QELBY-induced Enhancement of Exclusion Zone Buildup and Seed Germination

Abha Sharma[1], Dario Toso[1], Kurt Kung[1], Gun-Woong Bahng[2] and Gerald H. Pollack[1, 3]

[1]Department of Bioengineering, University of Washington, Seattle WA 98195

[2]Department of Mechanical Engineering, The State University of New York, Korea, Incheon, Korea 21985

[3]Name and institutional email address of the corresponding author:

Gerald H. Pollack; email:ghp@uw.edu

ABSTRACT

A hydrophilic powder, QELBY, from the feldspar family of clay minerals was investigated for its ability to form structured or exclusion-zone (EZ) water. We demonstrated microsphere-free zones around different fractions of the QELBY powder or its hydrated pellet. Averaging approximately 100 μm, these zones grew to a size similar to that formed in the vicinity of the Nafion standard. In the case of silica (control), only occasional microsphere-free zones of about 70 μm were found. Further, studies to investigate QELBY's energizing effect on germination and early sapling growth in brown chick-pea seeds showed at least a 2-3-fold increase in root-length and/or formation of shoots. This was seen in seeds bathed in QELBY supernatants or surrounded by QELBY powder outside the vials containing the seeds. This indirect effect was observed whether the QELBY was dry or hydrated.

I. INTRODUCTION

A highly hydrophilic material (QELBY®) developed by the Quantum Energy Company is manufactured through a special process from the feldspar family of clay minerals. Details and composition are presented in a Korean patent (KP 10-1172018)[1] and published elsewhere[2]. This hydrophilic material shows variety of interesting properties, mostly related to the enhancement of various cell-biological processes[3, 4]. Because of the diversity of enhancement effects, it is presumed that the mechanism is related to "something" present throughout the cell, the most likely substance being water. For example, one of the most important properties of QELBY is the absorption of ultraviolet light and emission of infrared light.[2] According to the explanation of water structure proposed by Pollack[5], this infrared light can be a source of energy for the formation of structured water, otherwise known as exclusion zone (EZ) water.

In addition to this optical feature, QELBY powder shows very high zeta potential as well as semi-conductive behavior[6]. This implies high surface polarity, which can be a seeding point for EZ growth. Both characteristics of QELBY- a nucleation site for EZ growth, and an infrared energy-generating substance for the formation of EZ water- can be anticipated to support the enhanced formation of EZ around QELBY powder grains.

In this report we explored whether an EZ forms around QELBY powder grains and compared it to the EZ that is formed around other hydrophilic substances. We also investigated the dynamics of EZ growth. As a control we used natural silica powder, chosen because silica is the major component of QELBY. The study was also undertaken to complement earlier studies on cellular models of health and disease with promising results.[3] Since EZs were observed around QELBY particles, we explored whether the water that was in contact with QELBY, when separated centrifugally from the powder, could still influence early plant growth, specifically seed germination and early sapling formation.

II. EXPERIMENTAL PROCEDURE

A. Powders and reagents

Powdered (particle size >30 µm) QELBY and silica (control) particles were provided to us as a generous gift from the Quantum Energy Company, Korea.

The suspension used for determining EZ size consisted of three different kinds of microspheres, polycarboxylate-coated 2 µm (Polysciences Inc; # 18327; 2.5% solids-latex); polystyrene-coated 2 µm (Polysciences Inc; # 19814; 2.5% solids-latex); and, hollow glass spheres 2 µm to 20 µm (Polysciences Inc; # 19823). The microspheres were suspended in deionized water (DI water) obtained from a Barnstead D3750 Nanopure Diamond purification system (type 1 HPLC grade (18.2 MΩ)). Carboxylate-coated and polystyrene-coated microspheres are negatively charged, whereas the hollow glass spheres are electrically and chemically neutral, and neutrally buoyant. The volume ratio of microsphere to water was 1:600 or 1:1000 depending on the kind of experiment performed, and kept constant to eliminate any effects that might arise from concentration differences.

B. Setup

Ordinary glass slides (Thermo Fisher Scientific) were used for examining pattern formation in QELBY- and silica-containing water droplets positioned with a 1-ml latex-free syringe. For the microsphere exclusion studies, we used a specially formulated glass-slide chamber built from a rectangular polycarbonate plastic block (48 mm x 26 mm), with a hollow cylinder cut out (15 mm diameter, 4 mm deep) in the center, secured to an uncoated glass slide (Thermo Fisher Scientific) at the bottom (Fig 1). Some studies were conducted in the 8-well chambered slide (8-well Permanox slide; Lab-Tek).

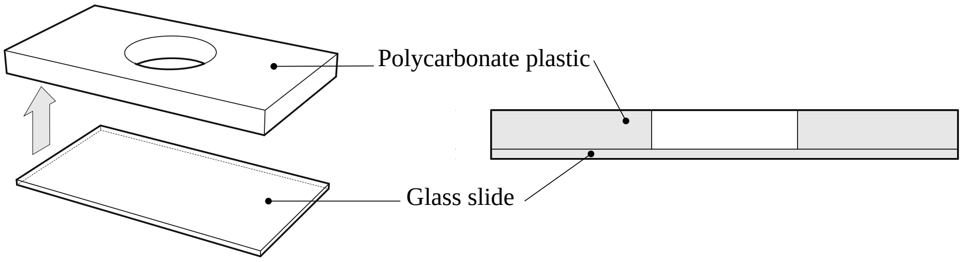


FIG.1. Diagrammatic representation of the glass-slide chamber.

C. Procedures

Pattern formation in water droplets. A 0.1% (weight by volume) suspension of QELBY was prepared in DI water obtained from a Barnstead D3750 Nanopure Diamond water purification system (type 1 HPLC grade, 18.2 MΩ). The suspension was stirred for 2 hours in a commercial blender and a portion was filtered using Sharkskin General-Purpose filter paper (8-μm to 12-μm pore size; Whatman) to remove the larger particles. A similar procedure was performed with a 0.1 % suspension of silica powder in DI water. However, due to the small size of the particles (less than 8 to 10 μm), the silica suspension was filtered with a 2.5 μm pore sized, ashless, Grade 42 Whatman® quantitative filter paper. The filtered and unfiltered QELBY and silica water suspensions were examined using the 'droplet evaporation method.'

Exclusion of microspheres in a particle suspension. A few particles of QELBY or silica powder were carefully placed at the bottom of the 8-well chambered Permanox or the polycarbonate plastic chambered glass slide. The chamber was gently filled with 250 μl of a diluted microsphere suspension (volume ratio of microsphere to DI water was 1:1000). Each type of microsphere listed above was tested. The interaction of microspheres with the particles was observed using an inverted microscope (Axio Observer A1, Zeiss). All image processing was done via ImageJ software.

Exclusion of microspheres in hydrated pellets. A 1% suspension of QELBY or silica powder (weight by volume) in DI water was vortexed overnight horizontally at room temperature. After centrifuging the mixture at 3000 rpm for 45 minutes, the supernatant was removed and labelled as QE-SUP or Si-SUP while the residue remaining behind was labelled as QE-RES or Si-RES, respectively. When compared with the white Si-RES, the QE-RES stacked as a tricolored residue (three hues of brown overlaid on one another). A few microliters of the well-mixed residue were pipetted to form tiny pellets (about 0.5-0.8 mm) on the chambered slide. The pellets were allowed to dry at room temperature for at least one hour. The chambered slide was placed on the stage of a Zeiss Axio Observer A1 inverted optical microscope and gently filled with 450 µL of the diluted microsphere suspension (volume ratio of the polycarboxylate-coated 2 µm microsphere to DI water was 1:600). The interaction of the microspheres with the QELBY (QE-RES) or silica (Si-RES) pellets was observed in the bright field mode with a 5X objective lens, which allowed visualization of the pellet in its entirety. Exclusion of microspheres in the vicinity of the two kinds of pellets was examined as a function of time. All image processing was done using ImageJ software.

D. Preparation of powder supernatants for UV-Visible Spectroscopy.

We placed a 1% suspension (weight by volume) of QELBY or silica powder in DI water contained in a 50 ml polypropylene tube. This was vortexed for 2h or overnight at room temperature and centrifuged thereafter at 3000 rpm for 45 minutes to obtain the powder supernatant (QE-SUP or Si-SUP). These were scanned over a wavelength range of 200-400 nm using the Cary UV-Vis-NIR 5000 model as per the manual instructions.

E. Seed germination and sapling growth.

Biological effects of the QELBY powder and their powder supernatants were examined via the "Contact" and "Non-contact" model experiments.

Contact model: In this model, we placed seeds in direct contact with 1-2ml of the powder supernatants (QE-SUP) prepared as described above in ***D***. Both the 2h and overnight-vortexed powder preparations were tested. For this study we selected regular brown chickpeas and those already "sprouted" under controlled conditions of light, temperature and humidity. Controls were set up with seeds/sprouts immersed in similar volumes of DI water or Si-SUP. Two types of containers -15 ml polypropylene, non-pyrogenic, high clarity tubes (Corning, Inc); as well as liquid scintillation glass vials (chemically inert, high clarity, from standard borosilicate glass) - were examined for their ability to support seed germination and sapling growth under regular conditions of laboratory light and temperatures of 22-23°C. Seeds were checked for germination (protrusion of radicle) after 1-2 days, and saplings were harvested after one week.

Non-contact model: Seeds selected for similar appearance and weights were rinsed and placed in DI water in vials. These were, in turn placed in plastic petri dishes that contained equal amounts of wet or dry QELBY (Fig. 2). Controls consisted of the same arrangement except that the petri dishes contained water or equal amounts of wet or dry silica powder. Set-ups were separated by a rack of aluminum foil or placed 12-14 inches from each other, and placed under standard conditions of laboratory light at temperatures of 22-23°C. Seeds were checked for protrusion of radicle (germination) after 1-2 days. Sprouted saplings were removed from the vials at the end of a one-week period, blotted gently with a soft paper towel and weighed immediately. A few experiments were harvested after a one-month period.

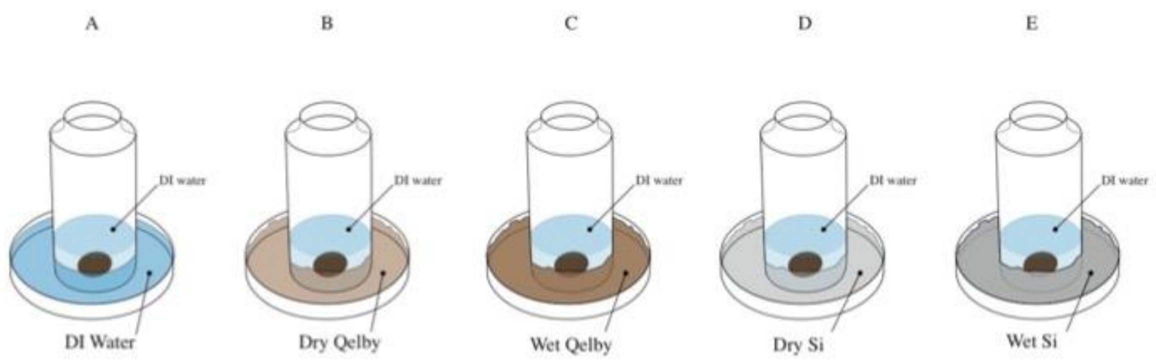


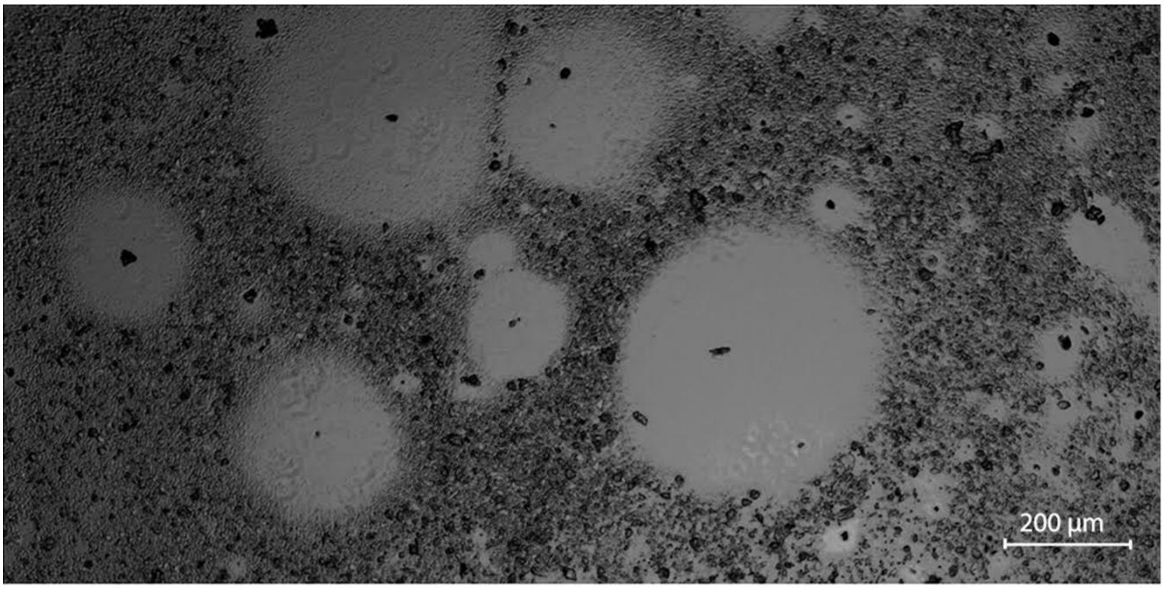
FIG. 2**.** Non-contact model: Diagrammatic representation of brown chick peas immersed in deionized water (DI) water contained in liquid scintillation vials. The vials were surrounded by one of several materials contained in petri dishes: DI water, dry/wet QE or Si.

F. Data analysis. Seed germination is normally described as a physiological process that begins with water imbibition by seeds and culminates with the protrusion of the radicle. The definition may change according to the length of the radicle, ranging from 1-5 mm[7]. In the current study, we recorded seeds that showed emergence of the radicle through its seed coat (testa) as being germinated. 'Percentage seed germination' was thus defined as the percentage of seeds with emerged radicles (1 mm) when compared to the total number of seeds used for experimentation. In some cases, we further substantiated these results by defining 'percentage of saplings with shoots' as the percentage of germinated saplings that developed a shoot in addition to the root.

III. RESULTS

A. Pattern formation in water droplets

Although droplet evaporation studies generally focus on the patterns remaining after full evaporation, we explored the patterns occurring at various stages during the evaporation process, moving the microscope stage minutely in order to observe different regions.



(a)

(b)

FIG. 3. Clear zones of several hundred micrometers were seen around single particles five minutes after placing droplets on the glass slide. (*A*) Representative Qelby powder droplet, still wet; (*B*) Representative silica powder droplet, still wet.

As depicted in Fig. 3, it was possible to observe clear zones of a few hundred microns around single particles in the evaporating droplets formed from the QELBY suspension. These clear zones persisted until the evaporation process was almost complete. Towards the end, a flow occurred, mixing all the particles together, leaving a typical “coffee-ring” pattern on the glass slide. However, even in the dried pattern it was still possible to recognize some clear areas (Fig. 4).

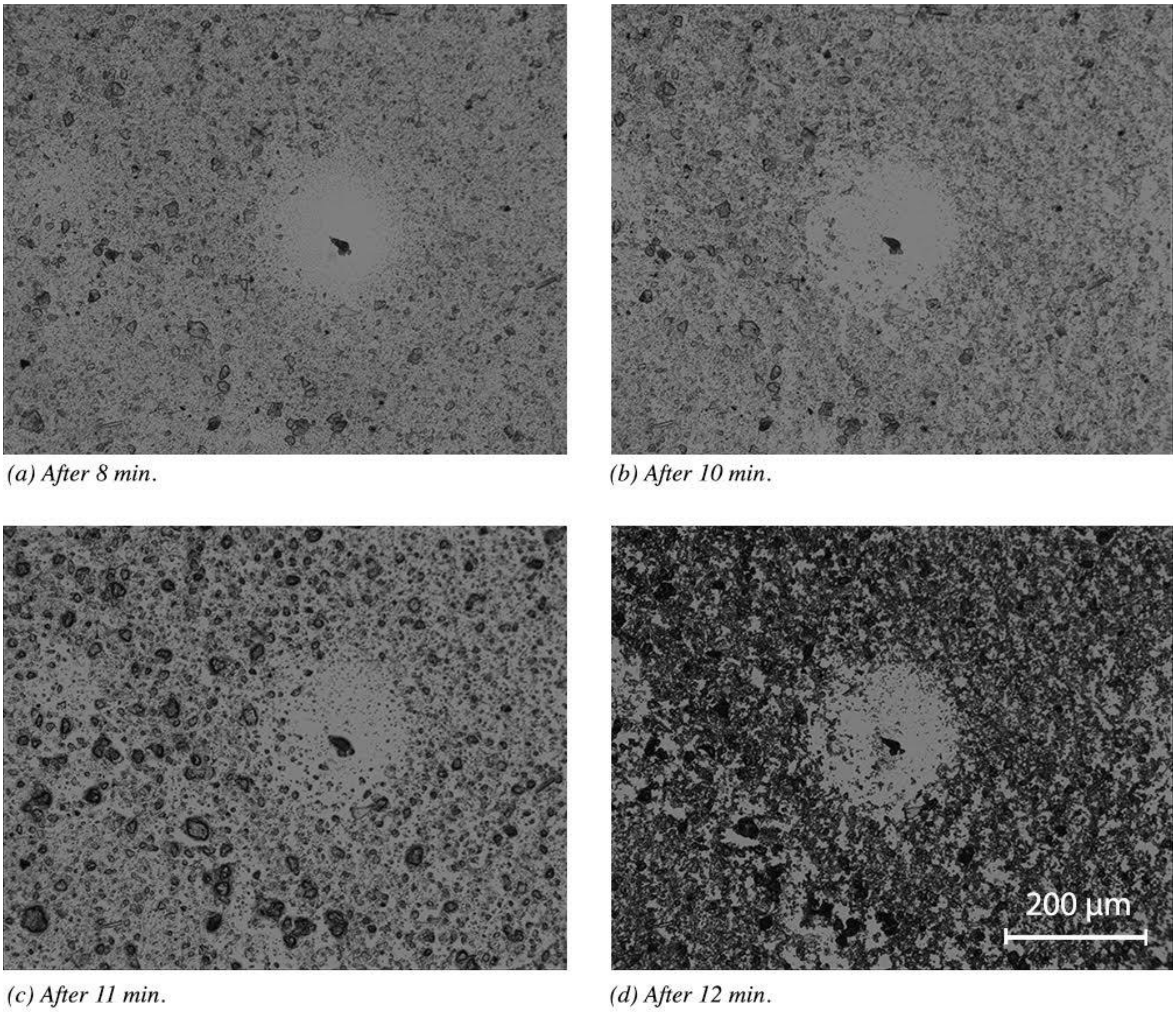


FIG. 4. Qelby powder droplet evaporation viewed over time until the complete evaporation after 12 minutes.

Clear areas around single particles were distinctly observed in 21 of 30 droplets of QELBY-containing water thereby suggesting a fundamental role for the QELBY interaction with water. In the case of droplets containing silica powder, clear areas were observed in only two of the 30 droplets and their sizes were less than 100 µm. A summary of results is shown in Fig. 5.

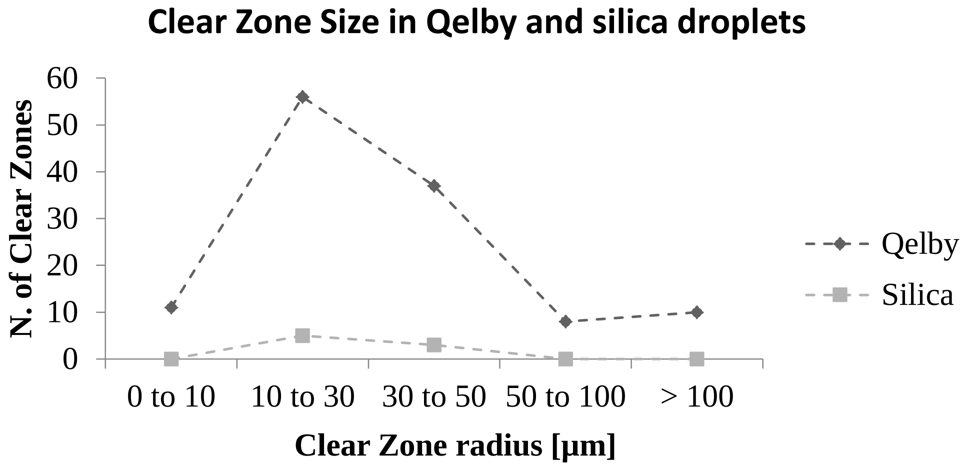
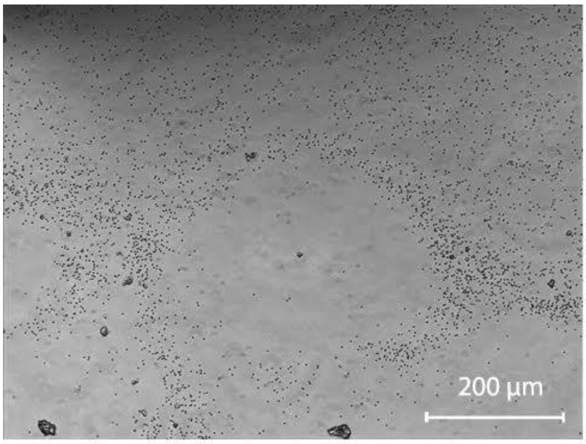


FIG. 5**.** Clear zone size distribution in a Qelby suspension compared to a reference silica suspension.

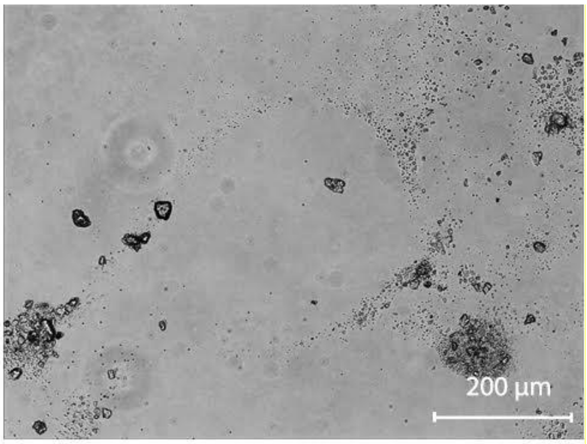
B. QELBY particles in microsphere suspensions

Similar to that observed in the droplet-evaporation experiment, QELBY particles placed in a suspension of microspheres often generated microsphere-free zones (Fig. 6). Microsphere exclusion was long lasting. We could observe clear zones for more than 24 hr. However, quantifying the fraction of particles generating exclusion was not easy because of the challenge of identifying individual particles. Some particles showed large microsphere-free zones, which resembled the particle-free zones described above (Fig. 3A) while others failed to show any exclusion of microspheres at all. Instead, the microspheres would actually gather on the surface of some of the QELBY particles. This diversity of behaviors implied that different fractions of the QELBY powder might show distinctly different features (*see* Fig. 9, below).



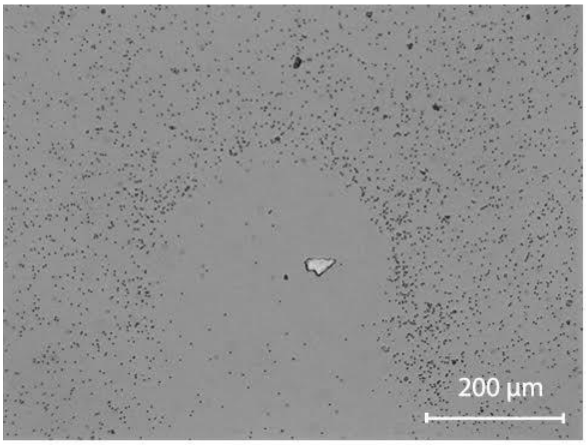
(a)

Qelby particles in polycarboxylate-coated 2-µm microsphere suspension (8-well chambered Permanox slide)



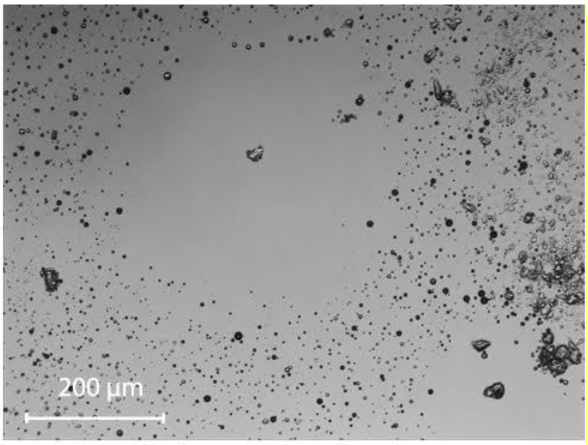
(b)

Qelby particles in polystyrene-coated 2-µm microsphere suspension (8-well chambered Permanox slide)



(c)

Qelby particles in polycarboxylate-coated 2-µm microsphere suspension (glass slide chamber)

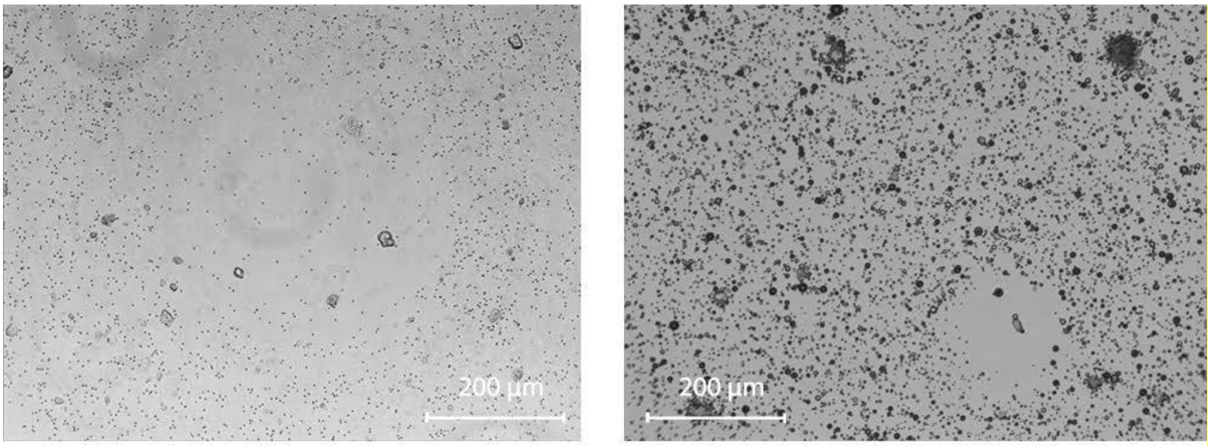


(d)

Qelby particles in hollow glass microsphere suspension (8-well chambered Permanox slide)

FIG. 6. Clear zones, free of microspheres, were generated around single Qelby particles in various chambers and microsphere types (a-d).

On the other hand, similar experiments with silica displayed only occasional microsphere-free zones; those that were observed were obviously smaller than those surrounding the QELBY particles (Fig. 7).



(e)

Silica particles in polycarboxylate-coated 2-µm microsphere suspension (8-well chambered Permanox slide)

(f)

Silica particles in hollow glass microsphere suspension (8-well chambered Permanox slide)

FIG. 7**.** Clear zones, free of microspheres, were generated around single Silica particles in microsphere suspensions of various types, but smaller and rarer than around Qelby particles.

The QELBY exclusion zones described above resembled those zones seen next to other hydrophilic surfaces such as Nafion. In fact, the exclusion zones in the vicinity of QELBY particles grew with a velocity comparable to that observed in the vicinity of Nafion (~1 µm/s) (Fig. 8).

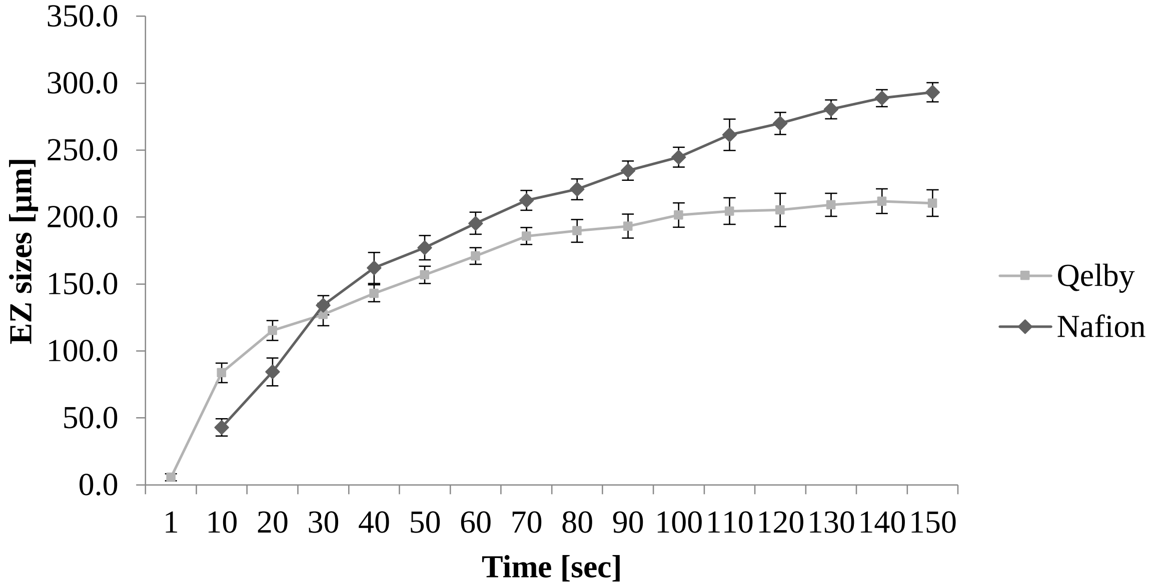
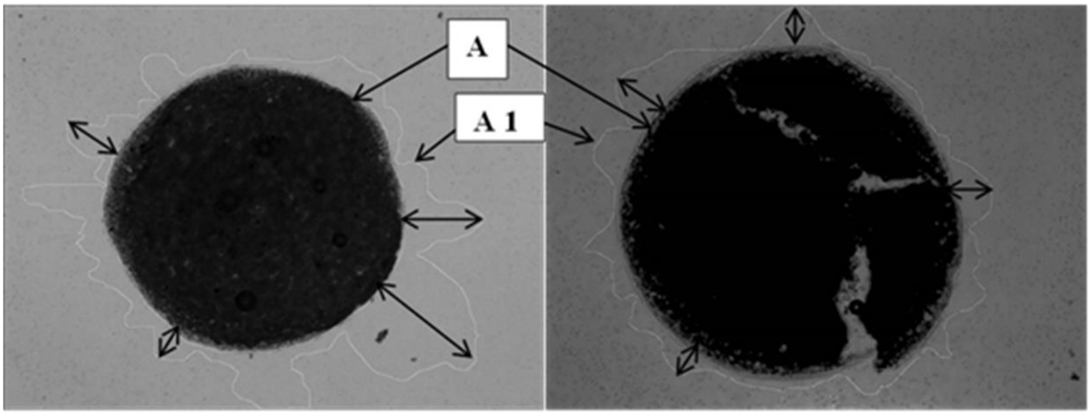


FIG. 8**.** Rate of exclusion zone formation around Qelby particles vs. next to Nafion. Qelby particles in polycarboxylate-coated 2-µm aqueous suspension, and Nafion TT110 in polycarboxylate-coated 2-µm microsphere suspension were placed separately in an 8-well chambered Permanox slide. The curves represent the means of three measurements.

C. Microsphere-free zones around hydrated pellets

Fig. 9 shows representative images of the microsphere-free zones formed adjacent to the QE-RES and Si-RES pellets (formulated as described in Procedures), following a 20-minute exposure to microspheres. Capturing images every four minutes confirmed reasonably stabilized EZs. ImageJ software was used to measure the sizes of the microsphere-free zones.



(a) **(b)**

FIG. 9**.** Representative images of exclusion zone development next to pellets of QE-RES (a) and Si-RES (b), also detailing the use of the Image J software to obtain area measurements. Double-sided arrows represent a few of the marked EZs.

Since the microsphere-free zones were typically non-uniform, some computation was necessary. We measured the area of the pellet (*A*) and that of the pellet plus microsphere-free zone (*A1*). Computing the difference and dividing that difference by the pellet circumference gave the mean EZ width. We performed this calculation at four intervals over the 20-minute span, and averaged the results. Experiments were repeated, each time with a different pellet sample. The mean EZ size for the QE-RES pellet was 107 ± 12 µm versus 74 ± 9 µm for the

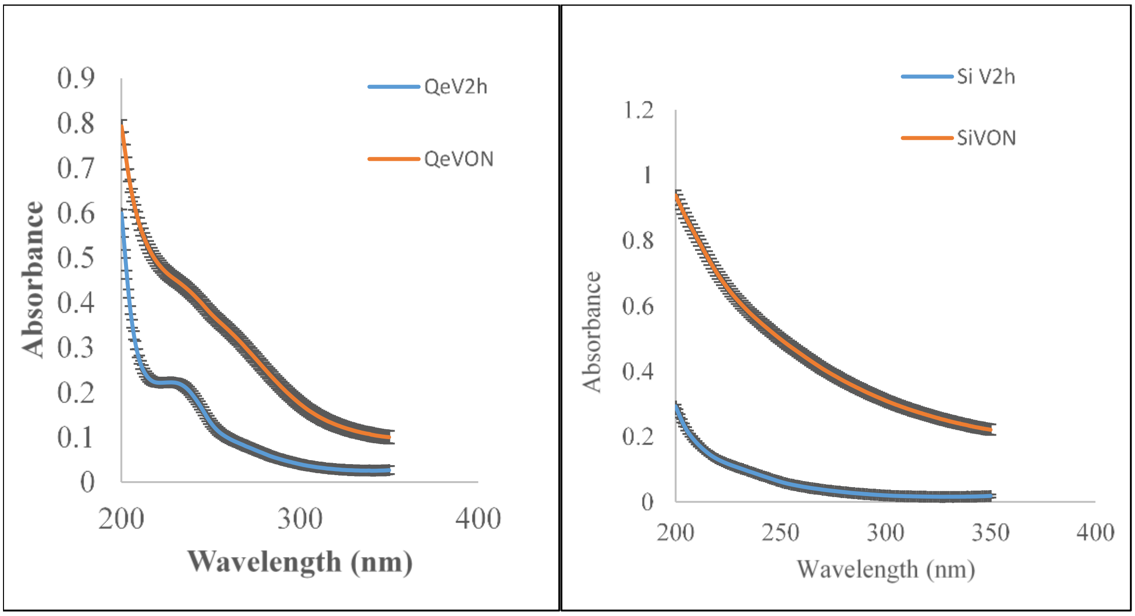
Si-RES pellet, which served as control. The modest SD indicated that the EZ appeared to be reasonably stable over time. The results are summarized in TABLE 1.

TABLE I: EZ width, measured with carboxylate microspheres across the QE-RES and Si-RES pellet interface (n=4 different pellet samples; * indicates *p* value ≤0.1)

	QE-RES pellet	**Si-RES pellet**
Average EZ size (μm) ± SD	107 ± 12*	74 ± 9*

D. UV-Visible Spectroscopy of powder supernatants

When compared with the silica supernatants, the QELBY supernatants showed a broad bulge between 240-280 nm. Overall, powder suspensions vortexed overnight had higher absorbance units than those vortexed for 2h only (n=10). Representative figures Fig. 10a and b depict absorbance measurements of QE-SUP and Si-SUP respectively.

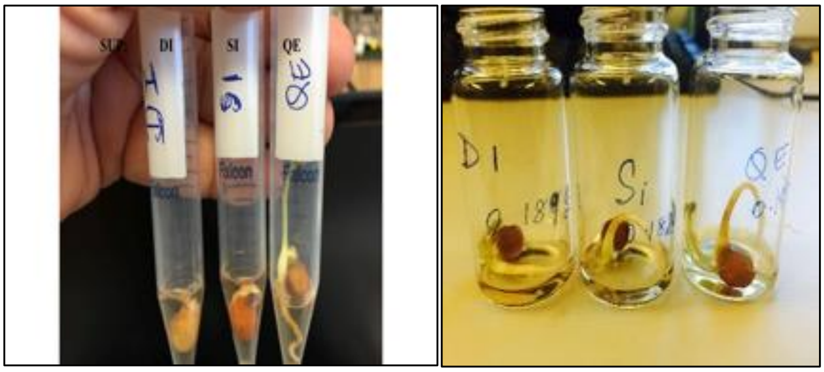


(a) (b)

FIG.10. Representative absorption spectra of the QE-SUP (a) and Si-SUP (b) powder supernatants in DI water. Powder solutions were vortexed (2h or overnight) and centrifuged at room temperature. Supernatants were scanned for absorbance measurements between 350-200 nm.

E. Contact model -Chick pea seeds

In order to determine the energizing role of QE, regular brown chick pea seeds were placed in direct contact with the different supernatants as described in the Experimental Procedures, and observed for seed germination and sapling growth. Irrespective of the type of container (n = 4; each kind), seeds immersed in QE-SUP were the first to germinate (n = 12). At the end of a week, while there was no significant difference in weight, young saplings in QE-SUP were at least 3-4 times longer than those in an equal volume of DI water or Si-SUP. (Fig. 11 *a* and *b*).



(a) DI Si-SUP QE-SUP (b) DI Si-SUP QE-SUP

FIG. 11. Representative images of early germination and growth observed in chickpea seeds immersed in equal volumes of DI water, Si-SUP, or QE-SUP in (a) test tubes or (b) liquid scintillation vials.

Sprouted Chick pea seeds: Germination amongst a population of seeds is not synchronous. Hence we minimized the variability by immersing chick pea seeds that were already sprouted under controlled conditions, as described in the Experimental Procedures. After selecting for similar weights and appearance, sprouted chick pea seeds were immersed in the powder supernatants for a week's growth in the two types of containers. Irrespective of whether the container was a tube or vial, we found no significant change in the weight of the seedlings. However, roots of sprouted chick pea seedlings were significantly longer when grown in QE-SUP (58%) versus Si-SUP and about 24% when compared with DI water (Fig. 12).

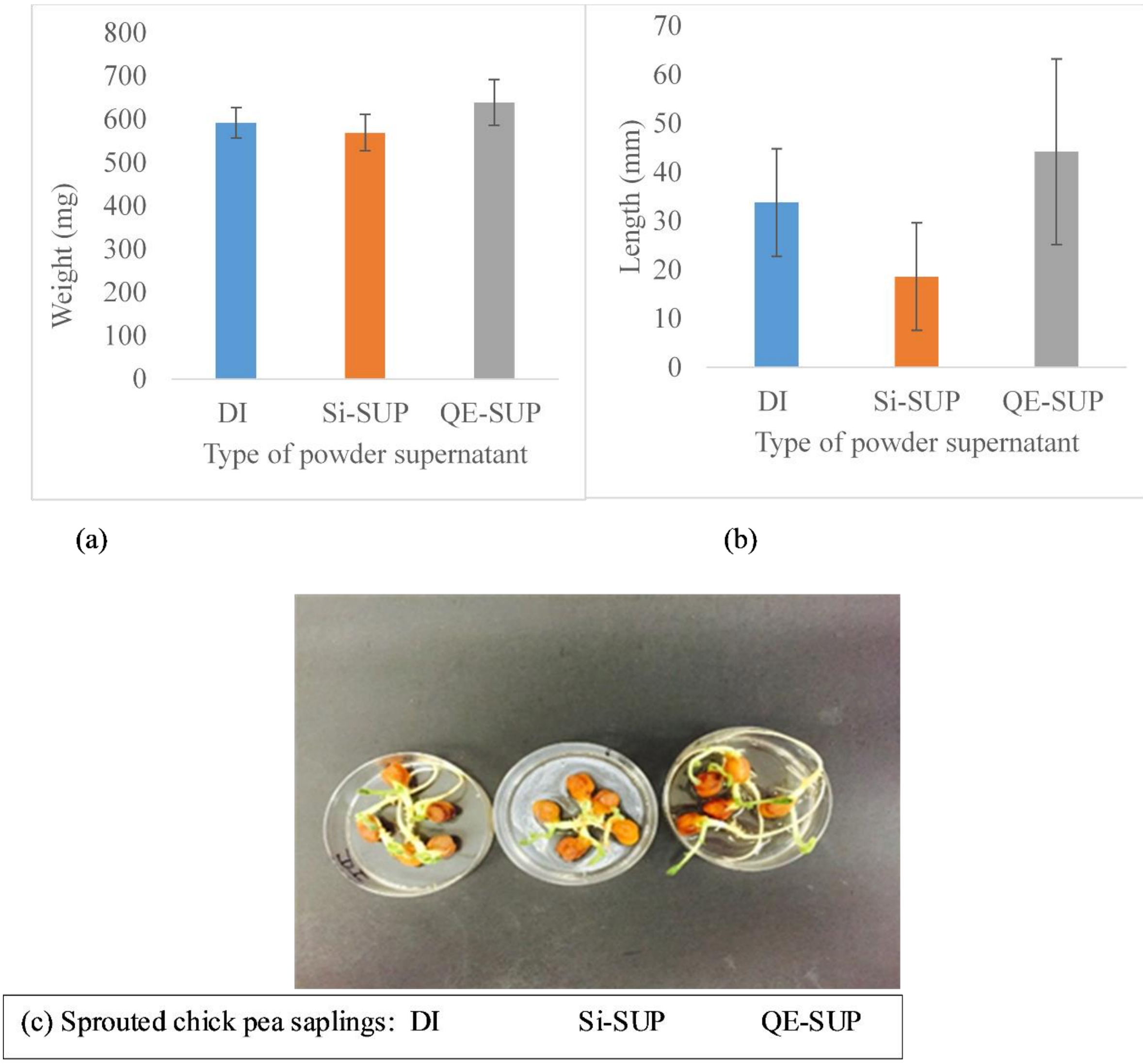


FIG. 12. Effect of powder supernatants-QE-SUP or Si-SUP on the (a) weight (mg) and (b) length (mm) of sprouted chick-pea saplings after one week (n = 8 seeds). (c) Pictographic representation of the sprouted chick pea saplings in different environments after one week.

F. Non-contact model -Chick pea seeds

In these experiments, the surrounding powder was not in direct contact with the seed; the vial wall separated the two. As depicted in Table II, while the percentage germination was only about 10-20% enhanced, the percentage of saplings with shoots was significantly higher (90-100%) in the presence of wet/dry QE versus Si powder. Saplings surrounded by wet/dry QE powder also had more adventitious roots and leaves. Quantitative evaluation of chick pea sapling weights at the end of a one-week growth period indicated a modest enhancement (~10-15%) when surrounded by wet/dry QE powder (FIG.13a). Root lengths and shoot lengths were higher in QE than Si (panels *b* and *c*), although SDs were too large to claim statistical significance.

TABLE II: Percentage seed germination and percentage of saplings with shoot growth in chick-pea seeds surrounded by wet/dry powder during a one-week growth period (*n*: 8-18).

Experimental conditions	**Silica powder**		**QELBY powder**	
	Dry	**Wet**	**Dry**	**Wet**
% germination	72	67	89	75
% of saplings with shoots	54	25	94	100

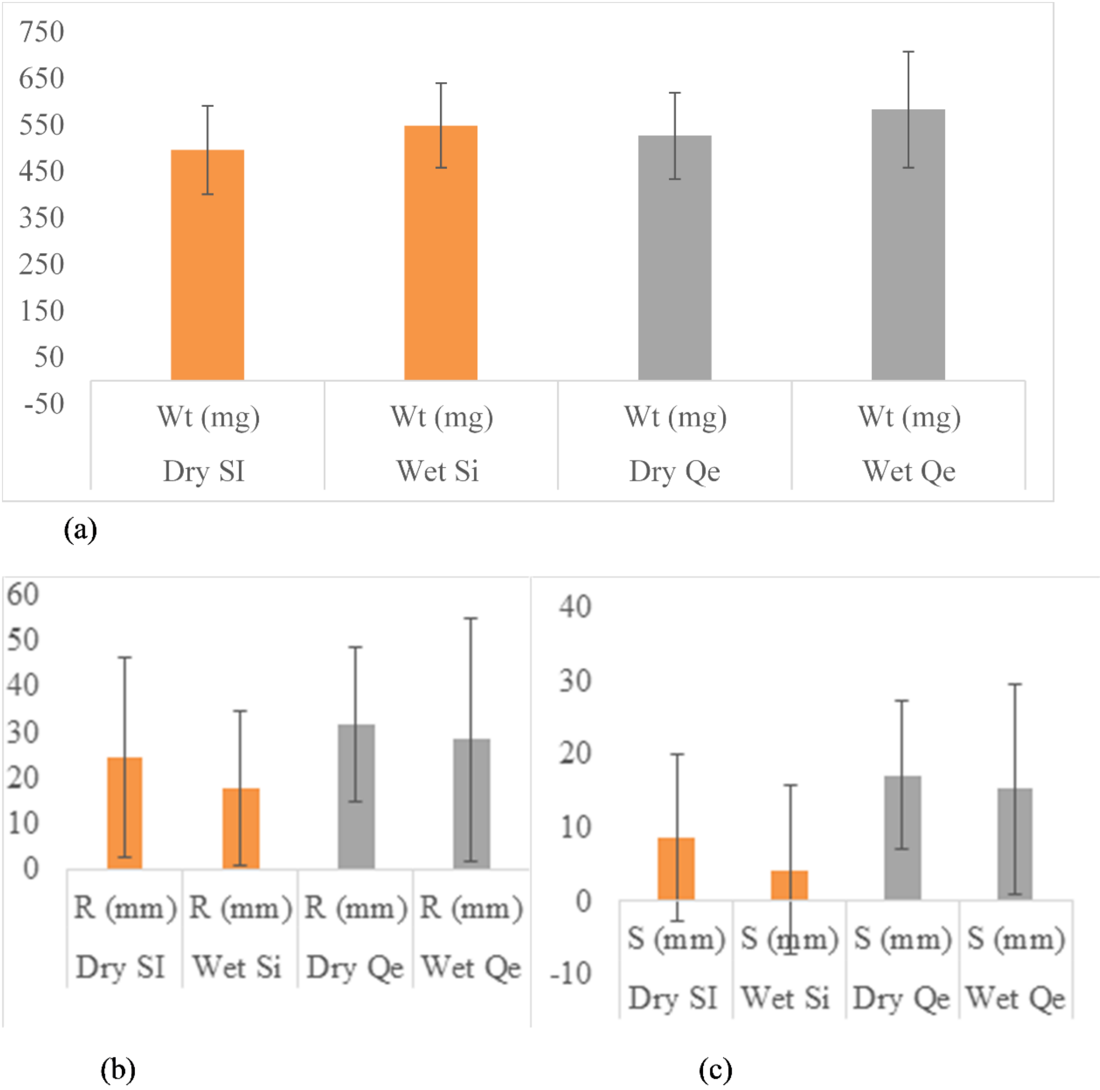


FIG. 13. Effect of dry and wet QE and Si powders on the (a) weight (mg) and (b &c) length (mm) of roots (R) and shoots (S) of chick-pea saplings after one week (n = 12-18).

A few experiments were prolonged over a month-long period. Results presented in FIG. 14 depict well-formed stems with abundant green leaves in chick-pea saplings surrounded by wet/dry QE powder relative to either DI water or wet/dry Si powder (FIG.14; n = 3-6).

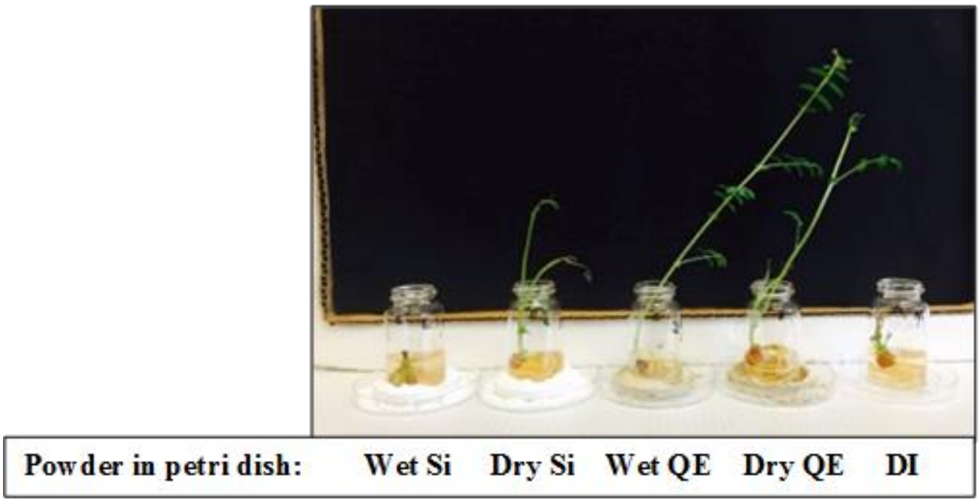


FIG. 14. Representative images of sapling growth in the non-contact model or the "seed in vial, powder in petri-dish" set-up during a 30-day growth period.

IV. DISCUSSION

The results confirm that QELBY powder builds EZ water, and powerfully acts to enhance plant growth. These effects were borne out by several independent methods.

In a droplet-evaporation method we observed clear, microsphere-free zones surrounding individual QELBY particles. The zones were generally on the order of several hundred micrometers in width. Similarly, particles examined in aqueous microsphere suspensions generated microsphere-free zones that lasted at least for the 24 hours of observation. Zone sizes were variable, reflecting the multi-component nature of QELBY particles.

These microsphere-free zones resemble the ones seen next to various hydrophilic surfaces, which serve to indicate the presence of EZ water[5]. In fact, typical EZ size was only slightly smaller than that seen next to Nafion, which has become a standard for studies of EZ development. Rate of growth was also comparable.

We also examined supernatants obtained from QELBY suspensions to determine whether EZ water was present. UV-VIS absorption spectra showed an absorption peak near 270 nm, which is indicative of the presence of EZ water[8]. This finding provided additional indication that QELBY particles nucleate EZ growth, and correlates with the substantial EZ-growth rate.

The reason why the supernatants are so effective in building EZ is not yet clear. Possibly some small mineral remnants present in the supernatants nucleate EZ buildup. Structure-building ions might do the same. Alternatively, small EZ fractions may survive centrifugation and wind up in the supernatant. Future studies may settle the issue. Given QELBY's capacity to nucleate EZ buildup, we explored whether exposure of QELBY to biological specimens might enhance natural biological processes. We exposed QELBY to regular and sprouted chick pea seeds and found that sapling growth was enhanced relative to controls. A similar trend was observed with radish seeds (data not shown). We speculate that QELBY was effective on the cells at the growing tip of the sapling roots, and resulted in their elongation. Enhancement was seen not only when the seeds were bathed in centrifuged QELBY supernatants, but also when QELBY powder was placed immediately outside the vials containing the seeds. This indirect effect was observed whether the QELBY was dry or hydrated.

This action-at-a-distance effect reflects the growing awareness that information can be transmitted between samples of water by electromagnetic signals. When vials containing certain suspended or dissolved materials are placed next to vials of pure water, the former can alter the water's structure, as inferred from characteristic changes of infrared absorption spectrum[9]. Further, vials of water placed near samples of DNA can be used to create new DNA with the same sequence as the original[10]. While the results obtained here do not go as far as those studies, they do show that growth-enhancing effects can occur without direct contact.

In sum, QELBY material, originating from natural clay, has the capacity to nucleate EZ buildup. And, possibly through the vehicle of EZ buildup, QELBY has an enhancing effect on plant growth, a feature with considerable practical value.

ACKNOWLEDGMENTS

The authors thank the Quantum Energy Co., Ltd, for providing a gift, which was used to help support this work, and the SAGST Foundation for providing grant support for this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

V. REFERENCES

1. J.D. Lee: Method for manufacturing of SiO2 containing ecofriendly and high functional fusion material powder. 1. Patent 10-1172018, Korea (2010).

2. G.W. Bahng and J.D. Lee: Development of heat-generating polyester fiber harnessing catalytic ceramic powder combined with heat-generating super microorganisms. Textile Research Journal. **84**, 1220 (2014).

3. J.D. Lee, Vergara, E.J.S, S.H. Choi, S.G. Hwang, and G.W. Bahng: Anti-inflammatory activity of Quantum Energy living body on lipopolysaccharide-induced murine RAW 264.7 macrophage cell line. *Bioceram Dev Appl*. **6**, 1 (2016).

4. H.T. Lee, D. Han, J.B. Lee, G.W. Bahng, J.D. Lee, and J.W. Yoon: Biological effects of indirect contact with QELBY® powder on non-macrophagic and macrophage derived cell lines. *J. Prev. Vet. Med.* ***40**, 1 (2016)*.

5. G.H. Pollack: The Fourth Phase of Water: Beyond Solid, Liquid, and Vapor. Ebner and Sons (2013).

6. J.D. Lee, A. Kulkarni, T. Kim, G.W. Bahng, S.J. Moh, Y.W. Yu, and S.H. Moh: Electrical properties of "Quantum Energy® Radiating Material" produced from natural clay minerals of South Korea. *Mater. Focus.* **3**, 1 (2014).

7. O. Munzuroglu and H. Geckil: Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in Triticum aestivum and Cucumis sativus. *Archives of Environmental Contamination and Toxicology*. **43**, 203 (2002).

8. J.M. Zheng, W. C. Chin, E. Khijniak, E. Khijniak, Jr, G.H. Pollack: Surfaces and Interfacial Water: Evidence that hydrophilic surfaces have long-range impact. *Adv. Colloid Interface Sci.*

127, 19 (2006).

9. V. Korenbaum, T. Chernysheva, A. Sergeev, V. Galay, R. Galay and S. Zakharkov: Long Term Order in Infrared Absorption Spectra of Water Subjected to Weak Electromagnetic Influence WATER **5**, 27 (2013).

10. L. Montagnier, J. Aissa, S. Ferris, J.L. Montagnier and C. Lavall: Electromagnetic Signals are Produced by Aqueous Nanostructures Derived from Bacterial DNA Sequences. *Interdisc. Sci. Comput. Life. Sci.* **1**, 81 (2009).