Effect of EDTA to growth and accumulation lead in Codiaeum varigatum

L Herlina1,4*, B Widianarko2 and H R Sunoko3

1 Student of Environmental Science, Doctoral Program, School of Postgraduate Studies, Diponegoro University, Semarang, Indonesia
2 Study Program of Food Technology, Faculty of Agricultural Technology, Soegijapranata Catholic University, Semarang, Indonesia
3 Faculty of Medicine, Diponegoro University, Semarang, Indonesia
4 Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Semarang, Semarang, Indonesia

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

Abstract
EDTA is used in soil remediation because of its ability to move metal in the soil matrix. EDTA solutions can dissolve heavy metals including Pb. Codiaeum varigatum is an ornamental plant in addition to having a high aesthetic, ecological and economic value. In this study analyzes the effect of EDTA on the growth and accumulation of metals in C. varigatum plants. The purpose of this study was to analyze the effects of EDTA on growth, photosynthetic pigment content and Pb accumulation in C. varigatum plants. Pot experiments using C. varigatum with lead 500 Pb kg-1 soil and different levels of EDTA (mM) 5, 10 and 20. The parameters studied included plant biomass, Pb accumulation in plant and photosynthetic pigment content measured after 30 days. The results showed that EDTA concentration of 20 mM caused an increase in Pb accumulation and a decrease in plant biomass and photosynthetic pigment content.

1. Introduction
Soil pollution by heavy metals is a global environmental problem [1] so that recovery steps need to be taken so that contaminated land can be reused for various actsives safety. Phytoremediation is one method that uses plants to remove, move, stabilize pollutants in the form of organic and inorganic compounds. This plant-based technology uses plants that are modified naturally and genetically to remove contaminants such as metals from the soil, sediment, or water [2,3,4,5]. The use of plants to clean the environment is the most rapidly developing and cost-effective component of environmentally friendly technology that has received great attention in recent years.

Lead is a nonessential element in metabolic processes and is toxic because it is very damaging to animals, plants, and humans [6]. Pb is naturally present in agricultural soils but their concentration can increase due to air pollution, as well as the use of animal waste, an organic fertilizers, phosphate fertilizers and pesticides containing lead arsenate[7]. Pb contaminated soil from several sources namely high rates of burning fossil fuels and mining, dust, and gas from various industrial sources. Long-term contamination without corrective action, high levels of lead on the soil will not return to normal [8]. Pb metal accumulates in the food chain through absorption at the primary producer level than through consumption at the consumer level [9]. Lead can reduce photosynthesis process, inhibit respiration,
affect germination and yield [10]. Pb stress can cause disruption to plants, such as inhibition of growth and stress at a certain level [8].

Phytoremediation can be increased by using a chelating agent which causes accumulation of heavy metals in plants. The use of chelating agents increases the absorption and translocation of heavy metals in tissues. Ethylene diamine tetraacetic acid (EDTA) is one of the most effective chelating agents to artificially increase solubility, complexation, and absorption of heavy metals [11,12,13,14]. Thus, the addition of EDTA into the soil induces absorption and translocation of heavy metals from the root to shoots of plants. For phytoremediation optimization EDTA chelating is used as explored by many researchers [13,14]. Metals that are soluble in chelating by forming metal-ligand bonds can free metals from soil particles or increase mobility in plant biology systems

The application of croton plants (C.varigatum) as an ornamental plant for phytoremediation purposes can be a good alternative for the recovery of contaminated land. Ornamental plants, if they have hyperaccumulator properties, can be applied for remediation of contaminated soil, in addition to ornamental plants can provide aesthetic quality in contaminated locations. The use of ornamental plants in remediation is sustainable, feasible and profitable [14]. The aim of the study was to analyze the effects of EDTA on growth, photosynthetic pigment content and Pb accumulation in C.varigatum (L) plants.

2. Methods
2.1. Soil preparation
The soil used is taken from the garden in Kaliserogo village, Gunungpati sub-district. The ground is drained and sieved. Soil physical and chemical characteristics were soil texture (sand 492%, silt 59.71% and clay 44.37%), pH 5.21, C-organic 1.15%, N-Kjeldahl 0.17%, P 0.12 mg/100mg, K 24.53 mg/100mg, CEC 15.41 cmol(+).kg⁻¹, permeability 2.35 cm/h, water content 10.25%, soil volume 1.12 g.cm⁻³ and specific gravity 2.05 g.cm⁻³

This Experiment was arranged in a complete randomized design with four level EDTA and four replications per treatment. After sieving, 1.0 kilograms of dry soil was put into plastic pots of size 19 cm x 14 cm, and soil samples mixed with vermicompost for each pot containing 1 plant. After 3 weeks of acclimatization period, plants were treated with Pb (NO3) 2 as much as 500 mg/kg of soil and EDTA (mM) 0, 5, 10, and 20 with four replications for each.

2.2. Determination of plant growth
After 30 days of treatments, plant growth was measured in fresh roots and leaves. All parts of the plants are dried in an oven at 70°C until the weight is constant and then weighed

2.3. Determination of photosynthetic pigments
Fresh leaves of 0.5 g were pounded with a mortar and then added 10 mL acetone 80 %. The absorbance of extracts at 645 nm, 663 nm and 470 nm for photosynthetic pigments were analysis by UV-Vis spectrophotometer (Lamda 25 erkin Elmer) and photosynthetic pigment unit in mg/g FW

2.4. Measurement of lead on plants
Lead content in plants is estimated through analysis of ash from plant parts. Dry samples from the roots and leaves were put in a porcelain cup and then slowly heated at 450 °C - 500 °C for 5-7 hours until white ash obtained. The ash is dissolved in 5 mL of HCl, heated to dissolved other remnants and filtered with filter paper. The determination of lead was measured by atomic absorption spectrophotometer (AAS, PerkinElmer AAnalyst 400) at 217 nm.

2.5. Bioconcentration and translocation factor
Bioconcentration factors indicate the efficiency of plant species accumulating metals into tissues that are from the surrounding environment. Translocation factors (TF) show the efficiency of plants to absorb and translocation them from roots to buds. This is calculated as follows:
BCF = metal content in plant tissue/ metal content in soil
TF = metal content in shoot/ metal content in root

2.6. Statistical analysis
Data on various parameters were analyzed by one-way ANOVA SPSS version 23 followed by LSD post-hoc test between the means of treatments to determine the significant difference at the level of p <0.05

3. Result and Discussion
3.1. Effect of EDTA on plant growth
Plant growth parameters such as fresh and dry weight of leave and root are shown in Fig 1. The results showed that Pb-EDTA significantly (P>0.05) to fresh and dry weight but the fresh weight of leaf not significantly of control.

3.2 Effect of EDTA on photosynthetic pigment
The results of different effects of Pb-EDTA doses on the photosynthetic pigment in Fig 2 show that the content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in leaves of C. varigatum in the treatment of 20 mM EDTA decreased significantly compared with those of the control, but that chlorophyll a in treatment with 5 mM EDTA was not significantly different from the control (p <0.05).

3.3. Lead content in the plant
With the increasing dose of EDTA, the Pb content at the root of C. varigatum increases is given in Fig. 3. EDTA application significantly increased Pb concentrations in the root and a leaf of plants. Dose EDTA 5 mM and 10 mM were not significantly different of control. In this study (table 1) the Pb concentration at the root was higher than that of leaves translocation factor less than 1.

The application of EDTA in the soil can push ahead of the transfer and transfer of metals also help in the translation of metals from roots to non-roots, increasing the bioavailability of heavy metals to accumulate in plants. Many studies report that better collection can damage the weight of metals in plants to a certain extent and also for plant growth.
Figure 2. Effect of EDTA on photosynthetic pigment

Figure 3. The effects of EDTA on Pb content in plants

Table 1. Effects of EDTA on Pb translocation and bioconcentration factor

| Treatments | TF    | BCF root | BCF leaf |
|------------|-------|----------|----------|
| Control    | 0.3025| 2.5527   | 0.7722   |
| 5 mM EDTA  | 0.3388| 5.6542   | 1.9154   |
| 10 mM EDTA | 0.4054| 9.0295   | 3.6603   |
| 20 mM EDTA | 0.3517| 6.5115   | 2.2898   |

Pb accumulation in plants causes a decrease in plant biomass because Pb inhibits cell differentiation [15] that Pb metal affects root extension but decreases shoot formation and affects plant biomass and germination. Plants absorb Pb and accumulate in plant tissues because they are not metabolism induce various toxic effects on the lives of organisms, including morphological, physiological and biochemical ones. Lead accumulation in roots is found in cell walls and lead stress causes a decrease in plant
productivity [16]. Pb accumulation causes a decrease in root growth, loss of apical dominance [17]. The main effect of Pb on plants is inhibition of root growth, this is caused by the inhibition of cell division that occurs at the root tip. Pb causes inhibition of cell division at the root of several plant species, including Sedum alfredii [18] decreases in length and at the root of dry mass under Pb toxicity. At certain concentrations, Pb can be toxic and interfere with plant growth. [15] states that Pb stress can cause disruption to plants, such as plant growth and stress at certain levels. Application of EDTA dose of 20 mM biomass in the roots and leaves decreases.

The application of EDTA increases Pb uptake by plants. [20] reported that a decrease in photosynthetic pigment might be caused by oxidative stress stimulated by the accumulation of Pb in the leaves. The concentration of chlorophyll reflects the photosynthesis ability of plant and is also used as an important indicator of evaluating the tolerant ability of plants-heavy metals [19]. High Pb absorption causes damage to the structure of chloroplasts and decreases in chlorophyll concentration.

The presence of Pb causes phytotoxicity by changing the permeability of cell membranes and reacting to active groups with of Pb enzymes involved in plant metabolism that reacts with ADP or ATP phosphate groups, by replacing important ions. Pb toxicity causes inhibition of ATP production, lipid peroxidation, and DNA damage, by overproduction of reactive oxygen species (ROS) [16]. This is like the [21] study in which increase in the lead, the amount of chlorophyll a, b and total content decreases in the sainfoin plants planted in soils containing lead 800 mg.kg-1. Lead has an effect on chlorophyll content, because lead inhibits chlorophyll synthesis by preventing the absorption of important elements of Mg and Fe, photosynthetic organs are also damaged due to increased chlorophyllase activity, under lead abundance also causes increased chlorophyll destruction in this condition chlorophyll a more influential than chlorophyll b [22] chlorophyll content, because lead inhibits chlorophyll synthesis by preventing absorption of important elements Mg and Fe, photosynthetic organs are also damaged due to increased chlorophyllase activity, under lead abundance also causes increased chlorophyll destruction in this condition chlorophyll a more influential than chlorophyll b [21,23]

EDTA improves heavy metal translocation, especially Pb, so it plays an important role in heavy metal phytoremediation. Limited translocation of heavy metals gets shoots due to blockages by Caspari tape [22], accumulation of plasma membranes, precipitation in interstellar space precipitation as insoluble salt [23] or deep compartment Cell vacuole and root rhizodermal.

4. Conclusion
It can be concluded that EDTA increases the absorption of Pb. Lead toxicity can cause reduced growth, biomass, photosynthetic pigments. The addition of EDTA significantly reduced growth, photosynthetic pigments and increased absorption of Pb. In this study TF less than 1.

Reference
[1] Bermudez G M A, Jason R, Pal R and Piñata M L 2012 J. Hazard Mater. 213 447
[2] Badr N, Fawzy and M Al-Qahtani 2012 World Appl. Sci. J. 16 1292
[3] Vithanage M, Dabrowska B B, Mukherjee, Sandhi A and Bhattacharya P 2012 Environ. Chem. Lett. 10 217
[4] Varun M, D’Souza R, Pratas J and Paul M S 2011 Bull. Environ. Contam. Toxicol 87 45
[5] Huang S Z, Han Y L, Yu S L, Gu J G and Zhang L L 2011 Environ. Bull. 20 2246
[6] Sainger P A, Dhanikhar R, Sainger M, Kaushik A and Singh R P 2011 Ecotoxicol. Environ. Saf 74 2284
[7] Srivastava D, A Singh and M Baunthiyal 2015 J. of Plant Science and Research. 2 123
[8] Tangahu B V, Abdullah S R S, Basri H, Idris M, Anuar N and Mukhlisin M 2011 International Journal of Chemical Engineering. 20 11
[9] Ashraf U and Tang X 2017 J.chemosphere 02 103
[10] Mohanty M, Pattnaik M M, Mishra A K and Patra H K 2012 Environ Monit Assess 184 1015
[11] Srinivasan M, Shivendra V S, F Paulo J C and Perumal V 2014 Botanical Studies 2014 55
[12] Bareen FE 2012 Environ Pollut 21 289
[13] Kambhampati M S 2013 Bull Environ ContempToxicol 91 310
[14] Mani D, Kumar C and Patel N K 2014 Int J Phytorem (Accepted in press)
[15] Sarma H 2011 J Environ Sci Technol 4 (2) 118
[16] Ghelich S I, Zarinkamar and Fatemeh 2013 Phyto J 2 20
[17] Gupta A K, Verma S K, Khan K and Verma R K 2013 Environmental Science and Technology, 47 (18) 10115
[18] Huang H, D.K. Gupta, Tian, X. Yang and T. Li. 2012. Environ Sci. Pollut. R. 19 1640
[19] Han Y L, Huang S Z, Yuan H Y, Zhao J Z and Gu J G. 2013 Ecotoxicology 22 1033
[20] Mahtab B, Habibi D, Kashani A, Paknejad F and Nooralvandi T Am-Euras 2011 J. Agric. & Environ. Sci., 10 (3) 440
[21] Mingorance M D, Leidi E O, Vald´es B and Oliva R 2012 Int. J.Phytorem. 14 174
[22] Arias J A, Peralta-Videa J R, Ellzey J T, Ren M, Viveros M N and Gardea-Torresdey 2010 Environ. Exp. Bot. 68, 139
[23] Ashraf U, Kanu A S, Mo Z W, Hussain S, Anjum S A, Khan I, Abbas RN and Tang X 2015 Environ Sci Pollut Res 22 18318