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Perchlorate and Agriculture on Mars

Christopher Oze [1,*], Joshua Beisel [2], Edward Dabsys [1], Jacqueline Dall [1], Gretchen North [2], Allan Scott [3], Alandra Marie Lopez [4], Randall Holmes [4] and Scott Fendorf [4]

1 Geology Department, Occidental College, 1600 Campus Rd., Los Angeles, CA 90041, USA; edabsys@oxy.edu (E.D.); jdall@oxy.edu (J.D.)
2 Biology Department, Occidental College, 1600 Campus Rd., Los Angeles, CA 90041, USA; jbeisel@oxy.edu (J.B.); gnorth@oxy.edu (G.N.)
3 Department of Civil Engineering, University of Canterbury, Private Bag 4800, Christchurch 8041, New Zealand; allan.scott@canterbury.ac.nz
4 Earth System Science, Stanford University, Stanford, CA 94305, USA; amlopez@stanford.edu (A.M.L.); randall.t.holmes@gmail.com (R.H.); fendorf@stanford.edu (S.F.)
* Correspondence: coze@oxy.edu

Abstract: Perchlorate (ClO_4^-) is globally enriched in Martian regolith at levels commonly toxic to plants. Consequently, perchlorate in Martian regolith presents an obstacle to developing agriculture on Mars. Here, we assess the effect of perchlorate at different concentrations on plant growth and germination, as well as metal release in a simulated Gusev Crater regolith and generic potting soil. The presence of perchlorate was uniformly detrimental to plant growth regardless of growing medium. Plants in potting soil were able to germinate in 1 wt.% perchlorate; however, these plants showed restricted growth and decreased leaf area and biomass. Some plants were able to germinate in regolith simulant without perchlorate; however, they showed reduced growth. In Martian regolith simulant, the presence of perchlorate prevented germination across all plant treatments. Soil column flow-through experiments of perchlorate-containing Martian regolith simulant and potting soil were unable to completely remove perchlorate despite its high solubility. Additionally, perchlorate present in the simulant increased metal/phosphorous release, which may also affect plant growth and biochemistry. Our results support that perchlorate may modify metal availability to such an extent that, even with the successful removal of perchlorate, Martian regolith may continue to be toxic to plant life. Overall, our study demonstrates that the presence of perchlorate in Martian regolith provides a significant challenge in its use as an agricultural substrate and that further steps, such as restricted metal availability and nutrient enrichment, are necessary to make it a viable growing substrate.

Keywords: perchlorate; Martian regolith; plant growth; metal bioavailability

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1. Introduction

Human habitation of Mars will have to prioritize and optimize shipments to provide resources for life, inclusive of food, water, and shelter. Rather than food delivery from Earth to Mars, one strategy is to produce food on Mars. As no life has been identified on Mars, plant seeds will need to be transported from Earth for food production. While plant seeds provide favorable payloads, seed germination and plant growth require either soils from Earth and/or hydroponic equipment, which will use significant space and increase infrastructure costs. One practical way to solve this agricultural problem is to utilize Martian regolith, as it is derived from rocks similar to those on Earth. Using Martian regolith would allow increased agricultural expansion in environmentally controlled spaces such as prebuilt structures or sealed/modified lava tubes [1–3]. Despite the potential of Martian regolith as a growing medium, Mars' planetary history has created toxic conditions that need to be addressed if food production is going to be successful.

No matter where landers and rovers have explored the surface of Mars, perchlorate (ClO_4^-) has been identified in the regolith [4,5]. Concentrations of perchlorate vary spatially and with depth, with values as high as 2 wt.% [6]. Perchlorate is detrimental to plant growth and a concern in contaminated soils [7–9] and an overview of perchlorate in soils, water, and food has been provided by Kumarathilaka et al. [10]. Perchlorate formation on Mars may have resulted from atmospheric chemical reactions of chlorine compounds with photochemically produced ozone [11–14] or additional non-gas phase processes, such as heterogenous reactions [15]. Following its generation and deposition on the surface, perchlorate interactions with regolith are capable of forming Mg− and Na− perchlorate salts in a process that has been occurring for at least 3 billion years [5,11]. With atmospheric circulation and wind transport of sediments, perchlorate salts are hypothesized to be present over the entire surface of Mars [16–18]. This presents a global problem to future human habitation of Mars.

Recent studies have begun assessing agriculture on Mars and the results have been varied. Martian regolith simulants, such as the Johnson Space Center (JSC-Mars-1A) simulant and Mojave Mars Simulant (MMS), have been shown to support plant growth with nutritional supplements, while Mars Global Simulant (MGS-1) was shown to not germinate even with added nutrients, and, even with nutritional supplements, the addition of Ca-perchlorate ($Ca(ClO_4)_2$) (2% w/v) or Ca-perchlorate tetrahydrate ($Ca(ClO_4)_2{\cdot}4H_2O$) (2% w/v) did not support plant growth [19]. Previously, Wamelink et al. [20] showed germination and plant growth using simulant JSC-Mars-1A, including nitrogen fixing plants; however, perchlorate was not included in the experiments. Moreover, addition of organic residues increased yields for *Lactuca sativa* L. var. *capitata* in MMS [21]. In a recent agricultural study [19], Martian regolith simulant and high concentrations of perchlorate (2 wt.%) were both determined to be detrimental to plant growth. Plant response to different and lower levels of perchlorate in Martian regolith requires further study. Additionally, how perchlorate influences the geochemistry of the regolith as well as how to potentially remove it needs to be addressed. Here, we present plant response to Martian perchlorate levels in both potting soil (as a control) and in simulated Martian regolith representative of the Gusev Crater on Mars. Additionally, we provide new geochemical evidence on how perchlorate may influence and affect the geochemistry of Martian regolith by increasing the release rates and bioavailability of metals and nutrients, such as phosphorous.

2. Materials and Methods

2.1. Regolith, Control Soil, and Additions

Synthesizing a targeted Martian simulant at volumes applicable for limited agricultural studies has been a constraint and limitation in previous studies. Here, we synthesized a regolith analogue representative of the Gusev Crater (i.e., located near the Columbia Hills region on Mars) via crushing, sieving, washing, and blending of New Zealand basalts and volcanic glass to reproduce the site's particle size, chemistry, and mineralogy (see Scott et al., 2017). A potting soil (Kellogg Garden Organics, consisting of recycled forest products, composted materials, and oyster shells plus lime to adjust pH with no additional fertilizer) acted as a control and was included to determine the effects of perchlorate on the plant treatments independently of other physical and geochemical properties of the Martian regolith simulant. Perchlorate was added to the Martian simulant and control potting soil prior to planting the seeds. Magnesium perchlorate ($MgClO_4$) was used due to its similarity to compounds observed on the Martian surface [22,23]. Perchlorate concentrations (0, 0.5, and 1 wt.%) are noted for each experiment in which these concentrations are lower than those in Eichler et al. [19] at 2 wt.%. Following the plant growth experiments, growing mediums with and without perchlorate were analyzed using the Whole Rock Analysis option by ALS Chemex.

2.2. Species Selection

Two plant species were tested including grain amaranth (*Amaranthus cruentus*, with seeds ~1 mm in length; mean seed mass is 0.008 g) and common bean (*Phaseolus vulgaris*, with seeds 8–10 mm in length; mean seed mass is 0.40 g). Common bean inoculated with *Rhizobium* bacteria was included as a third treatment to test the effect of nitrogen fixation on germination and growth. *Rhizobium* are nitrogen-fixing bacteria that form symbiotic relationships with legumes and allow them to fix nitrogen into the soil [24]. Seeds were sourced from Lake Valley Seed (bean) and Plants of the Southwest (amaranth). Inoculum for the second bean treatment was a blend from Gardens Alive Inc. which included *R. leguminosarum*, *R. phaseoli* and *Bradyrhizobium* sp.

2.3. Germination and Growth Experiment

Potting soil was placed in 12 × 10 × 10 cm pots, whereas, due to limited quantity, Martian regolith simulant was placed in 8 × 6 × 6 cm pots. Plant root growth was not restricted in either pot size. Amaranth pots received 8 seeds, bean pots received 3 seeds and pots were thinned to one representative plant after several weeks of growth. There were five pots per treatment resulting in 90 pots across the entire experiment. Plants were started in a greenhouse at Occidental College and then moved outside to a lightly shaded roof terrace during the second week of growth due to high temperatures in the greenhouse. All pots were well-watered throughout the experiments lasting 8 weeks and were started in June 2018. All pots in all treatments were scored on percent (out of 8 seeds planted for amaranth and 3 seeds for bean) and date of seed germination. Germination was determined by emergence of the cotyledons (seed leaves) above the soil surface. At the conclusion of the experiments, plants were destructively sampled and leaf area, fresh aboveground weight, and dry weight were recorded.

2.4. Biogeochemical Analysis

To assess metal/nutrient release and perchlorate retention within the soil, flow-through experiments of regolith simulant and potting soil samples with (1 wt.%) and without perchlorate were conducted. Filters were used to ensure that solids remained in the tube and were sealed with Teflon tape to prevent any leaks. Once the tubes were sealed, they were suspended, and a pump was used to move ultrapure water through them from below at a constant flow rate (~1.5 mL h^{-1}). The flow rate was based on those observed in similar soils on Earth while accounting for the difference in gravity on Mars [25]. After 8 h of initial flow, the water reached the top of the soil column and sampling was started. Each sample yielded 12–15 mL of solution and across 8 rounds of sampling 250 mL of solution was collected. 1.5 mL of solution for each trial was analyzed for pH. The remainder was stored in a Falcon tube for ion chromatography (IC) using a Dionex ICS 6000 for perchlorate analysis and for metal/nutrient (Na, Ca, Mg, P and K) analysis using an inductively couple plasma mass spectrometer (ICP-MS) Thermo XSeries II at Stanford University. Filters and syringes were changed after every sample to prevent contamination.

3. Results and Discussion

3.1. Germination and Plant Growth

Of the two plant species, only *Phaseolus vulgaris* (common bean) was able to germinate in Martian regolith simulant, and only without added perchlorate (Figure 1). *Amaranthus cruentus* (grain amaranth) failed to germinate in Martian regolith simulant, regardless of perchlorate concentration. Seed germination data were analyzed across all trials by using a germination rate index (GRI) as a composite of both germination rate and percentage of seeds (Figure 2A). Higher GRI values reflect greater, faster germination [26]. Here, GRI is the sum of G2/2 + G4/4 + G6/6 + G8/8 where G2, G4, G6 and G8 ×100 are germination percentages at 2, 4, 6 and 8 days, respectively. Seeds of both species germinated in every treatment in organic potting soil, but to varying degrees. Differences between the two bean treatments, bean and bean plus *Rhizobium* (nitrogen-fixing bacteria) were not significant ($p = 0.099$, t-test). Differences in GRI for amaranth in potting soil across all three perchlorate concentrations were significant (p-value = 0.011, 2-way ANOVA with Bonferroni pairwise testing).

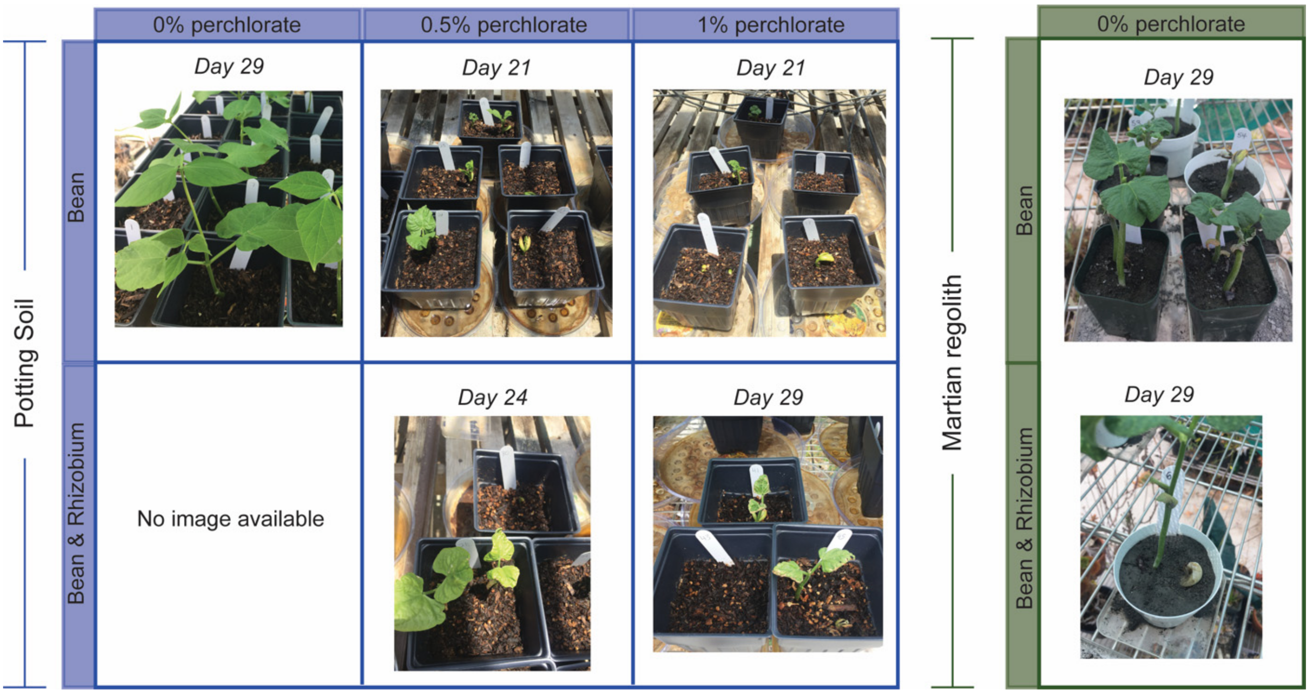

Figure 1. Examples of plant response in Martian regolith simulant and perchlorate during the 29-day growing period. The addition of perchlorate in Martian regolith prohibited plant growth across all treatments, while the addition of perchlorate in organic potting soil hindered but did not prohibit growth. Amaranth and bean plants in Martian regolith simulant with 0.5 and 1 wt.% perchlorate did not grow and amaranth also did not grow in 0% perchlorate in Martian regolith simulant (images are not included).

While germination occurred to some degree across all plants and soil treatments in the potting soil, the presence of perchlorate in the regolith simulant was found to completely inhibit germination. It is likely that there is a compounding effect of perchlorate and soil texture on seed germination in the regolith simulant. In potting soil (i.e., mainly organic matter with oyster shells), both amaranth and bean plants were adversely affected with increasing perchlorate, but most were able to germinate, which is consistent with other terrestrial perchlorate studies [8,27]. There are several possible reasons for the lack of success of amaranth, largely related to its small seed size. At less than 2% of the mass of bean seeds, amaranth seeds were probably more sensitive to the soil compaction and surface crusting that was observed in the regolith simulant after the addition of water. In addition, the low capacity for nutrient storage of amaranth seeds may be another limiting factor. Due to the high solubility of perchlorate, we expected the water to dissolve, mobilize, and remove the perchlorate from the soils; however, the effect of the perchlorate on the plants may not have diminished over the duration of the experiments.

Dry shoot biomass is an indicator of plant productivity and overall health. Plants in potting soil with 0% perchlorate had the highest biomass in both bean treatments (Figure 2B). Plants in potting soil for all perchlorate concentrations, as well as 0% perchlorate in Martian regolith simulant, all had similar values. Biomass was significantly reduced by the presence of perchlorate; however, the higher concentration of 1% perchlorate did not have a more negative effect than 0.5% perchlorate.

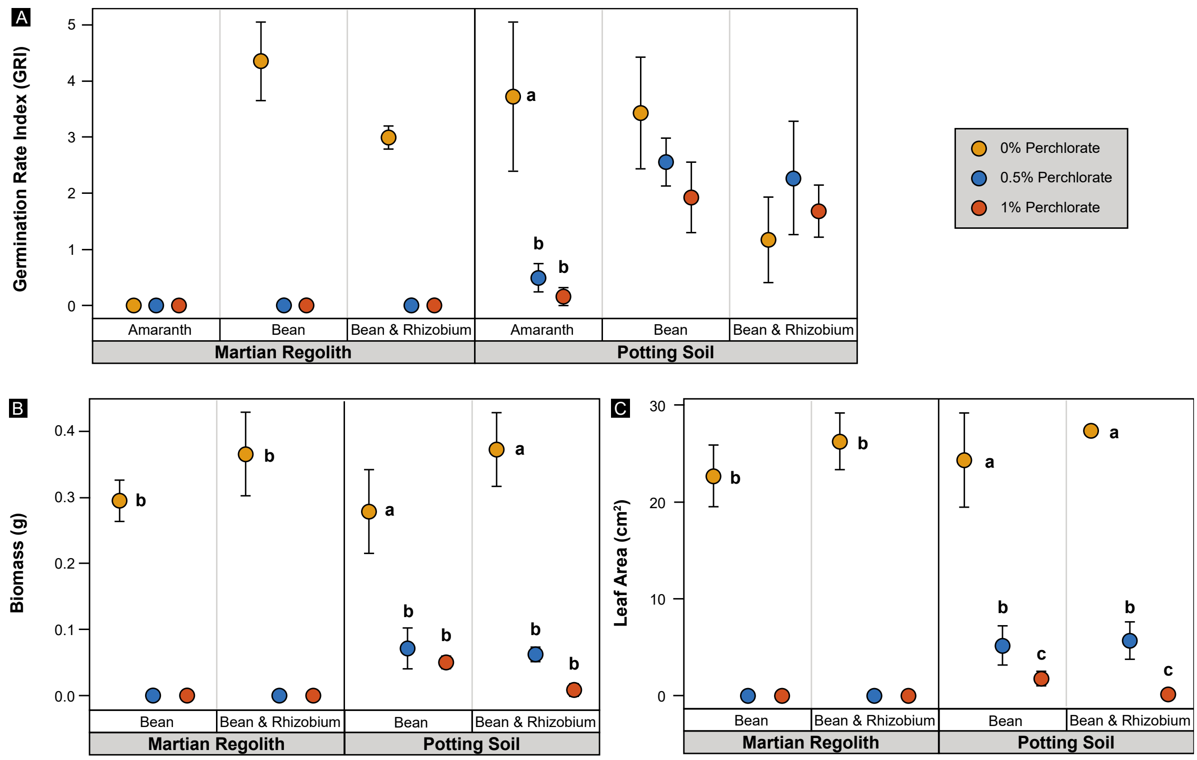

Figure 2. (**A**) Mean GRI value for each treatment; different letters denote a significant difference between perchlorate concentrations within amaranth ($p = 0.011$, 2-way ANOVA, followed by Bonferroni pairwise testing). (**B**) Dry shoot biomass; different letters represent significant differences between groups ($p < 0.001$, 2-way ANOVA, followed by Bonferroni pairwise testing). (**C**) Leaf area per plant; different letters represent a significant difference between groups ($p < 0.001$, 2-way ANOVA with Bonferroni pairwise testing).

Leaf area functions as a proxy for plant growth (Figure 2C). Within the potting soil treatments, the negative effect of perchlorate was significant, with increasing perchlorate resulting in decreasing total leaf area. The only two groups that did not show a significant difference were those in regolith simulant at 0% perchlorate and organic potting soil at 0.5% perchlorate.

The effect of *Rhizobium* on plant growth was difficult to discern; there were no significant differences between the bean plus *Rhizobium* treatment and the bean without inoculant. This may have been due to the limited growing time, as the effects of differences in soil nitrogen may not have been significant over the study period. There were no nodules on the bean roots, indicating that a symbiotic association was not established between the beans and the *Rhizobium.*

Overall, the presence of perchlorate was found to reduce plant productivity. Biomass was significantly reduced by the presence of perchlorate; however, the higher concentration of 1% perchlorate was not observed to have an increased effect compared to 0.5% perchlorate. Leaf area did not have a concentration-dependent relationship with perchlorate.

3.2. Perchlorate and Metal Release in Martian Regolith Simulant

From the plant growth experiments, perchlorate addition led to smaller leaf area. This could either be due to the perchlorate toxicity or indirectly by potentially modifying the release and/or availability of soil nutrients or metals [10]. Comparing organic potting soil and regolith simulant following the plant growth experiments suggests that the perchlorate modified the geochemistry of the regolith simulant (Table 1). No significant geochemical change occurred in the normal potting soils regardless of the introduction of perchlorate compared to the control. However, the Martian regolith simulant samples showed a relative increase in metal concentrations in the samples that included the perchlorate. This suggests that perchlorate is capable of modifying the geochemistry of soils, especially when no organic material is present.

Table 1. Geochemistry and Loss On Ignition (LOI) of simulant/soil with and without perchlorate.

Samples	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O	TiO_2	LOI
	Wt. %								
Martian regolith simulant	57.9	14.7	8.67	4.9	3.91	4.12	3.12	1.48	1.88
Martian regolith simulant(*Perchlorate*)	46.9	15.25	12.55	7.1	6.11	4.43	1.99	2.22	2.85
Potting soil	38.3	8.17	2.69	3.9	1.24	2.04	2.09	0.36	42
Potting soil (*Perchlorate*)	34.9	7.26	2.58	3.68	1.42	1.79	2.49	0.33	45.6

Soil column flow-through experiments support that metal release was enhanced by the presence of the Mg perchlorate salt, but primarily when organic matter was not present such as in the Martian simulant (Figure 3). The addition of Mg perchlorate modified Mg levels; however, no other metals were detectable in the perchlorate salt. Organic potting soil showed no change in metal release when perchlorate was present compared to the control. In the Martian simulant, increased metal leaching was observed when perchlorate was present, as the concentrations of metals released into solution were much higher compared to when there was no perchlorate. This modification of metal release does not appear to be result of changes in acidity as the pH values of soil solutions remained nearly constant (Figure 4). These observations begin to clarify that metal release might be related to a salt effect or oxidative release. With the perchlorate in Martian regolith, osmotic stress may make plant growth difficult. However, perchlorate could play an important role for metal release as it might be involved in exchange reactions or promote dissolution that releases metals. Finally, despite water flowing through the system with a volume of 0.25 L per soil column and a flow rate of 1.5 mL h^{-1}, ion chromatography (IC) analyses confirmed that perchlorate continued to be present in solutions throughout the duration of the experiments. Despite the high solubility of perchlorate, water alone (with a low flow rate) was not enough to completely remove high concentrations of perchlorate from the system. The idea of 'rinsing' perchlorate-containing regolith will have to be more thoroughly compared to our experiments and further assessment on how to completely remove or immobilize perchlorate from Martian regolith is required.

3.3. Implications for Regolith and Agriculture on Mars

Using Martian regolith will require significant processing and treatments in order for it to be usable for agricultural processes. One issue with regard to plant growth/germination not related to perchlorate is the cementing nature of the regolith with the addition of water. With desiccation and irradiation of the Martian regolith over billions of years as well as the nature of the particle sizes and minerals, it would be expected that Martian regolith would behave similarly (i.e., massive structure from cementation) or be more reactive with water to those in our experiments. In order to prevent cementation or crusting, the introduction of organic material, like those present in the control potting soil, may help alleviate some of the effects. More importantly, the development of soil structure will be crucial for providing textural and water-holding properties that will be beneficial for seed germination and seeding growth.

As this and other studies have shown [19,21], perchlorate concentrations in the range observed on the Martian surface can impair seed germination and plant growth. The mechanism for perchlorate uptake in plants is not well understood, but a variety of factors including physiological differences between species, pH, substrate, transpiration, and the presence of certain anions have been shown to affect uptake in plants [8,28–31]. This likely accounts for the differences between substrates and species shown in this study. Even at concentrations where germination and growth occur, plant tissues have been shown to take up perchlorate under relatively short growth periods [27,32,33], similar to our results. This suggests the possibility that phytoremediation, the use of certain specialist or genetically modified species of plants and/or bacteria to breakdown perchlorate and/or remove it from a substrate by uptake into tissues, may be a feasible remediation pathway for transforming or removing perchlorate [28].

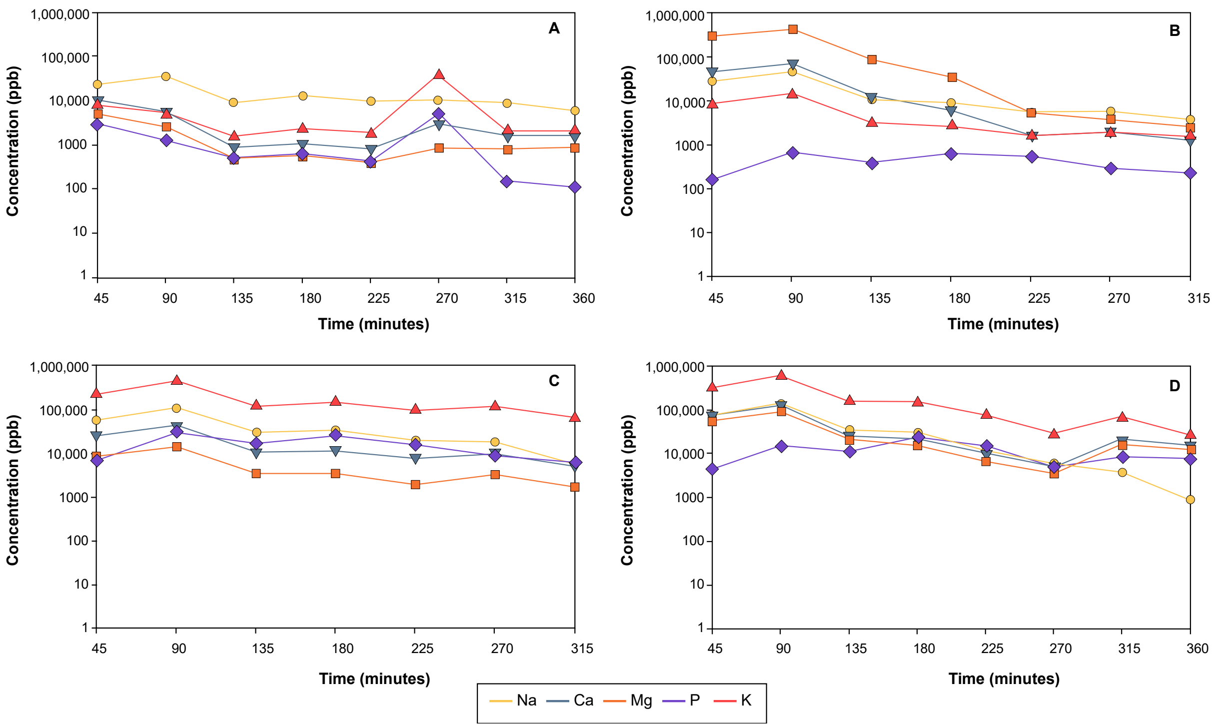

Figure 3. Soil column flow-through experiments recorded every 45 min on average to assess metal and phosphorous leaching related to Mg perchlorate additions in (**A**) Martian regolith simulant without perchlorate. (**B**) Martian regolith simulant with added perchlorate (1 wt.%) increased Mg levels compared to Martian regolith simulant without perchlorate. (**C**) Organic potting soil control without perchlorate. (**D**) Organic potting soil with perchlorate (1 wt.%) does not show changes in metal release compared to organic potting soil control without perchlorate.

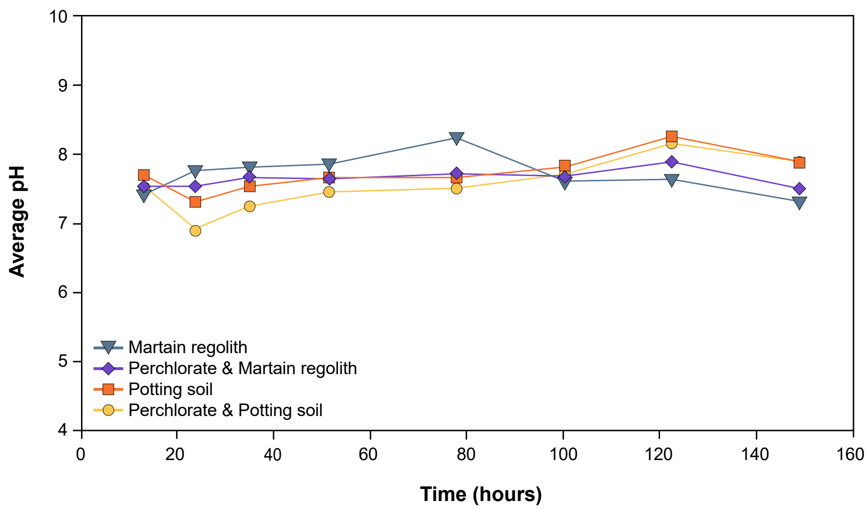

Figure 4. Measured pH as water flowed through soil/simulated samples. Consistency of average pH suggests changes in metal release (Figure 3) are likely not attributed to changes in pH and instead may be attributed to other factors.

From our observations, methods that promote perchlorate volatilization may present another feasible remediation pathway. Perchlorate left uncovered and not mixed with a soil/regolith was found to completely volatilize in less than 12 h. The amount of perchlorate in the soil/regolith that would volatilize would be significantly less because perchlorate was consistently present in the soil and simulant, even with 'rinsing' of the soil (i.e., the flow-through experiments). Overall, how to completely remove perchlorate requires further investigation and our results suggest that perchlorate volatilization, the introduction of organic material, and phytoremediation are three different approaches that would beneficially mediate the detrimental aspects of perchlorate in Martian regolith.

4. Conclusions

Providing adequate food for space exploration missions is challenging and will only become more difficult as we venture further away from Earth. As distances grow, food volume and the cost of transportation will both increase. As a result, the development of extraterrestrial agriculture techniques will be imperative to the success of long-term missions. Understanding the limits of Martian regolith as well as possible techniques for improving its viability as an agricultural substrate will be instrumental to successful Martian agriculture. Our results highlight the need to develop effective methods for perchlorate removal prior to using Martian regolith as a planting substrate; however, perchlorate is not the only issue at hand. Limitations to plant growth are directly related to chemical and/or physical stress which may or may not be related to the presence of perchlorate. For example, direct phytotoxicity, salt stress, metal release, or some combination of these variables will stress or inhibit seed germination and plant growth. Additionally, the massive structure created by the addition of water to the Martian regolith simulant will limit water infiltration and root penetration. Overall, removing perchlorate through volatilization or leaching, for example, is needed, coupled with regolith amendment with organic matter to promote structure and nutrient cycling. Future avenues of inquiry include the use of water rinses, phytoremediation, volatilization, and/or perchlorate-reducing bacteria to remove (or transform) perchlorate from the regolith, as well as plant studies to determine if plants grown in remediated Martian regolith are safe for consumption.

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