



Effects of an Electrokinetic Barrier to Inhibit Heavy Metal Absorption in *Rhizophora mucronata* Seedlings

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Abstract

Developing an electrokinetic barrier to hinder mangrove seedlings from heavy metal absorption is a novel technology for conducting mangrove reforestation within contaminated environments. In this study, the objectives were to discover a hydroponic solution that offers favorable plant responses for mangrove seedlings and to investigate this environment under a micro-electric field and/or a heavy metal (HM) presence. For 15 weeks, *Rhizophora mucronata* seedlings were grown hydroponically in containers encompassing unique conditions: the control, 1 ml nutrient solution (NS)/L, and 1.5 ml NS/L. Seedlings from 1.5 NS had the greatest mean regarding the number of roots ($p < 0.05$), but the 1 mL NS had the largest mean for root diameter ($p < 0.05$), along with thicker roots and more leaf development also observed. An electrokinetic experiment was performed to compare a direct current of 10 V/m and 20 V/m in a HM solution consisting of 1 ml NS/L with $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (400 mg/L), $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ (400 mg/L), and $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (1.5 mg/L). 10 V/m caused a statistically significant migration for Cd and Cr, whereas 20 V/m was required for Zn, respectfully. When comparing the nitrate to phosphate ratios and pH between HM and HM plus electric current (EC), the margins of difference were less substantial for 10 V/m than 20 V/m. It can be concluded that 1 ml NS/L and 10 V/m is preferable for future electrokinetic barrier design, but because HMs affect the pH values of hydroponic solution greater than natural soil conditions, the HM concentration must be reduced for mangrove tolerability accordingly.

Keywords : electrokinetic barrier; mangrove; heavy metal; hydroponics; ion migration

Introduction

Mangroves are a group of tropical-to-subtropical trees and shrubs that grow in intertidal regions due to their ability to withstand fluctuating salinities, as well as aerobic and anaerobic conditions. In developing countries alone, the ecological services and products provided by mangroves are equivalent to an economic value between 33,000-57,000 USD ha/year [1]. However, over the last 50 years, 20-35% of mangrove forests have been lost [2]. Anthro-pogenically, mangroves growing nearby

urban areas are impacted by pollution and heavy metals [3, 4]. While mature trees that have been subjected to heavy metal encroachment are capable of impressive bioaccumulation and tolerance [5], germinating seeds and young plants lack fully developed defense systems [6, 7], which lowers their rate of survival and the success of conducting mangrove reforestation projects in similar regions. Plant absorption of heavy metals (HM) and excessive biometals can lead to damage at the cellular level, which inhibits enzyme function, photosynthesis, respiration, and encourage defense mechanisms

that heighten stress [8-11]. Moreover, exposure to multiple HMs simultaneously amplifies the toxic effect to mangrove plants in comparison with single-metal contamination [12, 13]. Therefore, attempting to conduct reforestation in these areas is problematic.

Developing an electrokinetic barrier to hinder mangrove seedlings the opportunity from absorbing HMs is a novel technology for conducting mangrove reforestation within contaminated environments. As a form of electrokinetics, which is a type of environmental remediation that utilizes electric current (EC) to remove chemical species from soil [14], the main advantages are that minimum soil disruption is required, low amounts of waste material are produced, and it can be used in heterogeneous, fine-grained media [15]. For marine application, its potential to inhibit saltwater intrusion into fresh water has been studied [16].

The aim of this study was to address two issues. First, to discover a hydroponic environment that offers favorable plant responses for mangrove seedlings by comparing diverse conditions. Second, to investigate the characteristics of an aqueous saline environment under the presence of micro-electric fields and the changes when exposed to a HM intermixture of cadmium (Cd), chromium III (Cr), and zinc (Zn). These results from this study provide fundamental components to developing an intricate electrokinetic barrier experiment.

Materials and Methods

Growing Mangroves

Rhizophora mucronata seedlings of $35.0 \text{ cm} \pm 4.5$ were procured from the Bangpu Nature Education Center (Samut Prakan Province, Thailand). The propagules collected were deemed mature yet absent of any signs of root development. The plants were then grown hydroponically in a ventilated greenhouse under

natural conditions for 15 weeks that had an average temperature of 37°C and 65% humidity. Three plastic containers having dimensions of $34 \times 34 \times 14 \text{ cm}$ were selected as growing apparatuses for the mangrove propagules. Nine seedlings were grown per container, with each possessing a distinct solution for comparing morphology. The characteristics of each solution are presented in Table 1. Hydroponic experiments involving mangrove seedlings have been traditionally reliant on the Hoagland Solution for growing [17], which has to be prepared manually using fundamental chemicals. To reduce cost, time, and waste a commercially-made nutrient solution (NS) was purchased and used as an alternative (Sed Grow Tech, Thailand). Buoyant foam was utilized as a contraption to allow the seedlings to stand erect inside of the container while being elevated above the floor of the container, for accommodating root development. Pieces of foam were cut into sections to fit firmly inside of the container and holes were drilled through the foam to cradle the seedlings, which were then fastened into a stationary position using plastic cable ties (Figure 1). To reduce erroneous results, areas of the containers that were not blocked from sun exposure by the foam were covered with a non-transparent plastic bag to reduce both evaporation and algae growth. Tap water replenishments were added to maintain a consistent volume until a solution change was performed, which occurred once a week.

Morphological Growth Analysis

To reduce touching the plants, which lowers the risk of stress and damage, and ensure better accuracy, seedling growth was recorded once a week through photographic analysis. Pictures of the seedlings were taken using a 12 MP camera, from a smartphone. Every photograph included a ruler in the same plane of each component intended to be measured.

Table 1 Characteristics of the different hydroponic solutions

Parameters	Concentrations			Unit
	Control	1 mL NS	1.5 mL NS	
Volume of tap water	3.0	3.0	3.0	L
Sea salt	9.0	9.0	9.0	g/L
Nutrient solution (NS)	N/A	1.0	1.5	mL/L
Electrical conductivity	12.77	13.13	13.40	mS/cm

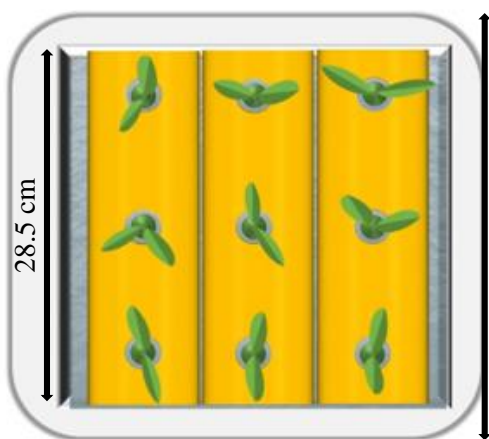


Figure 1 An illustration of the growing phase setup for the seedlings showing the buoyant foam inside of the container. Note that the sides of the container have a solution that is highly exposed to sunlight, which is where a plastic bag was used for covering

ImageJ software was used for calibrating the pixels from the photos into measurements of length, which then allowed the morphological changes of the seedlings to be recordable. The morphology of interest was the number of roots (and fine roots those < 2mm thick), root system diameter, and the number, length (L) and width (W) of the leaves. Additionally, any notable signs of stress for the seedlings were also recorded. To provide the most accurate results, the seedlings were left undisturbed apart from the routine morphology recordings, water replenishments, and solution changes. The data from each seedling was then compiled over a timeline to provide evidence pertaining to which NS would be most favorable for future applications.

Electrokinetic barrier

Electric current was assessed to see how it would affect NS, both with and without the presence of HMs. To carry similar variables to that of the plant growth experiment, the same size containers and volume of solution (3 L) for each setup was used. For the electrokinetic barrier setup, the containers consisted of graphite

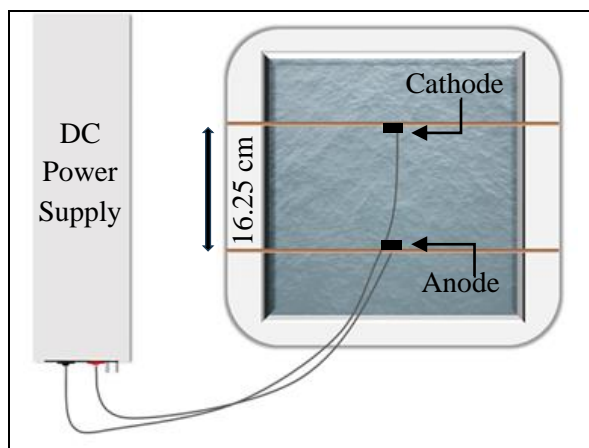
electrodes (10 x 3 cm) for the anode and cathode, with the attached wiring hung securely over a dowel in order to suspend them above the floor of the container, and a constant direct current (DC) of either 10 V/m or 20 V/m applied (PeakTech 6225 A PeakTech®, Germany), as shown in Figure 2. Due to results of the earlier tests, 1 mL NS/L saline water was used as the control, seedlings omitted, with a HM mixture of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (400 mg/L), $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ (400 mg/L), and $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (1.5 mg/L) to provide the contamination parameters. The HM concentrations selected are reflective of samples collected from the Chao Phraya River and the Upper Gulf of Thailand [18], which has been the regional focus for mangrove reforestation in this study. The six distinct conditions that were explored are presented in Table 2. Each trial lasted for 24 hours followed by sampling at the surface region to prevent mixing and analysis done in triplicates to verify consistency in the results. Cd, Cr, K, and Zn were sampled at the anodic, middle, and cathodic region separately, but nitrate (NO_3^-), phosphate (PO_4^{3-}), and pH were sampled as a composite because the earlier sampling led to solution disruption and inevitable mixing. The metals mentioned were analyzed by ICP-OES; (NO_3^-) and (PO_4^{3-}) values were obtained by using a spectrophotometer (Hach, DR900, United States); and the pH was able to be determined with a pH/Ion/Conductivity probe (SevenGo Duo pro, Mettler Toledo, Switzerland).

Statistics

Data in the figures and tables includes mean values \pm SD (standard deviation). The statistical differences of plant morphology grown under distinct hydroponic solutions were analyzed using the Kruskal-Wallis test. Non-parametric Dunn's test was used to provide a pairwise comparison between the three treatments. Findings for metals in the electrokinetic portion of the experiment used one-way ANOVA with Fisher's LSD test. All tests that had the significance determined utilized p-value < 0.05, respectively.

Table 2 Conditions of the six solutions. The plus and minus sign indicate the addition or omittance, respectively

Conditions	Solution 1 mL NS/ L saline water	N: 17.4 mg/L P: 4.2 mg/L K: 85.7 mg/L	Zn: 400 mg/L Cr :400 mg/L Cd: 1.5 mg/L	Electric Current (EC)
Control	+	+	-	-
Control HM	+	+	+	-
10 V/m	+	+	-	+
10 V/m HM	+	+	+	+
20 V/m	+	+	-	+
20 V/m HM	+	+	+	+

**Figure 2** An illustration of the electrokinetic barrier experiment that has applied EC

Results and Discussion

Seedling Morphology

All hydroponic setups induced growth for the *Rhizophora mucronata* seedlings (Table 3). Furthermore, no signs of stress that would have been associated from either the buoyant foam or cable ties were displayed, indicating that the design is suitable for future studies. Despite all this, seedling death was still unavoidable for all the setups, but the cause was independent from the methodology used, for the seedlings never established roots whatsoever. Starting with nine seedlings per container, the final number was 6 for the control, 7 for the 1 mL NS, and 7 for the 1.5 mL NS, respectively.

The average number of roots for the seedlings was 17.5 (control), 27.6 (1 mL NS), and 38.1 (1.5 mL NS). The difference between the control and 1.5 mL NS was significant ($p < 0.05$), but not between the 1 mL NS and 1.5 mL NS. In terms of the number of fine roots ($< 2\text{mm}$), no prominent variations between the

1 mL and 1.5 mL NS solutions were found ($p > 0.05$). For the diameter of the roots, the averages were 4.0 (control), 8.1 (1 mL NS), and 6.6 cm (1.5 mL NS).

The roots of the 1 mL NS group appeared thicker. Mangrove plants do not require aeration in the roots, but rather absorb oxygen in the lenticels and pneumatophores of the stems and leaves which are then delivered to the sub-merged roots. Oxygen can escape through the aerenchyma in the submerged roots in a process known as radial oxygen loss ROL. Longer and thicker roots are a good sign that the plant is delivering oxygen to the root tips in an efficient manner, as thin roots may be more prone to releasing oxygen due to having a higher surface area to volume ratio [19].

The difference in root length between the control and 1 mL NS was significant ($p < 0.05$), but not between the 1 mL NS and 1.5 mL NS. Regarding leaf development, the container of 1 mL NS had five plants with leaf manifestations (3 of which having fully developed leaves) and whose average leaf dimension was (L x W) 3.78 x 1.77 cm, while the 1.5 NS had two plants with leaf appearances (both having fully developed leaves), and whose average leaf dimension (L x W) was 3.60 x 1.40 cm. Leaves were absent in the control. While the ratio of leaf growth between the two nutrient solutions was not significant, the number of leaves was significant (Mann-Whitney test). However, one of the mangrove seedlings from the 1.5 NS group developed yellowish-green leaves with burnt spots, which may indicate that the stronger concentration causes stress or harm to the plants [20]. In connection, it could also explain why lower algae growth were detected (albeit unwanted) with 1.5 mL NS compared with the 1 mL NS solution.

Table 3 Results of the seedling morphology for the different hydroponic solutions (mean \pm SD), the different letters represent significant difference by the Kruskal-Wallis test and post-hoc Dunn's test ($p < 0.05$)

Recording Metrics	Control	1 mL NS	1.5 mL NS
Number of Plants	6	7	7
Total number of roots	17.5 \pm 4.4 ^b	27.6 \pm 5.7 ^a	38.1 \pm 10.2 ^a
Number of roots (< 2mm)	5.0 \pm 3.9 ^b	11.3 \pm 4.8 ^a	13.4 \pm 4.9 ^a
Root system diameter (cm)	4.0 \pm 0.6 ^b	7.8 \pm 1.5 ^a	6.2 \pm 1.2 ^a
Number of Plants with leaves	0	5	2
Leaf Length (cm)	-*	3.8 \pm 0.3 ^a	3.6 \pm 0.5 ^a
Leaf Width (cm)	-*	1.8 \pm 0.3 ^a	1.4 \pm 0.5 ^a

* No developed leaf observed.

Electrokinetics

Comparisons between the four different metals are presented in Figure 3. Detection of Cr was notably less than the other metals in the Control HM ($p < 0.05$) but inaccurately represented the actual concentration. Due to its semi solubility, the majority of oxidized Cr(III) settled to the bottom of the container, where sampling could not be realized.

For 10 V/m HM, Cd having low hydration properties were advantageous in migrating to the cathode region swiftly ($p < 0.05$), prior to competitive absorption by Zn and Cr [21, 22], but K was more likely grounded by the charges from copious Cl⁻. As the activation energy was met to mobilize a larger share of Cr, a significant increase was observed at the cathode ($p < 0.05$). Moreover, their competition for absorption at the cathode repulsed a high number of Zn ions [23], which caused its concentration to rise modestly at the anode ($p > 0.05$). As Cd reached the cathode first, Cr barricaded the ions from escaping, thus forming an inward repulsion that localized the concentration [24], but if failed to migrate in time before mass Cr mobilization, they then followed suit to that of Zn ($p > 0.05$), respectively.

20 V/m HM achieved the highest Cr detection ($p < 0.05$), while also altering some Cr(III) ions into Cr(VI). This was observed as the color of the solution changed from a light green (associated with Cr(III)) to an orange (associated with Cr(VI)). The most common Cr(VI) species in low pH is predominantly HCrO_4^- [23]. Being negatively charged, a portion of Cr ions once attracted to the cathode, began reverse migration. This, along with heterogenous Cr reduction offered Zn^{2+} migration opportunity, and the charge density near the cathode freed-up to expectedly permit Cd and K repulsion.

An immense buildup occurred on the cathode in both HM setups, qualitatively signifying that metal migration took place. Although K^+ does not easily precipitate, this phenomenon may also explain the reduced concentration as graphite released from it caused flocculation. As 20 V/m generated a larger buildup, the fixation of K and other metals was assumingly more prominent.

Indeed, the creation of Cr(VI) is concerning due to its greater health risk to the environment than Cr(III). Additionally, its justification for practicality is less pursuant based on the notion that a charge of 20 V/m has been shown to start affecting aquatic life through electrotaxis, whereas 10 V/m is considered to cause minimal impact [25, 26].

EC reduced nitrate and phosphate in all electrokinetic experiments when compared to the control (Figure 4). In terms of nitrogen to phosphate ratio (based on mg/L), the values are as follows: 4.12:1 (control), 12.50:1 (HM), 2.11:1 (10 V/m), 36.15:1 (10 V/m HM), 7.66:1 (20 V/m), and 186.17:1 (20 V/m HM), respectively. While 10 V/m caused a decline, due to the ions conditionally reacting and forming nitrogen gas products at the anode region [27], 20 V/m was less dramatic, as the high amount of hydroxide precipitates may have contributed to NO_3^- stabilization [28]. Interestingly, setups containing HMs showed higher concentrations of NO_3^- than its counterparts without it, which advocates that pH is not a determining factor in concentration decline [29]. In the HM setup, NO_3^- were attracted to the highly polarizing Cr ions (despite reduction from available OH ions) and migrated downward towards the bottom of the container, which caused a low concentration in similarity to

Cr(III) mentioned. The polarity of Cr in the 10 V/m HM hindered the rate of NO_3^- migration to the anode, which extended the time and concentration near the surface region. NO_3^- was more abundant for 20 V/m HM because of the combined effects of enhanced Cr mobility and negatively-charged Cr(VI) engaged in competition for anode access. Moreover, the immense buildup occurred on the cathodes previously mentioned likely also dampened its polarity repulsion of NO_3^- as well.

Phosphate reduction was more apparent. 20 V/m caused a reduction greater than 10 V/m, as the higher pH stabilized PO_4^{3-} for precipitation by the bioessential metals found in the hydroponic solution, but the concentration was still less impacted than being spiked with

HMs only. This was mainly contributed to Cr(III) capability of binding to phosphorus ions that exist at lower pH values than that of phosphate, which further reduced in concentration upon experiencing higher Cr mobilization by EC.

The high reduction of both ions are due to the fast migration capability from the hydroponic environment. The depletion of nitrogen and phosphorus would not have been as significant in soil because the ions would have been attracted to soil particles (causing binding) and the byzantine patterns involved with migration [30]. Furthermore, seawater contains traces of both ions [31], which in abundance can provide a source of ion replenishment to help compensate for the deficiency.

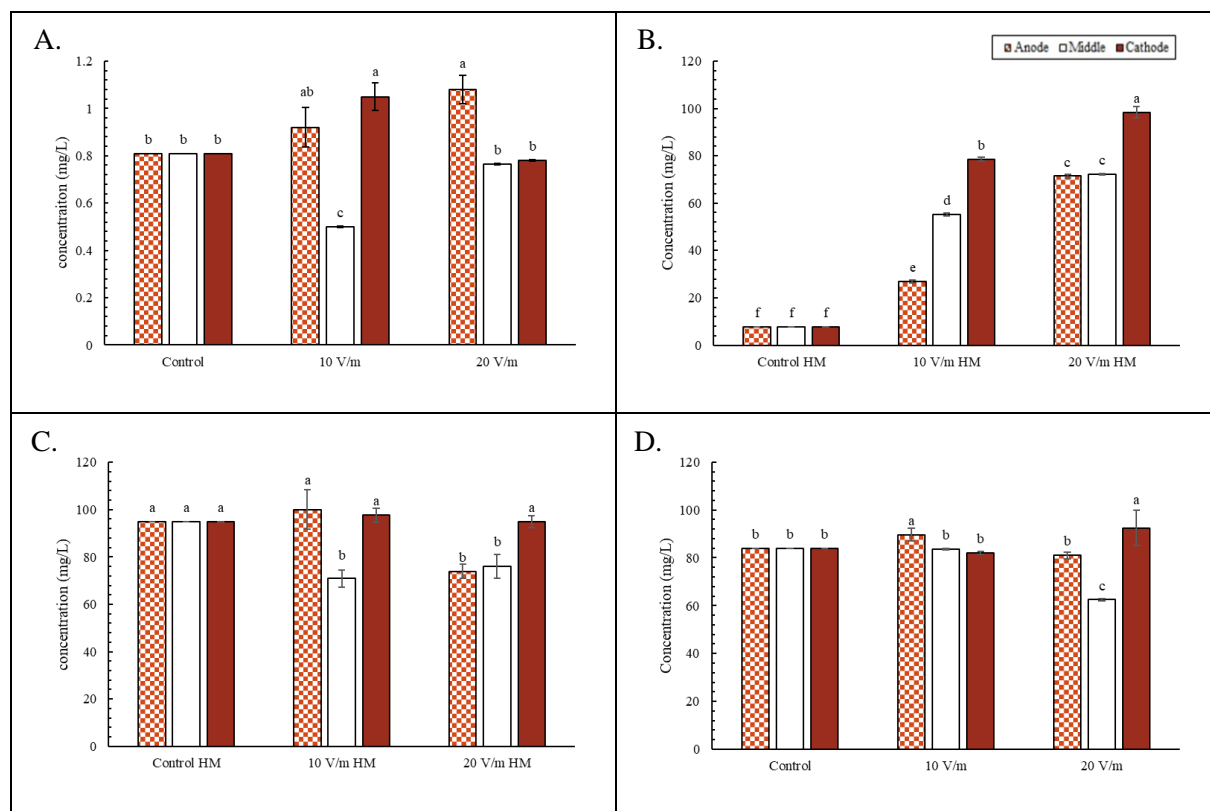


Figure 3 The migration of metals for (A) Cd, (B) Cr, (C) K, and (D) Zn after 24 hours. Error bars display the standard deviation. Columns with different letter symbolize statistical significance as per Fisher's LSD test ($p < 0.05$)

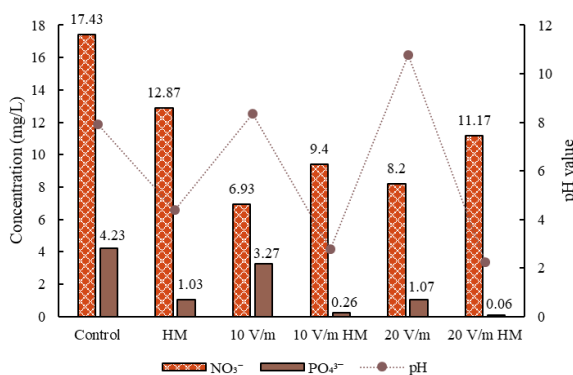


Figure 4 The concentrations of nitrate and phosphate (bars) and pH values (line) of the different setups

HMs and EC were both responsible for fluctuations in pH. A correlation was observed between the pH value of a solution and how it shifted after EC implementation. Essentially, EC exposure heightened alkalinity in the control, whereas the HM solution, which had a lower pH than the initial control, became even more acidic. While both outcomes consisted of OH⁻ and H⁺ being generated from the electrodes, the difference stemmed from hydrogen gas being emitted out of the EC setups (leaving behind OH⁻), whereas hydroxide precipitation from HM reduction was stabilizing the dissolved H⁺ from volatilization. This margin of dichotomy was consistent for all the setups utilizing EC, with results being more profound for 20 V/m than 10 V/m in both setups. Moreover, the highest and lowest values in the pH range for 20 V/m tend to exceed the tolerability for mangroves [32]. Adding several types of bases was attempted to raise the pH, but this warranted ion precipitation and hindered ion migration.

These findings may not necessarily depict that of soil, for it has a complex buffering capacity and particle heterogeneity which are of absence in the studied hydroponic solution. But it is likely that soil conditions would still adhere to similar trends, even if not as severe, and therefore should be noted for in soil applications.

Conclusions

Despite the larger number of roots observed from the mangrove seedlings in the 1.5 mL NS solution, the 1 mL NS solution was selected for future experiments as the

average root diameter was longer and thicker along with the leaf development being more prominent. 10 V/m caused a significant migration of ions for Cd, and Cr in the 1 mL NS/L, while 20 V/m was required for Zn, and K was ambivalent, respectively. While pH values are sensitive to both HM presence and EC, the setups incorporating 10 V/m provided an overall pH margin range less substantial than 20 V/m. Moreover, the ratio of nitrogen to phosphate for 10 V/m is also less than that of 20 V/m in all setups. Being that future investigation will involve *R. mucronata* seedlings being exposed to a HM hydroponic environment with EC applied, 10 V/m will be selected for future study in the electrokinetic barrier design. However, HM concentrations affect pH acidity more sensitively for a hydroponic solution than a soil, which has led to values too low for mangrove tolerability. Therefore, advisement is focused on raising the pH through a reduction in the initial HM concentration prior to starting the experiment. Additionally, the durability and placement of the electrodes (due to buildup) along with the avoidance of unintended electrokinetic phytoremediation requires further investigation prior to experiments beginning to incorporate live plants.

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