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# Al and Fe Heavy Metal Concentrations in the Vegetative and Root Parts of *Dicranopteris linearis*, *Nephrolepis bifurcata*, *Stenochlaena palustris* and *Acrostichum aureum* Grew in Highly Weathered Soil

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**Abstract** Phytoremediation is a plant function that degrades, eliminates, and inactivates potentially hazardous and toxic compounds in the polluted water, soil, and air. In phytoremediation mechanisms, phytodegrading enzymes break down the pollutants taken up by plants (phytodegradation). Additionally, the microorganisms found in the plant-related rhizosphere can also degrade the pollutants in the soil (rhizodegradation). The plants can extract the pollutants from the soil and accumulate them in their tissues (phytoextraction) or immobilise the contaminants in their root zone, remove the harmful compounds from the water and soil through transpiration into the atmosphere, and regulate the heavy metal pollutants across their root system (phytostabilisation). In this study, researchers characterised the development and aptitude of different fern species to remediate the heavy metal pollutants present in the highly weathered soil (i.e., laterite soil). For this purpose, soil samples from 3 different sites in Perak, Selangor and Johor were collected. The phytoremediation ability of 4 different fern species (i.e., *Dicranopteris linearis*, *Nephrolepis bifurcata*, *Stenochlaena palustris* and *Acrostichum aureum*) was determined to understand the pattern and distribution of heavy metal contamination in soil. The ICP-MS technique was utilised to determine the concentration of heavy metals. The results indicated the presence of a high concentration of heavy metal at many of these sites, while the concentration of Al and Fe ions varied. In addition, a significant difference ($P<0.0001$) in the concentration of heavy metals was discovered between fern species. All the interactions were detected using the Analysis of variance (ANOVA) technique. Phytoremediation is considered an alternative technique for reducing the heavy metal concentration in soil in light of various factors that lead to the accumulation of heavy metals. Furthermore, the remediation of numerous sites that are polluted with heavy metals, like landfills, is a cost-effective solution employing green technologies, like phytoremediation.

**Keywords** Green Technology, Phytoremediation, Laterite Soil Fern Species, Heavy Metal Sequestration Rate

## 1. Introduction

Many human activities have contributed to heavy metal pollution in recent years. A few researchers have noted that plants can be used to decrease the negative effects of heavy metal contaminants through their green technology [1]. Pollutants in the soil can be transmitted into the food chain, posing a threat to human health and well-being. In some circumstances, living creatures need various concentrations of heavy metals for certain biological functions. These heavy metals include Cobalt (Co), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), and Zinc (Zn). However, these heavy metals can be toxic at higher concentrations [2]. Other heavy metals like Arsenic (As), Cadmium (Cd), Mercury (Hg), and Lead (Pb) are considered very harmful since their beneficial effects on human health are unknown. Furthermore, many studies indicate that the rate of heavy metal sequestration in animal bodies can cause serious diseases.

However, these heavy metals are abundant in the landfill area. Landfills are waste disposal areas that significantly contribute to air and soil pollution. Most urban planners across the world are aware of these issues ascribed to urban land shortage [3]. As a result, the former landfills are transformed into residential areas for city dwellers. In one study [4], the researchers found that these regions produced much leachate owing to heavy metal pollution caused by landfill activities. Widespread anthropogenic activities have adversely affected human health and global biodiversity. Furthermore, waste production, particularly in developing countries, generated a substantial amount of leachate. Therefore, an effective and proper waste collection system is required to collect the raw leachate from the landfills to prevent the flow of this leachate into the soil, which would cause soil contamination and a high heavy metal sequestration rate [5].

Frequently, plants are used as green technology or as remediators to mitigate environmental damage. The use of plants to prevent soil erosion is known as phytostabilisation, in which plants are used to stabilise the soil. Heavy metals can be neutralised by planting metal-tolerating plants, which absorb and accumulate the toxic ions in their root systems or precipitate them in the rhizosphere [6]. These plants can immobilise the toxic chemicals in the soil and water, and reduce their access to the food chain. Hence, the cultivation of metal-tolerant native flora can provide a sustainable and effective solution to the problem of heavy metal contamination. In one study [7], the researchers studied the ability of a fern species (*Pteris vittata*) to absorb arsenic (As) from the soil and translocated it into different sections above the ground like leaves, where the US scientists discovered it. According to these findings, other types of ferns also can accumulate heavy metal ions. Hence, they attempted to study the potential of different fern plants as a natural remedy to tolerate the presence of toxic chemicals, particularly Al and Fe, in highly weathered contaminated soils. Some plant species would be investigated and classified as either endemic excluders, accumulators, and hyperaccumulators or other classes based on their ecotypes. The researchers have also determined their ability to extract heavy metal contaminants from the soil.

## 2. Materials and Methods

### 2.1. Soil Sampling and Analysis

In this study, about 1000 g of soil samples were collected from 12 points in 3 different locations in Peninsular Malaysia (i.e., Perak in North Peninsular Malaysia, Johor in Southern and Selangor in Central Peninsular Malaysia). Each sample was acquired at the depth of 0-200 mm using a soil auger (*Eijkelkamp Agrisearch*). These samples were sealed in polyethene bags, brought to the laboratory, and properly labelled. The samples were dried in a dry oven at 70 ℃ for 3 days. The dried soil samples were crushed and sieved using an agate mortar. Using a 2 mm mesh, the small stones and plant debris were separated. Thereafter, the samples were digested using the Microwave Digestion Ethos D instrument at room temperature (Milestone, 2001). This device is required to perform elemental analysis on samples such as metals, drugs, plants, dirt, or food. The ICP-MS (Perkin Elmer NexION 300X) is used to analyse the heavy metal concentrations in the soil samples.

### 2.2. Plant Sampling and Analysis

The dominant fern species used in the study were determined and performed tissue analysis. Four specimens of each fern species from similar sites were collected as the soil samples. After pressing and drying in the oven at 50-55 ℃ for 4 days, a small portion was preserved. Taxonomic literature previously described the pteridophyte plant samples based on their descriptions and keys. After collection, the plant samples were washed under the running tap water and rinsed and rewashed with deionised water. Leaves were separated from these samples for further use [7]. All samples were dried in the dry oven for 48 h at 70 ℃ before grinding into a powder with a mortar-pestle for heavy metal analysis. These samples were processed at room temperature and digested with Ethos D Microwave Digestion instrument (Milestone, 2001). Furthermore, the ICP-MS technique (Perkin Elmer NexION 300X) was utilised to analyse the concentration of heavy metals in the plant samples.

### 2.3. Determination of Heavy Metals in Soil Samples

Before they were analysed for the presence of heavy metals, they samples were digested [8]. The dried and ground (fine texture after being ground) soil sample (0.5 g) was mixed with concentrated nitric acid (10 ml) and degraded for this purpose. This mixture was heated for 30 mins at 175℃ using the Microwave Digestion Ethos D instrument. After digestion, the solution was extracted, diluted to a final volume of 50 ml with deionised water, and filtered. After diluting the filter (1:1) and dividing it into three 15 ml tubes (triplicates) for every heavy metal, the filtrate was analysed for the presence of heavy metals.

### 2.4. Determination of Heavy Metal Ions in the Vegetative and Root Samples

In this experiment, dried plant samples were weighed accurately (0.5 g) and placed in tubes. The plant samples were then treated with a mixture of nitric acid (6 ml of 65% $HNO_3$) and peroxide acid (2 ml of 30% $H_2O_2$ 30%). This mixture was heated for 30 mins at 200℃ using Microwave Digestion Ethos D instrument. After digestion, the solution was extracted, diluted to a final volume of 50 ml with deionised water, and then filtered. The filtrate was diluted (1:1), divided into 15 ml tubes (in triplicates for every heavy metal ion), and analysed for the presence of heavy metal ions using the ICP-MS.

### 2.5. Data Analysis

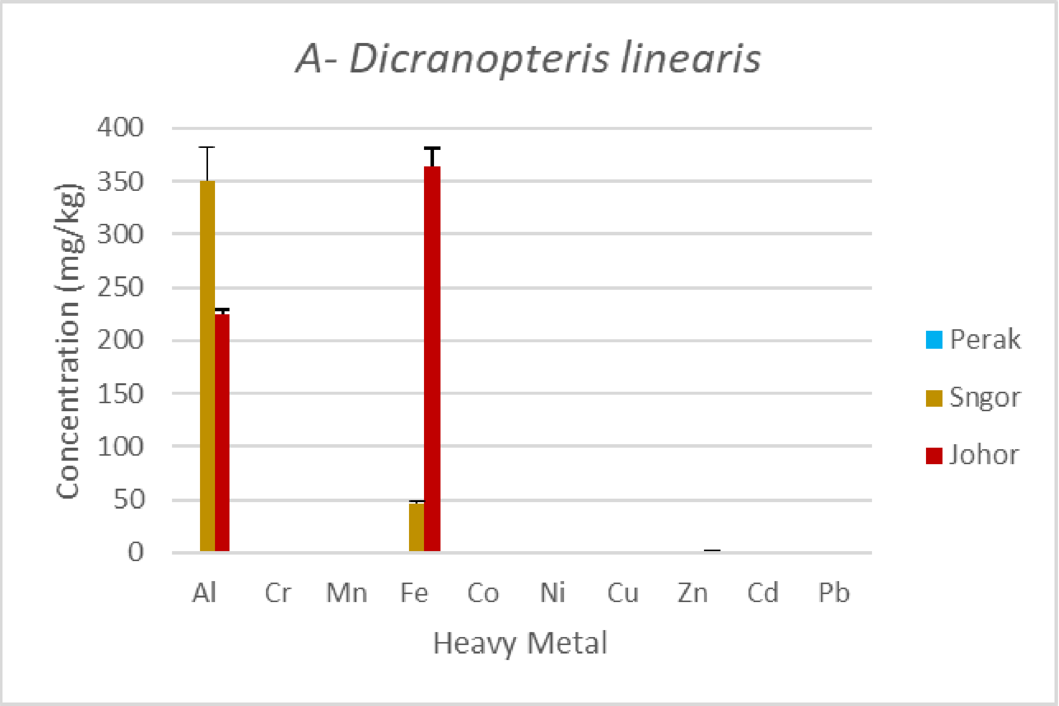
The experiments were conducted in triplicates, and the results were expressed as a mean of the 3 values. The data were further analysed using the Analysis of Variance (ANOVA) technique. The phytoremediation efficiency was calculated after determining the Bioconcentration Factor (BCF). The BCF value determined the efficiency of each fern species in absorbing a heavy metal into its tissues [9]. In one study [10], researchers described this technique in greater depth, where BCF = concentration of heavy metal in plant species (mg/kg) / concentration of heavy metal in soil (mg/kg).

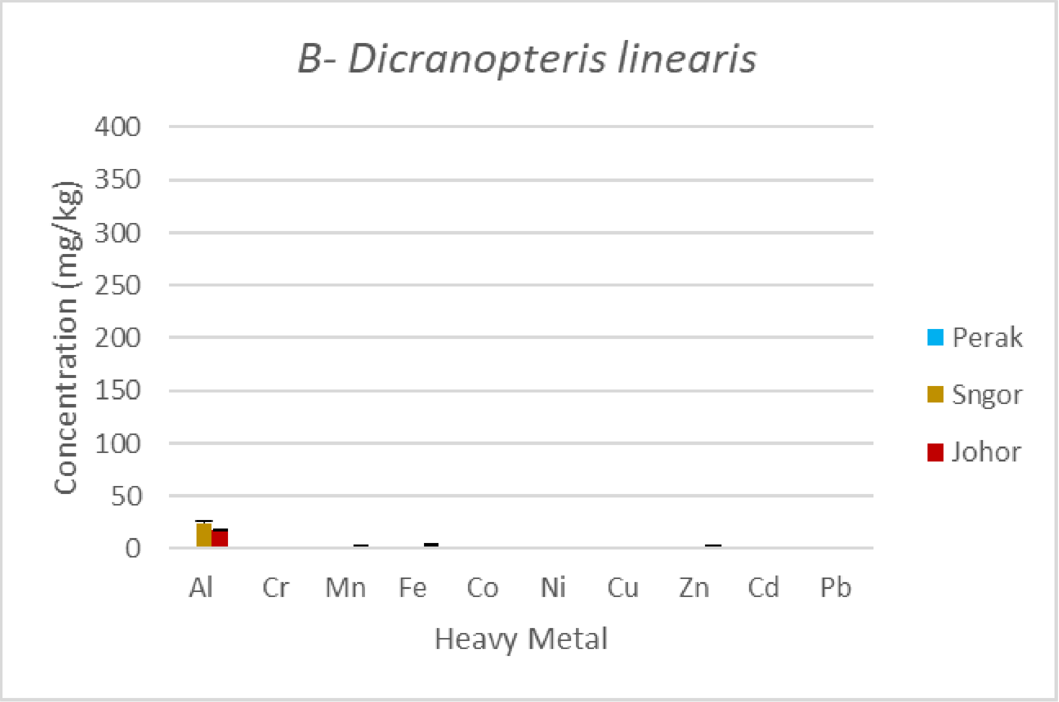
## 3. Results and Discussion

### 3.1. Heavy Metal Concentration in Soil and the Vegetative and Root Parts of the Fern Species

After studying the ANOVA results, a significant difference ($P<0.0001$) was discovered between the different heavy metal ions and the vegetative and root parts of the fern species, geographical location, and all other interactions. It was observed that each fern species had a different rate of heavy metal absorption based on the surrounding regions. This indicated that the vegetation and other environmental conditions could significantly affect the accumulation and quality of heavy metals in various geographical locations. High amount of Al and Fe ion concentrations were noted in the plantation areas of the residential, mangrove, and palm oil. This result revealed that the pollutants emitted by different industrial activities first reached aquatic and soil environments before being translocated to the plants [11]. It was also discovered that the root systems of the plants had a significant effect on the isolation and accumulation of heavy metal ions. Referring to Figure 1, Figure 2 and Figure 3, further analysis of the laterite soil samples collected from the 3 geographical localities (i.e., Perak, Selangor and Johor) showed that Al and Fe heavy metals were predominant in the soil and had the highest concentration of 350.460 mg/kg and 364.098 mg/kg, respectively. The results also showed that the vegetative components of *D. linearis* accumulated Al heavy metal at a concentration of 24.68 mg/kg, while they only accumulated trace concentrations of Fe. However, the root systems of *D. linearis* accumulated <50 mg/kg of Al and Fe. With regard to the *N. bifurcata* species, only trace concentrations of heavy metal ions in the vegetative components of the plant were detected; however, the root components contained a higher concentration of Al and Fe, i.e. 100 mg/kg and 350 mg/kg, respectively. The concentration of heavy metals was higher in the root of the plant due to the capability of the plant to extract heavy metals from the soil. This showed that this species had a high capacity for heavy metal extraction. The other 2 fern species, i.e., *S. palustris* and *A. aureum* also accumulated a trace concentration of heavy metal in their vegetative parts, whereas their root parts accumulated 50-250 mg /kg of Al and Fe. The results also showed that all fern species accumulated a relatively similar concentration of heavy metal, except *D. linearis*. The results showed that *N. bifurcata* displayed a high capacity for Fe sequestration, whereas *S. Palustris* showed a high capacity for Al sequestration.

Phytoremediation is the most ideal technique for remediating a metal-contaminated environment. A plant component with a higher concentration of an element is regarded as the element's accumulator. Phytoremediation is a cost-effective, innovative, and solar-driven technology that uses natural plants to remove and immobilise metal ions. Additionally, this technique does not require any fertilizer (thus, decreasing treatment cost) and also does not produce any additional waste that needs extensive treatment [12,13].


A- Dicranopteris linearis
Concentration (mg/kg)
400
350
300
250
200
150
100
50
0
Al
Cr
Mn
Fe
Co
Ni
Cu
Zn
Cd
Pb
Heavy Metal
Perak
Sngor
Johor


B- Dicranopteris linearis
Concentration (mg/kg)
400
350
300
250
200
150
100
50
0
Al
Cr
Mn
Fe
Co
Ni
Cu
Zn
Cd
Pb
Heavy Metal
Perak
Sngor
Johor

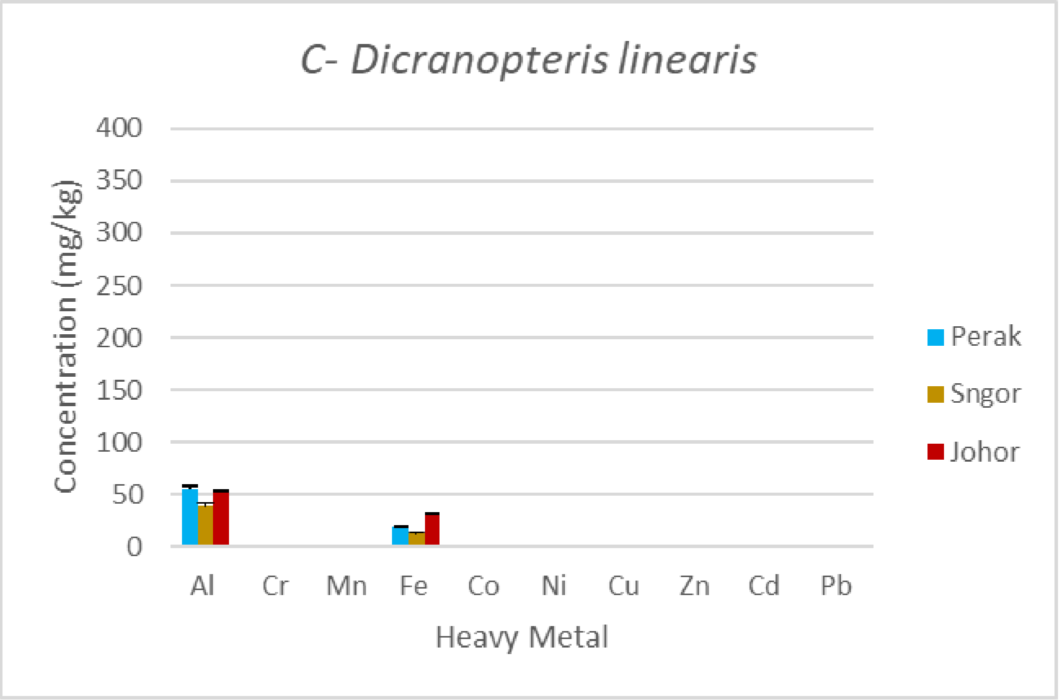
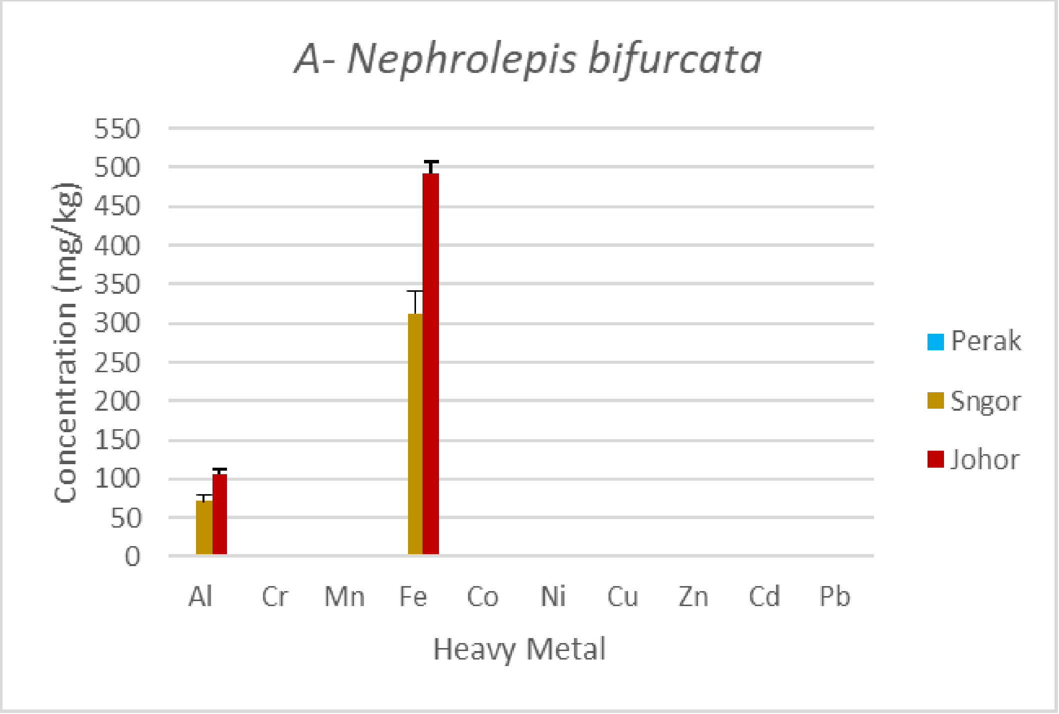
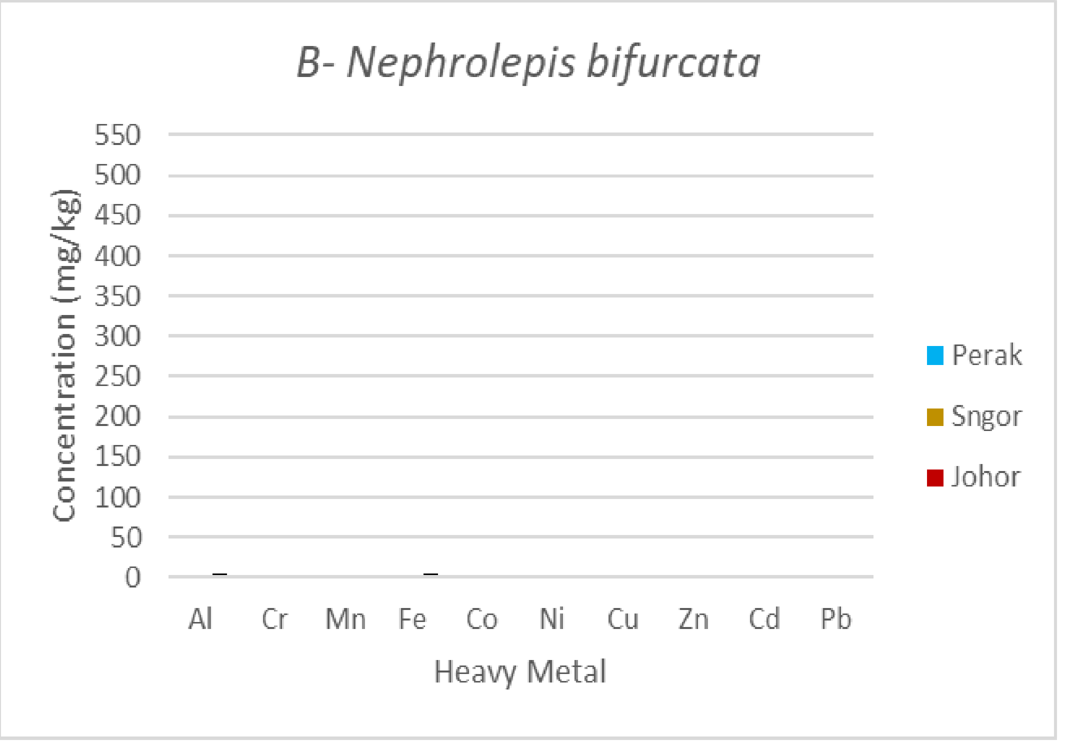



**Figure 1.** Al and Fe concentration for *D. linearis* distribution and accumulation specifically in laterite soil (A), vegetative (B) and root (C) parts at 3 different localities of Perak, Selangor and Johor





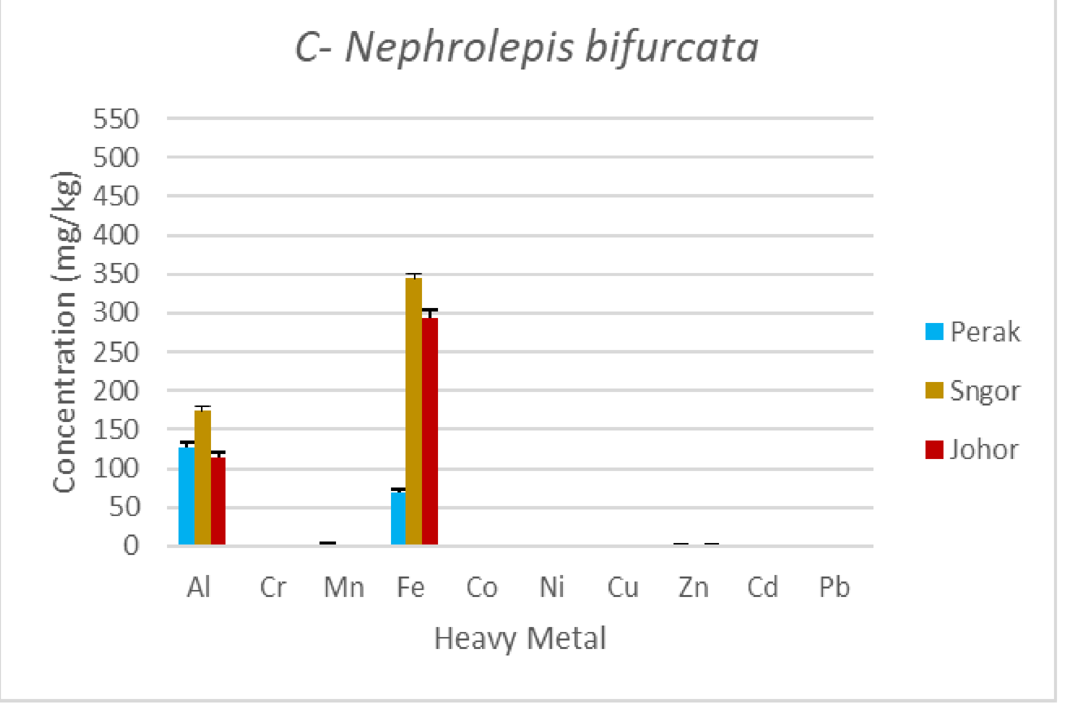
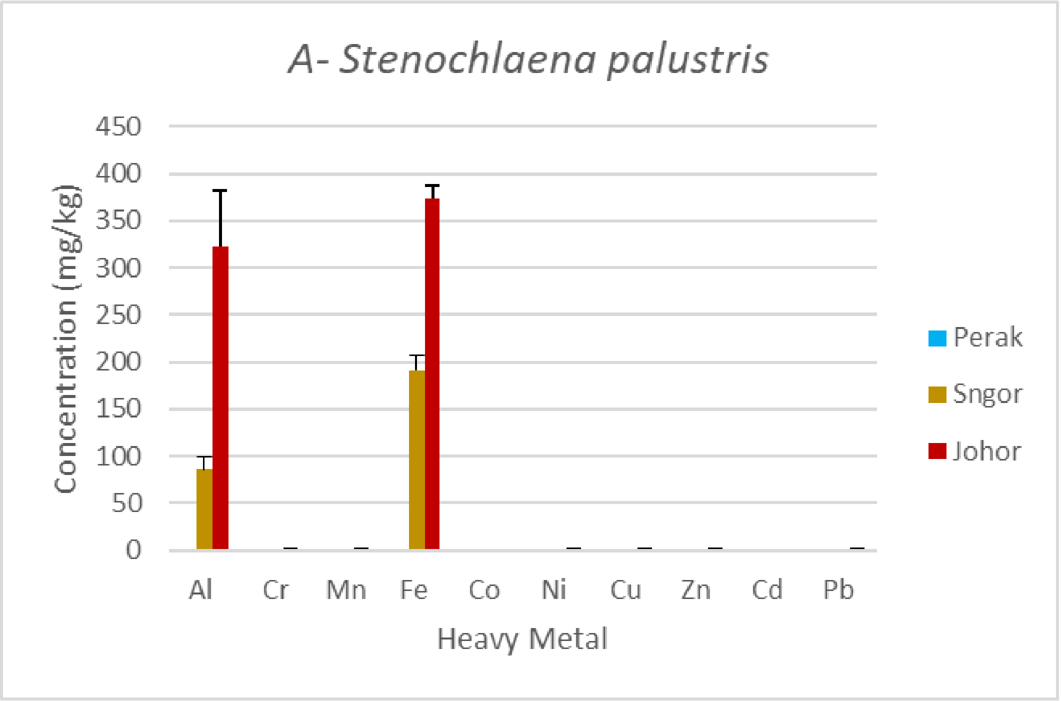
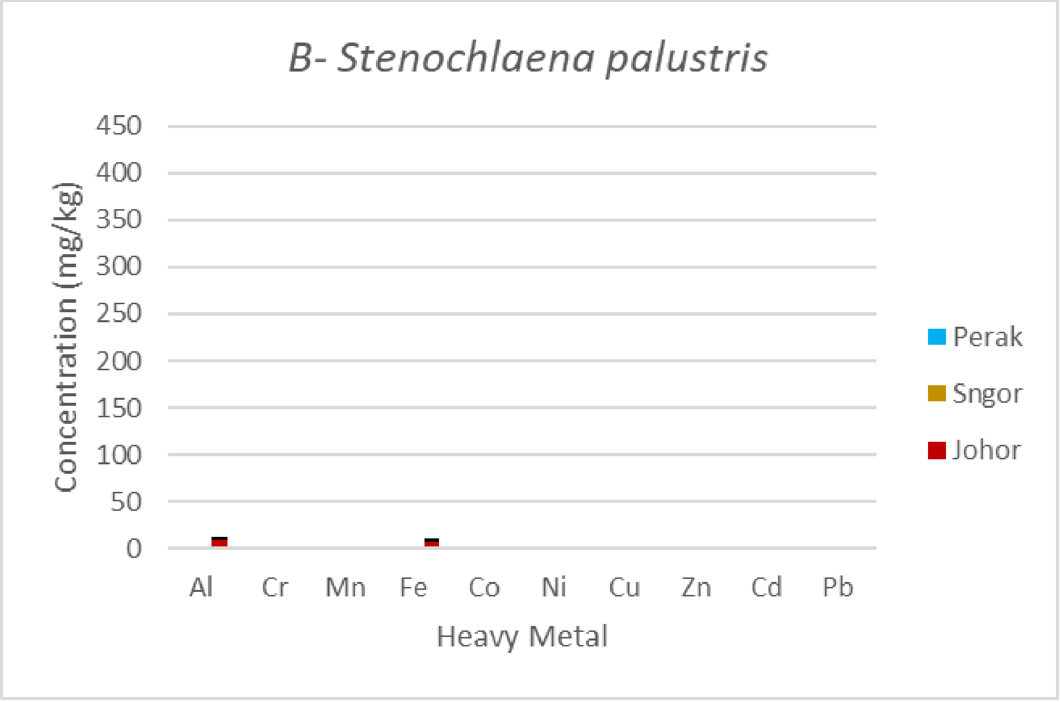




**Figure 2.** Al and Fe concentration for *N. bifurcata* distribution and accumulation specifically in laterite soil (A), vegetative (B) and root (C) parts at 3 different localities of Perak, Selangor and Johor


A- Stenochlaena palustris
Concentration (mg/kg)
450
400
350
300
250
200
150
100
50
0
Al
Cr
Mn
Fe
Co
Ni
Cu
Zn
Cd
Pb
Heavy Metal
Perak
Sngor
Johor


B- Stenochlaena palustris
Concentration (mg/kg)
450
400
350
300
250
200
150
100
50
0
Al
Cr
Mn
Fe
Co
Ni
Cu
Zn
Cd
Pb
Heavy Metal
Perak
Sngor
Johor

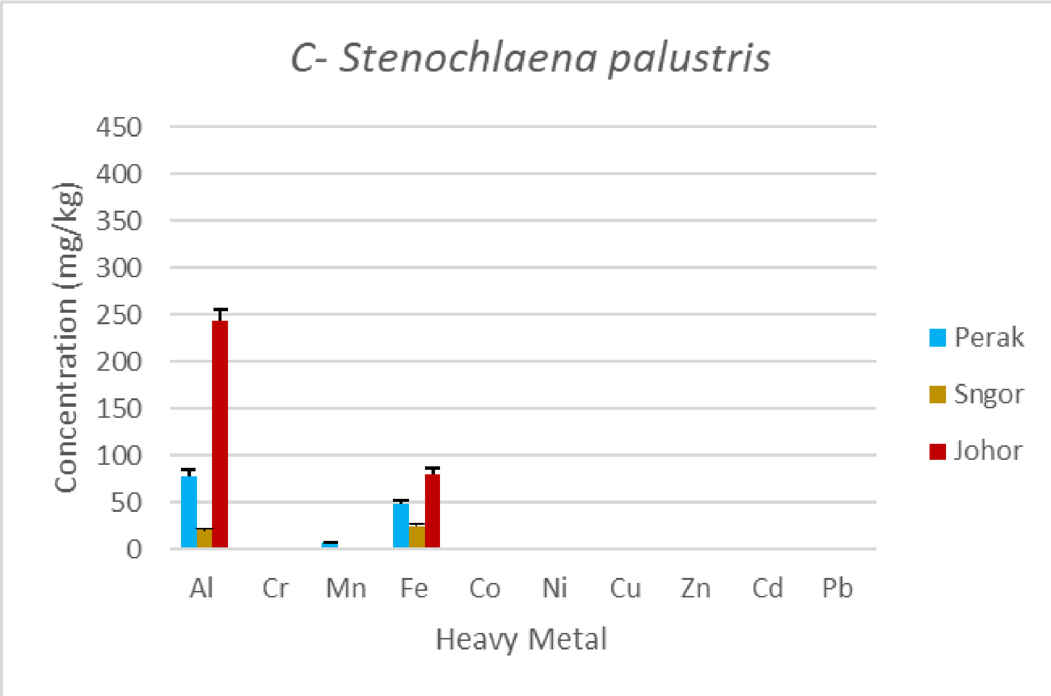



**Figure 3.** Al and Fe concentration for *S. palustris* distribution and accumulation specifically in laterite soil (A), vegetative (B) and root (C) parts at 3 different localities of Perak, Selangor and Johor

**Table 1.** BCF of *D. linearis, N. bifurcata, S. palustris* and *A. aureum* heavy metals sequestration rate

| **Fern** | **Heavy Metal** | |
|---|---|---|
| Species | *Aluminium (Al)* | *Iron (Fe)* |
| *D. linearis* | 1.18 | 1.26 |
| *N. bifurcata* | 1.09 | 1.55 |
| *S. palustris* | 1.18 | 0.70 |
| *A. aureum* | 0.79 | 1.24 |

According to Table 1, *D. linearis, N. bifurcata, S. palustris* were regarded as a potential bio accumulator of Al; whereas *D. linearis, N. bifurcata and A. aureum* were observed to bioaccumulate Fe. The results also showed that the different fern species showed a BCF rate of <2. Bioconcentration describes the efficiency of a fern species to extract the metal from the environment into its plant components. A species with a BCF value of >1 is a likely phytoremediation agent [14,15].

The ability of heavy metal ions in the soil to get immobilised in a different medium is dependent on the type of chemical, material, and metal speciation [16]. In contrast to different organic pollutants, which are oxidised to carbon (IV) oxide by microorganisms, most metals cannot be chemically or biologically degraded, and they remains active for an extended period of time after entering the soil [17]. However, their bioavailability and chemical structure often affect their mobility in soil. If the physicochemical composition of the soil is altered in any way, the pollutants become more mobile and are released into the atmosphere, causing severe damage [16]. It could be inferred that bio-concentration and translocation of the heavy metals are dependent on the bioavailability of heavy metals in the environment, both external (water and sediment related) and internal (animal and plant-related). Some floras can absorb and collect the metallic contaminants that are stored in their different plant components [18]. Hence, many plants can restrict their ability to absorb and accumulate harmful toxins. Even though some plants accumulate and translocate the pollutants from their roots to other plant parts, other indicator plants can regulate the movement of pollutants from the lower to the upper regions. The proportional intensity of the shoots indicates the uptake of the metal ions from the soil.

## 4. Conclusions

The results indicated that the different fern species, such as *D. linearis, N. bifurcata,* and *S. palustris,* could bioaccumulate Al ions and had a highest BCF value, while *D. linearis, N. bifurcata* and *A. aureum* bioaccumulated Fe. However, in comparison to the vegetative components, the root system of the fern species showed a higher rate of heavy metals sequestration. The results also showed that the laterite soil had a higher concentration of Al and Fe, while the ability of the species except *D. linearis* to accumulate heavy metal remained stable. *N. bifurcata* showed the highest Fe sequestration among the fern species, while *S. palustris* showed the highest Al sequestration.

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