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Phytoremediation of Lead Contaminated Land Using Vetiver zizanoides and Citric Acid as Chelating Agent

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Abstract. Environmental pollution due to hazardous waste in Karangdawa, Margasari, Tegal has an impact on soil and groundwater quality. Lead found in the soil exceeds the permissible standards. It is necessary to recover soil from Lead because it can enter food crops and accumulate in the human body. Phytoremediation is commonly used in land remediation because it's economical and doesn't cause secondary environmental problems. One of the ways to optimize Phytoremediation is by using chelating agents. The purpose of the study was to analyze the ability of Vetiver zizanoides and analyze the most optimum dose variation of Citric acid to uptake Lead to the plant. The ability of Vetiver zizanoides and Citric acid as metal chelators in Pb uptake was investigated for 28 days with Citric acid doses of 0.5 g/kg, 1 g/kg, and 2 g/kg. Analysis of Lead concentration in roots, stems, and soil using AAS. From the results of the study, Vetiver zizanoides was able to survive from Lead exposure up to 4979.8 ppm. Application 1 g/kg of Citric acid in the soil, increased the bioconcentration, and bioaccumulation compared to control plants. The results indicated that the addition of Citric acid as a chelating agent could increase the function of Vetiver zizanoides as a phytoremediation agent.

1 Introduction

Heavy metal contamination in soil poses a significant risk to human health and ecosystems. Heavy metals can be transferred to the food chain via edible plants, a significant threat to human health [1]. cases of environmental pollution in Karangdawa, Margasari District, Tegal Regency due to hazardous and toxic waste, have an impact on soil quality and groundwater quality. The main heavy metal content in polluted soil in Karangdawa is lead (Pb). Lead in the soil can enter the surrounding food plants and end up accumulating in the human body [2]. Lead in the human body can cause chronic poisoning, and damage to the central nervous system, bone marrow, and blood vessels [3]. So, it takes a technique to restore contaminated land. Phytoremediation is a technique for using plants to degrade pollutants from the soil [4].

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This technique is popular because it is economical and does not cause secondary environmental problems compared to land remediation using physical-chemical techniques.

However, phytoremediation requires a long period of land recovery on a large scale, because some heavy metals (Pb, Ni) have low bioavailability by plants [5]. Increased bioavailability of heavy metals by plants with the addition of metal chelating agents has been proven in previous studies. A common chelating agent used in phytoremediation is EDTA. EDTA is able to dissolve heavy metals into a soil solution that is easily absorbed by plants, but the EDTA complex is difficult to degrade and is still found in the soil after more than 5 months, and has the potential to increase the downward migration of heavy metals to groundwater [6]. In this study, Citric Acid was chosen as a natural chelating agent and is easily available in the market.

In the mechanism of heavy metal absorption by hyperaccumulator plants, some plants have the ability to synthesize organic acids which are used to chelate metals to be absorbed by plants [7], so research on citric acid for metal chelating has begun to be studied. In previous studies, citric acid was able to increase lead uptake in shoots 0.8 times greater than without chelating agents [8], and did not reduce plant dry biomass. However, citric acid can reduce soil pH [9], so it is necessary to know whether the use of citric acid will be optimal when applied to polluted soil from Karangdawa (which has a low pH). The success of phytoremediation depends on the choice of a combination of hyperaccumulators and chelating agents [10,11]. This study examines the ability of Vetiver zezanoides to uptake lead metal. This plant was chosen because it can survive and thrive in the study area. In addition, previous studies have shown that Vetiver zizanoides can accumulate up to 19,800 Pb in roots and 3350 mg Pb/kg dry weight in shoots in a hydroponic setting [12]. So, the purpose of this study is to find the ability of Vetiver zizanoides as a phytoremediation agent and the optimal dose combination of citric acid to uptake heavy metals (lead) into the plant body

2 Material and Methods

2.1 Study Location and Sample Analysis

Study location in Karangdawa Village, Margasari District, Tegal Regency. There are 2 sampling locations that represent polluted soil, namely location 1 (7°03'52.3" S and 109°01'35.1" E), location 2 (7°03'48.4" S and 109°01'36.7" E). The location Analysis of contaminated soil samples at the Environmental Engineering Laboratory, Faculty of Engineering, Diponegoro University, Semarang

2.2 Sampling Method

The sampling method of polluted soil is guided by SNI 8520: 2018. Sampling was carried out at 2 locations, then the soil was homogenized manually, and put into pots (4kg soil/pot).

2.3 Acclimatization

The vetiver (Vetiver zezanoides) was imported from Bogor, West Java, and acclimatized for 2 weeks.

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2.4 Toxic Test

Toxic test aims to determine the ability of plants to survive at the highest concentration values. This acute toxic test consists of a Screening Test and a Definitive Test. (25)

1. Screening Test; The screening test was carried out by exposing 5 individual plants to 10x the dose of Lead. The method is carried out by preparing 5 pots, each containing 5 plants (1 pot of 4 kg of soil). The soil used has a lead concentration of 0 mg/kg. The dose given in the toxic test is 1;10,100,1000,10000 mg/kg (logarithmic scale). Observations of dead plants were carried out 4 x 24 hours. The highest and lowest concentrations that cause plants to die above 50% are the reference doses tested in the Definitive test.
2. Definitive Test; The definitive test is a continuation of the screening test. The results of screening at the highest and lowest concentrations of the plant died 50%, divided into 5 lead dose ranges. The method was carried out by preparing 5 pots, each containing 5 plants (1 pot of 4 kg of soil). Furthermore, the concentration of Lead was added from the Screening results and observations were made 4 x 24 hours. From the number of dead plants, the median lethal concentration (LC50) or half of the plants in the research pot was determined.

2.5 Data Analysis

The study was conducted for 28 days (4 weeks), where observations were made on the parameters of plant growth rate, pH, soil temperature, air temperature, soil moisture, and plant transpiration rate.

The method of measuring the growth rate was obtained from the difference in plant length measured every day divided by the day the plant grew during the study. The pH measurement method is guided by SNI 03-6787-2002. Soil temperature measurements were carried out at 3 points in each pot using a soil thermometer. Measurement of soil moisture with a moisture meter was carried out before and 15 minutes after watering 100 ml of water in each pot. Measurement of transpiration rate using a photometer within 15 minutes. Analysis of lead concentration in soil and plants was carried out at the end of the study (28th day) using the wet destruction method by Atomic Absorption Spectrophotometry (AAS) following SNI 8910:2021.

2.6 Data Presentation

The presentation of the data was carried out by analyzing pH with the addition of citric acid using Microsoft Excel and SPSS 18.01 one way anova. Meanwhile, to analyze the addition of citric acid to the concentration of Lead in roots and leaves, the analysis of the values of BCF (Bioconcentration Factor), BAF (Bioaccumulation Factor), and TF (Translocation Factor) was used.

3 Result and Discussion

3.1 Preliminary Test Result

Pb concentrations in polluted soil in Karangdawa ranged from 5.5 mg/kg to 48.8 mg/kg.

3.2 Toxic Test Result

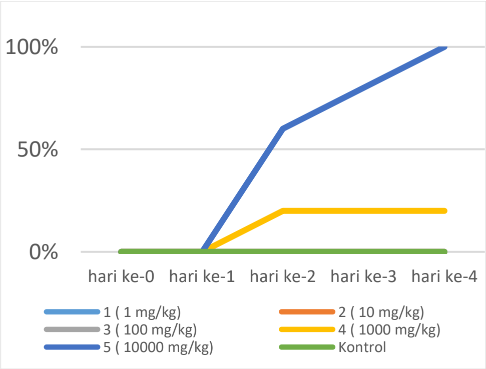
Toxic test is a preliminary test to determine the ability of Vetiver zizanoides to survive on polluted soil. The toxic test consisted of a screening test and a definitive test, each of which was carried out within 4 days (96 hours) with observations on plant mortality.

Screening test aims to find the initial range in which plants can grow on exposure to heavy metal Lead. In this screening test, Lead was added with a concentration of 10^0, 10^1,10^2,10^3,10^4. From the screening test (Table 7), it was found that the plants could not survive on lead contamination above 10000 mg/kg. Meanwhile, at a concentration of 1000 mg/kg Pb, vetiver was still alive despite the death of 20%. This basis is used for the definitive test.

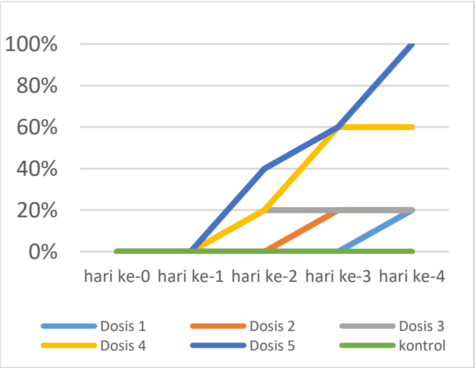
The Devinitive test uses a logarithmic concentration scale because organisms respond logarithmically to toxins. So, the experimental dose used is based on the toxic test in the concentration range of 10^3 and 10^4. The doses given are: 10^3 , 10^3,25 , 10^3,5, 10^3.75, and 10^4. From the results of the definitive test, the results are listed in Table 4 below.

Table 1. Result of Screening Test

Day	Screening test						Devinitive Test					
	Number of dead plants						Number of dead plants					
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Control 1	Dose 6	Dose 7	Dose 8	Dose 9	Dose 10	Control 2
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	1	3	0	0	0	1	1	2	0
3	0	0	0	1	4	0	0	1	1	3	3	0
4	0	0	0	1	5	0	1	1	1	3	5	0



(a)



(b)

Fig. 1. (a) Mortality rate on Screening Test (b) Death rate on Devinitive Test

In the observation of dose 1 where on the first day until the 4th day there was only 1 plant that died, and 1 plant experienced chlorosis. In the application of doses 2 and 3, there was a death of 1 plant. At the 4th dose (addition of Lead 5623.41 mg/kg) mortality was above 50% or 3 plants died and 2 plants were chlorosis. Meanwhile, at the application of dose 5 (10000 mg/kg Lead), all plants died on the 4th day. Determination of LD50 was analyzed by looking for the relationship between lead dose and the percentage of plant mortality. In the equation (Figure 13), the LD50 value is known to be 4979.8 ppm, meaning that the mortality rate of 50 percent of plants at a dose of lead is 4979.8 ppm.

The explanation of how Vetiver zizanoides can live under lead stress is: In the root system, Chandra, Dubey, and Kumar 2017 [2] stated that the root system takes inorganic compounds dissolved in water and nutrients. Roots also release exudates. Exudates consist of carbohydrates, vitamins, organic acids, enzymes, growth factors, and others that carry nutrients to the surrounding environment, especially for soil microorganisms that live around the roots [3]. Soil microorganisms form close contact with the root system which leads to the activation of potential metabolic interactions between roots and microorganisms. The mucus covering the root cap provides an additional substrate for the soil microflora [4]. Exudates consist of carbohydrates, vitamins, organic acids, enzymes, growth factors, and others [5,6] that carry nutrients to the surrounding environment, especially for soil microorganisms that live around the roots and some soil microorganisms that live in the root cap have functions as rhizofiltration, phytostimulation, and phytostabilization (movement of contaminants in the soil due to plant exudates) [7].

However, if the heavy metal content of the soil is very high, the mechanism that occurs is: Metal Lead and other heavy metals in the soil will have an effect of oxidative stress on plants, one of which is the formation of ROS [8]. ROS damage cell tissue and genes by interfering with the synthesis of ATP [9]. However, some plants have the ability to survive in an environment containing high heavy metals, by forming phytokelatins and metallotionins [10]. Phytochelatin synthesized from Glutathione protein functions in the binding of heavy metals to the soil [11].

Phytochelatins are low molecular weight thiols that bind metal ions using thiol groups as ligands. These phytochelatins are called natural chelators which consist of three amino acids – Glu, Cys and Gly and have various sizes with the general structure (-Glu-Cys)nGly (n = 2 to 11) [12]. Thiol group of Phytochelatin, Binds Pb to form a ligand/complex. While metallotionin is a protein that is responsible for cell damage [3]. Metallotionins are responsible for transferring excess Pb and other heavy metals from one plant part to another to minimize damage to one side of the cell [10]. The synthetic ability of Phytochelin and Metallotionin in each plant is different, depending on the carrier gene of each plant. These carrier genes can be complexing agents to form organic acids, amino acids, H+ ion-paired proteins, and metal-binding paired proteins [2]. This mechanism causes plants to survive high exposure to heavy metals. Based on this toxic test, Vetiver zizanoides is proven to be able to survive in environments with Pb content of less than 4979.8 ppm.

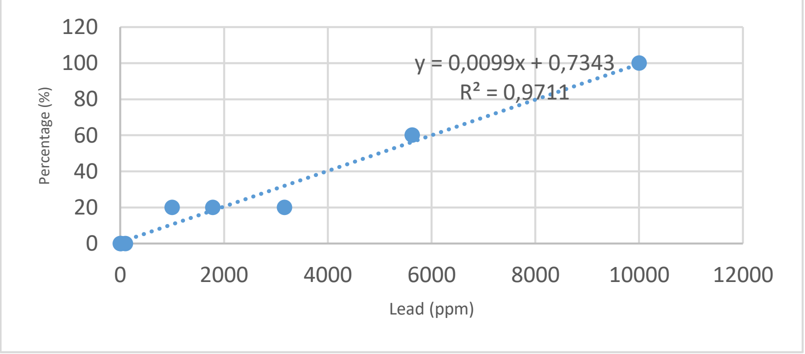


Fig. 2. Analisys of Lead Concentration on Plant Mortality.

3.3 Analysis of Plant Growth Rate

Plant growth from the first day until the 10th day did not show a significant increase in length because the plants were still adapting to the new environment. plants have not yet formed exudatekets, where exudates are enzymes, carbohydrates, and vitamins, which are released by plants that form a nutrient-rich environment for microorganisms (bacteria, actinomycetes, fungi) [13]. Exudate that has not formed around the roots reduces the accumulation of microorganisms into the root cap. In the root cap, there are Rhizobacteria bacteria, where the role of rhizobacteria can increase the growth of various plant organs, especially roots [2]. Bacteria that promote plant growth are known as plant growth-promoting bacteria (PGPP) [1]. Plant growth-promoting rhizobacteria known as PGPR and plant growth-promoting endophytic bacteria called PGPE are the two main types of soil bacteria that have been shown to act as plant growth-promoting bacteria [14]. During interaction with roots, endophytic bacteria and rhizosphere can perform several functions, such as increasing ammonia production; and synthesizing nutrients needed by plants, and producing plant hormones such as auxins, gibberellins, and cytokinins [10,15].

After day 15 the growth of the vetiver was not optimal. there is a decrease in growth rate at a dose of 2 g/kg. Analyzed for changes in soil pH, after the 15th day the soil pH reached 3, which caused a decrease in the growth rate. Vetiver can grow optimally in the pH range of 5.4 to 7.4 [16]. From the analysis, vetiver growing below pH 3 (after the 15th day) experienced a decrease in the growth rate of plant length, because the pH is not optimal for vetiver growth. But in addition to pH, the growth rate is also caused by the rate of transpiration. Transpiration rate is caused by differences in soil air temperature and ambient air.

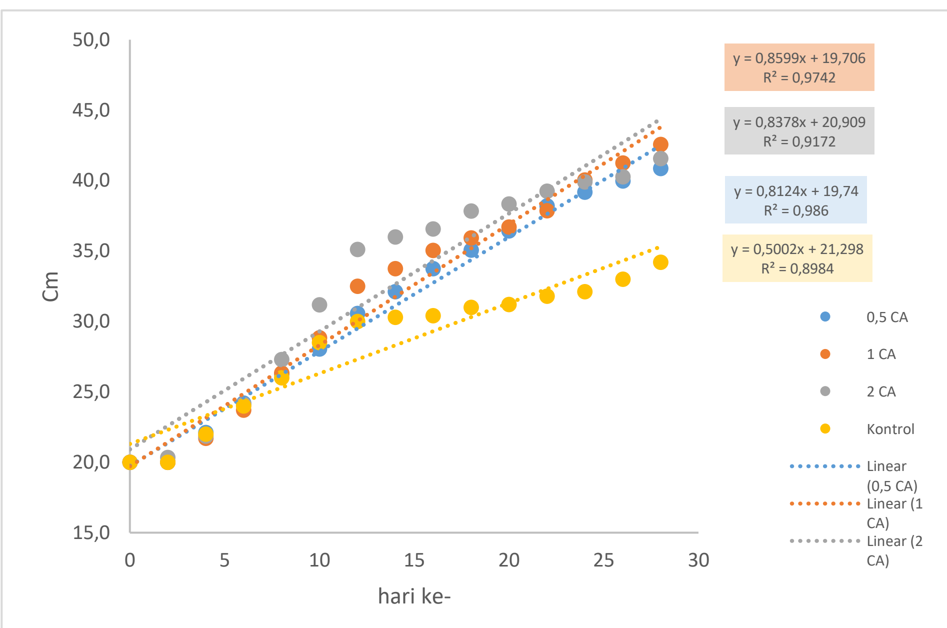


Fig. 3. Analysis of Lead Concentration on plant growth

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3.4 Analysis Dry Weight of Plant

At the end of the study, the dry weight of the plant was measured, to find the relationship between the addition of citric acid to the dry weight of the plant. The results of the average dry weight on roots and shoots of Vetiver zizanoides are presented in Figure 4.

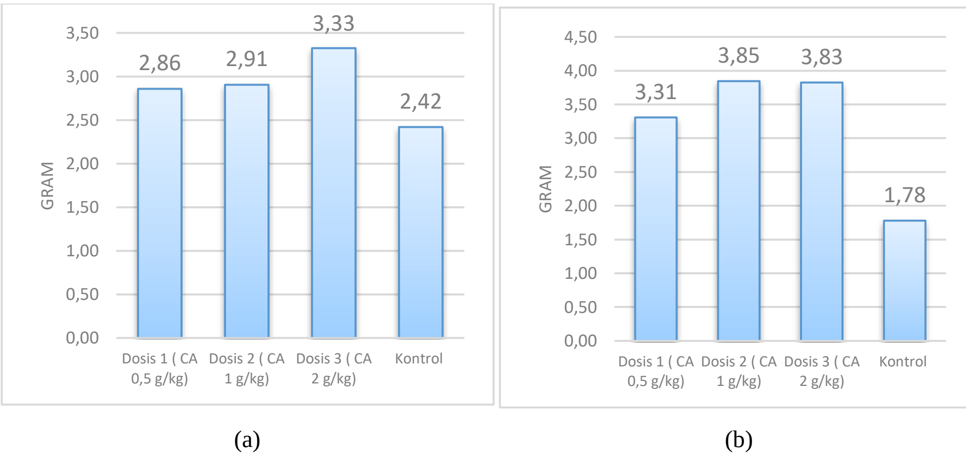

Fig. 4. (a) Average shoot dry weight ; (b) Average root dry w eight

The dry weight of the plant with the application of a dose of 2 g/kg Citric acid was the highest compared to other treatments. In the roots, the addition of citric acid at a dose of 1 g/kg resulted in a higher average dry weight of the plant. The application of citric acid around the roots will attract soil microorganisms to gather in the roots, and this will affect the absorption and stabilization of heavy metals in the roots. Microorganisms that gather more roots, result in better root growth [17], and good root growth will increase the area of absorption of nutrients and heavy metals to plant shoots.

3.5 Analysis to Soil pH

The research soil has a pH between 2.9-4.2. In the application of citric acid, the pH change was highest from the application of a dose of 2 g/kg. Citric acid which is a weak acid can lower the pH, the larger the dose of citric acid, the lower the pH. Previous research that mentions the addition of citric acid in small amounts over a certain period of time is a form of soil acidification to help chelate metals [18]. Soil pH is the most important factor affecting the mobility of metals in the soil, because it is directly related to the formation of dissolved complexes and adsorption/precipitation phenomena [19]. The decrease in pH is directly proportional to the increase in the concentration of citric acid.

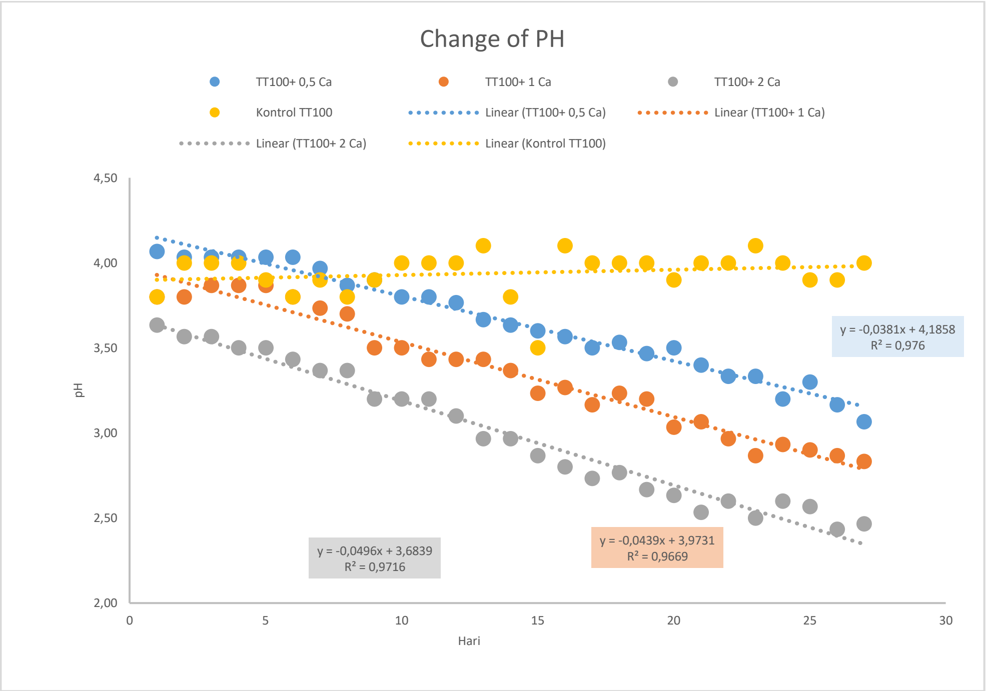

Fig. 5. pH change

In the decrease in soil pH by citric acid, the acidity of citric acid is obtained from the three COOH carboxyl groups that can release protons in solution in the soil. These three carboxyl acids release protons (H+) in the solution and produce citrate ions (COO-) [20]. These ions will bind to heavy metals (Pb) in the soil, which generally Pb has a charge of 2+ (13), resulting in a neutrally charged ligand/complex.or it can be explained that the hydrogen atom (H) is deprotonated (escaped) from the carboxyl group. The release of H+ ions (protons) with large ionization potential can enter the soil to replace the position of the cations. The release of protons from citric acid depends on the pKa value. Where the pKa value of citric acid for each carboxyl group is 3.13; 4.76; 6.4 [15]. If the soil pH is above the pKA value of citric acid, all protons can be released. However, if the soil pH is below pKA, less H+ is released, it means that COO- cannot form ligands with Pb2+ [21]. The conclusion from the analysis, that the higher the concentration of citric acid, the higher the decrease in pH (Figure 27). In a previous study, it was also mentioned that the application of citric acid with doses of 1.3 and 5 mmol on average reduced the pH to 0.49 in the 2 weeks of the experiment [22].

3.6 Analysis to Soil pH

Bioconcentration (BCF), Bioaccumulation (BAF), and Translocation Factor (FT) were used to study the ability of plants to accumulate heavy metals from the soil, and their efficiency in absorbing heavy metals from the soil. To determine the index/value, laboratory Lead measurements must be carried out. The results of the laboratory analysis of Lead concentration are as follows

The highest accumulation of Pb in roots was at the application of a dose of Citric acid (CA) 1 g/kg on polluted soil. Based on the results of the study, a dose of CA 1 g/kg was better in root Pb accumulation than CA 0.5 and 2 g/kg. This happened because the use of high doses of Citric Acid had a phytotoxic effect on plants [23], and too-small doses did not significantly increase the accumulation of Pb in the roots. However, compared to control plants, Pb accumulation in roots was higher in plants treated with citric acid. The role of citric acid in the accumulation of Pb in roots was described by D.P. Mellor 1965; the presence of citric acid is added to the soil, then COO- binds to Pb2+ to form a neutrally charged ligand that is not blocked by the charge of the root cell wall, so that it is easily absorbed by plants. Neutrally charged Pb and citric acid complexes will be absorbed to the root surface of the rhizodermis, then along with water to the upper plants through the Xylem. Xylem is a tube system that connects the leaves to the roots, the suction power in the xylem which is influenced by the rate of transpiration will attract water as well as the Pb-Citric Acid complex to the roots. While the accumulation of Pb in the shoots, polluted soil with a dose of citric acid 1 g/kg had the highest accumulation of Pb in the leaves. This is related to the optimal conditions that plants want to absorb metals, whereas, in previous studies, citric acid added in high doses and for a long time have a phytotoxic effect, including inhibiting growth and decreasing the ability to absorb heavy metals [18].

Table 2. Bioconcentration (BCF), Bioaccumulation (BAF), Translocation Factor (FT)

Treatment	Pb in root	Pb in shoot	BCF	BAF	TF
TT 100 + 0,5 CA	25,27	8,09	0,924 ± 0,358a	0,327 ± 0,383a	0,304 ± 0,174a
TT 100 + 1 CA	51,31	19,32	3,051 ± 1,302b	1,325 ± 1,000b	0,446 ± 0,326a
TT 100 +2 CA	34,42	9,32	2,019 ± 1,050b	0,575 ± 0,487a	0,415 ± 0,241b
TT 100	9,68	3,64	0,456 ± 0,335a	0,192 ± 0,127a	0,376 ± 0,242b

Note: Data is mean ± SD. Different lowercase letters in the same column show significant differences at the 0.05 level with Duncan's test

Bioconcentration is the entry of pollutants (Lead) directly from the environment (soil) into plant tissues. The value of BCF bioconcentration is known by comparing the contaminant concentration in the roots with the contaminant concentration in the soil. If the BCF value > 1, then Pb has the potential to accumulate in plants and can be considered a hyperaccumulator plant [25]. The BCF value polluted soil, citric acid increased the BCF value > 1 on the application of 1 g/kg citric acid while in control plants without citric acid, the BCF value < 1 means that Vetiver zizanoides is less capable as a Pb hyperaccumulator without chelating assistance. Pb is indeed one of the metals that have low bioavailability, settle in the soil, and are difficult for plants to absorb. The application of citric acid helps the absorption of Pb in the roots, as evidenced by the BCF value of more than 1. It means that Vetiver zizanoides are less able to absorb Pb, and the addition of citric acid makes the BCF value increase. The highest BCF value is at a dose of citric acid 1g/kg, this dose is the optimum dose for soil conditions that have an acidic initial pH (such as in contaminated soil of Karangdawa which has an initial pH range of 3-4). A dose of 2 g/kg citric acid applied for 28 days causes a decrease in pH and changes in ideal conditions for plants and soil microorganisms [18]. In addition, a pH that is too acidic will reduce bacterial colonies in the roots [19] thereby reducing the BCF value or the ratio of Pb concentrations in the roots and in the soil. This is also indicated by the growth rate of plant length which decreases after the pH is below 3 on the application of citric acid 2 g/kg after the 15th day.

From the analysis results, in control plants, BAF value < 1 means that Vetiver zizanoides plants are less able to accumulate Pb in shoots without a chelating agent. In polluted soil, the addition of citric acid 1 g/kg has a BAF value of more than 1. The shoot concentration was higher than the soil concentration with optimal absorption effectiveness at a dose of 1 g/kg Citric Acid.

In the chemical process, it was explained in the previous discussion that the COOH group of citric acid will release protons H+ and COO-, where COO- binds to Pb2+ to form a complex that can be absorbed by roots. Soil pH also affects the release of protons from citric acid. The effect of pH on the release of H+ (proton) depends on the pKa value. Where the pKa value of citric acid for each carboxyl group is 3.13; 4.76; 6,4, (15) if the soil pH is above the pKA value of citric acid, all protons can be released, if the pH is below the pKA, the H+ released will be small, meaning that COO- cannot form ligands with Pb2+. At polluted soil pH conditions below 3.13 (after the 15th day) caused protons to not be released, so that Pb did not form ligands/complexes with citric acid. resulting in the TF value < 1. The TF value is a comparison of the concentration in the shoots and roots, if the TF value > 1 then the plant has the ability as a hyperaccumulator plant for phytoextraction.

From the results of the analysis, the TF value of plants given citric acid compared to control plants had a higher TF value, but not significant because it had a TF value < 1. The higher Pb accumulation in the roots compared to the leaves in the application of Citric Acid was also influenced by the activity of microorganisms around the roots. Phytocelatin stimulates the formation of exudate in the roots, and this exudate makes microorganisms gather around the roots, and the application of citric acid around the roots causes more soil microorganisms to gather around the rhizofer. These microorganisms affect the absorption and stabilization of heavy metals in roots [6, 24]. The study of Cooper et al., 1999 and Kulli et al., 1999 stated that the addition of citric acid resulted in a significant increase in the solubility and uptake of plants for metals in the soil, but the effectiveness of citric acid in mobilizing metals to shoots was low because citric acid is composed of C and O which is needed by microorganisms for food sources so that the decreased pH is not necessary. Significant [25].

4 Conclusion

Based on the research, the following conclusions were obtained: Vetiver zizanoides are able to survive Pb stress up to 4979.8 ppm but are less able to remove Pb in the soil without chelating agents, not suitable for Lead Hyperacumulator. Second, the application of 2g/kg Citric Acid caused the highest decrease in pH compared to other treatments in polluted soil, but if it were applied continuously in polluted soil in Karangdawa, it would cause the soil to be very acidic and not the ideal conditions for Vetiver to grow. But the application of citric acid at a dose of 1 g/kg significantly increased the value of bioconcentration (BCF), and bioaccumulation (BAF) because it created an optimal pH for plants and microorganisms to uptake Lead to the roots and tops of plants (roots and leaves), so that Pb absorption rooted very high. The high absorption of Pb in the roots will increase the rhizofiltration function in soil and groundwater.

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