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Research Article

Study of Aromatic Plants on Cadmium Exposure Through Anatomical Structure, Shoot Root Ratio, Essential Oil and Heavy Metals

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Abstract

Background and Objective: Aromatic plants are used as phytoremediation tools for heavy metals because they are not food plants so they are safe for humans. However, there is little information about heavy metals. It is necessary to research to assess its resistance to cadmium through analysis of anatomical structure, shoot-root ratio, essential oil content and heavy metal absorption. **Materials and Methods:** Factorial block random design. The factor I: Types of aromatic plants: Vetiver (*Vetiveria zizanioides*), citronella (*Cymbopogon nardus*), citronella (*Cymbopogon citratus*), patchouli (*Pogostemon cablin*), basil (*Ocimum tenuiflorum*). Factor II: Cd concentration: 0, 85, 170, 255 and 340 ppm) three times. Parameters observed were root anatomical structure, root shoot ratio, essential oil content, metal content in the soil, heavy metal absorption in roots and shoots, bioaccumulation factor and translocation factor, as well as the amount of heavy metal extraction. **Results:** Showed that all types of experimental plants experienced root tissue damage due to exposure to Cd. Lemongrass and vetiver provide a high root-to-shoot ratio while yielding higher essential oils at high Cd concentrations. Vetiver grass, citronella, lemongrass, patchouli and basil showed a bioaccumulation factor value of <1 but vetiver grass, lemongrass and basil had a translocation factor of >1. Citronella plant showed a high heavy metal Cd extraction of 5958371 ppb. **Conclusion:** Lemongrass, citronella grass and vetiver grass are the best plants that represent other plants as phytoremediation tools. Judging from its ability to produce essential oils and absorption of heavy metal Cd.

Key words: Phytoremediation, aromatic plants, stress condition, heavy metals, cadmium, essential oils, resistance

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Contamination of farmland with toxic heavy metals can affect the productivity and safety of crops such as food crops and feed crops, which becomes a severe environmental problem[1]. Cadmium (Cd) is a heavy metal that is toxic to plants and animals[2]. The primary source of Cd in the environment is anthropogenic, including from agricultural activities such as chemical fertilizers, pesticides and industrial and mining sources[3]. The Cd is a highly toxic and water-soluble element with no biological function and is easily absorbed by plants due to its high bioavailability[4]. Studied the cadmium induces senescence symptoms in leaf peroxisomes and probably a metabolic transition of leaf peroxisomes into glyoxysomes and suggests that the peroxisomal proteases could participate in the metabolic changes produced by Cd[5].

The use of metal-accumulating plants to clean soil and water contaminated with toxic metals is the most rapidly developing component of this environmentally friendly and cost-effective technology[6]. Plant-based phytoremediation methodology is considered as one a secure, environmentally friendly and cost-effective approach for toxic metal remediation[7].

In recent years, there has been a growing interest in aromatic plants (some herbs) that are considered suitable for phytoremediation that is safe for the environment since the plant is mainly used for secondary products and essential oils from its leaves or roots[8]. Aromatic plants, also known as herbs and spices, have been used since antiquity as folk medicine and as preservatives in foods[9]. More than 400 plants that can accumulate heavy metals have been reported, Brassicaceae being the family with the most significant number of accumulator species[10]. However, information about aromatic plants as a phytoremediation tool for heavy metals, especially cadmium, is still lacking, especially lemongrass, citronella grass, patchouli and basil.

Assessment of soil contamination by metals has been widely done through plant analysis. Both wild plant species and cultivated species are often used as bioindicators for large-scale soil contamination and local contamination[11]. And one indication of plant resistance to heavy metals is through their growth, where fast-growing plants can be candidates as phytoremediation tools. According to Ebbs and Kochian[12] the ideal plant species for restoring soil polluted by heavy metals should be high biomass producing plants that can tolerate and collect pollutants. A further assessment of the plant's resistance to heavy metals, especially in aromatic plants, is its ability to produce high essential oils under heavy metal pressure. Thus, more research is needed on some other aromatic plants that have the potential to accumulate heavy cadmium metals and may enhance essential oils.

Based on the above description, it is necessary to test some aromatic plants such as vetiver grass, citronella grass, lemongrass, patchouli and basil as a phytoremediation tool and produce high essential oils. The purpose of the study was to evaluate the ability of these plants as cadmium heavy metal phytoremediation plants in terms of growth, root anatomy, essential oil content and cadmium metal uptake.

MATERIALS AND METHODS

Study area and sample collection: This research was carried out in the experimental garden of the Faculty of Agriculture, University of North Sumatra, Medan, from May to September, 2019. The materials used in this study were vetiver grass, citronella grass, lemongrass, then patchouli stem cuttings, basil stem cuttings and planting media (topsoil+sand, 1:1), polybag size 5 kg. Materials were obtained from Deli Serdang Regency, Medan, North Sumatra, Indonesia.

Methodology: The study used a Factorial Block Random Design, factor I was a type of aromatic plant consisting of vetiver grass (*Vetiveria zizanioides*), citronella grass (*Cymbopogon nardus*), lemongrass (*Cymbopogon citratus*), patchouli (*Pogostemon cablin*), basil (*Ocimum tenuiflorum*). Factor II was the concentration of heavy metal Cd consisting of 0, 85, 170, 255 and 340 ppm.

Before planting, prepare and weigh 10 g of soil to measure the pH of the initial soil. Then the content of cadmium heavy metals in the soil was analyzed. The soil to be used is dried, after which it is mixed with sand in a ratio of 3:1, then put into a polybag size of 5 kg. According to treatment, prepared and weighed cadmium (artificial) heavy metal derived from $3CdSO_4{\cdot}8H_2O$. The threshold of heavy cadmium metals applied to soils was 85 ppm (U.S. EPA, 1993). The cadmium was then immersed circularly in the root area ($\pm$5 cm from the plant's stem).

Then, five types of plants, namely vetiver grass, citronella grass, lemongrass, patchouli and basil are prepared. In the following way: The vetiver grass used in this study was taken from a 3 months old brood, carried out with cutting with a height of 20 cm and a root length of 8 cm. Patchouli planting material was obtained from patchouli cutting seedlings that were approximately 1 month old, the size of the cuttings consists of 3 segments and the leaves are trimmed first by leaving two leaves grown in a shady place, as well as procedures carried out for basil plants. Maintenance was carried out, including watering and pest and disease control. About 20 weeks after planting (WAP), the plant was removed slowly using polybags torn and then the roots were washed in a bucket of water (so that the roots are not damaged) and then observations are made.

Parameters: The parameters observed were soil pH, C-organic, root-shoot ratio, root anatomy, essential oil content, Cd metal content in the soil, Cd metal content at the root and Cd metal content in the shoot bioaccumulation, translocation factor and total Cd uptake. Then, calculate the percentage of data difference between planted with Cd and without Cd. Further calculated, the value of bioaccumulation factor (BCF), translocation factor (TF) and the amount of metal extraction were used to evaluate the efficiency of phytoremediation and calculated[13] by the following equation:

$$\text{Bioaccumulation factors} = \frac{\text{Metal concentration in the roots of plants}}{\text{Concentration of metals in the soil}}$$

$$\text{Translocation factors} = \frac{\text{Concentration of heavy metals in the plant shoot}}{\text{Concentration of heavy metals in the roots of plants}}$$

$$\text{Amount of metal extraction} = \text{Metal concentration in plant tissue} \times \text{biomass}$$

Statistical analysis: Data from the results of this study were also analyzed by using SAS 9.1.3. The treatment factors showed a real influence on the observed changes, followed by Duncan's multiple range test at a confidence level of 5%.

RESULTS

Root anatomy: The anatomical structure of the transverse slices of vetiver grass, citronella grass, lemongrass, patchouli and basil were shown seen in Fig. 2-5a-e. The image showed that the epidermis tissue was damaged due to Cd in all plants except vetiver grass compared to without Cd. While damage in the cortex tissue was also experienced by all types of plants with an increased concentration of Cd in the soil. The xylem elements in the form of trachea undergo thickening of their cell walls compared to the control treatment.

Root and shoot ratio: The results of various prints showed that the interaction between the aromatic plant type and the Cd metal concentration had a noticeable influence on the ratio of root and shoot. The test results of the average difference between each treatment were shown in Fig. 6. Vetiver grass provided the highest roots and shoots ratio at a concentration of 340 ppm. While in lemongrass plants and citronella grass, the highest response was shown by plants with a Cd concentration treatment of 255 ppm.

Essential oil content: Analysis of variance of aromatic plant type factors on the content of essential oils showed a noticeable influence. In contrast, the Cd metal concentration factor does not give a real influence as well as the influence of the interaction of the two factors was not real. Table 1 showed the average difference between the treatments of aromatic plant types where lemongrass plants have the highest essential oil content and differ markedly from others, except citronella grass which didn't show a noticeable difference. Table 2 showed that lemongrass plants and vetiver grass showed an increase in oil yields in the conditions of exposure to Cd, respectively 14.07 and 8.28%.

Heavy metal content: Table 3 showed the test results of different Cd content in the soil, at the roots and in the leaves caused by differences in Cd concentrations. At the root, the Cd content that received the Cd treatment 340 ppm was higher than the control but did not differ markedly from other treatments. While in the leaves, the highest Cd content was found in the Cd concentration treatment of 255 ppm and was markedly different from the control treatment but did not differ markedly from other treatments.

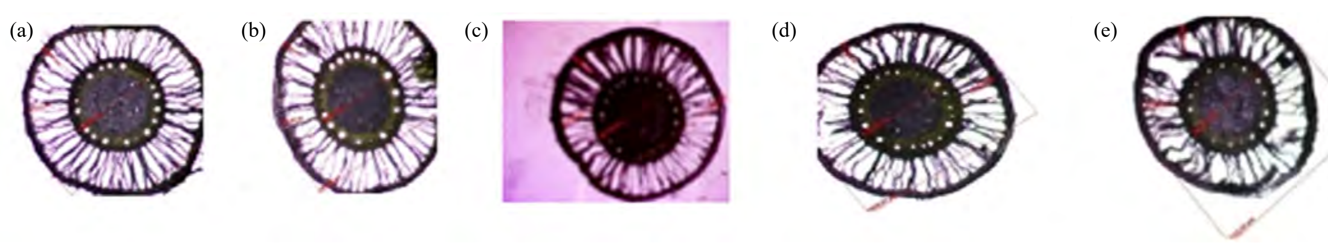

Fig. 1(a-e): Cross-section across the root end of vetiver grass (*Vetiveria zizanioides*) with Cd treatment of (a) 0, (b) 85, (c) 170, (d) 255 and (e) 340 ppm

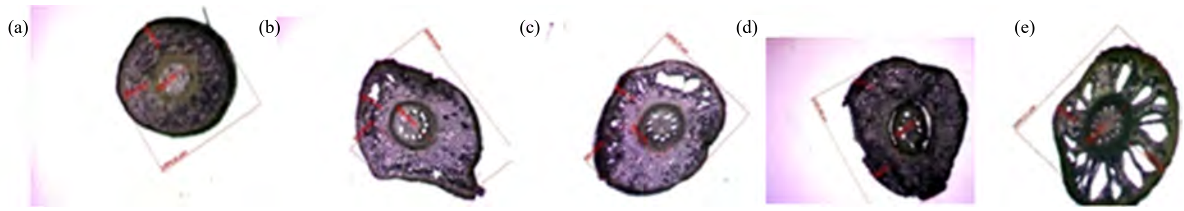

Fig. 2(a-e): Cross-section across the root end of the citronella grass plant (*Cymbopogon nardus*) with Cd treatment of (a) 0, (b) 85, (c) 170, (d) 255 and (e) 340 ppm

Fig. 3(a-e): Cross-section across the root end of the lemongrass plant (*Cymbopogon citratus*) with Cd treatment of (a) 0, (b) 85, (c) 170, (d) 255 and (e) 340 ppm

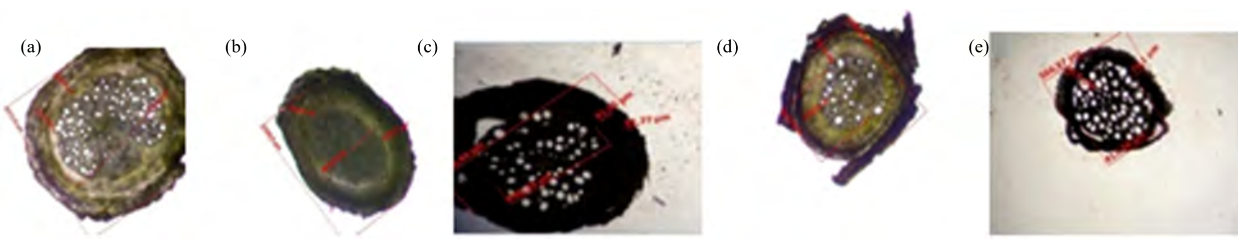

Fig. 4(a-e): Cross-section across the root end of the patchouli plant (*Pogostemon cablin*) with Cd treatment of (a) 0, (b) 85, (c) 170, (d) 255 and (e) 340 ppm

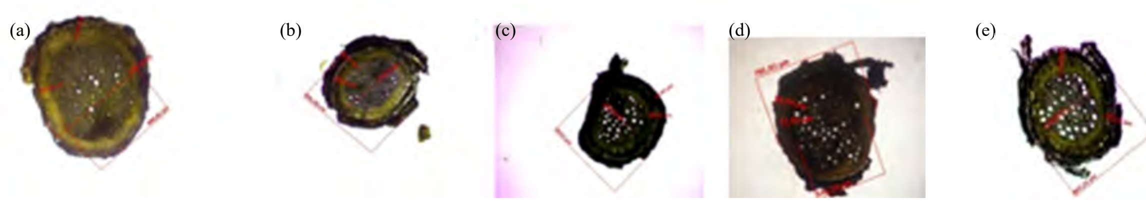

Fig. 5(a-e): Cross-section across the root end of the basil plant (*Ocimum tenuiflorum*) with Cd treatment of (a) 0, (b) 85, (c) 170, (d) 255 and (e) 340 ppm

Table 1: Average oil content (%) in some crops grown at age 20 WAP

Plant types	Oil content (%)
Vetiver grass	0.854^{c}
Citronella grass	1.995^{ab}
Lemongrass	2.182^{a}
Patchouli	1.375^{bc}
Basil	0.993^{c}

Numbers followed by the same letter in the column and row were not significantly different according to DMRT $\alpha = 0.05$ and ^{a,b,c}Means on the same row having different superscripts are significantly different ($p<0.05$)

The test results of the average Cd content in the roots and the leaves in Table 4 showed that patchouli plants have the highest Cd content in the roots, in contrast markedly to the Cd content in other aromatic plants. Similarly, the Cd content in patchouli leaves was also highest and differs markedly from the Cd content in vetiver grass leaves, citronella grass and lemongrass but was not different from basil.

Bioaccumulation factor and translocation factor: Based on the analysis of variance, the type of aromatic plant and the concentration of Cd metal have a real effect on the bioaccumulation factor. Still, the interaction of the two factors has no real effect. As for the translocation factor data, the type of aromatic plant, the concentration of Cd metal and the interaction of the two factors have a real influence on the translocation factor. Furthermore, Table 5 showed the difference in the average between aromatic plant types and bioaccumulation and translocation factors. Vetiver grass has the highest bioaccumulation factor value (0.85), which differs markedly from other plants, except with lemongrass, the value does not vary markedly. Translocation factor values range from 0.97-1.55, vetiver grass has the highest translocation factor value (1.55) in real difference from citronella grass and patchouli but not different from lemongrass and basil.

The test results differed on average by Cd metal concentration factors to bioaccumulation and translocation factors presented in Table 6. The results showed that at a concentration of 340 ppm, the highest bioaccumulation value (0.73) differed markedly from 0 ppm but was no different from other Cd concentration treatments. Furthermore, the highest value translocation factor at 0 ppm differs from 85 and 340 ppm but does not differ markedly from 170 and 255 ppm.

The interaction of translocation factors due to the influence of aromatic plant types and metal concentrations Cd was shown in Fig. 7. Vetiver grass has the highest translocation factor value at a concentration of 170 ppm, patchouli at a concentration of 255 ppm and others at a concentration of 0 ppm.

Table 2: Percentage change in the average oil content of some aromatic plants exposed to Cd and without Cd for 20 weeks

	Oil content (%)		
Plant types	Without Cd	With Cd	Percentage
Vetiver grass	0.80	0.87	8.28
Citronella grass	2.03	1.99	-2.03
Lemongrass	1.96	2.24	14.07
Patchouli	1.53	1.34	-12.49
Basil	1.56	0.85	-45.56

Table 3: Average Cd content in the soil, roots and leaves is due to different concentrations for 20 WAP

	Cd contents		
Cd concentration	Soil (ppb)	Roots (ppb)	Leaves (ppb)
0 ppm	373.97[c]	928.66[b]	645.58[b]
85 ppm	20599.81[b]	16063.50[ab]	7469.25[ab]
170 ppm	32611.71[b]	16051.45[ab]	8469.14[ab]
255 ppm	35381.04[ab]	16899.67[ab]	15730.00[a]
340 ppm	49644.58[a]	22080.30[a]	7365.82[ab]

Numbers followed by the same letter in the column and row were not significantly different according to DMRT $\alpha = 0.05$, [a,b]Means on the same row having different superscripts are significantly different ($p<0.05$)

Table 4: Average Cd content in the roots and the leaves on some aromatic plants for 20 weeks

	Cd contents	
Plant types	Roots (ppb)	Leaves (ppb)
Vetiver grass	2601.70[b]	2905.51[b]
Citronella grass	12247.66[b]	1608.13[b]
Lemongrass	6300.37[b]	2792.79[b]
Patchouli	34940.59[a]	19294.70[a]
Basil	16933.28[b]	10078.67[ab]

Numbers followed by the same letter in the column and row were not significantly different according to DMRT $\alpha = 0.05$, [a,b]Means on the same row having different superscripts are significantly different ($p<0.05$)

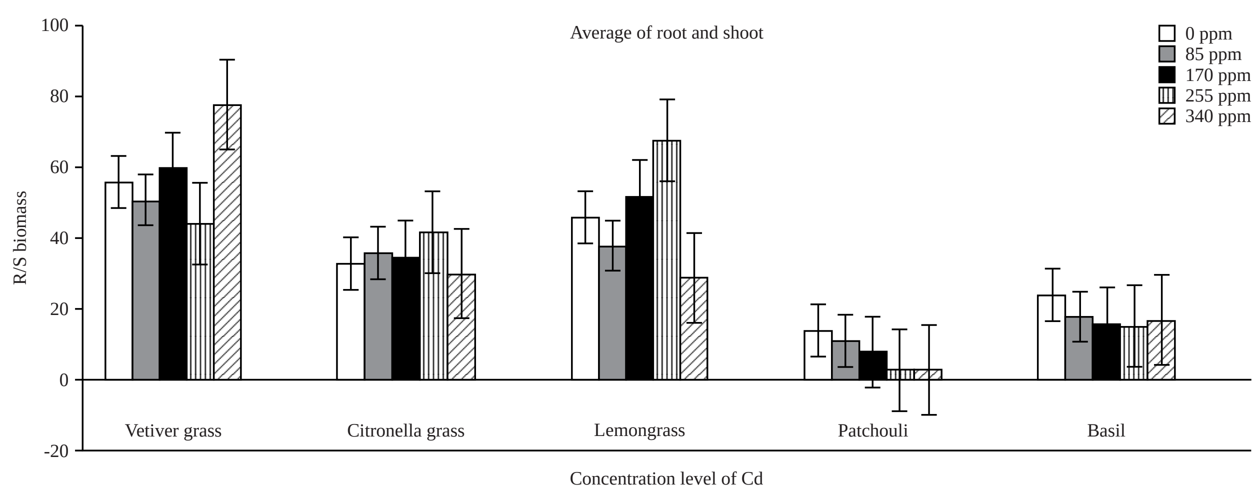

Fig. 6: Average root and shoot ratio in some types of aromatic plants at different level concentrations of Cd for 20 WAP

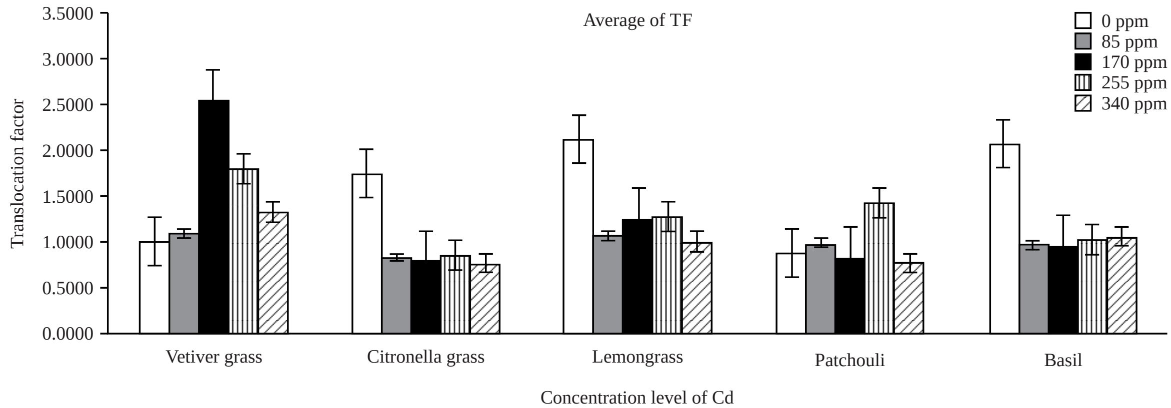

Fig. 7: Average of TF for some of type aromatic plants planted at a different level of Cd concentration for 20 WAP

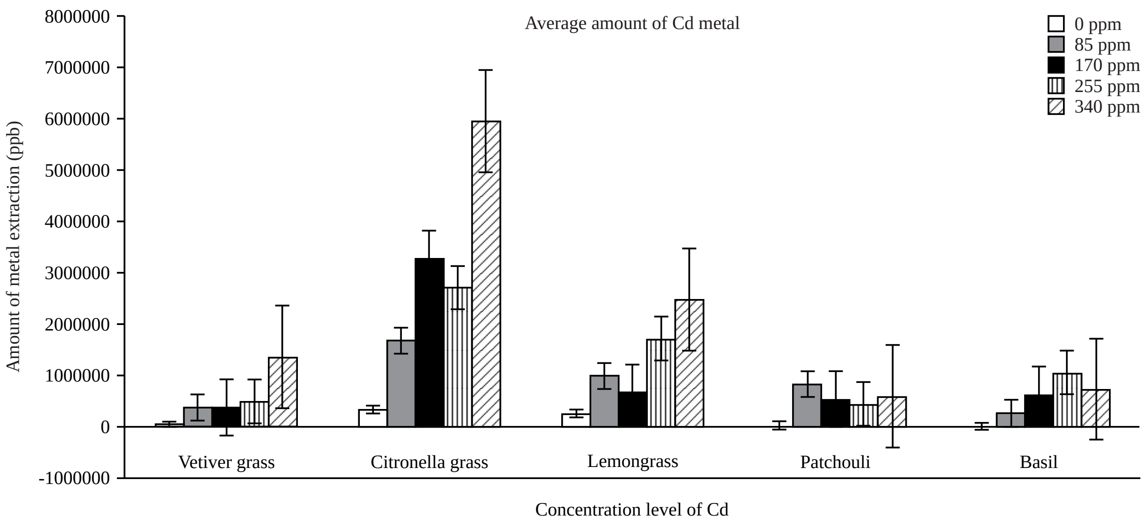

Fig. 8: Average amount of Cd metal extraction from several aromatic plants grown at different Cd concentration levels for 20 weeks

markedly from other plants, except with lemongrass, the value does not vary markedly. Translocation factor values range from 0.97-1.55, vetiver grass has the highest translocation factor value (1.55) in real difference from citronella grass and patchouli but not different from lemongrass and basil.

The test results differed on average by Cd metal concentration factors to bioaccumulation and translocation factors presented in Table 6. The results showed that at a concentration of 340 ppm, the highest bioaccumulation value (0.73) differed markedly from 0 ppm but was no different from other Cd concentration treatments. Furthermore, the highest value translocation factor at 0 ppm differs from 85 and 340 ppm but does not differ markedly from 170 and 255 ppm.

The interaction of translocation factors due to the influence of aromatic plant types and metal concentrations Cd was shown in Fig. 7. Vetiver grass has the highest translocation factor value at a concentration of 170 ppm, patchouli at a concentration of 255 ppm and others at a concentration of 0 ppm.

Amount of metal extraction: The variety prints showed that aromatic plant type factors, Cd metal concentrations and the interaction of the two factors had a noticeable influence on the amount of metal extraction. The different average tests showed that the highest amount of Cd metal extraction found in citronella grass could be absorbed in 5958371 ppb is very real with other combination treatments. The results of the different average tests were shown in Fig. 8.

Table 5: Average bioaccumulation and translocation factors in some aromatic plants for 20 weeks

	Average	
Plant types	BCF	TF
Vetiver grass	0.85[a]	1.55[a]
Citronella grass	0.53[bc]	0.99[b]
Lemongrass	0.72[ab]	1.34[ab]
Patchouli	0.35[c]	0.97[b]
Basil	0.41[c]	1.21[ab]

Numbers followed by the same letter in the column and row were not significantly different according to DMRT α = 0.05, [a,b,c]Means on the same row having different superscripts are significantly different ($p<0.05$), BCF: Bioaccumulation factor and TF: Translocation factor

Table 6: Average BCF and TF in some aromatic plants were planted at different concentrations for 20 weeks

	Average	
Cd concentration	BCF	TF
0 ppm	0.34[b]	1.56[a]
85 ppm	0.52[ab]	0.99[b]
170 ppm	0.68[a]	1.28[ab]
255 ppm	0.59[a]	1.27[ab]
340 ppm	0.73[a]	0.98[b]

Numbers followed by the same letter in the column and row were not significantly different according to DMRT α = 0.05, [a,b]Means on the same row having different superscripts are significantly different ($p<0.05$), BCF: Bioaccumulation factor and TF: Translocation factor

DISCUSSION

From the transverse slices of vetiver grass, citronella grass, lemongrass, patchouli and basil that received heavy metal stress treatment Cd, there were structural and ultrastructural cellular changes with increasing concentrations of Cd. The tried plants consist of monocot classes (vetiver grass, citronella grass and lemongrass) and dicot classes (patchouli and basil). Figure 1-5 showed that the epidermis tissue and cortex tissue were damaged. The tissues of xylem vessels increased in number (mainly appearing on patchouli and basil) and experienced thickening with increased Cd concentration. According to Nazar *et al.*[14], cadmium (Cd) is a toxic heavy metal that enters the environment through various anthropogenic sources and inhibits plant growth and development.

In contrast, channels played a significant role in transporting some Cd ions, mineral elements, water and other substances. Sebastiani *et al.*[15] said that a large amount of tracheal elements causes changes in hydrolytic capacity, increasing the risk of tissue damage. Changes in the anatomical structure of plants are adaptive responses of plants so that plants become tolerant to heavy metals. High Cd levels can trigger ROS onset (Reactive Oxygen Species). The ROS is a highly reactive molecule that can cause macromolecular damage and interfere with metabolism.

The durability of the tried aromatic plant is assessed through the ratio of root and shoot. Vetiver grass provided the highest root and shoot ratio and the highest response was found at a concentration of 340 ppm. While in lemongrass and citronella grass, plants with a Cd concentration treatment of 255 ppm showed the highest response. This can be attributed to data on the average dry weight of roots. The dry weight of citronella grass shoot at a concentration treatment of 255 ppm obtained the lowest average compared to other concentration treatments. This means that the citronella grass plant at a concentration of 255 ppm experiences tissue death large enough that the dead tissues contribute to the high C-organic content of its soil. Several studies have reported that Cd stress inhibited plant growth and biomass[3] and also affect plant absorption and translocation of essential nutrients by competition[16].

Biomass is a leading indicator of energy accumulation in plants. In this study, Cd pollution significantly increased the ratio of root and shoot biomass in vetiver grass at a concentration of 340 ppm and in lemongrass and citronella grass plants at a concentration of 255 ppm. In contrast, the ratio of root and shoot biomass in patchouli and basil plants decreased with an increase in Cd concentration in soil media. This showed that vetiver grass, lemongrass and citronella grass can be said to be plants that were resistant to Cd pollution. According to Zhang *et al.*[17], it was likely that the vetiver grass is a Cd excluder with 41-44 times more Cd in the root than in the shoot. Thus, it may allocate more energy to the roots for Cd absorption or maintenance of normal functions under Cd pollution, such as reducing water deficiency caused by excessive metal effects. Excluder plants can allocate more energy to the roots than buds.

Lemongrass plants have dense biomass containing essential vegetable oils that have potential applications in producing biofuels that can be extracted through steam distillation techniques. In the field, the appearance of plants that have dense leaves both on the soil exposed to and not exposed to Cd is lemongrass and citronella grass. This determined that its production remains high to the limit of the tried concentration. Aromatic plants have great potential for phytoremediation at sites contaminated with heavy metals. It has been observed that heavy metal pressure increases the percentage of essential oils of certain aromatic plants[18]. Plants exposed to heavy metal pressure show changes in the production of secondary metabolites, either suppressing or stimulating production. Changes in the composition of essential oils by heavy metal pressure are associated with the inactivation of enzyme-specific secondary metabolite metabolic pathways[19]. Table 2 showed that the vetiver grass crops and lemongrass provide a higher value of essential oils under conditions under pressure Cd. This means that lemongrass plants and vetiver grass have excellent adaptability under Cd pressure to grow well and even produce higher oil than without Cd. This also means that these two plants can be used as phytoremediation tools while also providing economic value. From the essential oils, it produces.

In Table 3, the Cd content in the soil ranging from a concentration of 85-340 ppb was higher and differs markedly from the concentration of 0 ppb. This suggested that the Cd metal applied to the soil is still left as Cd applications increase into the soil. At the same time, the metal content in the roots increased with the increasing concentration of Cd, at a concentration of 85-340 ppb higher and in contrast to the concentration of 0 ppb. This means that the plants trying to absorb heavy metals were quite effective. The leaves at a concentration of 255 ppm have the highest Cd content of 15730 ppb but do not differ markedly compared to the treatment concentration of 340 ppm. This suggested that the aromatic plants tried can absorb heavy metals into their shoot organs. While Table 4 showed that patchouli plants absorbed the highest Cd both at the root and in the title, section compared to other plants. This suggested that patchouli is a plant capable of absorbing high Cd metals within its organs. The importance of patchouli to produce essential oils makes this medicinal plant one of the main contributors to the Indonesian economy[20].

The BCF analysis was carried out to determine the degree of accumulation of heavy metals at the root. Plants are said to be accumulators: If the BCF value >1. An accumulator is a plant that can hoard high concentrations of metals in its plant tissues, exceeding the soil concentration. Table 5 showed that the value of bioaccumulation factors in the five types of plants ranges from 0.35-0.85, which were all less than 1. This means that in this experiment, vetiver grass crops, citronella grass, lemongrass, patchouli and basil absorb heavy metal Cd derived from the soil into the roots were still low. The TF analysis is used to calculate the translocation of heavy metals from roots to leaves.

According to Eissa[21], the value of TF had a category: If TF>1: Then followed the mechanism of phytoextraction. Phytoextraction is the absorption of heavy metals by plant roots which were then translocated to the stems and leaves. The translocation factor value for vetiver grass crops, lemongrass and basil had a value of more than one. The plants effectively absorbed the cheap Cd metal derived from the roots into their shoot, which is a phytoextraction mechanism. Figure 7 explained that the highest translocation factor value is found in vetiver grass crops at a concentration of 170 ppm. This means that at a concentration of 170 ppm, the vetiver grass plant effectively absorbs the heavy metal Cd from the shoot. This was to the study results, Aibibu *et al.*[22] which indicated that the vetiver tolerates high Cd concentrations and has an unusual ability to take heavy metals from its media treatment.

In phytoextraction technology, the amount of metal extraction is a good indicator for assessing the efficiency of phytoremediation[17]. In this study, the highest amount of Cd metal extraction is citronella grass which absorbs 5958371 ppb at Cd concentrations of 340 ppm and differs markedly from others means that citronella grass is an efficient plant as a phytoremediation tool. Research results from Gautam *et al.*[23]. In *Cymbopogon citratus* plants, lemongrass plants are tolerant plants. However, the Cd content in the shoot is higher than its threshold level, there is no severe phytotoxic effect of Cd on total reflected plant biomass that may be caused by Cd-induced synthesis of phytochelatin complexes that mask Cd poisoning.

CONCLUSION

From several assessments made on aromatic plants, namely vetiver grass, citronella grass, lemongrass, patchouli and basil, results concluded that certain plants didn't show consistent judgment on each parameter. From the assessment carried out, lemongrass, citronella grass and vetiver grass are the best plants that represent other plants as phytoremediation tools. Judging from its ability to produce essential oils and absorption of heavy metal Cd.

SIGNIFICANCE STATEMENT

This study reported that vetiver, citronella, citronella, patchouli and basil are resistant to heavy metal Cd and can also produce essential oils under stress conditions to clean polluted metal soil using inexpensive and inexpensive phytoremediation techniques and environmentally friendly. This research will help researchers to uncover critical areas of Aromatic Plants that many researchers cannot explore. Thus, a new theory can be obtained that to clean metal-contaminated soil can use phytoremediation.

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