Fern plant (*Pteris vittata*) as a phytoremediator of arsenic heavy metal and its effect to the growth and quality of Kale (*Ipomea reptans* Poir)

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Abstract. Kale (*Ipomea reptans* Poir) has high nutrition such as vitamin A, B and C as well as various minerals especially iron. Quality of animal feed must be clean from diseases and heavy metal contamination such as Arsenic. Arsenic in the soil can be reduced naturally using phytoremediator plants, one of which is *Pteris vittata*. The experiment was conducted at the Antirogo Green House, Sumbersari Distric, Jember Regency. Arsenic metal analysis was conducted in BARISTAND, Surabaya and BALITTANAH, Bogor on June 2019 to January 2020. The experimental design used Factorial Complete Random Design consist of 2 factors. The first factor consists of 4 levels of heavy metal arsenic treatment on the fern plant growth media, namely 0 ppm (D1), 5 ppm (D2), 10 ppm (D3) and 15 ppm (D4). The second factor consists of 2 levels of treatment time of fern: 1 month (P1) and 2 weeks (P2). Each of treatment repeated 4 times. The results showed that the interaction between planting time of fern and arsenic dosage showed no significant effect. The absorption power of ferns reaches 0.27 ppm to 4.589 ppm higher compared to the scale absorptions reaching 0.006 ppm to 0.155 ppm.

1. Introduction
Kale (*Ipomea reptans* Poir) is an annual plant which is an important vegetable in the Asian region. Kale planting can be favored because of its easy maintenance, short life and relatively cheap price. Kale including annual vegetable plants, short-lived and cultivation of water do not require a large area. Kale has quite high nutrients such as vitamins A, B and C and various minerals, especially iron which is useful for health [1]. Kale besides can be consumed by humans; kale also could be used as a forage for animal feed. Forage that has adequate nutritional numbers for ruminants that can support the growth of livestock. Animal feed, especially forage as a food, is one of the mainstay commodities of farmers in the area of livestock, requires good cultivation to increase production. Good handling of the cultivation of production, harvesting, post-harvest handling (processing), and marketing must be done optimally so that optimal income can also be obtained [2]. Good quality of forage must be clean from disease and heavy metal contamination of inside.

One type of heavy metal that is very dangerous for other living things is Arsenic (As) [3]. Arsenic content in ground water of several areas in Indonesia is still above the health safety standard limit according to WHO (World Health Organization) [4]. Likewise, arsenic is reported to be high in rice in some areas in Indonesia [5]. People exposed to arsenic have health implications, ranging from chronic...
arsenic poisoning to acute arsenic poisoning which is fatal [6]. Chronic arsenic exposure to southern Halmahera residents could giving impact to the allergies skin, keratosis and hyperpigmentation of the skin and cancer skin. Heavy metals toxic giving effects that can inhibit the work of enzymes that disrupt of body's metabolism that causes allergies, are mutagenic, teratogenic or carcinogenic to the humans and animals [7]. Arsenic exposure in the human body accumulates in nails and hair where levels match the arsenic levels in the environment [8].

Arsenic content of the soil was not found contaminated level content between 0.2 - 40 mg/kg, however contaminated contain level an average more than 550 mg/kg [9]. According National Standardization Agency [10], the maximum content of arsenic in food ranges from 0.25 mg/kg to 1 mg/kg. The absorption of heavy metals such as Arsenic (As) which could be ferns absorbed by 27.00 mg/kg of biomass [11] so that fern (Pteris vittata) is an alternative to reduce the absorption of heavy metal arsenic in metal-contaminated media. Pteris vittata is more efficient accumulating arsenic compared with Nephrolepis. Exaltata in the leaves of 38-54 mg/kg [7]. Environmental factor and temperature could be selected plant species to annual (non perennial) crops are given in [12]. Related environmental factor and temperature some plant species could adapt and appearance in tropical country [13]. Arsenic is predominant anaerobic soil, whereas arsenite dominates under aerobic conditions [14].

The purpose of this study was to determine the ability of Arsenic absorption by planting fern as a phytoremediator on water spinach cultivation areas contaminated with heavy metal As so that it can be clean of Arsenic metal and could be used as a consumption material that has the quality and nutritional content of sorghum that is safe for consumed.

2. Materials and methods

2.1. Time and place
The research was conducted from June 2019 to January 2020, at Antirogo Green House, Sumbersari District, Jember Regency. Arsenic (As) metal analysis conducted at the Soil Research Institute (ISRI) and BARISTAND.

2.2. Materials and instruments
Tools of this study used polybag size (50x50cm), analytical scales, shovels, ovens, blenders, erlenmeyers, bottles, plastic clips, paper ovens, meter, string plastic, and supporting tools for arsenic content analysis (spectrophotometry). The material of this study used seedlings fern. Kale seeds, aquades, soil, fertilizer, media and other materials and equipment that support this research.

2.3. Study methods
Methods of this study used complete random design which consisted of 2 factors. The first factor consists of 4 levels of heavy metal Arsenic dose treatment on fern growth of media, consisting of 0 ppm (D1), 5 ppm (D2), 10 ppm (D3), and 15 ppm (D4). The second factor consists of 2 levels time of the ferns treatment, namely 1 month (P1) and 2 weeks (P2). Each treatment was repeated 4 times.

2.4. Procedures of study

2.4.1. Making of media planting. Media of the fern planting was made by mixing the soil with compost in a ratio of 1:1. The planting medium was put on poly bag measuring 50x50 cm with a soil weight of 6 kg.

2.4.2. Making of arsen solution. Preparation of the solution by weighing in advance according to the concentration treatment, namely 0.5, 10, and 15 ppm. After weighing it, then making a solution by adding as much as 100 ml and homogenized. Homogeneous Arsenic solution is directly applied to the polybag in accordance with the treatment and stirred until the solution is dissolved into the soil. After
the application is carried out, it is allowed to stand for 2 days so that the Arsenic metal can be mixed into the soil.

2.4.3. Planting of Fern. Planting fern seeds was carried out after 2 days of giving Arsenic solution. Ferns planting conducted 1 month and 2 weeks before kale planting. Planting ferns consists of 3 plants per polybag. Fern seedlings taken from the pine forest area, Garahan District, Jember Regency with the same plant size and plant height of around 20-30 cm.

2.4.4. Planting of Kale. Planting fern or transplanting was done after 1 month and 2 weeks after planting the ferns in polybags measuring 50x50cm. The transfer of kale plants is done after the kale plants have 3 to 5 leaves. The number of planting in this study was carried out 2 seeds per poly bag.

2.4.5. Stitching. Stitching is done on kale, when seeds abnormal growth, wither and attacked by pests or diseases. This activity is carried out by replacing these plants with plants of the same age. The planting time is the first week after transplanting and is carried out in the afternoon so that the seedlings do not experience stress due to high temperatures.

2.4.6. Treatment. The treatment of kale plants includes watering which is done once a day in the afternoon, weeding is done by adjusting to the growing conditions of weeds around the medium in the polybag. Weeding is done manually by removing weeds that grow in polybags, done carefully so as not to damage the roots of kale or ferns. The next treatment is pest control which is done manually, namely by picking pests one by one.

2.4.7. Harvesting. Harvesting on kale plants is carried out at the age of 30 days. Whereas fern plants will be harvested simultaneously with water spinach plants.

2.4.8. Analysis of heavy metal arsenic in Sorghum and Ferns. Analysis of Arsenic (As) levels conducted at laboratory. Analysis of metal Arsenic in kale and fern plant tissue was conducted at the Soil Research Institute (BALITTAN) in Bogor using a spectrophotometer to determine Arsenal levels in kale plant tissue according of treatments.

2.5. Data analysis
Obtained of data from observations was collected using statistical analysis (ANOVA) and the Duncan Multiple Range Test (DMRT) advanced test with an error rate of 5%.

3. Results and discussion
Based on analysis of cassava plant tissue showed results below the threshold of 0.006-0.155 ppm because the higher the concentration given impact to the higher the absorption of Arsenil in sorghum seeds. This is due to the fact that each plant has different absorption abilities which are influenced by several factors, one of which is the concentration of each treatment of higher the metal concentration, the more metals of plant tissues could be absorption [15].

Based on influence of arsenic concentration to the plant height (Figure 1) shows that arsenic dosage has a significant effect to the plant height. From the result of study, the treatment of D1 (45.1 cm), D3 (39.6 cm), and D4 (39.2 cm) giving impact significant difference to the plant height, from the result highest average was 45.1 cm, which mean significantly different from D3 (39.6 cm), and D4 (39.2 cm) of treatment, but average of plant height D1 (45.1 cm), and D2 (41.1 cm) was not significant different.
Table 1. Results analysis of metal (arsenic) on soil, tissues kale and fern plants

| No | Analysis sample                  | Treatment | Arsenic concentration (ppm) |
|----|---------------------------------|-----------|-----------------------------|
| 1  | Antirogo soil                   | -         | <0.0006                     |
|    |                                 | D1P1      | 0.0060                      |
|    |                                 | D2P1      | 0.1180                      |
|    |                                 | D3P1      | 0.1200                      |
| 2  | Root, stem, leaf of fern        | D4P1      | 0.1550                      |
|    |                                 | D1P2      | 0.0006                      |
|    |                                 | D2P2      | 0.1200                      |
|    |                                 | D3P2      | 0.1800                      |
|    |                                 | D4P2      | 0.1500                      |
|    |                                 | D1P1      | 0.2700                      |
|    |                                 | D2P1      | 1.8750                      |
|    |                                 | D3P1      | 2.2670                      |
| 3  | Root, stem, leaf of fern        | D4P1      | 4.5890                      |
|    |                                 | D1P2      | 0.2800                      |
|    |                                 | D2P2      | 0.5130                      |
|    |                                 | D3P2      | 0.9670                      |
|    |                                 | D4P2      | 1.2270                      |

Following the results of Duncan’s further 5% test for all observed of the variables: Plant height

![Figure 1. Influence of arsenic concentration to the plant height](image)

Based on Figure 1, it shows that arsenic dosage has a significant effect to the number of kale leaves. Treatment D1 (42 leaves) had the highest average number of leaves giving significant different to the D2 (32 leaves). Treatment D3 (32 leaves), and D4 (29 leaves). The lowest average number of leaves was in treatment D4.

Based on the results of Figures 1 and 2, show that the higher the As metal, the plant height and the number of kale leaves are getting lower. According to Fayiga [12], plant height will increase along with the age of the plant; however, plant height will then be hampered due to metal stress. In the planting medium in the presence of metal stress, the plant height growth will be slightly lower when compared to plants on media without heavy metal stress. According to research conducted by Indrasti [16], the higher the concentration of heavy metals, the more inhibiting cell division and growth,
especially plant height. The high concentration of arsenic in the planting medium will also inhibit the compound which will then be used for the process of cell division and enlargement or differentiation in plant tissue. Hardiani [17] in their research also said that the inhibition of plant height was caused by the presence of death or anions from heavy metals which were absorbed by the roots and then entered into plants to become inhibitors of enzyme formation and would inhibit plant metabolic processes that will be used for cell division.

![Figure 2. Effect of Arsenic Concentration to the Number of Leaf](image)

**Figure 2.** Effect of Arsenic Concentration to the Number of Leaf

The low number of leaves on kale plants on soil contaminated with heavy metals causes leaf growth to slow down. Slowing leaf growth then has a negative impact on plant physiological processes related to the photosynthetic process of plants [18]. According to Dewi [19], the presence of contaminants in the form of arsenic in the high planting medium causes the plant height and the number of leaves on the plant to decrease. The presence of arsenic metal which exceeds the safe limit in the planting medium will cause a decrease in nutrient uptake by plants, thus inhibiting the cell division process and disrupting the plant photosynthesis process. The disruption in the photosynthesis process then results in a lack of photosynthate results, so that plant growth will be inhibited.

This is caused if the arsenic metal in the planting media has exceeded the quality standard, it will cause disturbance of plant growth, both the vegetative phase and the generative phase [20]. The highest arsenic absorption at a concentration of 0 ppm (without treatment) showed the best effect compared to giving other concentrations at sorghum plant. Absorbed arsenic levels have increased because plant growth will affect the ability to absorb. The greater the age of a plant, the greater its ability to absorb [21]. The factors that influence the absorption of heavy metals besides plant age, one of which is the concentration of heavy metals, the higher the metal concentration, the more metals that can be absorbed by plants [22]. According to Rezicca [23] the higher the metal concentration, the lower the plant uptake because the plant has the ability to tolerate toxic conditions.

Exposure to heavy metals in the human body through the soil-plant-food chain, so that in order to minimize the heavy metal arsenic content in cereals, a quality standard value of heavy metal arsenic in paddy soil is needed. The maximum limit of arsenic contamination in cereals based on SNI 7387: 2009 is 0.5 mg kg⁻¹ [22]. Pteris vittata planted before planting kale has a good impact as a phytoremediator to clean soil contaminated with arsenic by absorbing arsenic from the soil and transferring it to the body in a concentration greater than the arsenic concentration in the soil, and within 20 weeks the soil will be free from arsenic [24]. The longer the planting time of ferns in metal contaminated media, the metal absorption value also increases, both in hyperaccumulator plants or not [21]. It is also shown from the other research that the presence of ferns, the level of arsenic uptake by Sorghum (Sorghum Bicolor (L.) can be reduced [25].

4. Conclusion
Based on the results of the research that has been done, it can be concluded that the absorption power of ferns is higher, reaching 0.27 to 4.589 ppm, when compared to the absorption of water spinach which reaches 0.006 to 0.155 ppm. the presence of ferns, the level of arsenic uptake by kale can be
reduced so that the arsenic in kale has a low value and is safe for consumption.

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