

The Effectiveness of Nitrogen Fertilization in *Codiaeum variegatum* L. and *Sansevieria trifasciata* L. and the Effects on Pb Accumulation

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ABSTRACT

The use of $(\text{NH}_4)_2\text{SO}_4$ fertilizer is vital in increasing plant growth and reducing the Pb content in soil. This study determined the ability of *Codiaeum* and *Sansevieria* to accumulate Pb due to $(\text{NH}_4)_2\text{SO}_4$ fertilization and its effects on their growth rate. The ammonium fertilizer was applied three days after repotting according to these treatment levels: 0, 100, and 200 kg N/ha, denoted as P₀, P₁, and P₂, respectively. The height of *Codiaeum* decreased by 45.04%, 24.67%, and 13.70% in treatment P₀, P₁ and P₂, respectively, one week after repotting. The Pb concentrations in *Codiaeum* decreased with increasing doses of N fertilizer, while in *Sansevieria*, Pb concentrations increased with higher doses of N fertilizer. A reduction in the soil pH was seen with increasing dosage of N fertilization in *Codiaeum*, while the pH of the medium with *Sansevieria* was stable. Therefore, the effect of the different N doses, which causes a decrease in soil pH, was more significant in *Codiaeum* compared with *Sansevieria*. Furthermore, the highest value of TF was found in *Codiaeum* treated with 200 kg N/ha.

1. INTRODUCTION

Lead (Pb) is one of the heavy metal pollutants that is one of the most important worldwide ecological problems. It is heavy metal contaminant in the environment. Pb poisoning is an old and significant public-health problem throughout the world (Flora et al., 2006). Pb contamination in the plant environment has been shown to be highly toxic with affects on some processes such as depression of seed germination (Islam et al., 2007).

Most soil used for the purpose of agriculture obtains its heavy metals from agrochemicals through synthetic fertilizers and pesticides. Parkpian et al. (2003), and Atafar et al. (2010) reported that chemical fertilization increased the presence of heavy metals in soils. Also, Ogunlade (2011) reported that the use of heavy metals such as Cu, Pb, and Hg in various pesticides contribute to the increase in their concentration in soils and plants.

Additionally, soil naturally contains these heavy metals, some of which play vital roles in the physiological processes of plants. Examples include Fe, Cu, Zn, and Ni, but in relatively small amounts. An

excess of these metals is generally toxic to the plants. However, Cd and Pb are very poisonous, and, so far, their role is unknown in plant chemistry. According to (Lawlor et al., 2004), both elements are essential chemical contaminants in the environment and highly toxic to plants, animals, and humans. However, strategies implemented to clean up contaminated areas are generally costly (Longhurst et al., 2004), hence, there is a need to develop a cheap and environmentally friendly approach, such as fertilizing nitrogen using Ammonium Sulfate $((\text{NH}_4)_2\text{SO}_4)$.

In general, $(\text{NH}_4)_2\text{SO}_4$ is an inorganic salt with several uses, among which is as a soil fertilizer since it contains 21% nitrogen and 24% sulfur. When added to a planting medium, it forms Ammonium $(\text{NH}_4)^+$ and Sulfide $(\text{SO}_4)^{-2}$. In addition, the sulfide ions could bind with Pb^{+2} to form PbSO_4 , which plants easily absorb (Gurnita et al., 2017). Additionally, it has been shown through some studies that ammonium sulfate has the capacity to accumulate Pb content in *Brassica pekinensis* and *B. juncea* var. multiceps (Wang et al., 2008b; Xiong and Lu, 2002), and in bicolor sorghum leaves (Zhuang et al., 2009).

Phytoremediation is defined as the use of green plants to remove pollutants from the environment or to render them harmless. Phytoremediation can be applied to both organic and in-organic pollutants, present in solid substrates (e.g., soil), liquid substrates (e.g., water), and the air. One area of phytoremediation is phytoextraction. Salt et al. (1998), and Schmidt (2003) reported that the application of ammonium sulfate fertilizers to plants helps in increasing the efficiency of phytoextraction. According to Garbisu and Alkorta (2001), Quartacci et al. (2006), and Laidlaw et al. (2012), phytoextraction uses green plants to eliminate toxic metals from contaminated soil, making it an environmentally safe, relatively cheap and technically applicable strategy for the improvement of soils contaminated with heavy metals. However, its efficiency in hyperaccumulators is generally considered too slow due to low biomass production, slow growth rates and high metal specificity (Mulligan et al., 2001; Puschenreiter et al., 2001; Lesage et al., 2005).

The process of absorption and accumulation of heavy metals by plants starts with root absorption. Heavy metals must be in the form of a solution so that they can be absorbed by the roots of the plant. Heavy metals can reach the endoderm layer of the roots of the plant and then pass through xylem and phloem to the top of the plant. In this method, the plant attempts to avoid heavy metal contamination from its cells by storing heavy metals in certain organs, such as roots or leaves, so as not to inhibit the metabolism of tissue processes (Tangahu et al., 2011). Two of the plants that can absorb and accumulate heavy metals are *Codiaeum variegatum* and *Sansevieria trifasciata*. Both of these plants have been shown to be effective in removing Pb from polluted soil (Kumar and Saritha, 2014).

Application of inorganic fertilizers containing NH_4^+ , on plant mobilization of Pb is greatly increased even at low pH (Tu et al., 2000; Schmidt, 2003). Similarly, the application of nitrogen modifications effectively increased the accumulation of Pb (Lin et al., 2010). However, the influence of $(\text{NH}_4)_2\text{SO}_4$ on the improvement of Pb uptake was not very prominent.

This project studied the growth rate of *Codiaeum* and *Sansevieria* and their ability to accumulate Pb due to fertilization with $(\text{NH}_4)_2\text{SO}_4$.

2. METHODOLOGY

2.1 Time and place

This research was conducted in the greenhouse

of Agriculture Faculty, University of Islam Malang, East Java, Indonesia from September to November 2018. The altitude is 550 m above sea level, with a temperature of 20-29°C, at 112°06'-112°07' east longitude and 7°06'-8°02' north latitude.

2.2 Materials

The varieties of plants used in this study were *Codiaeum* with Gracepink and *Sansevieria*. The *Codiaeum* was two months old with height ± 30 cm and ± 40 cm for the *Sansevieria* with the same age range. Both plants were acclimatized to the greenhouse for one month before planting. Also, one week prior to planting, the soil was prepared in a 5 kg plastic bag mixed with $\text{Pb}(\text{NO}_3)_2$ at a dose of 350 mg/kg of soil. Then the N fertilizer using ammonium sulfate was applied 3 days after repotting according to these treatments -0, 100 kg N/ha (1.19 g $(\text{NH}_4)_2\text{SO}_4$ /polybag) and 200 kg N/ha (2.38 g $(\text{NH}_4)_2\text{SO}_4$ /polybag).

2.3 Parameters measured

The growth of *Codiaeum* and *Sansevieria* was evaluated starting one week after planting at intervals once a week, by measuring the plant height and leaf area. Plant height was determined by extending the last collared leaf upright. Plant heights were also measured for each individual plant before harvest. Leaf areas were measured with Leaf Area Meter (LAM). Also, the effect of N fertilizer in the accumulation of Pb was determined using an Atomic Absorption Spectrophotometer. The effects on growth rate were evaluated by measuring the total root length, root dry weight, soil pH, concentration of Pb on soil, crop and root, and the Translocation Factor (TF). According to MacFarlane et al. (2002), TF values are calculated to determine the transfer of accumulated metal from roots to the plant crop. It is calculated as follows:

$$\frac{\text{Pb on shoot}}{\text{Pb on root}}$$

2.4 Experimental design

The data analysis used in this study was the Factorial Random Block Design. The treatment have two factors. The first factor was a dose of nitrogen, consisting of three levels -0, 100, and 200 kg N/ha. (Wati et al., 2019). The second factor were the plants used *Codiaeum* and *Sansevieria*. Then, all the combination treatments were replicated three times, and each treatment consisted of three samples.

2.5 Statistical analysis

The statistical analysis was performed using SPSS V.17 software (SPSS Inc., Chicago, IL, USA). The data were expressed in the form of means±standard error, and the means were statistically compared using the Duncan’s multiple range test (DMRT) at the $p < 0.05\%$ level.

3. RESULTS AND DISCUSSION

3.1 Result

3.1.1 The effect of nitrogen dose to plant growth

The height of the *Codiaeum* decreased by 45.04% in treatment P₀, although not significantly different from P₁ (24.67%) and P₂ (13.70%) in one week after repotting. This decrease was due to falling leaves. It supposedly happens due to the influence of Pb on the media. Then from the second week onwards, *Codiaeum* started forming new leaves. However, *Sansevieria* showed more resistance to Pb, as the different doses of N had no influence on the plant height. Figure 1 shows that there was no significant change in plant height from the first to the seventh week.

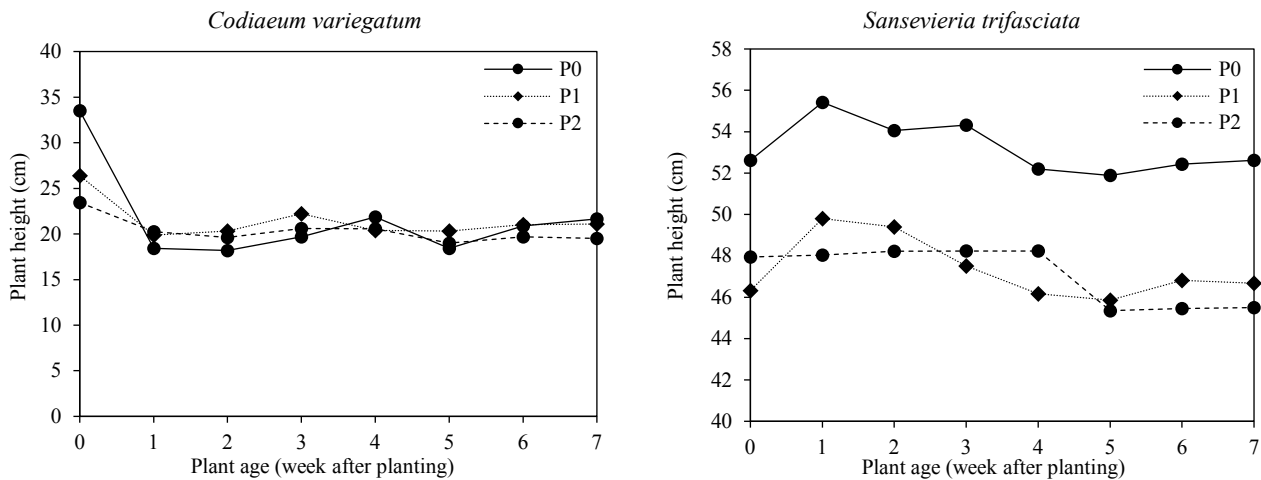


Figure 1. *Codiaeum variegatum* and *Sansevieria trifasciata* plant height development due to N fertilization. (P₀: control; P₁: dose of N 100 kg/ha; P₂: dose of N 200 kg/ha).

The mean of leaf area decreased by 21.19% (P₀), 21.62% (P₁), and 21.40% (P₃), respectively, for the three treatments for *Codiaeum* as shown in Figure 2. This was as a result of leaf loss due to soil exposure to Pb. However in *Sansevieria*, the reduction occurred only in the first week by 13.46% (P₀), 23.08% (P₁), and 17.82% (P₂). The decrease started with contraction

at the leaves tips, which eventually dried out. The different doses of nitrogen did not affect the leaf area. The N fertilizer in *Codiaeum* is not able to increase plant height and leaf area. The loss of leaves in the first week is more due to the influence of Pb on media. In *Sansevieria*, N fertilizer and Pb had no significant effect.

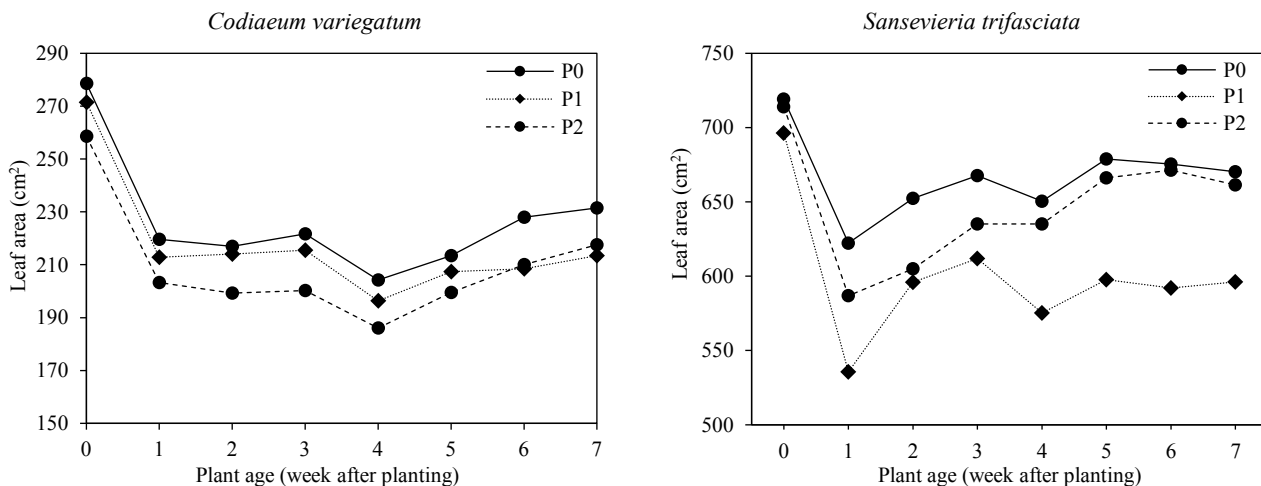


Figure 2. *Codiaeum variegatum* and *Sansevieria trifasciata* leaf area development due to nitrogen fertilizer (P₀: control; P₁: dose of N 100 kg/ha; P₂: dose of N 200 kg/ha).

3.1.2 The effect of nitrogen dose and concentration of Pb on crop, root and soil

In *Codiaeum*, the concentrations of Pb in the crop, roots, and soil decreased with increasing dose of N fertilizer. However in *Sansevieria*, the

concentrations of Pb in the crop increases with an increasing dose of N fertilizer. N fertilizer does not affect Pb uptake in roots and soil. N fertilizer in *Codiaeum* can reduce Pb uptake in crops, roots, and soil, but it is not effective for *Sansevieria* (Figure 3).

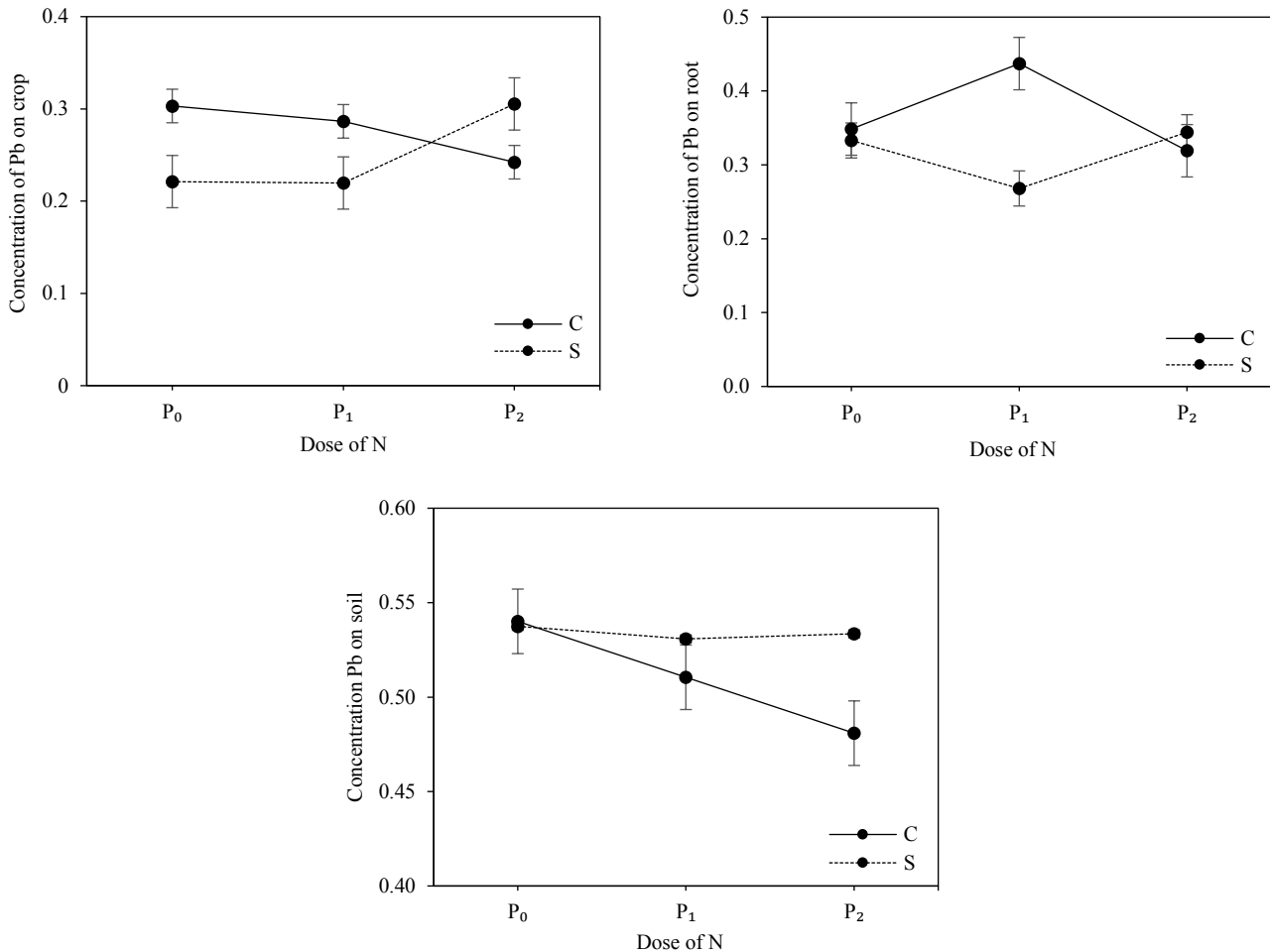


Figure 3. Concentration of Pb on crop, root, and soil due to dose of nitrogen fertilizer (P₀: control; P₁: dose of N 100 kg/ha; P₂: dose of N 200 kg/ha; C: *Codiaeum*; S: *Sansevieria*).

3.1.3 The effect of nitrogen dose with soil pH and tranlocation factor (TF)

The analysis of the *Codiaeum* soil shows that increasing the dose of N fertilization reduces the soil pH but tend to be stable in the medium planted with *Sansevieria* (Figure 4). Hence, the higher the fertilizer dose, the lower the pH value. The effect of N fertilizer doses with respect to decreasing soil pH was more significant in the *Codiaeum* compared with *Sansevieria*. Similarly, the highest TF value was found in *Codiaeum* treated with 200 kg N/ha fertilizer, as shown in Figure 4. This means that *Codiaeum* fertilized with 200 kg of N/ha was better for translating Pb from the root to the plant crop.

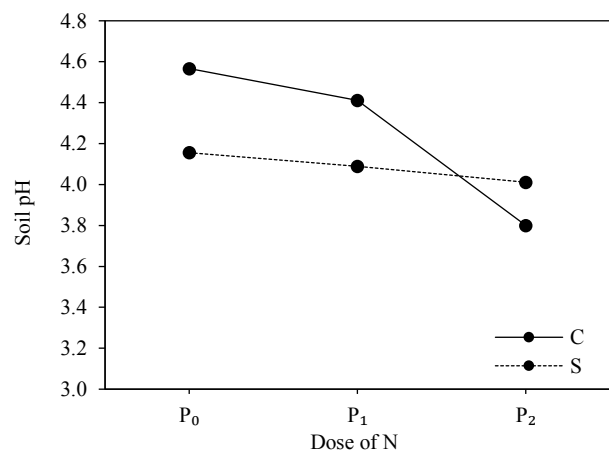


Figure 4a. Soil pH value due to dose of N fertilizer (P₀: control; P₁: dose of N 100 kg/ha; P₂: dose of N 200 kg/ha; C: *Codiaeum*; S: *Sansevieria*).

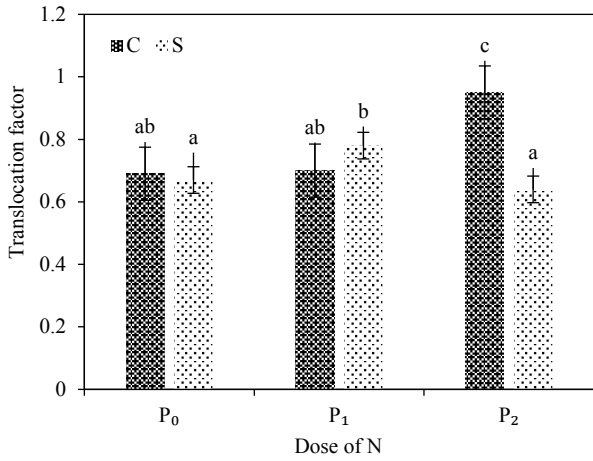


Figure 4b. TF value due to dose of N fertilizer (P₀: control; P₁: dose of N 100 kg/ha; P₂: dose of N 200 kg/ha; C: *Codiaenum*; S: *Sansevieria*) (cont.).

The relationship between the dose of N fertilizer and the absorption of Pb in the root and crop, as shown in Figure 5 tend to follow a quadratic pattern. The higher the dose of N fertilizer at a certain level, the greater the absorption of Pb in the root and crop, and after this level, the absorption of Pb decreases. Also, the optimal dose for *Codiaenum* was between 95-166.7 kg N/ha with root and crop absorption levels of Pb at 0.43875 ppm and 0.32753 ppm respectively. In addition, the optimal dose of *Sansevieria* was between 108.3-116.7 kg N/ha with root and crop absorption levels of Pb at 0.37383 ppm and 0.29292 ppm respectively.

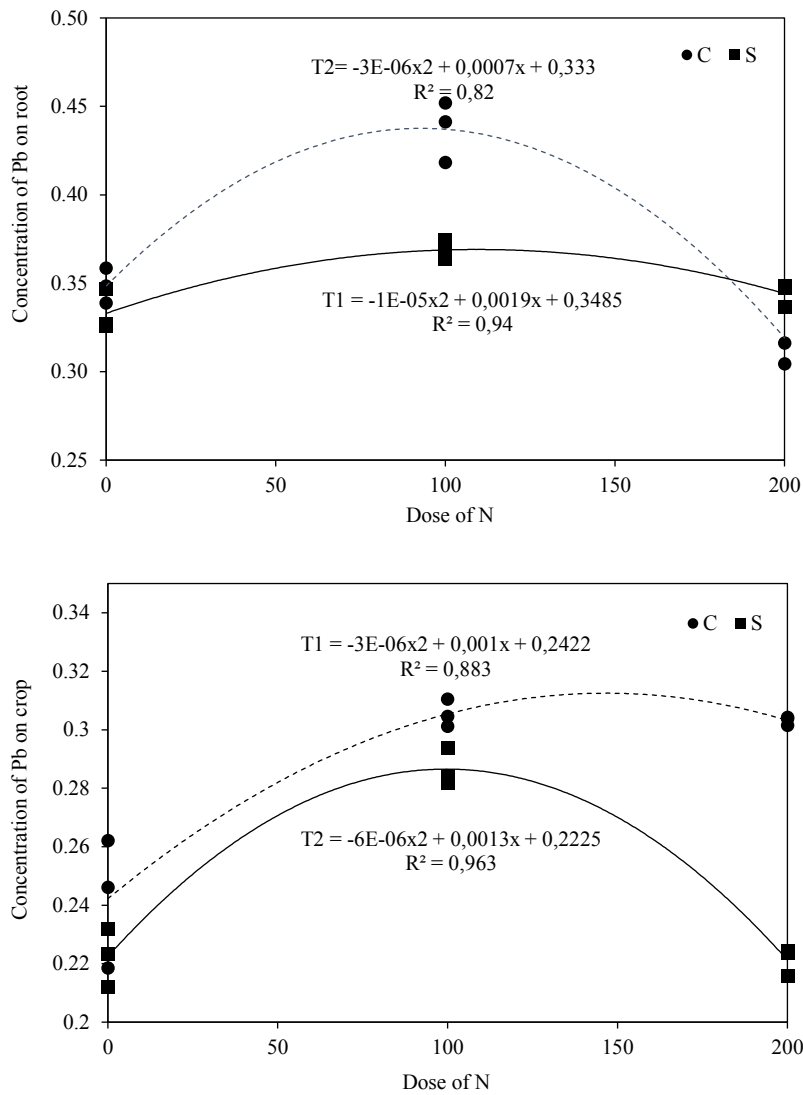


Figure 5. Graph of the relationship between the dose of N fertilizer and the absorption of Pb in roots and crops in *Codiaenum* and *Sansevieria*.

3.2 Discussion

Plants have a huge sensitivity to the presence of heavy metals in the growing media. The response shown by plants is strongly influenced by their species and the growth media, which was soil in this case. The effect of Pb largely depends on its concentration, soil properties, and plant species involved (Patra et al., 2004; Yilmaz et al., 2009). Additionally, there are many reports of toxicity of Pb in plants (Choudhury and Panda, 2005), including mitotic disorders (Jiang and Liu, 2000), inhibition of root and shoot growth (Liu et al., 2009), induction of leaf chlorosis (Pandey et al., 2007), reduction of photosynthesis (Xiao et al., 2008) and inhibition or activation of some enzymatic activities (Verma and Dubey, 2003; Sharma and Dubey, 2005; Liu et al., 2009). All these are mainly obvious in *Codiaeum* plant. However, the leaves of *Sansevieria* showed symptoms of dryness (necrosis) and wrinkles. Also, a study conducted by Sorrentino (2018) shows that the exposure of leaves to heavy metals causes an indirect decrease in its pigment concentration as a result of decrease in the rate at which Fe is transported to the leaves, resulting in the interruption of pigment synthesis.

Pb is absorbed by plants when the soil organic matter content and fertility are low. The soil composition, pH and cation exchange capacity (CEC) also affect the transfer of Pb from soil to plants. The Pb in this situation is separated from the bonding of the earth in the form of ions which move freely and then absorbed by plants through ion exchange. In addition, Pb are absorbed by plant roots when other heavy metals are unable to inhibit their presence. This causes the soil to be dominated by Pb cations, thereby reducing other cations in the root absorption complex. Furthermore, the absorbed Pb^{2+} from the roots into the plant inhibits the formation of enzymes and the process of plant metabolism, which includes the process of respiration which produces ATP used for photosynthesis. Hence, cell division (height, number and biomass) and reproduction are disrupted. According to (Alloway, 1990), the continuous occurrence of this, has a long term effect of reducing the quality of fragrant root plants growth, thereby affecting the general plant growth.

Soil pH is also important to plants and according to Brown et al. (1994), Dijkshoorn et al. (1983), and Zeng et al. (2011), who reported that pH is a key factor influencing the availability of heavy metals for root uptake. Zaccheo et al. (2006) revealed that the administration of $(NH_4)_2SO_4$ and $(NH_4)_2S_2O_3$

reduces soil pH by approximately two units, and the presence of metals increases in soil solution. Also, the higher the pH value, the lower the presence of Pb metal, but high pH affects the precipitation of metal complexes (Wang and Chen, 2006). Fertilizers with high content of NH_4^+ leads to the reduction in the soil pH. Also, the application of $(NH_4)_2SO_4$, being an oxidizing acid fertilizer, has the capacity to reduce the soil pH and increase the availability of heavy metals in the soil. These have been confirmed by many researchers (Eriksson, 1990; Lou, et al., 2005; Wang et al., 2008a). Furthermore, Schmidt (2003) suggested that the use of $(NH_4)_2SO_4$ could be considered a low cost phytoextraction strategy because it is proven that the dissolution of Pb and Cu occurs at lower pH values. Mainly, phytoremediation is a means of reducing heavy metal pollution which saves energy and is cost effective. Also, one of the applications of phytoremediation is phytostabilization. This is usually carried out to reduce contaminants in the soil, thereby reducing the movement levels of metals. Also, during phytostabilization, the concentrations of metals in the crop are lower compared with those in the roots (Ma et al., 2001). Based on calculating the concentrations of metals in the crop and roots, *Sansevieria* is considered a more tolerant plant against heavy metals or as a good candidate for phytostabilization strategies.

Generally, plants actively prevent the movement of metals from the root to the crown by sequestering the metal available in the roots, especially in the vacuole or cell wall (Gupta and Sinha, 2008). Additionally, the root has a system of preventing the transport of metal to the leaves, especially the non-essential metals, thereby leading to its accumulation at the root. According to Yoon et al. (2006), Pb is one of the non-essential metals plants tend to stack in the roots.

4. CONCLUSION

The *Sansevieria* is more responsive to N fertilization, hence, it could be considered as a more tolerant plant against heavy metals or as a good candidate for phytostabilization strategies.

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