.9% Cu.

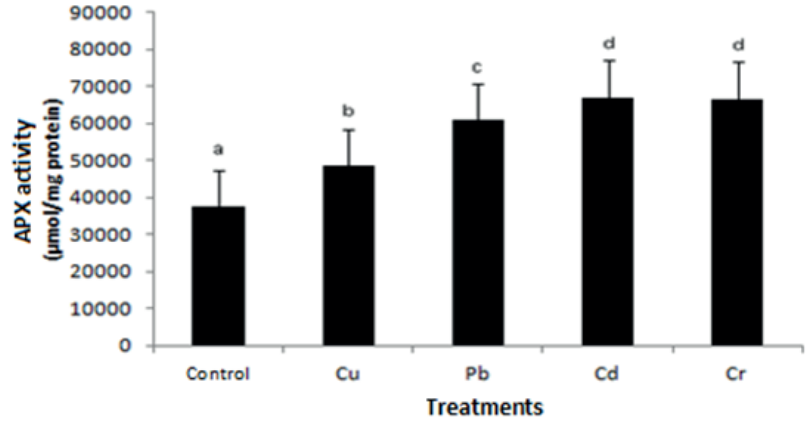

Figure 8: Activity APX *S. oleraceus* plants in heavy metal stress conditions. The notation a, b, c, d show a significant difference ($p \leq 0.05$) between treatments.

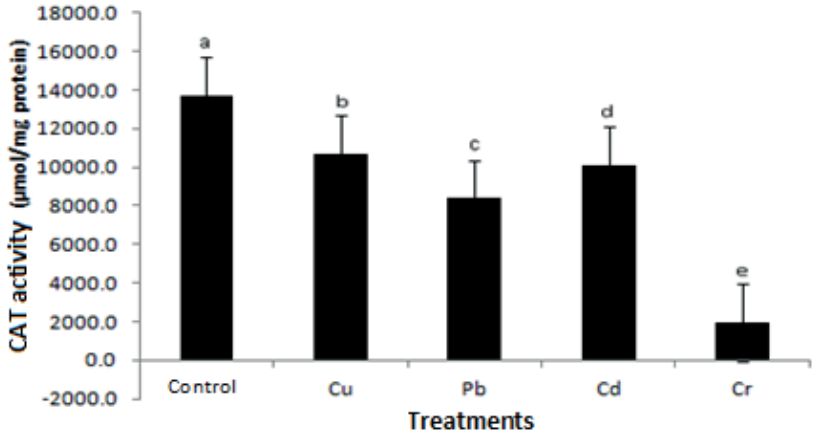

Figure 9: CAT activity *S. oleraceus* plants in heavy metal stress conditions. The notation a, b, c, d, e shows a significant difference ($p \leq 0.05$) between treatments.

Plant growth and development (Figure 1 to Figure 6) is the initial expression that can describe the environment. Cu were exposed for 30 days not affect the morphological appearance of *S. oleraceus*. This is possible because Cu including essential metals (micronutrients) needed by plants in the process of metabolism, in addition to the translocation of Cu from root to stem relatively low [21]. Pb causes the *S. oleraceus* leaves are green-yellowish, the same result is also shown *Eichornia crassipes* leaves exposed metal Pb [22]. Pb can cause damage to photosynthetic pigments such as chlorophyll, chloroplasts and carotenoids [23]. Cd inhibits the absorption of plant essensiat elements such as P, K, S, Ca, Zn and Mn so as to cause a reduction of nutrients in plants [24]. Cd metal causes the leaves *S. oleraceus* wrinkled and dwarf plants (stunting), because nutrition is not balanced so that growth is not optimal. The worst appearance of the leaves is the leaf *S. oleraceus* with exposure to Cr. Purple leaves and dry at the edges. Cr inhibit the uptake of P element, P element that decreases will lead to increased anthocyanin so that the leaves will be purple. Cr also causes root undergo plasmolysis and wilt, it is consistent with the results of research that found some dead plants.

The treatment of heavy metals Cu, Pb, Cd and Cr with a concentration of 10 mg $\cdot L^{-1}$ in the growing medium become barriers the growth of *S. oleraceus* plants. The presence of heavy metals in the plant growing medium so that the roots can damage the membrane will disrupt the process of absorption of water and nutrients, resulting in the need for water and nutrients are not being met so that the plants do not grow well. This can be seen from plant height, leaf area, as well as wet weight and dry weight of the treatment plant is lower than the control plants. Overall treatment had no significant effect on the number of *S. oleraceus*. Leaf formation occurs in the early phase of growth sprouts, thus the control plants and plants treated have the same capability in the production of the leaf. Giving the metal in the growth phase relative does not affect the number of leaves [25]. A decrease in leaf area due to its reduced parenchyma cells palisade and spongy parenchyma by heavy metals so that the size of a small leaf.

Antioxidative enzymes have an important role in the adaptation of plants when subjected to stress. Antioxidative defense responses of *S. oleraceus* towards heavy metal stress indicated by an increase or decrease in the activity of antioxidative enzymes. Presence of heavy metals in plants not only causes growth inhibition and oxidative damage in tissues of plants but also induces the activity of antioxidative enzymes to counteract the toxicity of heavy metals. Heavy metals into plants will react with proteins, lipids and other macromolecular membranes thus increasing membrane permeability to heavy metal. Increased permeability of the membrane leads to the income levels of heavy metals are more into the cells and the production of ROS is greater than the ability to detoxify the oxidative enzymes [26].

SOD enzyme activity in *S. oleraceus* with exposure to heavy metals has increased compared to controls. SOD is the first enzim working against ROS. SOD works transform superoxide (O_2) into H_2O_2 and O_2 with donating $2H^+$. Results showed that the most significant increase in SOD occurs in plants with exposure to Cr, SOD activity increased by 97.3% compared to controls. Cr is a strong oxidant that rapidly produce ROS in large numbers, so that the plant will produce more antioxidative enzymes to fight it.

S. oleraceus exposed to heavy metals also increased APX enzyme activity, but CAT enzyme activity decreased. APX and CAT enzymes convert H_2O_2into non-toxic compounds, namely water and oxygen. CAT decline is expected because O_2 can directly inhibit the action enim CAT [27]. APX is more effective in detoxify H_2O_2 than CAT. It is caused APX contained in the various cell organelles and cytoplasm and has a higher affinity due to ascorbic acid as a reduction agent, while CAT is limited only contained in the micro entities and the mitochondria and has a smaller affinity.

4. Conclusion

Stress heavy metals Cu, Pb, Cd and Cr in growing media causes impaired growth and *S. oleraceus* not optimal. The existence of these heavy metals cause high decrease in plant height and leaf area so wet weight and dry weight go down. The most significant decline in growth occurred in *S. oleraceus* exposed is Cr. Heavy metal stress also has significant impact on the activity of antioxidative enzymes in *S. oleraceus*. The enzyme

activity of SOD and APX increased, while the CAT enzyme decreases. Metals that has the most significant effect on the activity of antioxidative enzymes in plants *S. oleraceus* is Cr.

References

[1] J. Mukono and IP. Corie, "Toksikologi logam berat B3 dan dampaknya terhadap kesehatan [Toxicology B3 heavy metals and their impact on health]," *J. Kesehatan Lingkungan*, vol. 2, p. 129, 2006, in Bahasa Indoenesia.

[2] Darmono., "Logam dalam sistem biologi makhluk hidup [Metals in biological systems beings]," in *Logam dalam sistem biologi makhluk hidup [Metals in biological systems beings]*, pp. 125–126, UI Press, Jakarta, 1995, in Bahasa Indoenesia.

[3] F. Rokhmah, "Rokhmah F. Pengaruh toksisitas Cu terhadap pertumbuhan dan produksi padi (OryzasativaL.) serta upaya perbaikannya dengan pupuk penawar racun [Effect of Cu toxicity on the growth and production of rice (OryzasativaL.) as well as improvement efforts by the bidder fertilizer poison] [undergratuaded Thesis]. Bogor: Institut Pertanian Bogor; 2008,p. 31–55 [in Bahasa Indoenesia].".

[4] F. Hamzah, "Setiawan A. Akumulasi logam berat Pb, Cu, dan Zn di hutan mangrove Muara Angke, Jakarta Utara [Accumulation of heavy metals Pb, Cu, and Zn in Mangrove Forest Muara Angke, North Jakarta]. Jurnal Ilmu dan Teknologi Kelautan Tropis 2010; 2 (2):41–52 [in Bahasa Indoenesia]".

[5] C. Jonak, H. Nakagami, and H. Hirt, "Heavy metal stress. Activation of distinct mitogen-activated protein kinase pathways by copper and cadmium.," *Plant physiology*, vol. 136, no. 2, pp. 3276–3283, 2004.

[6] Rini. S. Kusdianti and Eva. T. Hafsah, "Analisis pertumbuhan tanaman kentang (SolanumtuberosumL.) pada tanah yang terakumulasi logam berat Cadmium (Cd) [Growth analysis of potato (SolanumtuberosumL.) on soil accumulated heavy metal Cadmium (Cd)]. Garut: STKIP; 2014, p.1–7 [in Bahasa Indoenesia].".

[7] I. Pratiwi, "Respon antioksidatif tanaman sorgun (Sorghum bicolor(L.) Moench) pada cekaman logam berat Cr (III) dan Cr (VI) [Plant antioxidative response Sorghum (Sorghum bicolor(L.) Moench) on heavy metals stress Cr (III) and Cr (VI)] [Undergratuaded Thesis]. Salatiga: Fakultas Biologi Universitas Kristen Satya Wacana; 2014,p.1–24 [in Bahasa Indoenesia].".

[8] D. Purwandani, "Pertumbuhan, kandungan klorofil, dan aktivitas nitrat reduk-taseSorghum bicolor(L.) Moench pada kondisi cekaman krom [Growth, chlorophyll content and nitrate reductase activity Sorghum bicolor (L.) Moench on condition Stress Krom] [Undergratuaded Thesis]. Salatiga: Fakultas Biologi Universitas Kristen Satya Wacana; 2014,p.1–19 [in Bahasa Indoenesia].".

[9] X. Yang, Y. Feng, Z. He, and P. J. Stoffella, "Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation," *Journal of Trace Elements in Medicine and Biology*, vol. 18, no. 4, pp. 339–353, 2005.

[10] D. Sharma, C. Chatterjee, and C. Sharma, "Chromium accumulation and its effects on wheat (Triticum aestivum L. cv. HD 2204) metabolism," *Plant Science*, vol. 111, no. 2, pp. 145–151, 1995.

[11] V. Pandey, V. Dixit, and R. Shyam, "Chromium (VI) induced changes in growth and root plasma membrane redox activities in pea plants," *Protoplasma*, vol. 235, no. 1-4, pp. 49–55, 2009.

[12] AK. Singh, "Effect of trivalent and hexavalent chromium on spinach (Spinaceaoleraceal.)," *Environ. Ecol*, vol. 19, pp. 807–810, 2001.

[13] Z. Zaimoglu, N. Koksal, N. Basci, M. Kesici, H. Gulen, and F. Budak, "Antioxidative enzyme activities in Brassica juncea L. and Brassica oleracea L. plants under chromium stress," *Journal of Food, Agriculture and Environment*, vol. 9, no. 1, pp. 676–679, 2011.

[14] X. Liu, C. E. Williams, J. A. Nemacheck et al., "Reactive oxygen species are involved in plant defense against a gall midge," *Plant Physiology*, vol. 152, no. 2, pp. 985–999, 2010.

[15] M. Heidari, "Antioxidant activity and osmolyte concentration of sorghum (Sorghum bicolor) and wheat (Triticum aestivum) genotypes under salinity stress," *Asian Journal of Plant Sciences*, vol. 8, no. 3, pp. 240–244, 2009.

[16] P. Ahmad, CA. Jaleel, MM. Azooz, and G. Nabi, "Generations of ROS and non-enzymatic antioxidants during abiotic stress in plants," *Botany research International*, vol. 2, pp. 11–20, 2009.

[17] K. Apel and H. Hirt, "Reactive oxygen species: Metabolism, oxidative stress, and signal transduction," *Annual Review of Plant Biology*, vol. 55, pp. 373–399, 2004.

[18] X. He, Y. Ruan, W. Chen, and T. Lu, "Responses of the anti-oxidative system in leaves of Ginkgo biloba to elevated ozone concentration in an urban area," *Botanical Studies*, vol. 47, no. 4, pp. 409–416, 2006.

[19] SM. Sitompul and B. Guritno, "Sitompul SM, Guritno B. Analisa pertumbuhan tanaman [Plant growth anatomy]. Yogyakarta: Universitas Gadjah Mada Press; 1995, p.376 [in Bahasa Indoenesia].".

[20] R. Sunkar, *Plant Stress Tolerance (Methods and Protocol*, Humana Press, 2010.

[21] A. Manara, "Plant Responses to Heavy Metal Toxicity," in *Plants and Heavy Metals*, vol. of *SpringerBriefs in Molecular Science*, pp. 27–53, Springer Netherlands, Dordrecht, 2012.

[22] S. Malaar, S. Sahi, JC. Paulo, and P. Venkatachalam, "Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [Eichhornia crassipes (Mart.)]," *Botanical studies*, pp. 55–54, 2014.

[23] R. K. Tewari, P. Kumar, P. N. Sharma, and S. S. Bisht, "Modulation of oxidative stress responsive enzymes by excess cobalt," *Plant Science*, vol. 162, no. 3, pp. 381–388, 2002.

[24] A. Metwally, V. I. Safronova, A. A. Belimov, and K.-J. Dietz, "Genotypic variation of the response to cadmium toxicity in Pisum sativum L.," *Journal of Experimental Botany*, vol. 56, no. 409, pp. 167–178, 2005.

[25] FB. Salisbury and CW. Ross, "Fisiologi tumbuhan [Plant physiology]. Jilid 1 Terjemahan Diah R. Lukman dan Sumaryo," in *Ross CW. Fisiologi tumbuhan [Plant physiology]. Jilid 1 Terjemahan Diah R. Lukman dan Sumaryo*, p. 241, ITB Press, Bandung, 1995, in Bahasa Indoenesia.

[26] A. K. Shanker, C. Cervantes, H. Loza-Tavera, and S. Avudainayagam, "Chromium toxicity in plants," *Environment International*, vol. 31, no. 5, pp. 739–753, 2005.

[27] Y. Kono and I. Fridovich, "Superoxide radical inhibits catalase.," *Journal of Biological Chemistry*, vol. 257, no. 10, pp. 5751–5754, 1982.