Accumulation and adaptation of perumpung (*Phragmites karka*) to iron ion stress in hydroponic media

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**Abstract.** Acid sulphate soils containing very high iron is a barrier to agricultural development in coastal areas. Also known, Perumpung (*Phragmites karka*) grows in coastal marshes. This experiment was conducted to determine the adaptation of the Perumpung to iron ion stress, and also its accumulation rate in hydroponic media. Hydroponic nutrient solution (referring to Hoagland solution) is used to support the growth of the Perumpung seedlings, and adding iron solution as a contaminant at five different concentrations of 0 (without addition), 25 mgL⁻¹, 50 mgL⁻¹, 100 mgL⁻¹ and 150 mgL⁻¹. After 45 days of treatment, the results showed that Perumpung which was placed in a addition iron solution of 150 mg/L contaminant was dead, while the growth of Perumpung at 25 mgL⁻¹ iron addition concentration was not disturbed. The rate of iron absorption was 100 mgL⁻¹ > 50 mgL⁻¹ > 0 mgL⁻¹ > 25 mgL⁻¹, respectively. Iron metal is stored in roots more than leaves/shoots.

1. **Introduction**

Acid sulphate soils that contain a lot of Fe, are found in many coastal areas. In Malaysia, acid sulphate soils mainly occur in the coastal plains of the west coast states of Peninsular Malaysia and Sarawak (Borneo Island). Acid sulphate soils are normally not suitable for the crop production unless they are properly ameliorated and their fertility improved [1]. Excessive Fe causes the growth of agricultural crops to be stunted or dead. The presence of Fe in the form of various minerals, especially pyrite, comes from the deposition of sulphate compounds around 35000 years ago [2]. When there was a decrease in sea level, deposits of pyrite and other iron minerals were formed in the soil. Then a potential acid sulphate soil is formed which has a pH of about 5. Furthermore, through the oxidation process, due to tillage, the pyrite compounds turn into sulfuric acid and iron ions. This oxidation process results in a decrease in soil pH to reach 3 [3].

Iron is an essential nutrient that plays a critical role in life sustaining processes. Due to its ability to gain and lose electrons iron works as a cofactor for enzymes involved in a wide variety of oxidation-reduction reactions (i.e., photosynthesis, respiration, hormone synthesis, DNA synthesis, etc.). This function makes iron an essential nutrient, and its deficiency causes iron chlorosis, which seriously constrains normal plant development. Iron toxicity in plants is indicated by bronzing characteristics, which have been observed in plants grown in greater than 100 mM iron solutions. Higher iron uptake by plants reduces protein synthesis in leaves [4]. Meanwhile, the plant has a mechanism to regulate the entry of heavy metals into the roots. Plants can present different tolerance mechanisms in response to excess of Heavy Metals, including a reduction in transport through the membrane, exclusion,
phytochelatins (PCs), metallothionein (MT) formation, chelation by organic acids and amino acids, and metal compartmentalization in subcellular structures [5].

The hydroponic method was chosen so that all iron is in the form of dissolved ions. As known, iron and other metals are present in soil in a number of different forms that determine its availability to plants. Therefore, the determination of total Fe does not give information about its mobility and lability [6]. Plants took some macronutrient, as both ferric (Fe3+) or ferrous (Fe2+) ion, although the latter is more common due to its greater solubility [7]. The chemical speciation or fraction of the metals was carried out using the method of Lo and Yang as modified by Asagba et al. [8]. Research has also been carried out on iron fractions in acid sulphate soils in Merauke, Papua, Indonesia. The available iron fractions range from 10-700 ug Fe/g, while the total iron content in the soil ranges from 3,000 to 50,000 ug Fe/g [9]. The determination of total soil metal content alone is not a good measure of bioavailability and not a very useful tool to quantify contamination and potential environmental and human health risks. Hence, total concentrations of metals in soils are a poor indicator of metal toxicity since metals exist in different solid-phase forms that can vary greatly in terms of their bioavailability [10, 11, 12, 13, 14].

Phragmites karka is a kind of swamp plant, including in the Poaceae or Gramineae family and the genus Phragmites. It has other names like Perumpung (Melayu) atau Reed (English) [15]. It has some characteristics such as robust, erect, strongly tufted, perennial grass with an extensive, creeping, branching rhizome or stolons up to 20 m long; stem (culm) 2-8 m tall and 1.5 cm in diameter, very stout, often wooly with hollow internodes and glabrous nodes [16]. Perumpung is able to absorb heavy metals from the soil and stored in the roots, stems and leaves. Research has been carried out on ex-mine land in Papua New Guinea (PNG), and the results show that Perumpung is capable of absorbing various heavy metals from the soil so that this plant can potentially be used as a phyto-accumulator [17]. Studies have also been conducted on P. australis (another type of reed that is the same as its genus) which has been found to be able to act as a phytoaccumulator for several types of heavy metals from secondary treated effluent [18]. Therefore, we conducted this research in order to find out the adaptability of Perumpung to the stress of iron ions in solution. Next, we want to know how much iron is absorbed by the Perumpung under normal conditions and iron stress conditions.

2. Material and Methods
All chemicals are analytical reagents. The source of iron ions or stock solution is Iron II Sulphate Heptahydrate (FeSO4.7H2O) from Merck in Aquadest 1000 mgL⁻¹. All solutions were prepared with distilled water in plastic bottles soaked in 0.1 molL⁻¹ HNO3 for at least 24 hours to reduce adsorption by the bottle wall. The modified Hoagland solution (pH 5.5 ± 0.2) was prepared with the following salts (molL⁻¹): Ca(NO3)2•4H2O,3.57×10⁻⁴; H3BO3, 2.31×10⁻⁵; KH2PO4, 9.68×10⁻⁴; KNO3, 2.55×10⁻⁴; MgSO4, 1.04×10⁻³; FeCl3, 6.83×10⁻⁵; MnSO4•H2O, 7.69×10⁻⁶; MoO3, 1×10⁻⁵; CuSO4•5H2O,1×10⁻⁵; and Zn(NO3)2•6H2O, 1×10⁻⁵ [19].

2.1. Hydroponic experiments
Perumpung cuttings are cut 10 cm long which was 2 cm down from the nodes and 8 cm above the nodes. The cuttings are put into a hydroponic solution without the addition of iron and then allowed to grow for 28 days, until the roots and shoots grow. Four seeds were planted in each bottle filled with Hoagland nutrition plus iron concentrations of 0 or blank, 25 mgL⁻¹, 50 mgL⁻¹, 100 mgL⁻¹, and 150 mgL⁻¹. So, all the seeds are 20 cuttings. Seedlings were treated for 45 days, given aeration for 30 minutes in the morning and 30 minutes in the afternoon. After 45 days, the seeds are harvested. Three seedlings were selected from each bottle with relatively uniformly growth.

2.2. Analysis of metal concentrations in plant samples
Each sample recorded its physical condition. Then, the root and stem samples are separated. The sample is washed with distilled water, then dried and labeled. Each sample were aerated for 3 days to dry and constant weight. Milled into fine powder. Powder samples weighing 5 grams were dissolved into a
mixture of 100 mL of HNO3 at 150 °C for 2 hours and continued with aquadest to 500 mL and heating for 1 hour. Fe concentration was measured by fire atomic absorption spectrometry.

2.3. Statistical analysis
Data was analyzed by one-way ANOVA using the Microsoft Excel 2016 software package for Windows. Data are presented as mean ± standard error (SE) (n = 3), and are tested by the Least Significant Differences (LSD) at the 5% level.

3. Results and Discussion

3.1. Physical Response by Perumpung
Perumpung’s root growth was inversely proportional to the increase in Fe concentration on the substrate. While plant (stem) growth highest at the substrate concentration of 25 mgL⁻¹. Plant’s growth was decrease proportionally to the increase of Fe concentration on the substrate. In detail, the response of plant’s growth to various Fe concentrations of substrate, can be seen in Table 1. Growth of Perumpung can be seen in Fig. 1.

Table 1. Effect of Fe Substrate Concentration on Root and Stem Growth.

| Fe conc of Sample (mgL⁻¹) | Length (cm)         |
|-------------------------|---------------------|
|                         | Root                | Stem                |
| 3.8                     | 10 – 13             | 8 – 58              |
| 25                      | 5 – 11              | 2 – 72              |
| 50                      | 5 – 8               | 11 – 41             |
| 100                     | 1 – 3               | 1.5 – 12.5          |
| 150                     | 1 – 2               | 1 – 3               |

Figure 1. Physical response of Prumpung which was planted in various Fe concentrations in the hydroponic substrate. A = Perumpung which was planted in 3.8 and 25 mgL⁻¹ of Fe conc.; B = in 50 mgL⁻¹; C = in 100 mgL⁻¹; D = 150 mgL⁻¹ (dead); E = Perumpung’s root was planted in 3.8 mgL⁻¹ of Fe conc.; F = Root in 50 mgL⁻¹; G = Root in 100 mgL⁻¹.
Perumpung growth has not been affected by the concentration of 25 mgL\(^{-1}\) Fe substrate. Most likely, Perumpung roots produce molecules whose properties can stabilize Fe in the substrate so that it is not absorbed further by plant roots. As stated by Eutropio et al. that preventing entry of metals in the cytosol through exudation of compounds by the action of plasma membrane may theoretically be the best defense strategy. Some plants, known as exclusionary plants, possess specialized mechanisms to reduce the entry of Heavy Metals in the roots. Malate, citrate, and oxalate have been identified as important chelating agents secreted by roots that are involved in plant resistance to Heavy Metals [5].

3.2. Fe Concentration in Roots and Stem

The lowest absorption of Fe by root at substrate concentration of 25 mgL\(^{-1}\) is 612 mgkg\(^{-1}\). The highest concentration of Fe in the roots at 100 mgL\(^{-1}\) Fe substrate concentration. The concentration of Fe in shoots was highest in the shoots which grew on the substrate of 100 mgL\(^{-1}\) Fe, while the lowest concentration of Fe in the shoots was at the substrate concentration of 25 mgL\(^{-1}\). In detail, can be seen in Table 2.

| Fe conc of Sample (mgL\(^{-1}\)) | Fe conc (mgkg\(^{-1}\)) | Roots | SD | Stem | SD |
|---------------------------------|-------------------------|-------|----|------|----|
| 3.8                             | 731\(^{a}\)              | 105.1 |    | 196\(^{a}\) | 33.6 |
| 25                              | 612\(^{a}\)              | 115.0 |    | 154\(^{a}\) | 65.1 |
| 50                              | 1720\(^{b}\)             | 239.6 |    | 249\(^{ab}\) | 60.7 |
| 100                             | 5235\(^{c}\)             | 924.2 |    | 307\(^{b}\) | 68.1 |

Absorption of Fe by roots at 3.8 and 25 mgL\(^{-1}\) substrate concentrations was not significantly different. While the absorption of Fe by stem at a concentration of 100 mgL\(^{-1}\) with 3.8 and 25 mgL\(^{-1}\) was significantly different.

The absorption of Fe at the concentration of samples 3.8 and 25 mgL\(^{-1}\) was not too different. While at the sample concentrations of 50 and 100 mgL\(^{-1}\), absorption of Fe was increase. Fe absorption in all samples was very high between 612 mgkg\(^{-1}\) to 5,235 mgkg\(^{-1}\), when compared with the absorption of Fe in Perumpung which grows on the soil. Research conducted by Rungwa et al., 2013 showed that accumulation of Fe in roots ranged from 436 to 1,861 mgkg\(^{-1}\). A single treatment, which is Fe, causes high absorption. In this case Fe is the only stressing factor. In addition, Fe is a micronutrient, which is needed by plants even in micro quantities. Iron needs by plants range from 2.5 to 4.5 mgkg\(^{-1}\) [20].

3.3. BCF, TF and BAC

The very high difference in concentration between Fe in the plant body and in the growing media (substrate) resulted in very high BCF and BAC values. While the value of Perumpung TF is relatively the same as the value of Perumpung TF which grows on the soil naturally. In detail, can be seen in Table 3.

| Fe conc of Sample (mgL\(^{-1}\)) | BCF | TF | BAC |
|---------------------------------|-----|----|-----|
| 3.8                             | 192.5 | 0.27 | 51.5 |
| 25                              | 24.5  | 0.25 | 6.2  |
| 50                              | 34.2  | 0.15 | 5.0  |
| 100                             | 69.8  | 0.06 | 4.1  |

| BCF = Bioconcentration Factor (Concentration of metal in Roots/Conc. of metal in soil). |
| TF = Transformation Factor (Conc. of metal in Stem/Conc. of metal in roots). |
| BAC = Biological accumulation coefficient (Conc. of metal in Stem/Conc. of metal in soil) |

Vaverkov and Adamcov [21] grouped plant species by their capability of heavy metal uptake and sensitivity to high metal pollution: hyperaccumulator plants BAC > 1, moderate accumulator plants 0.1 < BAC < 1.0, low accumulator plants 0.01 < BAC < 0.1 and non-accumulator plants BAC < 0.01. Plants
that accumulate very high concentrations of metals in any above ground tissue in their natural habitat are called hyper-accumulators [21].

The BCF value also show a high number which ranges from 24 to 192.5. This happens because the total amount of Fe present in the Perumpung’s growing media is all in the form of dissolved or available to plants. Unlike soil in natural conditions, where Fe is in various forms of fraction, where forms are available which are very few in number, compared to Fe in the form of fractions that are not available [17].

High TF values ranging from 0.06 to 0.27 indicate that P. karka can be used as a plant phyto-accumulator in the hydroponic system at dissolved iron concentrations equal to or below 50 mgL-1. Naturally, P. karka which grows on the ground also shows high ability in terms of iron translocation. Referring to research conducted by Rungwa et al. [17], the value of TF of Perumpung ranged from 0.02 to 0.23.

Based on the BAC value criteria according to Vaverkova and Adamcova [21], P. karka is classified as a hyper-accumulator. Almost the same as the results shown in BCF values. This can happen because the iron concentration in the substrate is low due to the iron fraction all of which are available. Another factor that also influences the high absorption is because iron is the only heavy metal that functions as a stressing agent. Therefore, Perumpung can be used as an iron accumulator with the condition that there is no interference with other heavy metals in the hydroponic substrate.

4. Conclusion
Perumpung which was placed in a addition iron solution of 150 mgL-1 contaminant was dead, while the growth of Perumpung at 25 mgL-1 iron addition concentration was not disturbed. The rate of iron absorption by roots was 100 mgL-1 > 50 mgL-1 > 0 mgL-1 = 25 mgL-1, respectively. The rate of iron absorption by stem was 100 mgL-1 > 50 mgL-1 = 0 mgL-1 = 25 mgL-1, respectively. Iron metal is stored in roots more than leaves/stem. Perumpung can be classified as a hyperaccumulator plant when planted in hydroponic media.

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**Further reading**

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