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Author/s:
Freidman, BL;Gras, SL;Snape, I;Stevens, GW;Mumford, KA

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Research Article

Effects of Freeze--thaw Phenomena on Controlled Nutrient Release: Application to Bioremediation[†]

Benjamin L. Freidman[1,2], Sally L. Gras[1,2,4], Ian Snape[3], Geoff W. Stevens[1], and Kathryn A. Mumford[1]*

[1] Particulate Fluids Processing Centre, Department of Chemical and Biomolecular Engineering, The University of Melbourne, Australia

[2] Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Australia

[3] Australian Antarctic Division, Kingston, Tasmania, Australia

[4] The ARC Dairy Innovation Hub, The University of Melbourne, Australia

Correspondence: Dr. K. A. Mumford, Building 165, The University of Melbourne, Parkville 3010, VIC, Australia

Email: mumfordk@unimelb.edu.au

Graphical abstractControlled release nutrient materials can enhance biodegradation at petroleum hydrocarbon contaminated sites in cold regions. Freeze-thaw cycling can alter the nutrient release profile as well as the material integrity; suggesting that suitable materials must be applied in these extreme environments. This study supports the application of Nutricote™, Polyon™ and Zeopro™ to cold region bioremediation.

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Abstract

Cold region soils are often devoid of sufficient nutrients essential for timely bioremediation of petroleum hydrocarbon contaminants. While materials that release nutrients in a controlled manner have been shown to stimulate biodegradation over extended time periods, the physical and chemical response of these materials to repetitive freeze--thaw stresses typical of cold regions remains poorly understood. This study reports the performance of four controlled release materials in water (Osmocote^{TM}, Nutricote^{TM}, Polyon^{TM}, Zeopro^{TM}) exposed to freeze--thaw cycling or control temperature of 4 °C. Additionally Zeopro^{TM}-activated carbon mixtures are investigated for application to permeable reactive barriers. Osmocote^{TM} experienced higher nutrient release under control conditions compared with samples exposed to freeze--thaw after 20 days. Nutrient release from Nutricote^{TM} and Polyon^{TM} was similar under freeze--thaw and control conditions. Zeopro^{TM} delivered low nutrient concentrations into solution in both freeze--thaw and control samples with calcium phosphate dissolution accelerated in the presence of activated carbon. Osmocote^{TM}, Nutricote^{TM} and Polyon^{TM} revealed strong resistance to breakdown under freeze--thaw. Zeopro^{TM} experienced only partial disintegration, while significant break-up of activated carbon occurred under freeze--thaw. A physical and chemical understanding of the response of multiple fertilisers can guide the selection of materials for biostimulation and biodegradation of petroleum hydrocarbons in environments exposed to freeze--thaw cycling.

Abbreviations: CRN, controlled release nutrient; **FESEM**, field emission scanning electron microscopy; **GAC**, granular activated carbon; **ICP-OES**, inductively coupled plasma-optical emission spectrometry; **PHC**, petroleum hydrocarbon; **PRB**, permeable reactive barrier

Keywords: Cold regions, Fertilisers, Granular activated carbon, Petroleum hydrocarbon contaminants

1. Introduction

Petroleum hydrocarbon (PHC) spills can have a significant impact on ecosystems in cold regions, as extreme climates can result in changing sensitivities to pollution [1--4]. Additionally, spilt PHCs can impact upon the physicochemical properties of soil in cold regions, influencing freeze--thaw processes, frost heave, thermal and moisture regimes, as well as soil pH and nutrient availability [2].

Bioremediation offers one of the most cost effective and environmentally sensitive management approaches to the clean-up of PHC contaminated sites [3]. Bioremediation utilises microorganisms to accelerate the natural degradation of PHCs by altering environmental factors *in situ* [3]. In cold regions, poor nutrient supply, low temperatures, low soil moisture, and the increased viscosity of PHCs can inhibit the onset of biodegradation [2, 5--7]. The addition of nutrients has been shown to increase cell growth rate and maintain cell metabolic function, leading to increased PHC degradation [8]. In most cases, nitrogen, phosphorus, potassium and sulphate, as well as other trace elements like iron, are delivered to enhance the degradation of PHCs [3, 6].

While multiple studies suggest the positive implications of nutrient addition for bioremediation [9, 10], oversupply has been shown to inhibit microbial metabolic function [8, 11]. For example, biodegradation within PHC contaminated Arctic soil has been found to be higher when amended with 100 mg N kg^{--1} soil rather than 200-300 mg N kg^{--1} [8]. At sub-Antarctic Macquarie Island, PHC degradation was maximised at 604 mg N kg^{--1} soil H_2O with depressed activity above 1800 mg N kg^{-1} soil H_2O [11].

Generally, microbial activity in soil is optimal at a water potential of -0.01 MPa, although this can vary between microbes with fungi being more tolerant to negative water potentials than bacteria [12]. Similarly, biodegradation by bacteria is optimal in the pH range 5--9 with fungi often shown to contribute to biodegradation in more acidic soils [8, 13]. In addition to the indigenous microbial population, plans for nutrient addition also need to consider factors such as soil particle size, moisture content and PHC concentration [14].

As nutrient levels must be sustained at sufficient concentrations throughout the entire bioremediation operation, controlled release nutrient (CRN) materials are preferred. CRN materials enable nutrients to be released over extended time periods, leading to increased control over the rate and pattern of release while eliminating the need for multiple treatments [15]. The release of each nutrient or ion is expected to depend on: aqueous solubility, diffusivity through the particle coating, interaction with other ions, particle water content and temperature within the particle [16].

A number of commercial fertilisers have been considered as CRN materials for bioremediation. Osmocote™ fertiliser successfully enhanced biodegradation in PHC contaminated beach sediments [17] and nutrient release was steady from MaxBac™ fertiliser at varying moisture conditions [7]. The response of selected CRN materials to recurring freeze--thaw stresses, as well as the weight of overlying soil, is a critical factor in assessing the suitability of these materials for bioremediation in cold regions. Rupture of the coating of encapsulated CRN materials has been shown to occur where the pressure build-up inside a granule is greater than the resistance of the particle, suggesting coating strength may be important in areas of freeze--thaw cycling [16].

The study compliments other recent CRN material investigations at low temperatures [18, 19] and aims to examine the physical and chemical response of CRN materials to freeze--thaw cycling. The CRN materials varied in coating composition to assess freeze--thaw susceptibility and were evaluated against a non-polymer coated material, Zeopro™. Granular activated carbon (GAC), used to adsorb PHCs, was also mixed with Zeopro™ to better understand the application of CRN materials to permeable reactive barriers (PRB) [18, 19]. This is the first time that physical and chemical assessment of a broad range of polymer and non-polymer CRN materials, encompassing application to PHC capture and degradation in groundwater, has been conducted for suitability testing to inform cold region bioremediation.

2. Materials and Methods

2.1 CRN materials

Four commercial CRN materials were investigated (Table 1). These products were selected based on features that may assist in their application in cold climates and conditions of repeated freeze--thaw.

The mineral fillers dispersed into the ethylene-vinyl acetate coating of Nutricote™ have been reported to provide resistance to cracking [20, 21] while the Polyon™ coating is reported to provide flex capacity [21] as well as solvent resistance [19]. Osmocote™, Nutricote™ and Polyon™ granules were sieved to 5 × 8 US mesh (4000--2380 µm) before use. Zeopro™ H (8 × 16 US mesh (2380--1190 µm)) operates by dissolution of the calcium phosphate coating, exchanging calcium with ammonium and potassium in the pores (Table 1) [18]. In Zeopro™-GAC mixtures, the GAC used was chemically activated with phosphoric acid and derived from pine wood (Pica, USA). The GAC was sieved to 8 × 16 US mesh (2380--1190 µm).

2.2 Experimental design

CRN materials were exposed to 100 freeze--thaw cycles across 50 days within a cooled incubator MIR-153 environmental testing chamber (Sanyo). 1 g (dry weight) of CRN material was placed in a 50 mL polyethylene tube with 45 mL of deionised water (resistivity of 18.2 mΩ; Milli-Q, Millipore). In Zeopro™-GAC samples, 1 g of Zeopro™ and 1 g of GAC (1:1, w/w) was combined in a 50 mL polyethylene tube. Deionised water was sterilised by autoclaving at 121 °C for 30 min and cooled to 4 °C prior to use. All CRN and GAC materials were sterilised with ethylene oxide (Steritech).

Each 12 h freeze--thaw cycle consisted of 8 h at --9 °C and 4 h at 9 °C [22], with a 25 min period required for the temperature to ramp up and ramp down between the desired settings. Polystyrene foam was used to insulate the bottom 25 mL of each 50 mL polyethylene tube to ensure one-dimensional top-down freezing. Control samples were refrigerated at 4 °C. To avoid potential inaccuracy associated with repetitive extractions from the same sample, a single 50 mL polyethylene tube was dedicated to each sampling interval. Sampling was conducted at 0.5, 1, 2, 4, 8, 14, 22, 30, 40 and 50 days.

At all sampling intervals, the supernatant was decanted and the pH was recorded prior to nutrient analysis. Freeze--thaw and control samples were analysed in triplicate for each sampling interval.

2.3 Nutrient flux and water potential

Release rates (mmol/g CRN per day) of total inorganic nitrogen (total NH_3 and NO_3^-) were estimated between 4 and 40 days. Nitrogen is the primary limiting nutrient to the biodegradation of PHCs in cold regions and therefore determining its flux is important for assessing the stability of nutrient release from CRN materials under freeze--thaw. This timeframe minimised biases associated with the initial hydration of CRN granules (less than four days) [15] and the termination of nutrient release associated with a zero concentration difference between the CRN granules and the receiving solution (more than 40 days) [16].

Water potential was determined to guide the application of CRN materials to soil and groundwater systems [23]. Water potential (φw) was calculated as:

$$\varphi w = \varphi s + \varphi p \qquad (1)$$

where φs is the solute potential and φp is the pressure potential. The solute potential was determined by the van't Hoff equation:

$$\varphi s = -iCRT \qquad (2)$$

where i is the number of ions present per formula unit in water, C is the molar concentration, R is the universal gas constant and T is the temperature (K). As pressure potential takes no explicit account of the effect of freezing on the total energy change in an open container, it is inferred that φp in all samples is equal to zero [23]. As CRN materials were examined at saturation, soil water potential (MPa) can be determined from total inorganic nitrogen values [11].

2.4 Analysis of nutrients in solution

Water samples were acidified with 1 % nitric acid and analysed for potassium (K^+) and calcium (Ca^{2+}) using a Varian 720-ES inductively coupled plasma-optical emission spectrometry (ICP-OES) [18, 19]. Calcium was only measured in Zeopro™ and Zeopro™-GAC samples as nutrient release is regulated by dissolution of the

calcium phosphate coating [18]. Solutions were filtered (0.45 µm) prior to analysis by ICP-OES.

Total ammonia (NH_3 and NH_4^+) was analysed by colorimetry using the salicylate method [18, 24]. Samples were measured at 660 nm using UV-vis spectrophotometry (Cary, Agilent). Water samples were analysed for nitrate (NO_3^{--}), phosphate (PO_4^{3--}) and sulphate (SO_4^{2--}) using ICS-1000 ion chromatography with an IonPac® AS14 anion-exchange column (Dionex™). NO_3^- and SO_4^{2-} are not impregnated within the Zeopro™ lattice and therefore were not measured in Zeopro™ and Zeopro™-GAC samples.

2.5 Freeze--thaw particle analysis

CRN and GAC particle imaging was conducted using field emission scanning electron microscopy (FESEM) (Philips XL30 FEG, Melbourne Advanced Microscopy Facility, The University of Melbourne) [19]. Multiple granules were fixed to 12 mm Ø mounts and sputter coated with gold for 2.5 min (Dynavac).

Particle size analysis of CRN materials and GAC exposed to freeze--thaw was conducted by sieving [25]. A 1 g sample was shaken in a sieve stack of decreasing mesh sieve (4000, 2360, 1700, 850, 600, 300 and 106 µm) for 10 min (Endecotts). Sieving of control samples was also undertaken for comparison with freeze--thaw samples between 0 and 50 days. The particle size distribution was calculated as the amount of material passing (wt. %) each sieve as a cumulative procedure [25].

2.6 Statistical analysis

Paired two-sample *t*-tests were conducted to compare potential differences in nutrient release under freeze--thaw and control conditions (Microsoft Excel). Freeze--thaw and control sample means were compared at 50 days across all nutrients analysed. Additionally, paired two-sample *t*-tests were applied to compare nutrient release from Zeopro™ and Zeopro™-GAC samples at 50 days. Where no significant difference in nutrient release between freeze--thaw and control samples was observed, this indicated that the CRN material could successfully recommence the release of nutrients in a controlled manner following exposure to physical stresses induced by freezing.

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3. Results

Osmocote™

Osmocote™ samples exposed to control conditions at 4 °C demonstrated higher nutrient release than samples exposed to freeze--thaw across 50 days. The release of total NH_3 was found to be significantly higher (t = --6.94, p = 0.01) in control samples than freeze--thaw samples (Fig. 1a). Total NH_3 release in control samples was particularly pronounced following 20 days, rising to 56.04 mmol/L at 50 days (Fig. 1a). Similarly the release of NO_3^{--} from control samples spiked following 20 days, however was not found to be significantly different between freeze--thaw and control samples (t = --1.86, p = 0.10) (Fig. 1a). Total nitrogen flux was estimated at 0.603 mmol/g Osmocote™ per day in freeze--thaw samples and 2.018 mmol/g Osmocote™ per day in control samples (Table 2). Water potential, as calculated from total nitrogen flux, was --0.027 MPa in freeze--thaw samples and --0.093 MPa in control samples (Table 2). Sample pH was recorded in the range suitable for bioremediation, increasing from 5.1 to 5.6 across 50 days under freeze--thaw while control samples increased from 5.0 to 6.1 [8].

Release of PO_4^{3--} was found to be significantly higher (t = --16.01, p = 0.002) in control samples than in freeze--

thaw samples (Fig. 1b). Similar to nitrogen, PO_4^{3--} release in control samples was accelerated following 20 days to a concentration of 13.31 mmol/L at 50 days while freeze--thaw samples did not exceed 3 mmol/L at 50 days (Fig. 1b). The release of SO_4^{2--} (t = --17.61, p = 0.001) and K^+ (t = --3.95, p = 0.03) was also found to be significantly higher in control samples than in freeze--thaw samples (Fig. 1b and c). These results suggest that the diffusion of all nutrients from Osmocote™ may be hindered by freeze--thaw processes.

FESEM and sieving indicated that the surface structure and grain size of Osmocote™ was unchanged following exposure to freeze--thaw cycling (Figs. 2a and b and 3a).

3.2 Nutricote™

Nutrient release from Nutricote™ was largely similar between freeze--thaw and control samples across 50 days. A pH range of 5.8 to 6.4 in freeze--thaw and control samples was observed. These pH values demonstrate that a more suitable pH range for microbial respiration was achieved using Nutricote™, when compared with the pH range for Osmocote™.

Release of total NH_3 under freeze--thaw occurred rapidly within the first 15 days, slowing to a final concentration of 20.13 mmol/L at 50 days (Fig. 4a). Control samples generated a similar release pattern (t = 1.55, p = 0.13) to a final concentration of 15.98 mmol/L at 50 days (Fig. 4a). Freeze--thaw and control sample NO_3^- values were also analogous across the 50 days (t = 2.98, p = 0.25) (Fig. 4a). Total nitrogen flux from freeze--thaw samples was estimated at 0.858 mmol/g Nutricote™ per day while the flux from control samples was estimated at 0.606 mmol/g Nutricote™ per day (Table 2). Water potential was --0.039 MPa in freeze--thaw samples and --0.028 MPa in control samples (Table 2).

Freeze--thaw cycling was not shown to significantly increase PO_4^{3--} release rates above control sample values (t = 1.82, p = 0.11). PO_4^{3--} concentrations remained <5 mmol/L under freeze--thaw and control conditions across the course of the experiment (Fig. 4b). SO_4^{2--} release in freeze--thaw samples was observed to be double that of control samples (t = 3.96, p = 0.03). No significant difference in the release of K^+ from freeze--thaw and control samples (t = 0.94, p = 0.22) was observed, with the pattern of release similar to that of PO_4^{3--} (Fig. 4c).

FESEM revealed the development of pores (~0.5-2 µm Ø) in the Nutricote™ coating under freeze--thaw at 50 days (Fig. 2c and d). Similar to Osmocote™, the retention of Nutricote™ in a 2360 µm sieve confirmed that these pores did not disrupt physical integrity (Fig. 3a).

3.3 Polyon™

Similar to Nutricote™, total NH_3 release from Polyon™ was not found to be significantly different between freeze--thaw and control samples at 50 days (t = 0.85, p = 0.24). While freeze--thaw samples demonstrated accelerated NO_3^{--} release following 20 days, the large variability of this data suggests that freeze--thaw may not have had a significant effect on release (t = 1.97, p = 0.09) (Fig. 5a). Similar to Nutricote™, NO_3^{--} was shown to be the main nutrient constituent of Polyon™. Total nitrogen flux was 0.813 mmol/g Polyon™ per day and 0.494 mmol/g Polyon™ per day from freeze--thaw and control samples, respectively (Table 2). Water potential was --0.037 MPa in freeze--thaw samples and --0.023 MPa in control samples (Table 2). Freeze--thaw and control sample pH were in the range of 5.5-6.2.

Release of PO_4^{3--} remained <4 mmol/L in both freeze--thaw and control samples (t = 1.34, p = 0.15) (Fig. 5b). Similar to Nutricote™, SO_4^{2--} (t = 6.65, p = 0.01) and K^+ (t = 4.61, p = 0.02) release was significantly higher

when exposed to freeze--thaw cycling (t = 6.65, p = 0.01) (Fig. 5b and c). Although SO_4^{2--} and K^+ concentrations were higher in freeze--thaw samples, the Polyon™ surface did not show cracking or disintegration between 0 and 50 days (Fig. 2e and f). The particle size of Polyon™ was shown to be unchanged across 50 days (Fig. 3a).

From the results observed, it is important to state that the weight ratio of nutrients for encapsulated CRN materials Osmocote™, Nutricote™ and Polyon™ will likely change as the nutrient core is depleted (Table 1). This has important implications for the nitrogen flux, CRN material integrity and longevity of nutrient release to PHC degraders. These factors are explored in recent works [19].

3.4 Zeopro™

Zeopro™ demonstrated a near neutral pH across the experiment (6.7--7.5) with significantly lower nutrient release rates than in Osmocote™, Nutricote™ and Polyon™. This can be attributed to the low solubility of calcium phosphate in the deionised solution and the proportion of exchangeable nutrients within the Zeopro™ lattice (Table 1). Total NH_3 remained <0.01 mmol/L, with release rates similar across freeze--thaw and control samples (t = --2.61, p = 0.06) (Fig. 6a). Total nitrogen flux was <0.001 mmol/g Zeopro™ per day across freeze--thaw and control samples (Table 2). As a result, water potential was below --0.001 MPa in freeze--thaw and control samples (Table 2). The release of K^+ was not shown to be elevated following exposure to freeze--thaw (t = --1.66, p = 0.12) (Fig. 6a).

PO_4^{3--} concentrations increased steadily in freeze--thaw and control samples to 0.005 mmol/L at 50 days (Fig. 6b). Release of Ca^{2+} was found to be significantly higher (t = --7.71, p = <0.01) in control samples than in freeze--thaw samples. Ca^{2+} in control samples rose steeply following 20 days to 0.068 mmol/L at 50 days (Fig. 6b). Interestingly, despite Zeopro™ comprising a calcium phosphate coating, PO_4^{3--} concentrations were significantly lower than Ca^{2+} concentrations in freeze--thaw and control samples (Fig. 6b). A 30 % increase in the <1000 µm sieved fraction of Zeopro™ was recorded following 30 days, suggesting minor break-up with exposure to freeze--thaw (Fig. 3b). Imaging of Zeopro™ can be found in previous literature [22].

3.5 Zeopro™-GAC

The presence of GAC was shown to have a strong influence on the nutrient release and ion exchange properties of Zeopro™ (Fig. 7a). Zeopro™-GAC samples recorded a pH of <3 under freeze--thaw and control conditions. At 50 days, Ca^{2+} concentrations between 2.7--3.2 mmol/L in the Zeopro™-GAC samples suggest that GAC had a significant influence on Ca^{2+} release from Zeopro™ (t = --27.95, p = <0.01) (Fig. 7b).

PO_4^{3--} concentrations exceeding 20 mmol/L in all samples can be attributed to the chemical treatment of GAC with phosphoric acid and the subsequent release of these ions upon contact with deionised water (Fig. 7b). The pronounced and variable influence of GAC on PO_4^{3--} concentrations in solution prevented accurate determination of PO_4^{3--} release from Zeopro™ in the presence of GAC.

In Zeopro™-GAC samples, total NH_3 concentrations were stable in freeze--thaw and control solutions over 50 days (Fig. 7a). Total NH_3 in all samples was in the range of 0.03 to 0.04 mmol/L (Fig. 7b). The total NH_3 in solution produced a water potential of --0.005 MPa in freeze--thaw samples and --0.006 MPa in control samples (Table 2). Significantly higher concentrations of total NH_3 were also observed under freeze--thaw (t = --21.84, p = <0.01) and control conditions (t = --4.81, p = 0.02) when compared with Zeopro™ samples without GAC

(Fig. 6a). Concentrations of K^+ in Zeopro™-GAC samples were >0.1 mmol/L after one day with concentrations of 0.151 and 0.230 mmol/L in freeze--thaw and control samples at 50 days, respectively (Fig. 7b). The unchanging total NH_3 and K^+ values across 50 days, and the heightened nutrient concentrations when compared with Zeopro™ alone, suggest that Zeopro™ was rapidly depleted of nutrients following exposure to the impurities released from GAC.

FESEM revealed disintegration of GAC under freeze--thaw (Fig. 2g and h). Sieving of GAC resulted in 9.8 % of GAC passing the 106 µm sieve at 50 days compared with <1 % passing the 106 µm sieve in control samples at 50 days (Fig. 3c). The Zeopro™ grain size was unchanged in the presence and absence of GAC (Fig. 3b).

4. Discussion

The rapid release of nitrogen from Osmocote™ following 20 days suggests a lag period in release followed by accelerated diffusion through the particle coating, as has been reported elsewhere [20, 21, 26, 27]. This accelerated diffusion from Osmocote™ is reflected in a high negative water potential that may invoke osmotic stress on PHC degraders [12, 28]. However, the high microbial respiration measured at -0.98 MPa in Macquarie Island soils suggests that Osmocote™ may be suitable where the tolerance of microorganisms aligns with the nitrogen flux from the CRN material [11, 13]. Lower nitrogen release from Osmocote™ under freeze--thaw may be associated with the influence of ice, resulting in low diffusion across large portions of the experimental timeframe [23]. Limited PO_4^{3-} delivery from Osmocote™ may be associated with the slow dissolution of calcium phosphate in water, comprising a large proportion of the total phosphate from Osmocote™ [20, 29].

The exposure of Osmocote™ granules to freeze--thaw was shown to constrain nutrient release, suggesting that the longevity of nutrient release may be heightened in areas of freezing ground. In agreement with previous studies however, control sample data suggests that the accelerated release of nutrients from Osmocote™ may occur with exposure to high soil moisture, as would occur during summer melt periods [20].

Nutricote™ and Polyon™ demonstrated steady nutrient release rates across freeze--thaw and control temperature ranges. These results show that Nutricote™ and Polyon™ demonstrate a higher resistance to fluctuations in temperature than in Osmocote™ [21]. Ongoing release from Polyon™ under freeze--thaw and control conditions opposed reports of cessation in nutrient release below 5 °C [27]. Freeze--thaw and control sample water potentials proximal to --0.01 MPa, coupled with the reported longevity of release at 4 °C [19], support the suitability of Nutricote™ and Polyon™ for cold region bioremediation [12].

The ability of Osmocote™, Nutricote™ and Polyon™ to maintain particle structure and size suggests that these CRN materials may provide controlled and sustained nutrient release for bioremediation in cold regions. However, high nitrogen values from Osmocote™ suggest that nutrient release across extended timeframes is unlikely to be sustained; as has been observed from similar polymeric resin coated materials in Antarctic PRBs [18]. As nitrogen is commonly the limiting nutrient for the natural attenuation of PHCs [6, 28], the selection of Osmocote™ may enforce costly, repeat treatments to address the extended timescales required for bioremediation at low temperatures. Alternatively, while Nutricote™ and Polyon™ remain untested in cold regions, the observed consistency in nutrient release and resistance to disintegration offers promise for delivering a defined nutrient concentration to promote bioremediation [14]. However, over long time periods at saturation, concaving and collapse of the polymer shells has been observed, generated by dissolution of the fertiliser core [19].

The slow release of total NH_3 and K^+ from active exchange sites suggests that Zeopro^{TM} can effectively deliver nutrients required for bioremediation in cold regions [19, 30]. Higher concentrations of K^+ in solution, compared to total NH_3, can be attributed to the exchangeable concentrations in the zeolite (Table 1), and the increased affinity of NH_4^+ on zeolite at low temperatures [31]. With ~12 freeze--thaw cycles per year in the top 30 cm of Antarctic soil (S. Ferguson, Australian Antarctic Division, unpublished observations), ongoing release of total NH_3 and K^+ at 100 freeze--thaw cycles demonstrates the long-term suitability of Zeopro^{TM} to promote PHC degradation. However, it must be noted that while this experiment was conducted in deionised water, cations in groundwater and particle colonisation by microorganisms may alter the release rates of total NH_3 and K^+ from Zeopro^{TM} [32].

The total NH_3 and K^+ values reported for Zeopro^{TM} can be largely attributed to ion exchange with Ca^{2+}, available through dissolution of the calcium phosphate coating [18]. The higher Ca^{2+} release reported in Zeopro^{TM} control samples may be associated with the longer timeframes the particles spent in the aqueous phase, as reported for Osmocote^{TM}, compared to freeze--thaw samples. Significantly higher concentrations of Ca^{2+} to concentrations of PO_4^{3-} in untested Zeopro^{TM} suggests that other Ca^{2+} bearing minerals may be present on the Zeopro^{TM} surface. Ca^{2+} bearing minerals such as calcite on Zeopro^{TM} can inhibit the dissolution of calcium phosphate [30, 33]. This common ion effect has shown to result in low PO_4^{3--} release from Zeopro^{TM} [33], evident in freeze--thaw and control samples, and may limit the delivery of PO_4^{3--} to PHC degraders. Partial disintegration of Zeopro^{TM} compares with an increase in the proportion of size fractions up to 500--1000 µm in Antarctic PRBs [34].

The heightened release of Ca^{2+} from Zeopro^{TM} in the presence of GAC can be attributed to the release of phosphoric acid and resulting low pH [19]. Increased Ca^{2+} concentrations resulted in accelerated ion exchange with total NH_3 and K^+, resulting in complete exclusion of total NH_3 and K^+ from the Zeopro^{TM} pores. High total NH_3 and K^+ concentrations in freeze--thaw and control samples suggest that exposure to low pH environments may significantly affect the timeframe of release from nutrient amended zeolites. As natural attenuation is often not a feasible approach towards the management of PHC contaminated sites in cold regions [5, 6], sustained nutrient release is critical for achieving bioremediation targets.

The observed break-up of GAC under freeze--thaw has significant implications for the hydraulic performance of passive groundwater remediation technologies [34--36]. Additionally, release of chemical activation products such as phosphoric acid from GAC may introduce secondary contaminants and disrupt microbial communities in soils and groundwater.

5. Concluding Remarks

The effectiveness of CRN materials for bioremediation is dependent upon their ability to deliver a sufficient concentration of nutrient to PHC degraders over an extended period of time. Osmocote^{TM} demonstrated high and uncontrolled nitrogen release, revealing a lower suitability to cold region bioremediation. Alternatively Nutricote^{TM} and Polyon^{TM} were shown to deliver nutrients in a controlled manner with largely overlapping release patterns under freeze--thaw and control conditions. The exchange of Ca^{2+} with total NH_3 and K^+ in the Zeopro^{TM} pores produced consistent nutrient release across the experimental timeframe. Where Zeopro^{TM} and GAC were contacted, heightened calcium phosphate dissolution resulted in depletion of total NH_3 and K^+ from Zeopro^{TM}. Freeze--thaw induced significant particle size reduction of the GAC. These findings provide guidance

for CRN material selection and implementation for bioremediation of PHC contaminated soils and groundwater in cold regions.

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Figure 1 The nutrient release profile of Osmocote™. Total ammonia and nitrate release from freeze--thaw and control samples (a). Phosphate and sulphate release from freeze--thaw and control samples (b). Potassium release from freeze--thaw and control samples (c). Error bars represent ± standard deviation.

Figure 2 FESEM micrographs of CRN materials under freeze--thaw cycling. Osmocote™ surface at 0 days (a) and 50 days (b). Nutricote™ surface at 0 days (c) and 50 days (d); Polyon™ surface at 0 days (e) and 50 days (f); GAC particles at 0 days (g) and 50 days (h). The white arrows (h) indicate the fracturing of GAC under freeze--thaw. Imaging of Zeopro™ has been conducted previously [22].

Figure 3 Particle size analysis of Osmocote™, Nutricote™ and Polyon™ under freeze--thaw cycling and control conditions at 50 days (a). Particle size analysis of Zeopro™ at 4, 14, 30 and 50 days under freeze--thaw cycling, and Zeopro™ at 50 days under control conditions for comparison (b). Particle size analysis of GAC at 4, 14, 30 and 50 days under freeze--thaw cycling, and GAC at 50 days under control conditions for comparison (c). Note: Limited sample volumes for particle sizing prevented replicate analysis.

Figure 4 The nutrient release profile of Nutricote™. Total ammonia and nitrate release from freeze--thaw and control samples (a). Phosphate and sulphate release from freeze--thaw and control samples (b). Potassium release from freeze--thaw and control samples (c). Error bars represent ± standard deviation.

Figure 5 The nutrient release profile of Polyon™. Total ammonia and nitrate release from freeze--thaw and control samples (a). Phosphate and sulphate release from freeze--thaw and control samples (b). Potassium release from freeze--thaw and control samples (c). Error bars represent ± standard deviation.

Figure 6 The nutrient release profile of Zeopro™. Total ammonia release from freeze--thaw and control samples (a). Phosphate and sulphate release from freeze--thaw and control samples (b). Potassium and calcium

release from freeze--thaw and control samples. Note: Nitrate and sulphate are not impregnated within the Zeopro™ lattice. Error bars represent ± standard deviation.

Figure 7 The nutrient release profile of Zeopro™ in the presence of GAC. Total ammonia and potassium release from freeze--thaw and control samples (a). Phosphate and calcium release from freeze--thaw and control samples (b) Note: *y*-Axes are not aligned due to large concentration differences and to aid data interpretation. Error bars represent ± standard deviation.

Table 1 Characteristics of CRN materials

Material	Particle chemistry	(NO_3^-, total NH_3/P/K/S/Ca) (wt. %)	Previ
Osmocote™ (Scotts Australia, Australia)	Organic resin coating (dicyclopentadiene and glycerol ester).	1.2, 1.1/0.7/2.4/3.7/0.2	
Nutricote™ (JCAM AGRI, Japan)	Thermoplastic resin coating (polyolefin and copolymers) and ethylene-vinyl acetate.	5.2, 7.8/4.8/9.1/5.0/--	Unass
Polyon™ (J.R Simplot, USA)	Ultrathin polyurethane membrane coating.	8.8, 7.2/6.0/11.0/6.0/--	Unass
Zeopro™ H (ZeoponiX, USA)	Calcium phosphate coating ($Ca_{10}(PO_4)_6(OH)_2$) (<10 %) a natural clinoptilolite ore (>90 %) impregnated with ammonium and potassium.	--, 0.1/0.1/0.6/--/0.2	

Nitrate and sulphate are not present in Zeopro™

Table 2 Flux of total nitrogen and water potentials from CRN materials under freeze--thaw and control conditions.

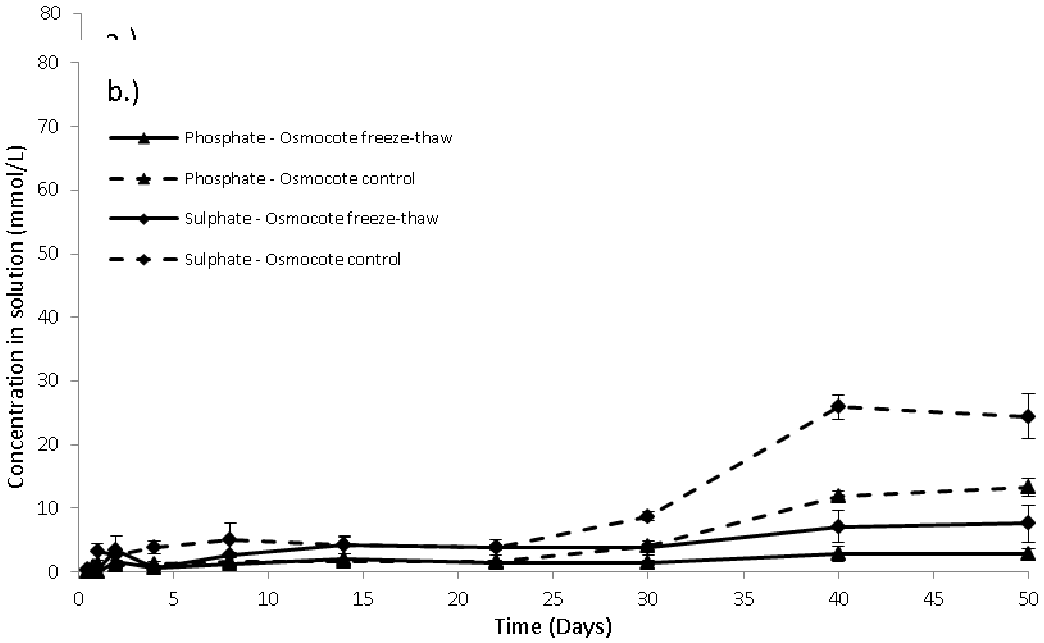
CRN material	Total N (mmol/g CRN per day)	Water potential (MPa)
Osmocote™		
Freeze--thaw	0.603	--0.027
Control	2.018	--0.093
Nutricote™		
Freeze--thaw	0.858	--0.039
Control	0.606	--0.028
Polyon™		
Freeze--thaw	0.813	--0.037
Control	0.494	--0.023
Zeopro™		
Freeze--thaw	0.000057	--0.0000013
Control	0.000082	--0.0000019
Zeopro™-GAC		

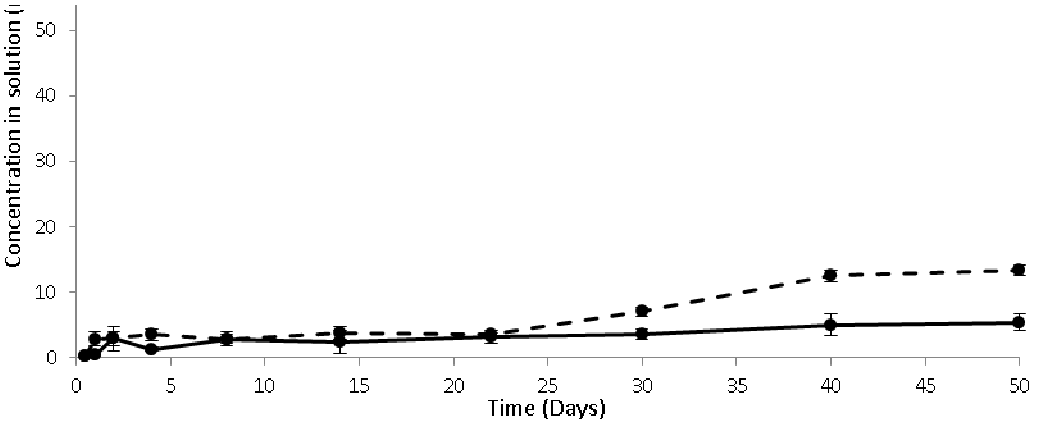
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Freeze--thaw	0.000215	--0.004878
Control	0.000268	--0.006169

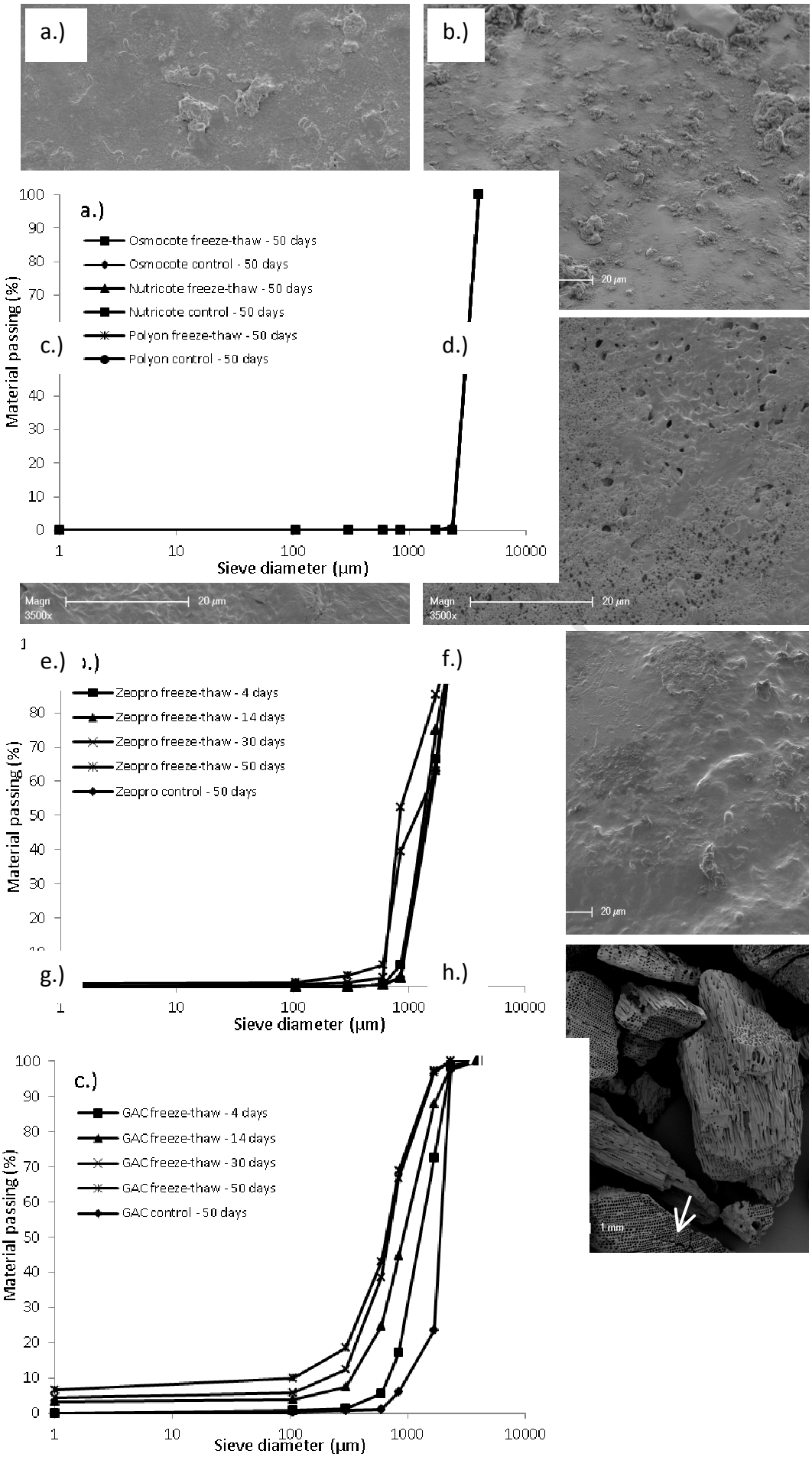
Nitrate did not contribute to the total nitrogen flux from Zeopro™ and Zeopro™-GAC samples.

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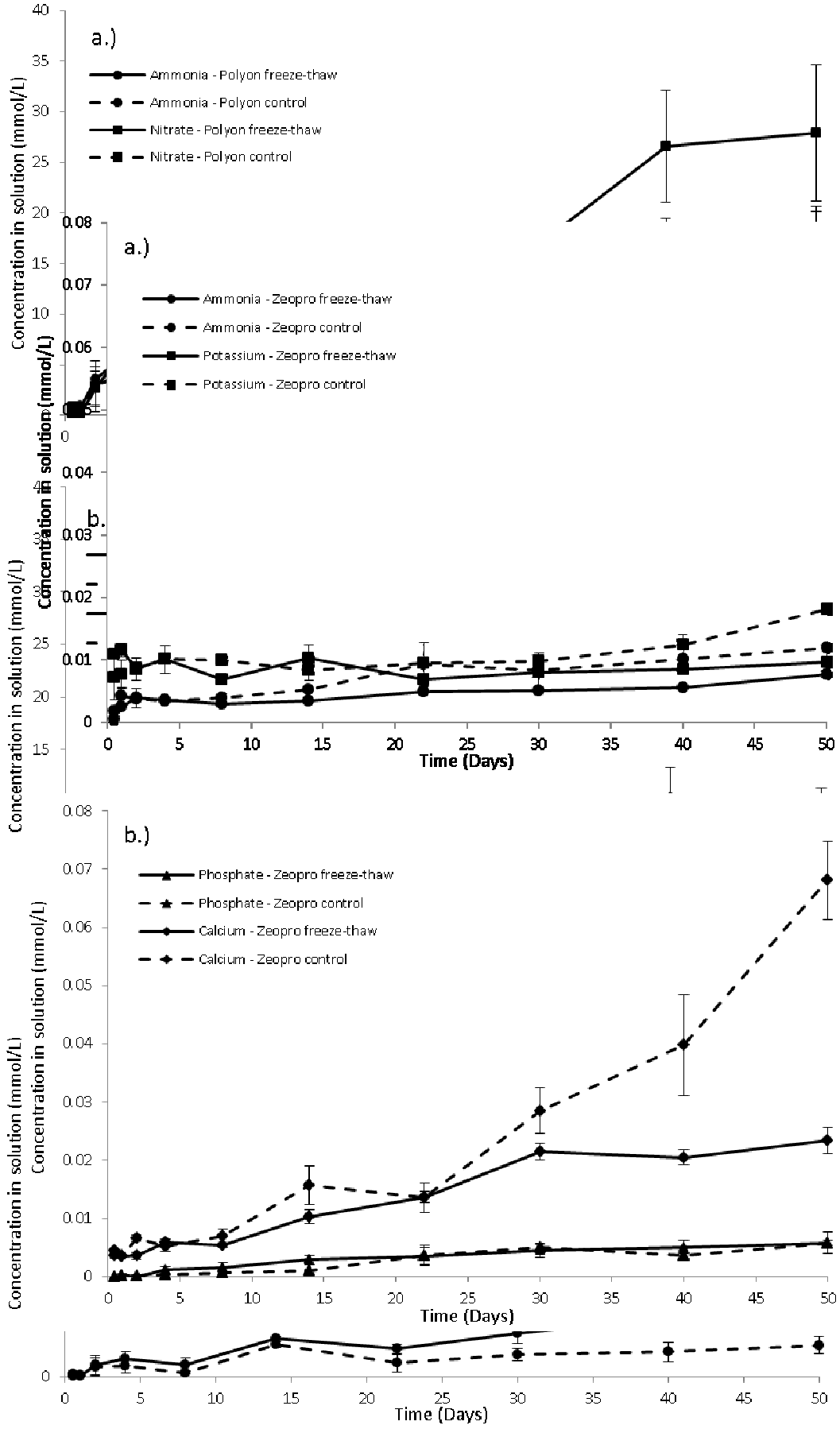

b.)
Phosphate - Osmocote freeze-thaw
Phosphate - Osmocote control
Sulphate - Osmocote freeze-thaw
Sulphate - Osmocote control
Concentration in solution (mmol/L)
Time (Days)

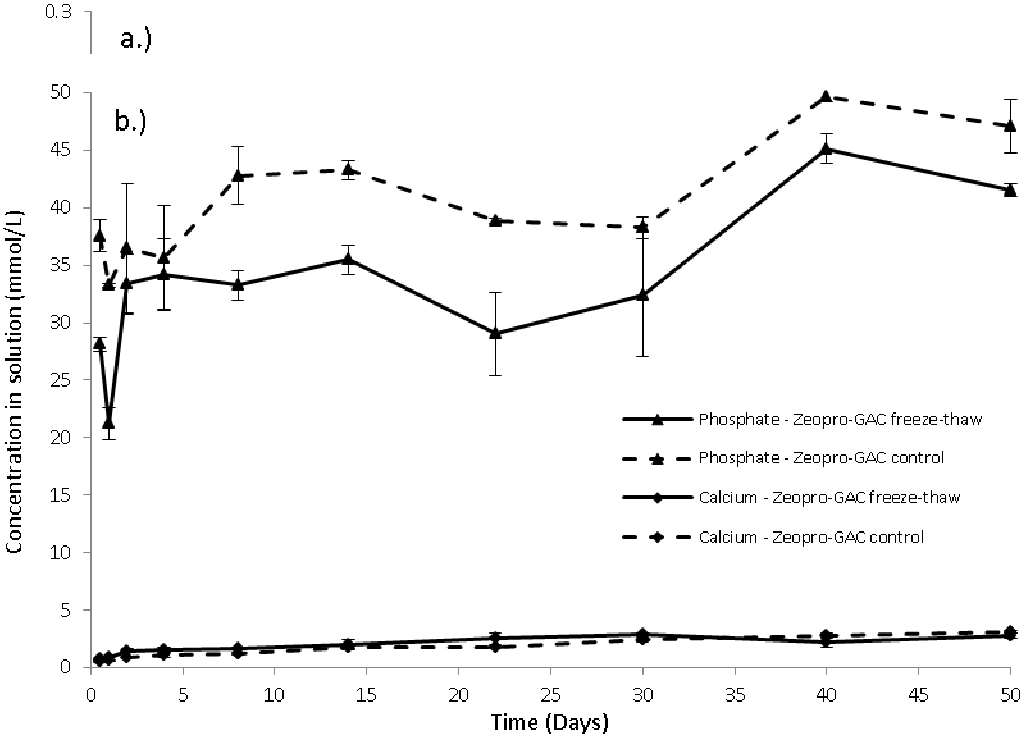

Time (Days)

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a.)
b.)
a.)
Osmocote freeze-thaw - 50 days
Osmocote control - 50 days
Nutricote freeze-thaw - 50 days
Nutricote control - 50 days
Polyon freeze-thaw - 50 days
Polyon control - 50 days
Material passing (%)
Sieve diameter (µm)
c.)
d.)
20 µm
Magn
3500x
e.)
f.)
Zeopro freeze-thaw - 4 days
Zeopro freeze-thaw - 14 days
Zeopro freeze-thaw - 30 days
Zeopro freeze-thaw - 50 days
Zeopro control - 50 days
g.)
h.)
1 mm
c.)
GAC freeze-thaw - 4 days
GAC freeze-thaw - 14 days
GAC freeze-thaw - 30 days
GAC freeze-thaw - 50 days
GAC control - 50 days

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a.)
Ammonia - Polyon freeze-thaw
Ammonia - Polyon control
Nitrate - Polyon freeze-thaw
Nitrate - Polyon control
Concentration in solution (mmol/L)
a.)
Ammonia - Zeopro freeze-thaw
Ammonia - Zeopro control
Potassium - Zeopro freeze-thaw
Potassium - Zeopro control
Concentration in solution (mmol/L)
b.
Concentration in solution (mmol/L)
Time (Days)
b.)
Phosphate - Zeopro freeze-thaw
Phosphate - Zeopro control
Calcium - Zeopro freeze-thaw
Calcium - Zeopro control
Concentration in solution (mmol/L)
Time (Days)
Time (Days)



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