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# Aquatic Macrophytes for Phytomining of Iron from Rice Based Acid Sulphate Wetland Ecosystems of Kuttanad

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**Phytomining is an effective plant based technology suitable for the cleanup of aquatic ecosystems contaminated with toxic metals. Acid sulphate soils of rice based wetland ecosystems of Kuttanad faces acute problem of Fe toxicity coupled with extreme acidity. Phyoremediation potential of aquatic macrophytes viz., *Hydrocotyl asiatica, Bacopa monnieri, Eichornia crassipes* and *Cyperus pangorei* under graded doses of Fe ranging from zero to 3000 mg per kg of soil was evaluated in a pot culture experiment. On 45th day after planting the plants were harvested and analysed for elemental concentration and soil samples for different forms of Fe. The total Fe content of the soils showed a mean value of 7.05% and available Fe 346 mg kg$^{-1}$. Among the aquatic macrophytes, *Eichhornia crassipes* was found to be the best extractor of Fe as evidenced by its highest shoot and root dry matter production, Fe content in both shoot and root, Fe extraction efficiency, translocation index and Fe accumulation factor.**

(***Key words***: *Aquatic macrophytes, Phytomining, Phytoremediation, Translocation index*)

Phytomining is an effective plant based technology suitable for the cleanup of aquatic ecosystems contaminated with toxic metals. Kuttanad, the unique rice based wetland ecosystem of Kerala, lying 0.5 to 2.2 m below MSL constituting 14000 ha of acid sulphate soils and 39,000 ha of acid saline soil faces the acute problems of Fe and Al toxicity coupled with extreme acidity. These problems are in their severest extent in acid sulphate soils compared to acid saline soils. But the acid sulphate soils are also to be put under cultivation to cope up with the food requirement of the nation. Rice is the only food crop that can survive under such situations and produce reasonably under proper scientific management practices. Even then Fe toxicity and associated yield decline is very common in this tract.

Rice is cultivated here in "polders" surrounded by ring bunds/canals through which water is flown. It is a general practice in this tract to drain out the water from paddy fields to the ring bunds/canals surrounding the rice fields and the same water is used later for wetting the polders. This water is highly contaminated with the toxic metals, especially Fe, released from the paddy fields. Any amount of Fe that can be mined out of the soil/water will be a boon to farmers. Exploitation of phyoremediation potential of few promising aquatic macrophytes for the removal of Fe from acid sulphate wetland ecosystems are discussed below.

## MATERIALS AND METHODS

### Soil characterization

The study was carried out in acid sulphate soils of *Kuttanad* extending from 9° 17′ to 9° 40′ N latitude and 76° 19′ to 76° 33′ E longitude, Kerala, India. Out of the 50,000 ha of rice soils, acid sulphate soils constitute 14,000 ha (Typic Sulfaquent). Surface (0-15 cm) soil samples in bulk (Typic Sulfaquent) collected from Vechoor village of Kottayam district, Kerala were used for the study. The soil samples were analysed for their general chemical constituents and the specific chemical parameters like total, dithionite citrate extractable (free), DTPA extractable (available) and water soluble Fe as described by Page *et al.*, (1982). The soils were characterized by high acidity (pH-3.4) and moderate salinity (EC-1.3 dS m$^{-1}$) with OC–2.2%, DTPA extractable Fe-346 mg kg$^{-1}$, total Fe-5.2%, KCl extractable Al-88 mg kg$^{-1}$ and $CaCl_2$ extractable S-2840 mg kg$^{-1}$. Surface water is having a pH of 5.4, EC-1.1 dS m$^{-1}$, Fe-1.2 mg L$^{-1}$, Al-1.1 mg L$^{-1}$ and S-13.5 mg L$^{-1}$.

### Selection of hyperaccumulators

The entire area was surveyed and actively growing natural flora were collected, dried and processed for chemical analysis to determine their elemental composition as per standard procedures described by Kalra (1998). Bioconcentration quotient for Fe ($BQ_{Fe}$)

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was calculated by dividing the Fe concentration in plant with that in soil. The aquatic macrophytes having a $BQ_{Fe}$ >10 either in shoot/ root were selected for pot culture study.

**Pot culture experiment**

The aquatic macrophytes viz., *Hydrocotyl asiatica, Bacopa monnieri, Eichornia crassipes* and *Cyperus pangorei* having a BQ for Fe >10 (Table 1 and 2) either in shoot or root were grown in pots containing 5 kg acid sulphate soil under graded doses of Fe viz., @ 0, 300, 600, 900, 1200, 1800, 2400 and 3000 mg per kg of soil in the form of $FeSO_4.7H_2O$. On 45th day after planting, plants were harvested, separated in to shoot and root and fresh weights were recorded. The dried and powered plant samples were analysed for elemental composition (Kalra, 1998) and soil samples for different forms of Fe (Page, 1982). Statistical analysis was done as described by Gomez and Gomez (1984).

**Calculated indices**

The following indices were worked out using the formula given below.

$$\text{Accumulation Factor} = \frac{\text{Metal content in treated plant} - \text{Metal content in absolute control}}{\text{Amount of added metal}}$$

$$\text{Fe extraction efficiency} = \frac{\text{Fe extracted by treated plant} - \text{metal extracted by control}}{\text{Fe applied to soil as per treatment}}$$

$$\text{Translocation index} = \frac{\text{Metal concentration in shoot}}{\text{Metal concentration root}}$$

(Ghosh and Singh, 2005)

## RESULTS AND DISCUSSIONS

**Fe content of soil and water of the study area**

The data on different forms of Fe in soil, and Fe content of water are presented in Table 1. The total Fe content of the soils showed very high spatial variation and it ranged from 4.51 to 8.15% with a mean content of 7.05%. In general, Kuttanad soils are rich in Fe due to the presence of pyritic deposits, and the permanent acidic soil reaction maintained higher levels of active forms of Fe in soil. Major portion of the total Fe exist in free form in these soils indicating that a large amount of Fe is free to interact with other ions and often results in Fe toxicity.

**Bioconcentration quotient ($BQ_{Fe}$) of aquatic macrophytes for Fe**

The Fe content of aquatic macrophytes, $BQ_{Fe}$, and DTPA extractable Fe of the soil used for pot culture are presented in Table 2. *Hydrocotyl asiatica, Bacopa monnieri, Eichornia crassipes* and *Cyperus pangorei* were selected for the study since these plants have a $BQ_{Fe}$ > 10 *either in shoot or root.* The BQ was highest for *Eichhornia crassipes for both shoot and root.*

**Fe content of soil and water**

Data pertaining to DTPA extractable Fe of soil at harvest are presented in Table 3. At zero level of Fe, *Eichhornia crassipes* showed the lowest value for DTPA extractable Fe and it maintained this trend to the highest level of Fe indicating its high efficiency to extract Fe from acid sulphate soils. Even under very high rates of Fe application, *Eichornia crassipes* was able to reduce the available Fe (DTPA extractable) of soil depicting its high extraction efficiency. The Fe content of water in these pots was in traces only (less than 0.01mg $kg^{-1}$) and did not show any significant variation among plants and treatments. The high extraction ability of the plants might not have allowed accumulation of soluble Fe in water. But the decrease observed in soil available Fe was comparatively low, even though the plants have removed large quantity of Fe from soil. The high content of total Fe in soil and its mineralization and high solubility under acidic soil conditions have maintained the equilibrium between total and available Fe, resulting values near to original values for available Fe in soil.

***Table 1**. Different forms of Fe in soil, and Fe in water of acid sulphate wet land ecosystems of Kuttanad (average of 60 samples)*

| Particulars | | Total Fe (%) | Free Fe (DCE) (%) | DTPA extractable mg $kg^{-1}$ | Water soluble mg $kg^{-1}$ |
|---|---|---|---|---|---|
| Soil | Mean | 7.05 | 6.43 | 346 | 23 |
| | Range | 4.51-8.15 | 3.92-7.65 | 300-870 | 15-38 |
| Field Water | | 0.9 mg $kg^{-1}$ | | | |
| Canal water | | 0.77 mg $kg^{-1}$ | | | |

DCE = Dithionite citrate extractable

***Table 2**. Iron content of soil, selected aquatic macrophytes and their $BQ_{Fe}$*

| Species | Shoot Fe | Root Fe | $BQ_{Fe}$ Shoot | $BQ_{Fe}$ Root | DTPA extractable Fe of soil |
|---|---|---|---|---|---|
| | mg kg$^{-1}$ | | | | mg kg$^{-1}$ |
| *Hydrocotyle asiatica* | 1765 | 4604 | 5.10 | 13.3 | 310 |
| *Bacopa monnieri* | 2375 | 4644 | 6.86 | 13.42 | 310 |
| *Eichornia crassipes* | 12865 | 8405 | 37.18 | 24.29 | 310 |
| *Cyperus pangorei* | 2780 | 6400 | 8.03 | 18.49 | 310 |

$BQ_{Fe}$ = Bioconcentration quotient for Fe

***Table 3**. DTPA extractable Fe content of soil (mg kg$^{-1}$) on 45th day after planting*

| Rates of Fe (mg kg$^{-1}$) | *Hydrocotyle asiatica* | *Bacopa monnieri* | *Eichhornia crassipes* | *Cyperus pangorei* |
|---|---|---|---|---|
| 0 | 307 | 304 | 303 | 304 |
| 300 | 309 | 307 | 305 | 306 |
| 600 | 312 | 310 | 305 | 309 |
| 900 | 317 | 312 | 306 | 312 |
| 1200 | 318 | 314 | 307 | 312 |
| 1800 | 318 | 317 | 307 | 315 |
| 2400 | 321 | 319 | 309 | 316 |
| 3000 | 325 | 321 | 311 | 318 |

**Dry matter production and Fe content of aquatic macrophytes**

Perusal of the data (Table 4) revealed that an increase in shoot and root dry matter production with graded doses of Fe was observed up to 1800 mg Fe kg$^{-1}$ for *Hydrocotyle asiatica* and 2400 mg kg$^{-1}$ for *Bacopa monnieria,* while *Eichhornia crassipes* and *Cyperus pangorei* maintained positive relation up to the highest level tried, ie., 3000 mg Fe kg$^{-1}$. These two plants showed significant variation in shoot and root dry matter production under graded levels of Fe and *Eichhornia crassipes* recorded the highest values for both. But such a positive relation with levels of Fe was not observed in shoot:root ratio except for *Hydrocotyle asiatica* where the ratio was found to increase up to 1800 mg Fe kg$^{-1}$ and decreased thereafter.

Evaluating the data on Fe content in aquatic macrophytes (Table 5), it was observed that all the plants except *Bacopa monnieri* showed an increase in both shoot and root Fe content with levels of Fe. All the tested plants except *Eichhornia crassipes* accumulated more Fe in root compared to shoot.

**Fe extraction by plants**

Comparing the Fe extraction by plants (Table 6), it was observed that all the tested plants showed positive response to levels of Fe. But *Bacopa monnieri*, maintained the positive relation with graded doses of Fe, only up to 1800 mg kg$^{-1}$ and *Hydrocotyle asiatica* up to 2400 mg kg$^{-1}$, while *Eichhornia crassipes* and *Cyperus pangorei* responded positively up to 3000 mg Fe kg$^{-1}$. *Eichhornia crassipes* was the highest extractor of Fe in both experiments. *Hydrocotyle asiatica* and *Eichhornia crassipes* have accumulated more Fe in shoot compared to root while *Cyperus pangorei* showed more accumulation in root. *Eichhornia crassipes* accumulated the highest quantity of Fe in both shoot and root revealing it as the best extractor for Fe in acid sulphate soils.

On assessing the removal of applied Fe, it was observed that *Eichhornia crassipes* alone showed very high extraction efficiency (Fig 1). Though the quantity of Fe removed increased with levels of Fe, the Fe extraction

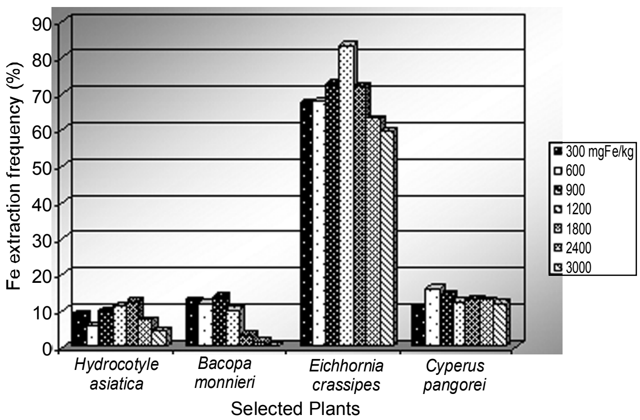


***Fig. 1.** Extraction efficiency of applied Fe by aquatic macrophytes %*

***Table 4***. *Dry matter production by aquatic macrophytes (g plant$^{-1}$) on 45th day*

| Rates of Fe (mg kg$^{-1}$) | *Hydrocotyle asiatica* | | | *Bacopa monnieri* | | | *Eichhornia crassipes* | | | *Cyperus pangorei* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | S:R | Shoot | Root | S:R | Shoot | Root | S:R | Shoot | Root | S:R |
| 0 | 26.5 | 2.0 | 13.3 | 11.5 | 5.2 | 2.2 | 25.1 | 15.2 | 1.7 | 12.7 | 14.7 | 0.86 |
| 300 | 28.8 | 2.2 | 13.1 | 11.9 | 6.4 | 1.9 | 27.5 | 17.3 | 1.6 | 15.6 | 16.5 | 0.95 |
| 600 | 30.1 | 2.3 | 13.1 | 12.0 | 6.5 | 1.9 | 30.2 | 19.8 | 1.5 | 20.3 | 20.3 | 1.0 |
| 900 | 32.0 | 2.3 | 13.9 | 12.3 | 6.8 | 1.8 | 34.9 | 22.3 | 1.6 | 21.4 | 21.5 | 0.99 |
| 1200 | 33.3 | 2.3 | 14.5 | 12.4 | 7.0 | 1.8 | 36.0 | 24.2 | 1.5 | 21.8 | 22.7 | 0.96 |
| 1800 | 36.9 | 2.4 | 15.4 | 12.7 | 7.1 | 1.8 | 41.1 | 27.3 | 1.5 | 22.9 | 23.9 | 0.96 |
| 2400 | 26.7 | 2.5 | 10.7 | 13.0 | 7.5 | 1.7 | 42.5 | 28.1 | 1.5 | 24.3 | 24.5 | 0.99 |
| 3000 | 19.0 | 1.8 | 10.6 | 9.7 | 4.2 | 2.3 | 44.2 | 30.7 | 1.4 | 25.7 | 25.3 | 1.01 |
| CD (0.05) | 2.11 | NS | | NS | NS | | 1.28 | 1.17 | | 1.51 | 2.14 | |

S:R = Shoot : Root ratio

***Table 5***. *Iron content of plants (mg kg$^{-1}$) on 45th day of planting*

| Rates of Fe (mg kg$^{-1}$) | *Hydrocotyle asiatica* | | *Bacopa monnieri* | | *Eichhornia crassipes* | | *Cyperus pangorei* | |
|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root |
| 0 | 1624 | 4616 | 2135 | 4653 | 10210 | 7536 | 1441 | 6400 |
| 300 | 1861 | 11824 | 2299 | 9434 | 14571 | 9981 | 1531 | 7381 |
| 600 | 1989 | 12062 | 3211 | 9875 | 18200 | 11515 | 1616 | 8630 |
| 900 | 3485 | 12883 | 4324 | 11247 | 20928 | 13102 | 1745 | 9628 |
| 1200 | 4563 | 16345 | 3709 | 12290 | 26972 | 16415 | 1836 | 10500 |
| 1800 | 6418 | 19527 | 4364 | 7582 | 29540 | 16820 | 3412 | 12250 |
| 2400 | 6927 | 22036 | 2691 | 7497 | 32840 | 17359 | 3945 | 14400 |
| 3000 | 7218 | 25636 | 2618 | 7425 | 36700 | 17440 | 4090 | 15940 |
| CD (0.05) | 32.8 | 48.5 | 21.4 | 17.6 | 26.7 | 22.5 | 20.3 | 22.9 |

***Table 6***. *Iron extraction by plants under graded doses of Fe (mg plant$^{-1}$)*

| Rates of Fe (mg kg$^{-1}$) | *Hydrocotyle asiatica* | | | *Bacopa monnieri* | | | *Eichhornia crassipes* | | | *Cyperus pangorei* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| 0 | 43 | 9 | 52 | 25 | 24 | 49 | 256 | 114 | 370 | 18 | 94 | 112 |
| 300 | 54 | 26 | 80 | 28 | 60 | 88 | 401 | 172 | 573 | 24 | 122 | 246 |
| 600 | 60 | 28 | 88 | 39 | 84 | 123 | 550 | 228 | 778 | 33 | 175 | 208 |
| 900 | 112 | 30 | 142 | 53 | 123 | 176 | 730 | 292 | 1022 | 37 | 207 | 244 |
| 1200 | 152 | 38 | 190 | 45 | 86 | 131 | 971 | 397 | 1368 | 28 | 238 | 266 |
| 1800 | 237 | 47 | 284 | 55 | 54 | 109 | 1214 | 459 | 1673 | 56 | 293 | 349 |
| 2400 | 185 | 54 | 239 | 35 | 56 | 91 | 1396 | 488 | 1884 | 68 | 354 | 422 |
| 3000 | 137 | 46 | 183 | 26 | 31 | 57 | 1622 | 535 | 2157 | 78 | 404 | 482 |
| CD (0.05) | 7.80 | 4.52 | | 3.26 | 4.88 | | 12.1 | 8.12 | | 5.21 | 6.86 | |

S:R = Shoot : Root ratio

***Table 7**. Translocation index for Fe in aquatic macrophytes under graded doses of Fe*

| Rates of Fe (mg $kg^{-1}$) | *Hydrocotyle asiatica* | *Bacopa monnieri* | *Eichhornia crassipes* | *Cyperus pangorei* |
|---|---|---|---|---|
| 0 | 0.351 | 0.458 | 1.35 | 0.230 |
| 300 | 0.157 | 0.236 | 1.45 | 0.200 |
| 600 | 0.16 | 0.325 | 1.58 | 0.190 |
| 900 | 0.271 | 0.384 | 1.50 | 0.18 |
| 1200 | 0.279 | 0.302 | 1.64 | 0.170 |
| 1800 | 0.328 | 0.576 | 1.75 | 0.280 |
| 2400 | 0.314 | 0.359 | 1.89 | 0.270 |
| 3000 | 0.282 | 0.352 | 2.10 | 0.260 |

***Table 8**. Accumulation factor for iron in shoot and root under graded doses of Fe*

| Rates of Fe (mg $kg^{-1}$) | *Hydrocotyle asiatica* | | *Bacopa monnieri* | | *Eichhornia crassipes* | | *Cyperus pangorei* | |
|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root |
| 300 | 0.79 | 24.0 | 0.54 | 15.9 | 14.53 | 8.15 | 0.30 | 3.27 |
| 600 | 0.61 | 12.4 | 1.8 | 8.70 | 13.31 | 6.63 | 0.29 | 3.71 |
| 900 | 2.06 | 9.18 | 2.43 | 7.32 | 11.9 | 6.18 | 034 | 3.58 |
| 1200 | 2.45 | 9.77 | 1.31 | 6.36 | 13.52 | 7.39 | 0.33 | 3.41 |
| 1800 | 2.66 | 8.28 | 1.23 | 1.63 | 10.73 | 5.15 | 1.09 | 3.25 |
| 2400 | 2.20 | 7.25 | 0.23 | 1.18 | 9.43 | 4.09 | 1.04 | 3.33 |
| 3000 | 1.86 | 7.00 | 0.16 | 0.92 | 8.83 | 3.30 | 0.88 | 3.18 |

efficiency showed an increase up to 1200 mg Fe $kg^{-1}$ and later it decreased. The high extraction efficiency of *Eichhornia crassipes* might be due to its better Fe extraction and accumulation mechanisms compared to other plants. The general mechanisms behind Fe extraction by hyperaccumulators are either acidification of soil solution by excretion of protons or organic acids or chelation of $Fe^{3+}$ by plants. The efficiency of *Eichhornia crassipes* for Fe extraction from constructed wetlands had been already reported by Jayaweera *et al.* (2007).

The data on translocation index (TI) of Fe (Table 7) also support the high Fe extraction efficiency of *Eichhornia crassipes*. Only *Eichhornia crassipes* showed values of TI above 1.0 definitely due to the influence of higher Fe content and higher shoot biomass compared to root.

Comparing the Fe accumulation factor for shoot and root (Table 8), *Eichhornia crassipes* alone showed a higher value for shoot while all other tested plants showed higher values for root. In all the cases, accumulation factor decreases with increasing levels of Fe. All the above factors have clearly indicated the suitability of *Eichhornia crassipes* as the ideal phytoextractor since it has removed more Fe by the shoot itself and had better extraction efficiency. Though *Eichhornia crassipes* is a noxious aquatic weed, the study revealed the possibility of its use for decontaminating the water bodies contaminated with Fe. The study also indicated the future line of work for exploring the possibility of utilizing *Eichhornia crassipes* for the removal of Fe from the effluents discharged to the constructed lagoons.

## CONCLUSIONS

From the present investigation it was concluded that *Eichhornia crassipes was* the best phytoextractor of Fe from acid sulphate soils of Kuttanad, Kerala as evidenced by highest shoot and root dry matter production, Fe content in both shoot and root, Fe extraction efficiency, translocation index and Fe accumulation factor.

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