

A simple membrane-based approach for assessing the diffusion kinetics of mineral foliar fertilizers

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ABSTRACT: The present work explores a practical strategy for real-time assessment of foliar-based mineral fertilizers diffusion. Thereby, a dialysis tubing membrane was employed as a leaf cell-proxy for tracing the release of three commercial foliar fertilizer formulations and their correspondent primary compounds, $MnCO_3$, $Mg(OH)_2$, and Cu_2O at the field-recommended concentration, by simply measuring changes on conductivity and pH during 96 h. It enabled either modulating fertilizer diffusion kinetics, as well as comparing the commercial formulations to its primary compound ones. Hence, the proposed approach stands out as a fast and effective tool for addressing relevant information about the performance of foliar fertilizer formulation, one of the bottlenecks of programs developing new fertilizer formulations.

Key words: foliar fertilizer, diffusion kinetics, dialysis tubing, membrane.

Foliar spraying of fertilizers is often employed in the nutritional management of crops either in open fields or controlled environments (Fernández and Brown 2013). This practice entails operational-related advantages, accurate nutrient supply timing, avoidance of fertilizer interaction with the soil chemical matrix, also as the expected positive side-effects it may promote, e.g., higher yields and tolerance to biotic and abiotic stresses (Mateus et al. 2021; Stover et al. 1999).

Although usually taking place passively (Burkhardt et al. 2012; Eichert et al. 1998; Schönherr 2006), the absorption of fertilizers is also subjected to the dynamic interactions with the complex matrix and diversity of leaf surface structures, such as the cuticle membranes surrounding the epidermis, the stomata, and veins (Fernández et al. 2021). Since this process may change either across species or formulation, understanding and tracking the pathway of the foliar-applied fertilizers (e.g., nutrient uptake or translocation) within plant leaf tissues might be difficult (Fernández and Brown 2013; Fernández et al. 2021), usually requiring the use of costly, time-consuming, complex, or nonbroadcast techniques, i.e., mass spectrometry, stable isotopes, or benchtop and synchrotron-based *in vivo* X-ray fluorescence spectroscopies (Fenilli et al. 2007; Gomes et al. 2019; Li et al. 2019; Machado et al. 2019; Read et al. 2019).

Herein, a practical strategy for a real-time assessment of diffusion kinetics of mineral foliar fertilizers was explored by tracking their release throughout a dialysis tubing sunk on deionized water as a function of changes in conductivity. Since the conductivity is proportional to ionic strength in a solution, the higher the values, the higher is the ion diffusion. Furthermore, the hydrophilic and semipermeable membrane composition of the dialysis tubing enables simulating the passive diffusion process, and therefore it is suitable for mimicking the uptake of foliar fertilizer through permeable fractions of the cuticle, leave stomata and trichomes, for example (Bienfait and Scheffers 1992; Fernández et al. 2021; Tingey and Stockwell 1977), as illustrated in Fig. 1.



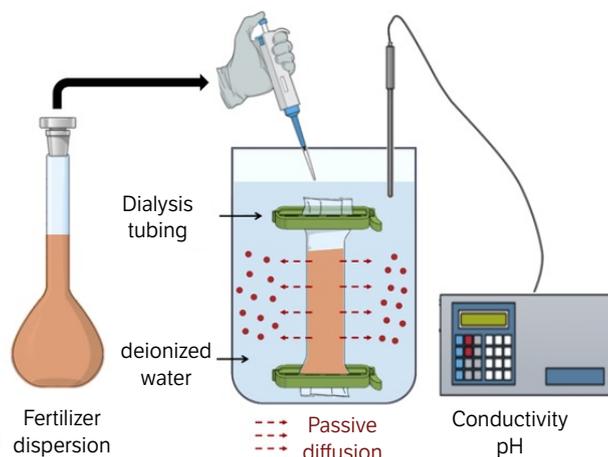


Figure 1. Schematic representation of dialysis tubing-based setup employed herein as a leaf-cell proxy to assess the dynamics of foliar fertilizers.

For this, dispersions of three commercial foliar fertilizer formulations (products A, B, and C), as well as their correspondent primary compounds, respectively MnCO_3 , $\text{Mg}(\text{OH})_2$, and Cu_2O , were prepared at field-recommended concentration, as described in Table 1. The Mn, Mg, and Cu content were the same on both commercial and primary compounds. These target elements were chosen due to their scarcity on the weathered soils of tropical weathered regions, such as Brazil (Castro et al. 2021; Fageria and Stone 2008).

Table 1. Elemental content and concentration of three commercial formulations, products A, B, and C, as well as their correspondent primary compounds, MnCO_3 , $\text{Mg}(\text{OH})_2$, and Cu_2O employed in the experiments. The elemental concentration is the same either for commercial formulations or the primary compound ones.

Compound	Elemental basis	Concentration ($\text{mol}\cdot\text{L}^{-1}$)
Product A – MnCO_3	Mn^{2+}	0.0353
Product B – $\text{Mg}(\text{OH})_2$	Mg^{2+}	0.2675
Product C – Cu_2O	Cu^+	0.0032

Fifteen milliliters of each prepared dispersion were added within a 10 cm-long dialysis tubing membrane (Spectra/Por, No. 3, Spectrum Labs, USA) previously prepared by a 30-min soaking in deionized water at room temperature. These membranes are composed of hydrophilic-like standard grade regenerated cellulose (RC) reconstituted from cotton linters, and do present a 3.5 kD molecular weight cut-off and working pH 2–12 limits range. A total of three ($n = 3$) independent replicates were set per group, and both edges of the tubing were locked using proper dialysis clamps. Ultrapure water, whose electric conductivity was smaller 18.18 than $\text{M}\Omega\cdot\text{cm}^{-1}$, was used as control.

Thereafter, the prepared dialysis tubings were immediately transferred to glass beakers containing 250 mL of deionized water, with the upper edge sealed by polyvinyl chloride film and kept under continuous shaking on a magnetic stirring bar throughout the assays. The external water was refilled daily to maintain a constant volume and replaced 40 h past the beginning of the experiment to assess whether the water input into the system might affect fertilizer release dynamics.

The conductivity and pH of the water outside the dialysis tubing were monitored during 96 h using a handheld conductometer (Mettler Toledo, SevenGo Duo/SG23, Switzerland) and a benchtop pHmeter (Tecnal, TEC-2, Brazil). The equipment was calibrated right before each measurement using a set of reference solutions (84 and $1413 \mu\text{S}\cdot\text{cm}^{-1}$) for conductivity (Dinâmica, 697450/2149-90, Brazil) and buffer solutions (pH 4.01 and 7.00) as pH standards (Digimed, SM-S1B, Brazil).

Figure 2 presents the average intensity of cumulative conductivity of the water surrounding the dialysis tubing containing the commercial formulations and their correspondent active ingredients supplied alone, i.e., MnCO_3 , $\text{Mg}(\text{OH})_2$, and Cu_2O , monitored for 96 h. It reveals the conductivity values were found higher in the commercial products rather than primary compounds. It also depicts that the water replacement 40 h past the beginning of the experiments did not induce expressive changes in conductivity, thus suggesting the elemental concentration outside the tubing had reached a chemical equilibrium point at that point.

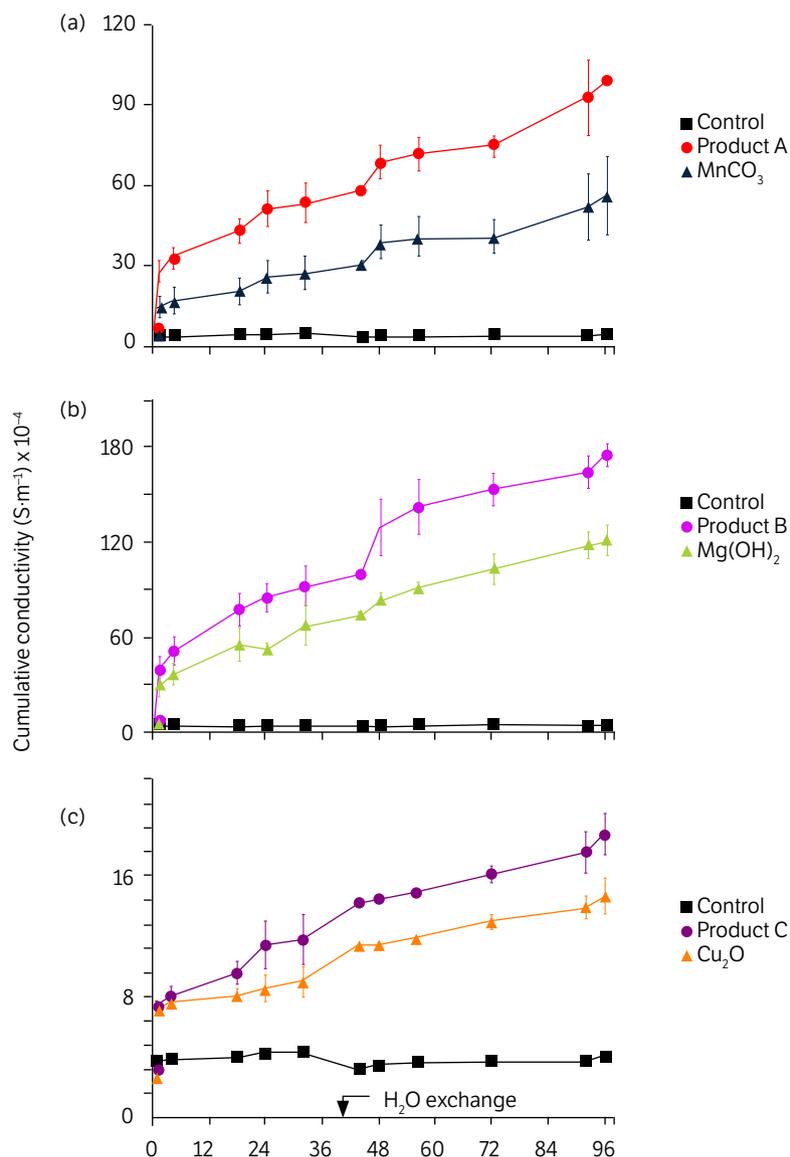


Figure 2. Cumulative conductivity monitoring of the water surrounding dialysis tubing containing three commercial products, (a), (b), and (c), as well as their correspondent primary compounds, MnCO_3 , $\text{Mg}(\text{OH})_2$, and Cu_2O .

Note. The elemental content on both commercial compounds and the corresponding original molecule were the same. Ultrapure water ($\rho < 18.18 \text{ M}\Omega\text{-cm}^{-1}$) was used as a control, and the values were not cumulative. The arrow denotes the water exchange in the membrane-containing beakers 40 h past the beginning of the experiment. The values account for the average value of three ($n = 3$) of independent replicates, and the error bars indicate the standard deviation.

In this scenario, Fig. 3 shows that the fitting curves that better described the conductivity kinetics behavior ($R^2 > 0.87$) of the compounds before and after water exchange were expressed by a sigmoidal-shape logistic function, presented in Eq. 1.

$$\text{Conductivity} = C_{\max} + \frac{(C_{\min} - C_{\max})}{\left(1 + \left(\frac{x}{x_0}\right)^p\right)} \quad (1)$$

where C_{\min} is the initial conductivity average value, C_{\max} is the final conductivity average value, x_0 is the inflection point (center) and p is the power.

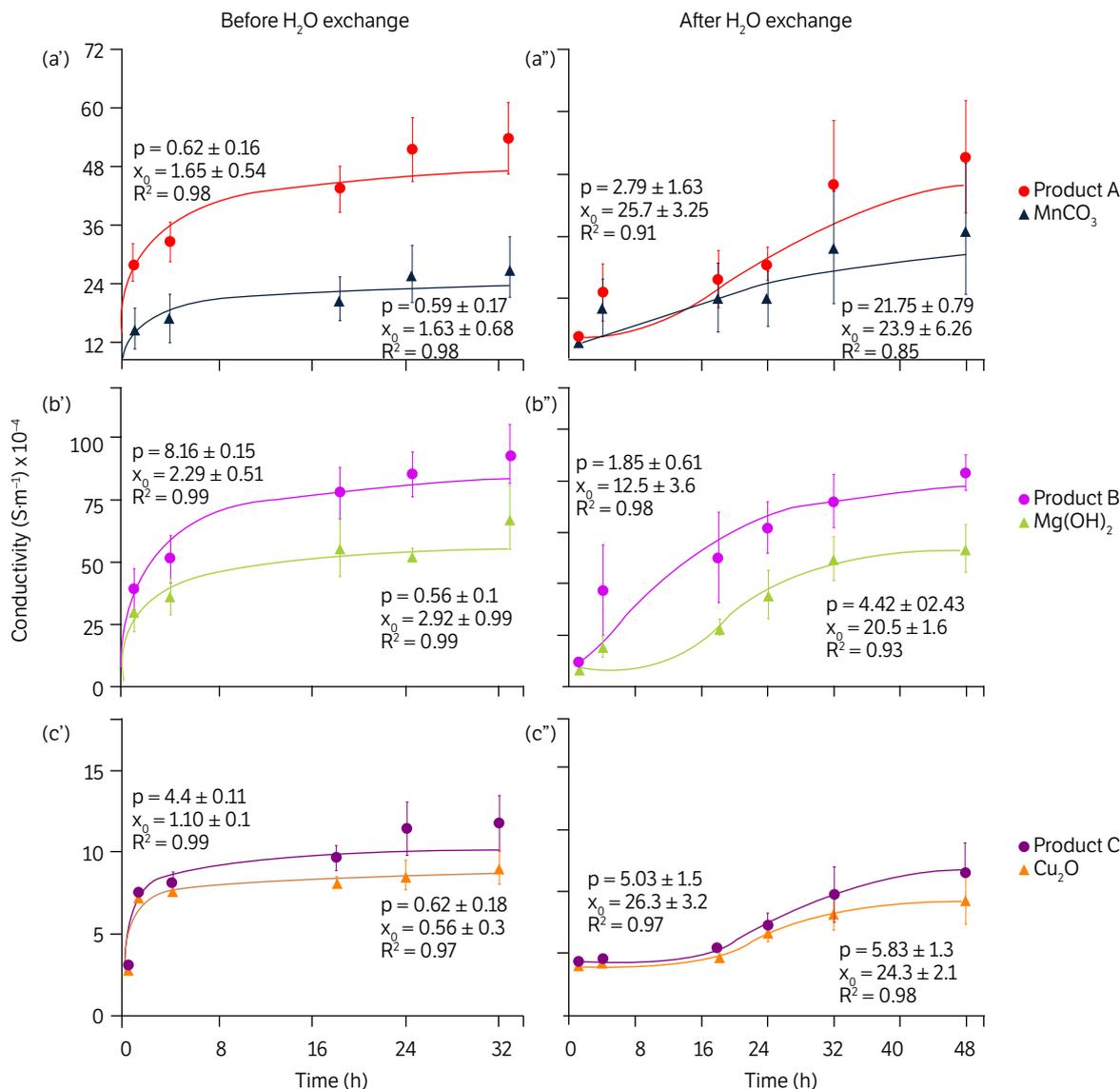


Figure 3. Experimental data and fitted curves for the 96-h conductivity intensity monitoring of water surrounding dialysis tubing containing three commercial products, (a), (b), and (c), as well as their correspondent primary compounds, MnCO₃, Mg(OH)₂, and Cu₂O.

Note. Before (') and after (") water exchange of the membrane-containing beakers. The fitted curves were obtained using the function described in Eq. 1, and parameters are described. The values account for the average value of three ($n = 3$) of independent replicates, and the error bars indicate the standard deviation.

The adjusted kinetic curves show that the diffusion of the material through the dialysis tubing before the water exchange (Fig. 3 a'-c') were similar in both commercial products A and MnCO₃, whereas it was significantly higher for the products B and C compared to their primary compounds, Mg(OH)₂ and Cu₂O.

The midpoint of sigmoid, i.e., the x_0 factor of Eq. 1, which refers to the moment at which the growth rate starts decreasing, reveals that both formulations and primary material ones achieve stability within a similar time frame in all cases, except for product C, that reached the midpoint *ca.* 50% faster compared to Cu_2O . Besides, the kinetics curves just started to stabilize a few hours after the beginning of the measurements.

Conversely, the release of the materials (denoted by p-value) after the water exchange (Fig. 3 a”-d”) is increased in both products A and C, as well as for MnCO_3 , $\text{Mg}(\text{OH})_2$, and Cu_2O , whereas it decreases for product B. In all cases, the sigmoid midpoint was achieved later than those before the water exchange, which might be related to a lower concentration of dispersions inside the dialysis tubings, and, therefore, a longer time requirement for reaching a chemical equilibrium.

On the other hand, the recorded pH values presented a similar range among the formulations and pristine materials for all the compounds and did not significantly vary along the monitoring period, since the coefficient of variation remained under 5% in all cases, therefore suggesting that pH was not affected by the fertilizer releasing.

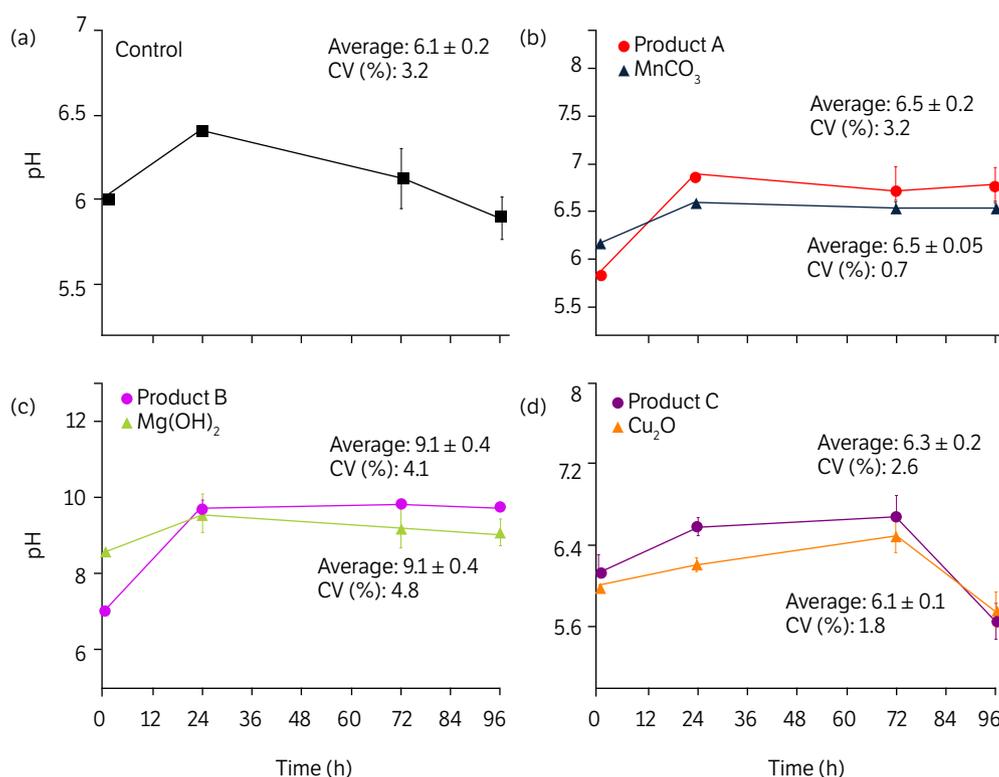


Figure 4. The pH monitoring of the water surrounding dialysis tubing containing three commercial products, (a), (b), and (c), as well as their correspondent primary compounds, MnCO_3 , $\text{Mg}(\text{OH})_2$, and Cu_2O for 96 h.

Note. The average and coefficient of variation for both products and primary materials are described. Ultrapure water ($\rho < 18.18 \text{ M}\Omega \cdot \text{cm}^{-1}$) was used as the control (a). The values account for the average value of three ($n = 3$) of independent replicates, and the error bars indicate the standard deviation.

Considering the primary materials, i.e., MnCO_3 , $\text{Mg}(\text{OH})_2$, and Cu_2O , it is noteworthy that these compounds present low solubility in water, and, thus, are likely to presume that most dispersed content was not diffused through the dialysis tubing, remaining trapped within them. In this scenario, the higher conductivity values recorded on the commercial products may be related to formulation properties and the effect of adjuvants.

Therefore, this experimental setup stands out as a simple and effective tool for assessing the dynamics of ion release from fertilizers by simulating a foliar application in a dialysis-membrane cell-proxy. Although it might be limited to mimicking the hydrophilic tissues, one could account as a fast approach for probing the composition dynamics of several fertilizers. Thus, further studies might take advantage of combining the present approach with established determination techniques,

such as inductively coupled plasma optical emission (ICP-OES) and energy dispersive X-ray fluorescence spectrometry (EDXRF), to address relevant information about the performance of foliar fertilizers formulation prior to its field application.

AUTHORS' CONTRIBUTION

Conceptualization: Montanha G. S., Carvalho H. W. P. and Dias M. A. N.; **Methodology:** Montanha G. S. and Carvalho H. W. P.; **Investigation:** Montanha G. S.; **Writing – Original Draft:** Montanha G. S.; **Writing – Review and Editing:** Montanha G. S., Dias M. A. N. and Carvalho, H. W. P.; **Funding Acquisition:** Carvalho H. W. P.; **Resources:** Carvalho H. W. P. and Dias; **Supervision:** Carvalho H. W. P.

DATA AVAILABILITY STATEMENT

The data will be available upon request to the authors.

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