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Estimation of Lead and Iron Concentration in Fern (*Stenochlaena palustris* (Burm. F. Bedd)) Grown under Different Conditions of Peatlands

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Abstract **Background of the research:** Fern with excessive or lacking of Fe as a result of peat burning was considered toxic to be consumed. **Purpose:** The aim of the study was to evaluate the metals bioaccumulation of lead (Pb) and iron (Fe) in Fern (*Stenochlaena palustris* (Burm. F. Bedd)) grown on peatlands. **Methodologies:** Three classifications of peatlands were used in this study: unburnt peatlands, peatlands that burned once and peatlands that burned several (2-3) times. To examine the estimation, the research was conducted at Pakning, Bukit Batu District, Bengkalis Regency, Riau Province, Indonesia. Determination of Pb and Fe metals in the roots, stems and leaves of Fern growing on burnt peat was analyzed using ANOVA. **Principal results:** The result obtained showed that the concentration of Pb on *Stenochlaena palustris* decreased in the peatland burned, however, Fe concentration increased at once burned and burned several times. Bioconcentration Factor (BCF) result showed that Pb (II) and Fe (II) were > 1 (one) and translocation factor (TF) result showed < 1. **Major conclusions:** The results showed significant differences in roots, stem, and leaves of *Stenochlaena palustris* that grew on unburnt peatland, once burned and burned several times. **Contributions to the field:** This study provides empirical evidence of Lead and Iron Concentration in Fern (*Stenochlaena palustris (Burm. F. Bedd*)) grown under different conditions of peatlands. **Important aspects of the study:** Based on the BCF, *Stenochlaena palustris* was classified as a bioaccumulator's plant. **Research limitations/ implications:** The analysis only examines Fern as a bioaccumulator and the TF of *Stenochlaena palustris* (Burm. F. Bedd) against Pb (II) and Fe (II) ions. **Practical implications:** This practically emphasized that a lower Acceptable Daily Intake (ADI) value decreases the possibility of Fern being consumed.

Keywords Metal Bioaccumulation, *Stenochlaena Palustris*, Atomic Absorption, Spectrophotometer, Peatlands, UV-Vis Spectrophotometer, TF

1. Introduction

Riau Province Indonesia has the largest peatland in Indonesia, covering 4,360,740,2 hectares [1]. Bengkalis Regency has the second largest peatland area in Riau, which is around 803,891.1 hectares spread over 16 villages [2]. One of which is Pakning. Most of the Pakning area is filled with oil palm plantations with an area of 614 hectares. The community's economy, which is mostly built from the plantation sector, has resulted in the conversion of peatlands to plantations. In fact, this peatland can be used as a source of food. One of the plants that can grow on peatlands is *Stenochlaena palustris* (Burm F. Bedd)). *Stenochlaena palustris* is a type of fern that is commonly consumed by local communities.

According to the Ministry of Agricultural Affairs [3], each peatland has different characteristics depending on the physical, chemical and biological properties as well as the type of sediment underneath. According to Coggins et al. [4], the more frequent fires occur, the accumulation of lead in Fern will increase. Lead absorption by Fern is largely influenced by environmental factors, one of which is motor vehicle fumes. Fern that contains excess lead will be toxic and harmful to health. The lead concentration generally is about 0.5-3.0 parts per million/ppm.

Sediment that is low in nutrient concentration will affect the nutrition of Ferns that grow on the sediment. Iron (Fe) is one of the elements found in the soil as a two-valent cation in the form of a solution. The function of Fe is as a constituent of chlorophyll, proteins, enzymes and plays a role in the development of chloroplasts. Fern with excessive or lacking Fe was considered toxic to be consumed. According to Mackinnon [5], only the stems and leaves are taken from Fern stems. Thursina [6] showed that the Fe concentration in the mantle was 336.4 ppm. On the other hand, the speed of accumulation depends on the characteristics of the leaf surface, wind speed, and rainfall [7]. Hence, the study aims to examine the metal concentration of lead and iron in *Stenochlaena palustris* that grow on ex-burnt peatland in Pakning, Bengkalis Regency, Riau, Indonesia.

2. Research Methods

The equipment used in this study were uv-vis spectrophotometer (Spektronik 20 Milton Roy), cuvette (Milton Roy), Atomic Absorption Spectrophotometer (Perkin Elmer 3110), Instrument Scales (Mettler AE200), Thermometer, Hot Plate (Digital Magnetic Hot Plate Stirrer-IKA C-MAG HS 7), Desiccator (CSN SIMAX), Oven (Gallen Kamp Hotbox Oven Size 1), Furnace (Gallen Kamp) and glassware other commonly used in the laboratory.

The material used in this study was a sample of Fern (*Stenochlaena palustris* (Burm. F. Bedd)), nitric acid (HNO_3), iron (II) ammonium sulfate $[(NH4)_2Fe(SO_4)_2.6H_2O]$, hydroxylamine hydrochloride ($NH_2OH.HCl$) 10%, 1,10 phenanthroline monohydrate ($C_{12}H_8N_2.H_2O$), ammonium acetate buffer ($NH_4C_2H_3O_2$), Whatman No.42 filter paper and distilled water.

The samples of Fern were taken from the three locations that have been determined: point I on unburnt peatlands, point II on peatlands that burned once and point III on peatlands that burned several (2-3) times in Pakning, Bukit Batu District, Bengkalis Regency, Riau Province, Indonesia (Figure 1). Samples of ferns were taken, namely the young male Fern at each sample point. This sample is taken by pulling Fern to the roots of Fern. The samples that have been taken are put in a large plastic container and labeled according to the sampling location (Figure 2). Furthermore, the samples of Fern were taken to the Instrument Laboratory of the Faculty of Mathematics and Natural Sciences, Riau University and analyzed using an Atomic Absorption Spectrophotometer.

Source: Google Maps

Figure 1. Map of research location

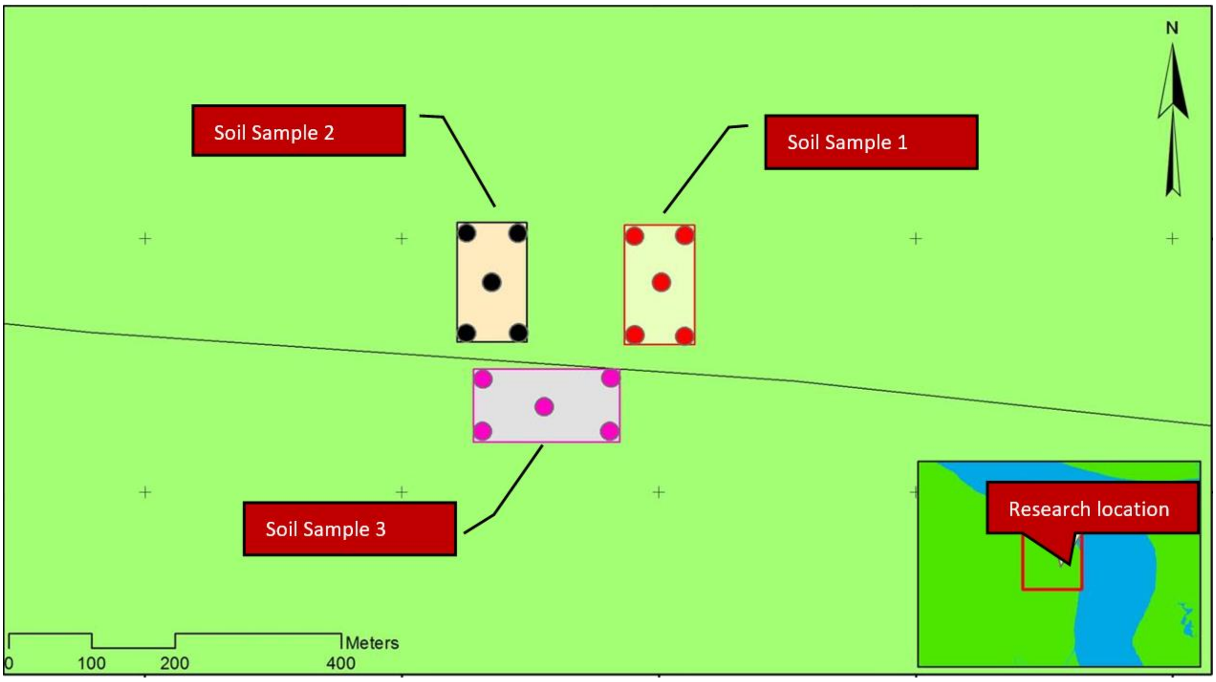

Figure 2. Map of sampling points

The samples were air-dried at room temperature, then cleaned of adhering soil, separated from roots, stems, and leaves and cut into small pieces. Furthermore, the samples were analyzed to determine the concentration of lead (Pb) and Iron (Fe) in the sample.

To examine bioaccumulation process, the absorbance results of lead and iron were used to determine the value of the bioconcentration factor (BCF) and the value of the translocation factor (TF). Bioconcentration factor (BCF) is the ability of Fern to accumulate certain metals with respect to the concentration in the soil substrate. Translocation factor (TF) is the ability of metal transfer from roots to Fern leaves. The formulas to determine BCF and TF were shown as follows:

BCF = metal concentration in mg/Kg

soil metal concentration mg/KG

TF = metal concentration in leaves mg/Kg

metal concentration in root mg/KG

To determine the concentration of the sample in water, a sample of 0.5 grams was put into a porcelain dish whose constant weight was known. The cup containing the sample was weighed and put into a drying oven at a temperature of ± 105℃ for ± 1 hour. Then the cup was cooled in a desiccator for ± (30 minutes) and weighed. The treatment was repeated until a constant weight was obtained.

Moreover, to examine sample dissolution, the sample was weighed as much as (5 grams) then the sample was dried in a furnace at a temperature of 400℃ for 4 hours, and then the sample was put into a 100 mL Erlenmeyer. A total of 10 mL of 65% nitric acid was added to the sample and heated. When the sample is not dissolved, nitric acid is then added again and reheated until the sample is completely dissolved. Erlenmeyer was removed and allowed to cool. The solution was then filtered through Whatman No. filter paper. 42. The solution obtained from the destruction is put into a 50 mL volumetric flask and adjusted to the mark with the addition of distilled water. The results of the destruction were used for the analysis of lead (Pb) and iron (Fe).

To determine the concentration of lead (Pb) was by using Atomic Absorption Spectrophotometer (AAS). Samples from the destruction were taken as much as 5 mL (1:1) mL in each sample. Furthermore, the Pb concentration in the solution was analyzed using Atomic Absorption Spectrophotometer (AAS) with a wavelength of 283.3 nm.

To determine the concentration of iron (Fe) was conducted by using the Fenanthroline method in four examinations. The first was the determination of wavelength. The absorption of standard iron solution (concentration 5 parts per million/ppm) was put into a 100 mL volumetric flask, then 10 mL of ammonium acetate (pH=4.8) was added, 2 mL of hydroxylamine hydrochloride solution, and 2 mL of Phenanthroline complexing reagent. Then distilled water was added up to the limit mark, as was the case with the blanks, then homogenized. Then the absorbance was measured at a wavelength of 450-550 nm with 5 nm intervals. The maximum absorbance value obtained shows the optimum wavelength and a graph is made between absorbance and wavelength.

The second was determination of color stability time. The Determination of color stability time, and absorption of standard iron solution (concentration 5 ppm) was put into a 100 mL volumetric flask then added 10 mL of ammonium acetate (pH=4.8), 2 mL of hydroxylamine hydrochloride and 2 mL of Phenanthroline complexing reagent. Then distilled water was added up to the limit mark, as well as the blanks, and then homogenized. The absorbance of the sample was measured every 5 minutes for 1 hour at a wavelength of 510 nm. Stable absorbance value at a certain time indicates that at that time there is color stability. Then graphs of absorbance against time intervals are made.

The third was to determine calibration curve. Samples were taken as much as 1, 3, 5, 7, 9 and 11 ppm which were put into a 100 mL volumetric flask. Then 10 mL of ammonium acetate (pH=4.8) was added, 2 mL of hydroxylamine hydrochloride solution and 2 mL of Phenanthroline complexing reagent. Then the sample is diluted to the mark, as well as the blank. The sample was shaken until homogeneous and allowed to stand for a period of time obtained from the determination of color stability. The absorbance was measured at the optimum wavelength with a spectrophotometer (measurement time was adjusted to the time limit interval on color stability). A standard solution calibration curve between absorbance to standard solution concentration is made.

The fourth was to determine iron concentration. In this analysis, 10 ml of the digested sample was put into a 100 mL volumetric flask. Then 10 mL of ammonium acetate (pH=4.8) was added, 2 mL of hydroxylamine hydrochloride solution and 2 mL of Phenanthroline complexing reagent. Then the sample was shaken and diluted to the limit mark, as was the case with the blanks. The sample was allowed to stand for several minutes (until the formation of perfect color) then the absorbance was measured according to the color stability time at a wavelength of 510 nm. The iron concentration was determined using the ratio of the sample absorption to the standard on a standard calibration curve and carried out up to three repetitions [8].

The data obtained from this study were calculated using standard curve linear regression equations that were displayed in the form of tables and graphs. Determination of Pb and Fe metals in the roots, stems and leaves of Fern growing on burnt peat was analyzed using ANOVA.

3. Results

3.1. The Concentration of Water

The gravimetric method is used to analyze the metal concentration in water. Table 1 shows that the highest concentration was found in *Stenochlaena palustris* that grew on unburnt peatland, namely in the roots, stems and leaves, each 67.97%, 35.84%, and 46.64%. Meanwhile, the lowest concentration was in Fern that grew on burnt peat several times, namely in the roots, stems, and leaves, each 53.78%, 22.96% and 37.70%.

Table 1. Measurements of water concentration in Fern (*Stenochlaena palustris* (Burm. F. Bedd)).

Sample	Water concentration (%)
UbS root	67.97
UbS stem	35.84
UbS leaf	37.70
BS1 root	57.36
BS1 stem	32.86
BS1 leaf	40.00
BSn root	53.78
BSn stem	22.96
BSn leaf	37.70

Information: UbS= unburnt soil; BS1= once burnt soil; BSn = multiple (2-3) times burnt soil

This shows that the metal concentration in water in *Stenochlaena palustris* has decreased along with the increasing frequency of fires due to the better ability to absorb and pass water in unburnt peatlands compared to peatlands that have been burned several times, as well as shrinkage in soils that have been burned once and several times. According to Purbowaseso [9], the shrinkage of concentration is caused by the loss of ground cover vegetation, which reduces the rainwater barrier function. Previous research showed that the concentration of *Stenochlaena palustris* on unburnt peatland is 66.21% while on often burned peatland is 12.12% [10]. Table 1 showed that the concentration in the roots of *Stenochlaena palustris* is higher than that of the stems and leaves. The roots absorb water from the soil. Meanwhile, another research [11] showed that the concentration in the leaves contained in *Stenochlaena palustris* was 8.56% and the concentration in the stem was 7.28%.

3.2. The Concentration of Lead (Pb)

The analysis revealed that the frequency of fires can affect the lead concentration (Pb) in *Stenochlaena palustris* (Burm. F. Bedd). The results of the chemical analysis of lead (Pb) in *Stenochlaena palustris* (Burm. F. Bedd)) growing on ex-burnt peatland with different frequencies was shown in Figure 3.

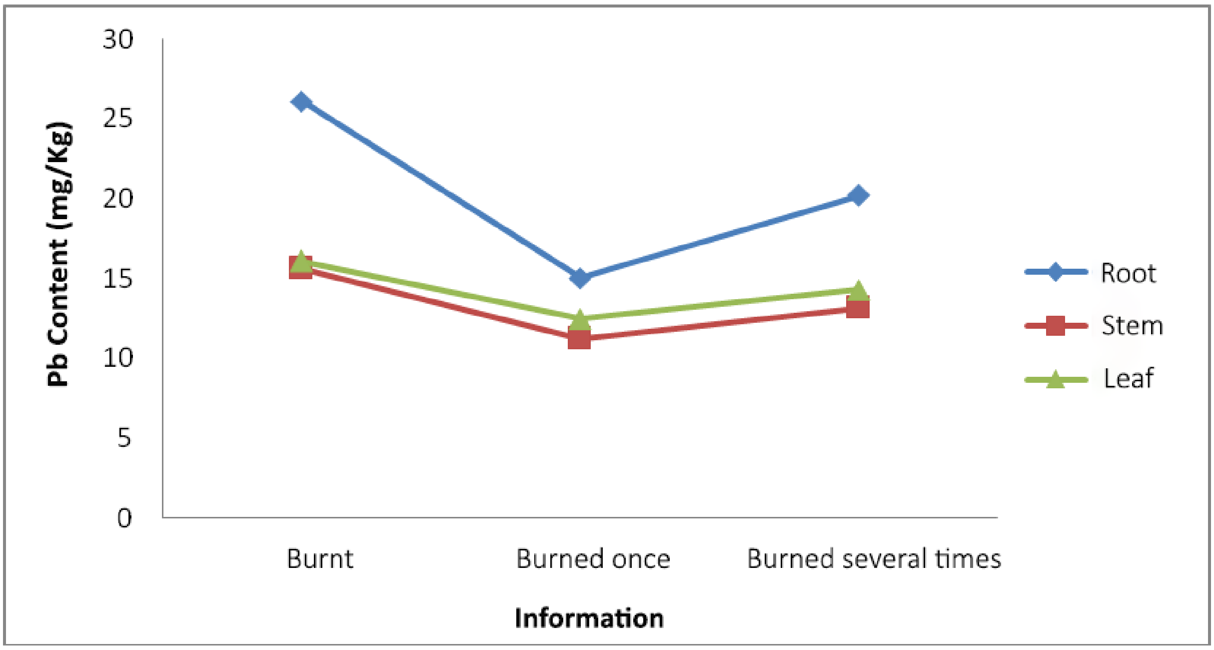

Figure 3. Pb concentration in the roots, stems, and leaves of *Stenochlaena palustris* (Burm.F. Bedd) growing on never, once and several times burnt peatland

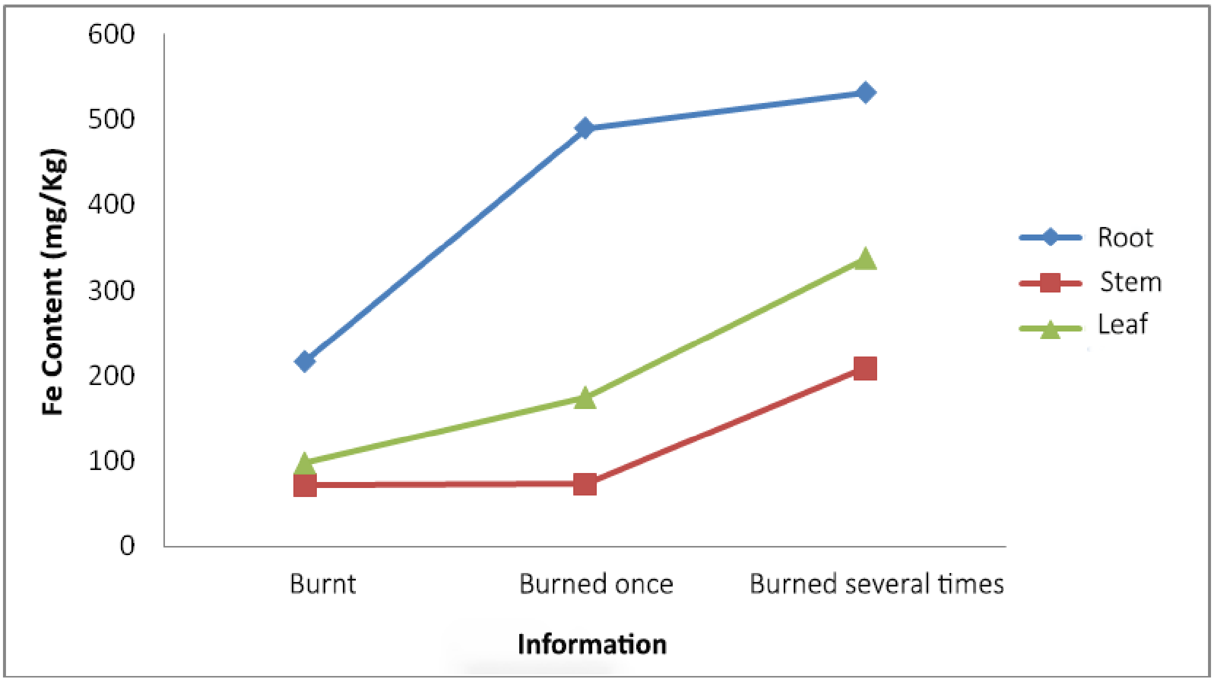

Figure 4. The concentration of iron (Fe) in the roots, stems and leaves of *Stenochlaena palustris* (Burm.F. Bedd) which grows on never, once and several times burnt peatland

In Figure 3, it was shown that the lead (Pb) concentration in the unburned soil is high and there is a decrease in the peatland once it is burned. Subsequently, the peatland was burned again several times. The metal concentration of lead (Pb) in the roots is higher than in Fern stems. In this context, previous research has shown that Fern roots are in direct contact with the soil [12]. Fern can be mediator of the spread of heavy metals as the entry point in which metals were absorbed into Fern through roots and leaves. The accumulation of metals from solution into Fern tissues is more likely in water with high concentration of metals. The more metals are absorbed, the more metals accumulate in *Stenochlaena palustris*.

3.3. The Concentration of Iron (Fe)

The results of the analysis of Iron (Fe) on *Stenochlaena palustris* found on unburnt peatland, burned once and burned several times was shown in Figure 4.

The concentration of iron (Fe) increases with frequent fires. The value of anchovies found on unburnt peatlands had the lowest value, while those found on burnt peatlands had the highest value. When a fire occurs again, over time there will be an accumulation of iron (Fe). Fern growing again absorbs more and more iron then fires again. Furthermore, Fe settles in the soil and accumulates again and so on so that there is an increase in the iron concentration (Fe) in the soil once burned and increases again in Fern found on peatland several times. The results were consistent with previous research demonstrating that the availability of iron (Fe) in the soil is largely due to iron (Fe) being easily soluble in water [13].

3.4. Bioconcentration Factor (BCF) of Pb and Fe

Based on the results of the analysis using the AAS (Atomic Absorption Spectrophotometer) method, the value of the bioconcentration factor (BCF) was shown in Table 2.

Table 2. Bioconcentration Factor for Pb and Fe

Sample	Metal	BCF
UbS	Pb	1.2389
	Fe	1.1039
BS1	Pb	1.6616
	Fe	1.8176
BSn	Pb	1.3692
	Fe	1.7909

Information: UbS = unburnt soil; BS1 = once burnt soil; BSn = multiple times burnt soil

Table 2 shows the differences in BCF of each sample. Lead metal (Pb) has a BCF in samples of *Stenochlaena palustris* growing on never, once and several times burnt peatlands, each of 1.2389, 1.6619 and 1.3692, respectively. Meanwhile, the BCF value of iron on never, once and several times burnt peatland has increased with successive values of 1.1036; 1.8176 and 1.7909. So, Fern has the potential as a bioaccumulator of lead (Pb) and iron (Fe) metal ions. However, BCF < 1 means that the pollutant is indicated not to accumulate in Fern [14].

3.5. Translocation Factor (TF) of Pb and Fe

The results of the analysis of Pb and Fe metal translocation factor values was shown in Table 3.

Table 3. Translocation factor for Pb and Fe

Sample	Metal	TF
UbS	Pb	0.8291
	Fe	0.4526
BS1	Pb	0.7076
	Fe	0.3574
BSn	Pb	0.6168
	Fe	0.6347

Information: UbS = unburnt soil; BS1 = once burnt soil; BSn = multiple times burnt soil

The calculation of the value of the translocation factor was carried out to determine the ability of *Stenochlaena palustris* to translocate metals from roots to all parts of Fern [15]. The results of the calculation of the value of the translocation factor of lead (Pb) contained in unburnt peatland, burned once and burned several times in a row, namely 0.8291; 0.7076 and 0.6168. The lowest value of translocation factor was found in *Stenochlaena palustris* that grew on peatlands that had been burned several times and the highest value was found in Fern growing on unburnt peatlands.

Ferrous metal (Fe) has a translocation factor value of 0.4526; 0.3574 and 0.6347. The lowest value of translocation factor was found in *Stenochlaena palustris* that grew on peatlands once burned and the highest values were found in Ferns that grew on peatlands that were burned several times. From the translocation factor data obtained, it shows that Fern is not a hyperaccumulator plant, because the value of the translocation factor must be more than one (Tf > 1) [16].

3.6. Acceptable Daily Intake (ADI)

The limits of daily consumption of Pb and Fe in *Stenochlaena palustris* was shown in Table 4. WHO [17] determined the ADI for the permissible weight of Pb in Fern that grows on never, once and several times burnt peatland, exceeding the maximum limit of 13.56 grams, respectively; 18.13 grams and 15.68 grams. In Table 4, it was shown that the highest ADI was in the *Stenochlaena palustris* which was found on once burned peat and the lowest was on unburnt peatland. The iron ion that can enter the body per day is about 15 mg. The average concentration of Fe in *Stenochlaena palustris* found in never, once and several times burnt peatland was 170.17 mg, Kg^{-1}; 218.07 mg, Kg^{-1} and 546.47 mg, Kg^{-1}. According to the Belitz & Grosch [18] standard which stipulates the ADI for Fe, the maximum weight of *Stenochlaena palustris* that are safe for consumption are 88.18 grams, respectively; 68.78 grams and 27.45 grams. The results also found the lowest ADI value in Fern grown under different conditions on peatlands.

Table 4. Acceptable Daily Intake (ADI) Pb and Fe

Sample	Pb (gram)	Fe (gram)
UbS	13.6	88.2
BS1	18.1	68.8
BSn	15.7	27.5

Information: UbS = unburnt soil; BS1 = once burnt soil; BSn = multiple times burnt soil

Overall, the results are consistent with previous research in examining the metal bioaccumulation resulted from a burnt environment [19, 20] and geologically different conditions [21]. The results are also relevant to the effect of metal bioaccumulation in ecological and physiological aspects [22], especially in peatland environment [23-25]. The findings are also in line with studies highlighting bioaccumulation of metals as pollutants are persistent in the environment and their effect on the food chain, and public health because of their toxicity [26-30].

4. Conclusion

The results showed significant differences in the lead (Pb) and iron (Fe) concentration in roots, stems and leaves growing on burnt peatland. The BCF value > 1 (one) indicates that Fern can be used as a bioaccumulator and the TF of *Stenochlaena palustris* (Burm. F. Bedd) against Pb (II) and Fe (II) ions is highest in burnt peatlands. The results also showed ADI value of Pb in Fern that grew on never, once and several times burnt peatlands in a row, namely 13.56 grams; 18.13 grams; 15.68 grams and for Fe, which is 88.18 grams; 68.78 grams and 27.45 grams. This practically emphasized that a lower ADI value decreases the possibility of Fern to be consumed.

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