Accumulation of iron by perumpung (phragmites karka) and its adaptation in various pH of hydroponic solution

Y Risma*2,5, A Ahmad1,3, Raya1 and B Ibrahim4

1Chemistry Department, Mathematics and Natural Science Faculty, Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, Makassar 90245, Indonesia
2Doctoral Program of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Indonesia
3Laboratory of Research Centre and Developing of Sciences, Faculty of Mathematic and Natural Sciences, Hasanuddin University, Jl. Perintis Kemerdekaan KM 10, Makassar 90245, Indonesia
4Department of Soil Science, Faculty of Agriculture, Hasanuddin University, Makassar 90245, Indonesia
5Department of Chemistry, Mathematics and Natural Science Faculty, Cenderawasih University, Kelurahan Yabansai, Distrik Hemarm, Kota Jayapura 99352, Indonesia

*Email: ahyarahmad@gmail.com; These authors contributed equally to the work

Abstract. Iron is included as micronutrient which is needed in limited quantities by plants. Extraction from the soil is carried out through ion exchange between roots and solution in the soil. While the transport mechanism from the root to the leaves is carried out through xylem by a capillary mechanism. Iron concentration in acid sulfate soils is excessive, so it becomes toxic to plants. This research has been carried out to determine iron accumulation by Perumpung in various pHs in hydroponic solutions. The research was also carried out to determine the level of adaptation in the acidic pH and stress of iron ions at a concentration of 50 mg Fe/L. Perumpung used in this study has been seeded on hydroponic media for 3 weeks. The experiment was carried out for 3 and 5 weeks. The nutrient solution used according to the standard Hoagland Solution then added iron ions to a concentration of 50 mg Fe/L. Then 4 variations of pH were made 3, 4, 5, 6 plus two variations as a comparison, according to Hoagland's solution without the addition of iron (blank) and Hoagland's solution with the addition of iron but the pH was not regulated (control). The results of this study showed the highest level of adaptation which grew on the blank, then followed by pH 5.6 (control), pH 6, pH 5, and pH 4. Perumpung at pH 3 dead. The highest level of iron accumulation in roots was occurred at pH 5, followed by pH 6, then pH 4, and the control. The accumulation of iron in roots was higher than in shoots. Perumpung was suitable to be used as an iron accumulator at pH 5, while for cultivation it was suitable to be planted at pH 6.

1. Introduction

Acid sulfate soils contain lots of iron compounds and their pH is extremely low [1]. If planted with cultivated crops such as rice, the growth will be disrupted, so crop failure often occurs. To solve the problem of sulfate acid soil, several methods are often used, such as the addition of ameliorant, by irrigating, the use of sulfate-reducing bacteria, and using soil remediation techniques. Remediation, also known as phyto remediation, is relatively cheaper and more economical, but relatively requires a
longer time, because it follows the growing time of plants used as metal accumulators or remediators.

Perumpung (*Phragmites karka*) is one of the plants that thrive in swamp areas or wetlands. This plant also able to grow in conditions containing total iron in the soil to 214.288 mg Fe/kg [2] and 100 mg Fe/L in hydroponic solution [2], and 100 mg Fe/L in hydroponic solution [3].

Plants have several strategies to absorb Fe from soil. The first strategy is used by non-graminaceous plants, which absorb free iron (II) as their main source of iron [4, 5, 6]. In contrast to graminaceous plants such as barley, rice, and corn, they use a second strategy in which iron is absorbed in the form of chelating [6, 7, 8]. However, Li et al. reported that it turns out that Maize plants also use the first strategy [6].

If the pH of the hydroponic substrate is below normal, it will cause a decrease in nutrient availability [9], including iron, which also causes damage to plant roots [10].

2. Materials and methods

2.1 Materials

All chemicals were analytical reagents. The source of iron ions or stock solution was Ethylenediaminetetraacetacetaferrate(2-) or Iron(II)-EDTA (C_{10}H_{12}N_{2}FeO_{4}) from Merck.

All hydroponic solutions were prepared with distilled water in plastic bottles soaked in 0.1 mol.L^{-1} HNO_{3} 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 (mol.L^{-1}): Ca(NO_{3})_{2}.4H_{2}O, 3.57×10^{-4}; H_{2}BO_{3}, 2.31×10^{-3}; KH_{2}PO_{4}, 9.68×10^{-4}; KNO_{3}, 2.55×10^{-4}; MgSO_{4}, 1.04×10^{-3}; FeCl_{3}, 6.83×10^{-5}; MnSO_{4}.H_{2}O, 7.69×10^{-6}; MoO_{3}, 1×10^{-3}; CuSO_{4}.5H_{2}O, 1×10^{-3}; and Zn(NO_{3})_{2}.6H_{2}O, 1×10^{-3}; H_{2}SO_{4}, KOH, and Aquades.

2.2 Methods

2.2.1 Hydroponic Experiments. Hydroponic solutions were made according to the standards of Hoagland's solution [11] plus the amount of Fe (II)-EDTA until it reaches a concentration of 50 mg.Fe.L^{-1}. H_{2}SO_{4} was added to make a pH 4 solution and pH 5. KOH was added to make a pH 6 solution.

Preparation of Seedlings. Seedlings were taken from the Tanjung Bunga Makassar area. Seedling in the form of cuttings originating from the stem. The stem length of about 2 to 2.5 m consists of 5-7 segments. The selection of seeds was taken from the middle section which consists of 4 segments then cut into 2 segments. The stem cuttings were then soaked in distilled water overnight. The next day, the seeds were put into the hydroponic solution. The seeds are planted, the lower stem segments are soaked in the solution. Seedling for 3 weeks so the plants can adapt well. Then 36 cuttings that grow well and are relatively uniform were selected. Preparing of 12 cups measuring was 300 mL as a nursery container. There were 4 (four) variations of pH, namely 3, 4, 5, and 6 and 2 (two) comparators, namely blank (Hoagland solution) and control (Hoagland solution plus Fe 50 mg Fe / L). Making the hydroponic solution was done by adding Fe-EDTA to Hoagland's solution so that the iron concentration reached 50 mg Fe/L. pH regulation was done by adding concentrated sulfuric acid or potassium hydroxide. There were two experimental groups, the first group is 18 cuttings (6 groups) grown in the hydroponic solution for 3 weeks (21 days), and the second group was the same number harvested after 5 weeks (35 days). Stirring is done every morning and evening so that there was aeration. After 21 and 35 days, measurements of shoots, leaves, and roots were measured.

2.2.2 Samples analysis. Each sample recorded its growth and physical condition. Then, the root and stem samples are separated. The samples were washed with distilled water, then dried and labeled. Each sample was aerated for 3 days to dry and constant weight. Milled them become a fine powder. Then measured using an X-Ray Fluorescence (XRF) Spectrometer device.
3. Results and discussion

3.1 Relationship between Adaptation of Perumpung and pH of Hydroponic Substrate Solutions

Observations and measurements of the growth of shoot cuttings were carried out at weeks I, III, and V. Perumpung planted on the substrate with pH 3, all died at week 2 - 3. As many as 2 cuttings were planted on pH 4 substrate, dead on week 4 – 5. Growth of tall plants plant buds planted on substrates with pH 4 and 5 stops increasing in week 5. If it sorted from the highest to the lowest that the growth rates of the test plants were as follows: Control > P6 > P5 > P4 > P3. In summary, the growth of Perumpung is shown in Table 1 below.

![Table 1. Summary of Perumpung’s Growth](image)

| Samples | Substrate pH | Week – I SLI (cm) | NLP | Weeks – III SLI (cm) | NLP | Weeks – V SLI (cm) | NLP | Total Growth (cm) |
|---------|--------------|------------------|-----|---------------------|-----|-------------------|-----|------------------|
| P3      | 3.0          | 2.0              | 3   | 0.0                 | 0   | 0.0               | 0   | 2.0              |
| P4      | 4.0          | 2.0              | 3   | 0.7                 | 2   | 0.0               | 2   | 2.7              |
| P5      | 5.0          | 3.5              | 3   | 2.6                 | 3   | 0.0               | 2   | 6.1              |
| Co (Control) | 5.6  | 4.6              | 3   | 3.1                 | 3   | 2.6               | 3   | 10.3             |
| P6      | 6.0          | 4.2              | 3   | 2.8                 | 3   | 2.3               | 3   | 9.2              |

SLI = Shoots Length Increase;
NLP = Number of Live Plants

Perumpung’s highest adaptation was in the control solution and pH 6 substrate, then decreases as the pH decreases. Some of the contributing factors are the rise or fall of pH results in a decrease in the level of nutrient availability and disrupt the absorption of nutrients by the roots [12], because the root of the plant is partially or completely damaged [10] [9]. In general, plant growth in the optimal hydroponic system is pH 5.5 to pH 6 [12]. As a comparison, the Rose plants that grow in the hydroponic system, the most optimal growth was at pH 8 after 5 days of planting and pH 6 after 20 days of planting [13].

3.2 Relationship between Iron Absorption in Perumpung Root and pH of Hydroponic Solution

Iron accumulation by roots in the third week showed the highest yield in control plants followed by plants at pH 5, 6 and 4. While at week 5, the pattern changed. The highest accumulation by roots was at pH 5, then 6, 4 and lowest in control plants. In full, data on iron accumulation on roots can be seen in Table 2 below.

![Table 2. Iron Accumulation Data by Perumpung’s Root on the 3rd and 5th Weeks.](image)

| pH  | R-3 (g Fe/kg) | R-5 (g Fe/kg) |
|-----|---------------|---------------|
| 4.0 | 2.284         | 16.096        |
| 5.0 | 6.930         | 32.656        |
| 5.6 | 7.015         | 10.295        |
| 6.0 | 6.822         | 18.970        |
At 3rd week, the absorption of iron by the root of the Perumpung was influenced by pH. In this case, it can be said that the optimal pH for iron absorption was 5.6. The farther away from the optimal pH, the iron absorption decreased. However, this was not the case after 5th week, where the highest absorption occurred in shoots planted at pH 5. The value far exceeds the accumulated value was at pH 4, 5.6 and 6. At pH 4, iron absorption was blocked by root damage and disruption of plant growth. At pH 5.6 or control, it was strongly suspected that the shoots had produced root exudates in the form of compounds rich in carboxyl and hydroxyl groups so that the iron in the substrate is stabilized [14] [15]. Whereas at pH 6, iron deposits occurred in the substrate because there is an addition of Potassium hydroxide when the solution was made.

![Figure 1. Graph of Iron Accumulation in Roots and Shoots at the 3rd and 5th Weeks.](image)

### Table 3. Iron Accumulation Data by Perumpung’s Shoot on the 3rd and 5th Weeks.

| pH | S-3 (g Fe/kg) | S-5 (g Fe/kg) |
|----|--------------|--------------|
| 4.0 | 0.507        | 0.607        |
| 5.0 | 0.532        | 0.689        |
| 5.6 | 0.664        | 1.625        |
| 6.0 | 0.015        | 0.043        |

3.3 Relationship between Iron Accumulation in Shoots with Perumpung’s Fertility

An interesting thing emerged in this experiment, where shoot fertility was related to iron absorption. The indicator of fertility that we use in this experiment is the growth rate of shoots. The data is shown in the following Tables 3 and 4.

Iron accumulation in shoots (stems and leaves) of Perumpung has a different pattern from the accumulation of iron in the roots. Iron accumulation in shoots on the 21st day and 35th day showed the highest yield in pH 5.6 or control substrate solution that followed by pH 5 and pH 4 solution. While the planted shoots in substrate solution pH 6 showed the lowest results. The phenomenon of iron absorption by shoots (after passing through the roots) was related to the fertility of the shooter. Except for the substrate solution pH 6, the lowest level of iron absorption by shoots occurred because of the deposition of iron in hydroponic containers, as well as precipitation on the roots of plants.
Table 4. Relation of Shoots Growth Rate with Iron Accumulation

| Sample   | pH Solution | W-5 | TF    | BAC   |
|----------|-------------|-----|-------|-------|
| P3       | 3.0         | 2.0 | -     | -     |
| P4       | 4.0         | 2.7 | 0.038 | 12.14 |
| P5       | 5.0         | 6.1 | 0.021 | 13.78 |
| PC0 (Control) | 5.6    | 10.3| 0.158 | 32.50 |
| P6       | 6.0         | 9.3 | 0.002 | 0.86  |

Caption:
W-5: The shoots growth at 5th Week;
TF: Transfer Factor (Iron Concentration in Shoot / Iron Concentration in Root);
BAC: Biological Accumulation Coefficient (Iron Concentration in Shoot / Iron Concentration in Soil or Solution)

A good metabolic process was characterized by relatively high growth resulting in the highest TF and BAC values. This is caused by iron transport through Xylem and transport from cell to cell [9] [6] takes place better in control plants. For phyto-remediation purposes, pH 5 was more suitable. Whereas in plants that grow at pH 6, this was not the case, even though the growth was relatively good. This was due to the accumulation of iron which was quite low at the root and the resulting in the shoot was not being griped enough so that it only sent enough iron to the bud. For cultivation purposes, pH 6 was more suitable that was of course with the addition of bases such as KOH or Ca(OH)₂ which act as nutrients.

4. Conclusion
At week 3 after planting, absorption of iron by the root of the Perumpung was as follows: pH 5.6 (control)> pH 5> pH 6> pH 4; while absorption by the shoots was as follows: pH 5.6 (control)> pH 5> pH 4> pH 6. At week 5 after planting, absorption of iron by the root of the Perumpung was as follows: pH 5> pH 6> pH 4> pH 5.6 (control). While absorption by shoots was as follows pH 5.6 (control)> pH 5> pH 4> pH 6 (the sequence is the same as at week 3). Perumpung was suitable to be used as an iron accumulator at pH 5 while for cultivation and it was suitable to be planted at pH 6.

Acknowledgments
Ministry of Research and Higher Education of the Republic of Indonesia, through the BUDI Postgraduate Scholarship

References
[1] Y B Rumsanta A Ahmad I Raya and B Ibrahim 2018 Distribution Profile of Iron Fractions in Acid Sulfate Soil from Secondary Swamp Forest - Merauke Papua," International Journal of Science, Environment and Technology vol. 7 no. 4 p. 418 – 1426
[2] S Rungwa G Arpa H Sakulas A. Harukuwe and D Timy 2007 "Assessment of Phragmites karka as Possible Phytoremediation Plant Species for Heavy Metal Removal from Mining Environment in PNG A Case Study on Closed Namie Mine Wau," in 7th HUON Seminar, Lae
[3] Y B Rusmanta A Ahmad I Raya and B Ibrahim 2019 "Accumulation and adaptation of perumpung (Phragmites karka) to iron ion stress in hydroponic media," in ICMMBT 2018 Bogor

[4] M N Hindt and M. I. Guerinot 2012 "Getting a sense for signals: regulation of the plant iron deficiency response," *Biochim Biophys Acta*, pp. 1521-1530

[5] R. Ivanov T Brumbarova and P. Bauer 2012 "Fitting into the harsh reality: regulation of iron-deficiency responses in dicotyledonous plants," *Mol Plant*, no. 5, pp. 27-42

[6] S Li X Zhou J Chen and R Chen 2018 "Is there a strategy I iron uptake mechanism in Maize?," *Plant Signaling & Behaviour*, vol. 13, no. 4, pp. 9-12

[7] K Higuchi K Suzuki H Nakanishi H Yamaguchi N K Nishizawa and S Mori 1999 "Cloning of nicotianamine synthase genes, novel genes involved in the biosynthesis of phytosiderophores," *Plant Physiol*, no. 119, pp. 471-480

[8] M Takahashi H Yamaguchi H Nakanishi T Shioiri and N Nishizawa 1999 "Cloning two genes for nicotianamine aminotransferase, a critical enzyme in iron acquisition (Strategy II) in graminaceous plants," *Plant Physiol*, no. 121, pp. 947-956

[9] I W Wiraatmaja 2016 Pergerakan Hara Mineral dalam Tanaman, Denpasar: Prodi Agroteknologi Unud

[10] R E Haling R J Simpson R A Culvenor H Lambers and A E Richardson 2011 "Effect of soil acidity, soil strength and macropores on rootgrowth and morphology of perennial grass speciesdiffering in acid-soil resistancepce," *Plant, Cell and Environment*, no. 34, p. 444–456

[11] H Wang and Y 2009 Jia, "Bioaccumulation of heavy metals by Phragmites australis cultivated in synthesized substrates," *Journal of Environmental Sciences*, vol. 21, no. 10, pp. 1409-1414

[12] L. Morgan 2016 "Perfecting the pH of Your Hydroponic Nutrient Solution." [Online]. Available: https://www.maximumyield.com/perfecting-ph/2/1212. [Accessed 22 June 2019].

[13] H J Kim Y S Cho O K Kwon M W Cho J B Hwang S D Bae and W T Jeon 2005 "Effect of Hydroponic Solution pH on the Growth of Greenhouse Rose," *Asian Journal of Plant Sciences*, vol. 4, no. 4, pp. 348-355

[14] B Usharany and N Vasudevam 2016 "Root exudates of Cyperus alternifolius in partial hydroponic condition under heavy metal stress," *Phcog Res*, vol. 9, no. 3, pp. 294-300

[15] F J Eutropio A C Ramos M S Folli-Pereira N A Portela J B dos-Santos J M da Conceicao A A Bertolazi F F Firme S B de-Souza A J Cogo and N Rasool 2016 "Heavy Metals Stress and Molecular Approaches in Plants," in *Plant Metal Interraction*, A. Parvairs, Ed., Elsevier, pp. 531-544.