

Evaluating plant micronutrient retention using an electric potential around the root zone in Rice (*Oryza sativa* L.) by using iron as an index ion

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Abstract

Deficiency in micronutrients lead to poor health and performance in plants and hidden hunger in many developing countries. Unavailability of micronutrients around the root zone is a major reason accounting for poor performance of crops. Especially, in rice, retention of plant nutrients is challenging due to water logged conditions and adding electric potential to retain nutrients would be a beneficial method to retain micronutrient around the root zone. Therefore, this study was designed to evaluate the effect of direct electric potential (EP) in retaining soil nutrient around the root zone using iron as an index ion. The experimental setup consisted of two concentric iron mesh pots, inner pot connected to the negative end and outer pot connected to the positive end. An electric potential of 5.0 V was supplied with the direct current of 0.1 A for 8 hours per day. Soil was characterized for available iron content around three zones at the time of panicle initiation and time of harvest using ICPMS plant growth and performance was measured. Soil-available Fe content was higher around the negative electrode than the positive electrode at the time of panicle initiation. Plant height, above ground biomass, and paddy yield were significantly lower in WEP plants than the NEP plants ($P < 0.05$). WEP plants showed a 45% reduction in paddy yield compared to the NEP plants. Results confirms that iron provided in the presence of an electric potential increases the iron concentration around the root zone and enhances the Fe absorption. This underpins the use of electric potential to retain micronutrients especially, cations around the root zone ultimately enhancing their availability for plants.

1. Introduction

Supplying balance nutrients in sufficient level is a key aspect in increasing crop production in the modern agricultural systems. As same as macro nutrients, trace minerals also account for huge impact on growth and performance of plants as well as human nutrition. Micronutrient deficiencies in plants lead to poor growth, disease susceptibility, vulnerability to stress and eventually low yield (Ali et al., 2018; Hajiboland 2012; Grusak, 2001)

Major staple grains such as rice, wheat are calorie-rich but levels of several micronutrients are insufficient to meet human minimum daily requirements (Hirschi 2009). Micronutrient deficiencies account for hidden hunger in developing countries leading to many health risks. Enzymes, proteins, and other biological compounds that perform important metabolic functions in humans do not function well without micronutrients (WHO, 2020). Even for plants the same rule applies as if one of the essential plant nutrients is deficient, plant growth will be poor even when all other essential nutrients are abundant. Therefore, to get the maximum yield both macro and micro nutrients should be given adequate amount. Hence, improving retention of micronutrients in the root zone will enhance the absorption of nutrients to the crops in adequate level and thereby increase the crop yield and the quality.

During past decades different approaches have been adopted to overcome the poor retention of micronutrients in soil to facilitate better absorption to plants other than genetic engineering. Use of organic fertilizer like compost amendments and biochar increase the availability of micronutrients (Adejumo, Owolabi & Odesola, 2016). Slow-release fertilizers have low solubility and can provide a gradual nutrient supply for a long period of time which improves the nutrient uptake efficiency of fertilizer and reduces leaching (Escobar et al., 2004; Trenkel, 1997). Foliar application of micronutrients proved to be effective in crop growth and performance (Aziz et al., 2019) but the efficacy in high rainfall areas can be insufficient.

Breazeale, McGeorge, & Breazeale, (1951) and Breazeale & McGeorge (1953) demonstrated that cation uptake could be stimulated by the application of controlled direct voltage and suggested that cation uptake by plants is an electrical phenomenon. The application of electricity is also known to stimulate plant growth (Costanzo 2008; Black et al., 1971). Majority of previously reported studies have been conducted in a way that the plant itself acts as one electrode (Bratton and Henry 1977). At present, studies are more concerned about creating an electrical field around the plant (Gogo et al., 2016; Dannehl et al., 2009). These studies are more focused on increasing plant secondary metabolites with the effect of direct current. In this study we wanted to evaluate the micronutrient retention in the root zone and absorption in to the plants by giving direct electric potential. Therefore, we selected iron (Fe) as the test micronutrient. Although iron is considered as a micronutrient, its toxicity or deficiency become more evident/visible and it will be easier to draw a conclusion about how providing an electric potential would affect nutrient retention especially, the cation around the root zone. Therefore, the objective of this study is to determine the effect of direct electric potential in retention of soil Fe around the root zone and thereby increase the iron uptake of rice.

2. Materials And Methods

2.1. Experimental setup

This study was conducted in a greenhouse at Sri Lanka Institute of Nanotechnology, Homagama, Sri Lanka using local rice variety: BW 367.

Treatments used for the pot study were; plants without direct electric potential (EP) around the root zone (NEP) and without EP around the root zone (WEP). 5.0 V of EP with maximum direct current of 0.1 A was supplied for 8 hours until the end of the growing season. The iron mesh was purposely selected to prepare the electrodes to provide excess iron (Fe) to the root zone as rice shows visible symptoms in growth and yield when subjected to Fe toxicity. The inner pot assigned as negative electrode while the outer pot was considered as the positive electrode (Figure 01-A).

2.2 Soil sampling, characterization and extraction of Fe

Representative initial soil sample was collected, air-dried, sieved (2-mm) and analysed for basic soil properties and available nutrients. Soil pH (1:2.5 soil: solution) were measured using pH meter (PL-700 PV). Available Nitrogen was measured by extracting using Potassium chloride (KCl) and extract was distilled and the distillate was titrated against 0.01 M HCl. Soil available phosphorus (P) was extracted by sodium bicarbonate and P concentration in extracts was determined by the Molybdate blue method (Olsen *et al.*, 1954) Soil organic carbon content was determined by Walkley and Black method (Walkley & Black, 1934).

Soil samples were collected from three zones: near root region (x), near negative electrode (y) near positive electrode (z) (Figure 01-b) from both control and treatment pots at the time of panicle initiation (40 days) and the end of the harvesting season (120 days). 2.5 g of air-dried soil were extracted with 0.1 M HCl (1:10 w/w) and filtrate (Osiname, Schulte & Corey (1973) was analysed for plant available Fe content using Inductive coupled plasma mass spectrophotometry (ICP-MS-Agilent 7900).

Plant height was measured weekly. Above and below ground biomass, paddy yield, number of seeds per panicle and unfilled grains per panicle were measured at the end of the growing season.

At the end of the growing season plants were up rooted and air dried. Total Fe amount in plant material was determined by dry ash method (Plank, 1992) with slight modification. Samples were ashed at 600 °C in the muffle furnace and residue was then dissolved in Aqua Regia and diluted with distilled water. Finally, the total iron concentration was determined using ICPMS.

2.3 Statistical analysis

Data were analyzed using t-tests for the comparison of two samples, by SAS (ver. 9.4). The level of significance for all statistical analyses was set at P=0.05. Non normal data was transferred to its log value and then subjected to statistical analysis. All analysis were done in triplicate.

3. Results And Discussion

The pH of initial soil was slightly acidic and organic matter content was 2.43 %. Available nitrogen content was 2.21 mg/100 g of soil and available phosphorus content was 6.75 mg/kg of soil (Table 01).

Table 01: Soil chemical parameters

pH	5.46 ± 0.47
Available nitrogen in soil (mg/100 g soil)	2.21 ± 0.03
Available phosphorus in soil (mg/kg soil)	6.75 ± 1.96
Organic carbon content (%)	1.41 ± 0.07

The errors represent the standard error of measurements for 3 replicates (n = 3)

Fe was chosen as the target cation as toxicity symptoms could be much related to the hypothesis under study. Lower Fe doses can lead to deficiency symptoms while higher concentrations may result in toxicity symptoms. Fe can be absorbed as Fe²⁺, Fe³⁺ and as Fe-chelate, and its absorption is metabolically controlled by plant (Kobayashi, Itai & Nishizawa, 2014). The occurrence of Fe toxicity is associated with a high concentration of Fe²⁺ in soil solution (Ponnamperuma, Bradfield & Peech, 1955). Rice plants usually maintain 60–300 ppm of Fe, while iron-deficient plants may have 10–30 ppm of Fe. Under toxic conditions the plant iron concentration may rise to 400–1000 ppm (Vose, 1982). At the panicle initiation time, Fe²⁺ concentration in soil was higher than the optimum concentration of 30 ppm reported by Portch & Hunter (2002). Available Fe concentration was high in WEP pots than NEP pots at the panicle initiation time (Figure 02-a) and it has shown a steep increase towards the negative electrode (as Fe ions are positively charged) whereas in NEP pots all regions showed more or less similar concentrations (500-620 mg/kg soil).

Iron concentration around the plant stem region is quite similar in both WEP and NEP pots (524.0, 585.3 mg/kg soil respectively). However, concerning the release of Fe, it was visible that among the two treatments, the concentration was higher in WEP plants (Figure 02-a). Ions like Fe and Manganese (Mn) are reduced in waterlogged soil to the well water-soluble Fe²⁺ and Mn²⁺ ions increasing the concentration of these two elements in the soil solution (Page & Feller 2015). As rice is grown in waterlogged conditions, availability of Fe and Mn become high. However, at the end of the experiment, Fe concentration has decreased in both WEP and NEP soil but WEP soil concentration remained high (680 mg/kg soil) (Figure 02-b). Fe concentration reduction can be described with the increase absorption of Fe by the plant and absorption is significant when an electric potential is applied.

Plant height was (Table 02) significantly (p<0.05) higher NEP plants (nearly 19% increase) compared to WEP plants. Although below ground biomass did not show a significant decrease in WEP plants, it is comparatively low compared to the NEP plants suggesting iron toxicity in the WEP plants. NEP plants showed a 44.5% increase in paddy yield compared to WEP plants. Besides, the stunted growth and poor yield were observed due

to iron toxicity in the WEP plants. Both treatments may have not given the higher yield due to iron toxicity but the severity is high in WEP plants. All these results confirm that the electric potential around the root zone enhances the Fe uptake to the plant where plant shows toxicity symptoms. High concentrations of Fe in soil solution also decrease the absorption of other plant nutrients, especially due to chelating effect (Schmidt, Buckhout, & Thomine, 2019; Olaleye *et al.*, 2001). Therefore, poor growth and performance can be attributed by poor availability of essential nutrients.

Table 02: Biometric measurements of WEP and NEP rice plants at the time of harvest

Parameter	NEP	WEP
Plant height ($\times 10^{-2}$ m)	64.3 \pm 1.5 a	54.1 \pm 1.8 b
Above ground biomass ($\times 10^{-3}$ kg)	3.62 \pm 0.09 a	2.34 \pm 0.07 b
Below ground biomass ($\times 10^{-3}$ kg)	2.71 \pm 0.62 a	1.71 \pm 0.32 a
Paddy yield kg/ha	156.13 \pm 19.04 a	86.12 \pm 10.85 b

The errors represent the standard error of measurements for 3 replicates (n = 3). Different letters indicate a significant difference ($p < 0.05$) between treatments. NEP=without electric potential, WEP=with electric potential

Fe translocation was higher in vegetative parts compared to the seeds in both WEP and NEP plants (Figure 03). Especially rice roots showed highest translocation of Fe. This observation was analogous to what observed with winter wheat (Stieger & Feller 1994) and rice (Rascio *et al.* 2008). Winter wheat showed high translocation of Fe and Mn in vegetative parts and glums compared to seeds (Stieger & Feller 1994) and rice showed high translocation of Cd (Rascio *et al.*, 2008). These findings confirm that the control of redistribution processes via the phloem is important for the composition of harvested grains. Seeds in WEP plants showed a translocation of Fe in to seeds to a lesser extent compared to the seeds in NEP plants. This can be due to the same protective mechanism that the plant had undergone to overcome iron toxicity in edible portion. Elevated concentrations of metal ions such as Zn, Ni, Co or Cd cause increased content of ions in leaves and glums least affecting grains suggesting again a control of heavy metal delivery to the grains via the phloem. Retention of Fe in higher amounts in roots can also be to overcome oxidative stress in iron toxic conditions (Becker & Asch, 2005).

In the present study, it is confirmed that WEP plants showed toxicity symptoms of Fe due to high availability in soil (Figure 02) and high absorption (table 02) thus it is evident that the 5 V potential or the current (100 mA) provided was not the reason for poor growth of rice plants supplied with electric potential.

Previous studies showed radish plants supplied with electric current up to 1000 mA were healthy and had not shown any visible damage (Dannehl *et al.*, 2009).

Table 03: Cationic element concentration around positive and negative electrode regions in WEP plants

Element	Positive electrode mg/kg soil	Negative electrode mg/kg soil
Ca	79.35 \pm 28.36 a	318.48 \pm 114.48 a
Mn	8.25 \pm 1.54 b	21.95 \pm 5.92 a
Cu	2.12 \pm 0.16 a	2.12 \pm 0.04 a
Zn	53.51 \pm 6.48 b	77.99 \pm 2.50 a
Co	0.11 \pm 0.01 b	0.30 \pm 0.03 a
Ni	0.28 \pm 0.03 a	0.32 \pm 0.05 a
Mg	10.02 \pm 1.02 a	27.92 \pm 8.71 a

The errors represent the standard error of measurements for 3 replicates (n = 3). Different letters indicate a significant difference ($p < 0.05$) between treatments. WEP= with electric potential

Even the time duration for the supply of potential and current is not the reason for poor growth as Black *et al.* (1971) has conducted studies employing 4 and 12 hours per day with two splits and 24 hours.

Not only Fe (Fe^{2+} / Fe^{3+}), other cations such as Calcium (Ca), Magnesium (Mg), Manganese (Mn), Zinc (Zn), Nickel (Ni), Cobalt (Co) and Copper (Cu) determined by ICPMS were higher near the negative electrode (Table 03) in the WEP pots. Meaning that cation is retained near the root zone making them available to the plants (Table 03). These cations play a major role in the plant life cycle. Therefore, this approach is useful in retaining micronutrients along the root zone for better absorption. Many cations are micronutrients which are essential for plant growth and

development. Plants need Ca^{2+} provide structural support, provide stress protection and also act as a messenger molecule (Thor 2019). Mg is particularly important to plants, with some 75% of leaf Mg involved in protein synthesis and 15–20% of total Mg associated with chlorophyll pigments (White & Broadley, 2009). Zinc deficiency in plants retards photosynthesis and nitrogen metabolism (Das and Green, 2016).

Ni is essential for viable seed production (Brown, Welch, & Cary, 1987). Mn^{2+} and Cu^{2+} acts as an essential element in enzyme activity and accounts for reduced yield in deficient conditions (Manivasagaperumal *et al.*, 2011; Hänsch & Mendel 2009). Co has been recently found be essential micronutrient which enhances enzyme activity especially in legumes (Akbar *et al.*, 2013)

4. Conclusion

The current study confirms that a plant provided with an external voltage having a minimum current has increased the iron concentration around the negative electrode region making them more available for plants while in an NEP plant, all regions showed more or less similar concentrations of Fe. Plants provided with an external voltage showed higher iron translocation in all parts except the seed and a decrease in growth and performance due to iron toxicity. However, absorbed total Fe concentration in WEP plants was higher than NEP plants except in the seeds. This confirms that this approach can be used to increase retention of micronutrients around the root zone and thereby increase plant uptake to overcome the nutrient losses. Further studies should be carried out involving macronutrients instead of targeting a trace nutrient.

Declarations

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Conflicts of interest

The authors declare that they have no conflicts of interest pertaining to this study

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Figures

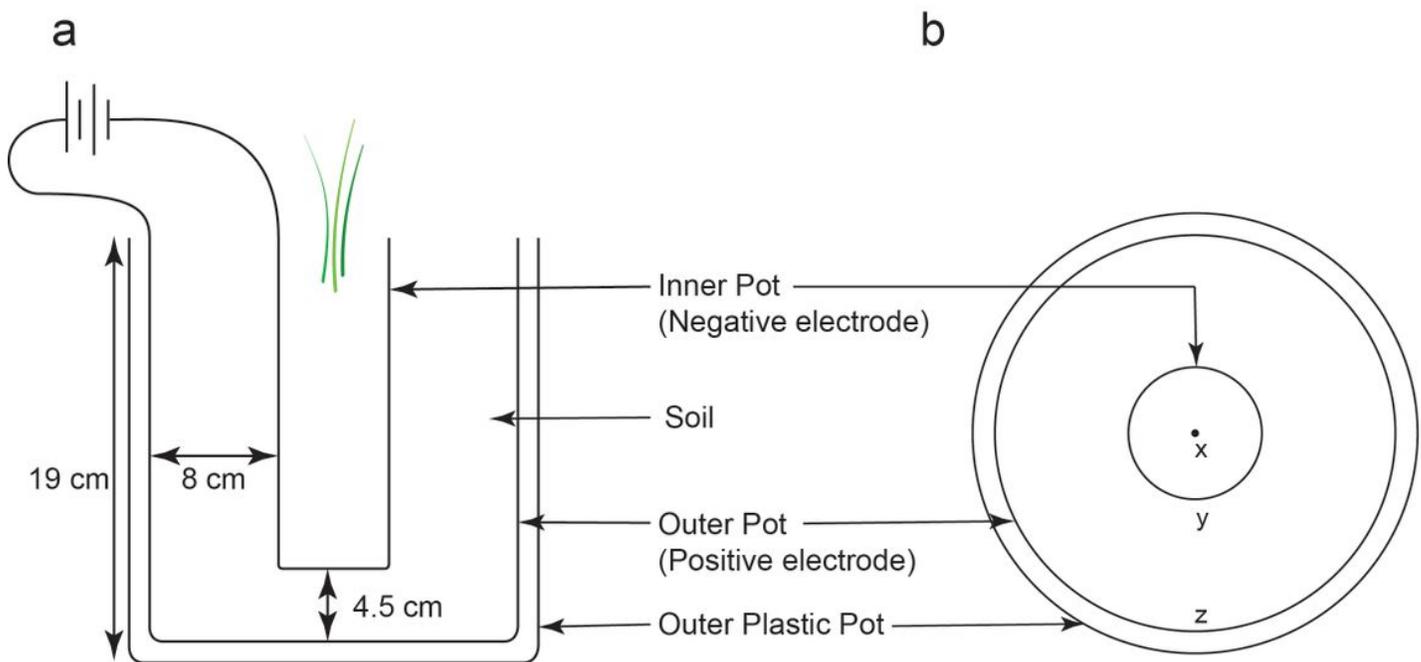


Figure 1

Schematic diagram of the experimental setup; a: longitudinal section of the experimental set up containing inner pot connected to negative end and outer mesh pot connected to positive end, b: soil sample collection points x= near the root of the plant, y= near negative electrode, z= near positive electrode.

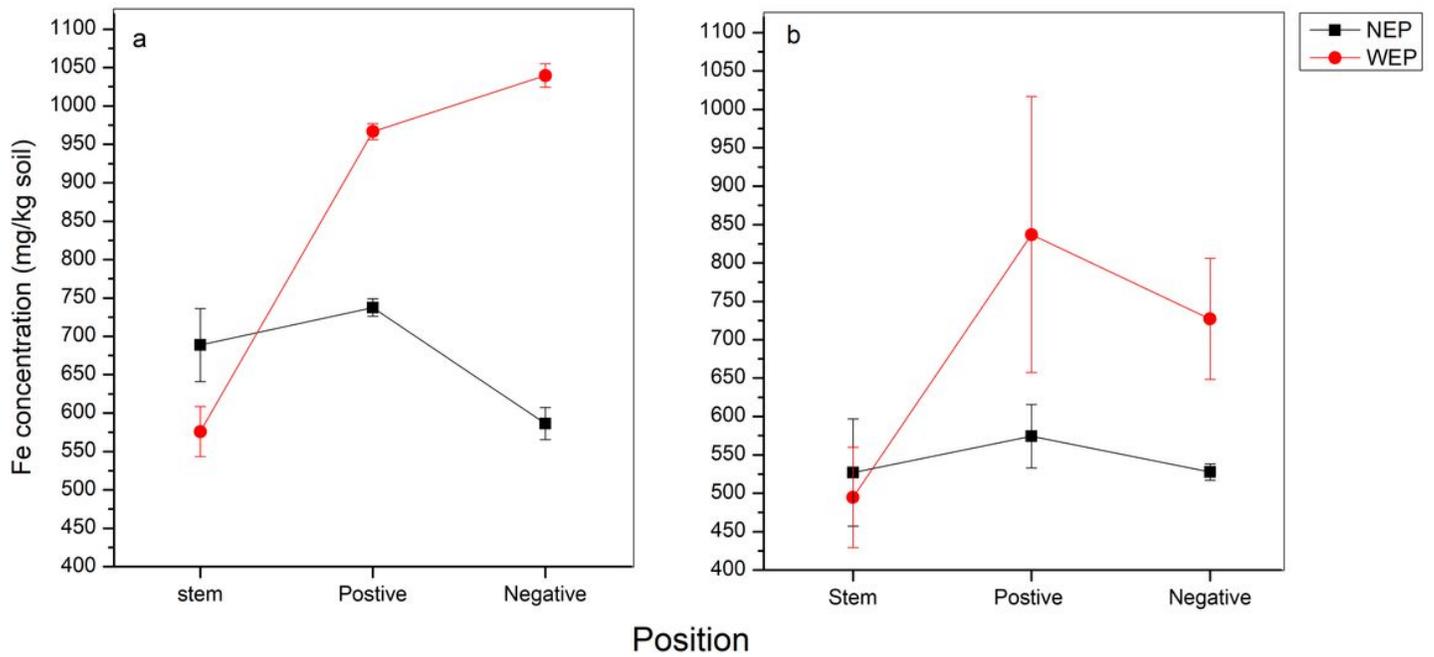


Figure 2
 Iron concentration in soil a: at the time panicle initiation, b: at the end of the life cycle. The error bars represent the standard error of measurements for 3 replicates (n = 3). NEP= without electric potential, WEP= with electric potential

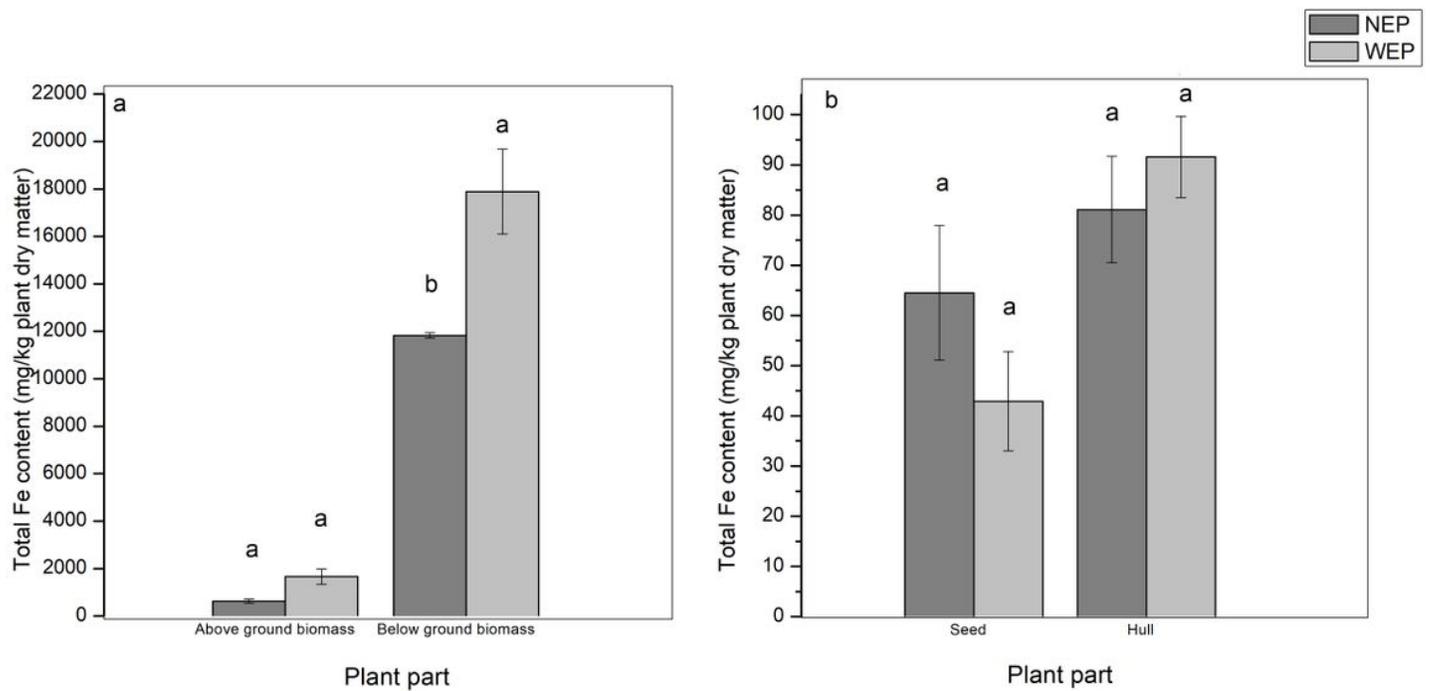


Figure 3
 :Total iron content in plant parts: a: above ground biomass and below ground biomass of WEP and NEP plants, b: in seed and hull of WEP and NEP plants. The error bars represent the standard error of measurements for 3 replicates (n = 3). (Different letters indicate a significant difference (p < 0.05) between treatments) NEP= without electric potential, WEP= with electric potential

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