



# **Effect of Mycorrhizal Inoculation on Growth and Nutrient Uptake by Leek (*Allium porrum*) fertilized with Inorganic N Combined with Saturated Polonite**

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# Forward

Before embarking on my journey through the master's program in Agroecology at the Swedish University of Agricultural Sciences, I was introduced to the world of Agricultural Science Education during my bachelor studies. This initial exposure allowed me to delve into the intricacies of crop and animal production while equipping me with the skills to inspire future agricultural scientists as a teacher.

As an agricultural science teacher, I encountered a significant challenge in kindling a deep interest in farming among my students, particularly in the Ghanaian context where agriculture was often associated with small-scale, labor-intensive, and traditional practices. To bridge the gap, I resorted to showing my students images and videos of mechanized, large-scale farming as it's practiced in more developed countries.

This exposure ignited a critical question within me: "How can agroecological farming principles be applied on a large scale?" It was this question that fueled my journey into the Agroecology program.

Throughout my studies, I had the privilege of gaining practical experience on various farms in Sweden and across the globe. These experiences fostered a profound appreciation for the complexities of diverse farming systems. They also provided a unique vantage point, allowing me to understand the perspectives of both farmers and farm laborers. What became increasingly evident was that agroecological farming, whether small or large scale, is not solely an environmental matter; it necessitates a comprehensive approach that considers social and economic aspects.

My quest for answers led me to explore the fascinating world of biodiversity and ecosystem services within agroecosystems. This exploration unveiled the delicate balance of relationships between ecosystem members, which, in turn, fosters resilience to environmental perturbations. I was particularly drawn to the concept of improving agricultural input efficiency while mitigating the environmental repercussions of over-reliance on inputs.

This interest steered me toward the investigation of circular systems that facilitate nutrient recycling within food systems, demonstrating their potential benefits for both farmers and the environment. As I delved deeper into this topic, I became determined to find practical applications for the principles I had learned.

Through diligent inquiry, I was fortunate to connect with my current supervisor and collaborate with Alnarp Cleanwater Technology AB. Together, we embarked on a research journey to explore the effects of mycorrhizal inoculation on the growth and nutrient uptake of leek (*Allium porrum*) when fertilized with inorganic nitrogen combined with saturated polonite.

This research study throws light to innovative alternatives to traditional farming inputs, promising sustainable food production, the promotion of circular economies, and the

advancement of more ecologically sound agricultural practices, whether on a small or large scale.

The culmination of this research represents a step forward in my ongoing quest to address the challenges of agroecology and sustainable agriculture, especially in regions like Ghana. It is with great enthusiasm that I share the findings and insights that have emerged from this scientific exploration.

## Abstract

The continuous rise in global population continues to increase the demand for food. Meeting this rise in demand requires an increase in agricultural input, such as applying fertilizers, including phosphorus (P). This results in a surge in demand and acquisition of P from non-renewable phosphate rocks from natural reserves in areas such as Morocco, USA and Russia. Rise in political unrest within Morocco and between Russia and Ukraine pose threats to global trade and supply. Continuous accumulation of P in soil due to P application increases the risk of aquatic eutrophication. Inoculation with arbuscular mycorrhizal fungi (AMF) can potentially improve plant P uptake and reduce fertilizer input dependence. The need to apply principles of the circular economy such that recycled P fertilizers used for crop production are also used responsibly in crop cultivation is crucial for phosphorus-importing countries, sustainable food production, food security and the environment. This study, therefore, aimed to determine the effect of mycorrhizal inoculation on growth, Nitrogen (N) and P uptake by host plant (leek) fertilized with P-saturated polonite combined with either ammonium sulfate or nitrate.

The study involved an incubation and a cultivation experiment. The incubation experiment was designed to determine the effect of nitrogen application using three levels of ammonium sulfate (200 mg/pot, 400 mg/pot, and 600 mg/pot), each supplying 50, 100, and 150% of 140mg N/L and ammonium nitrate (370.06 mg/pot) supplying 150% of 140mg N/L on soil pH, and phosphorus availability in the soil and from 9g per pot of saturated polonite (PO) added. The cultivation experiment was designed to determine AMF inoculation on growth and P uptake by leek (*Allium porrum*) fertilized with 9g of saturated polonite, 400 mg/pot of ammonium sulfate and 247.06 mg/pot of ammonium nitrate per standardized rate of N application (140 mg N/L soil).

AMF inoculation significantly and positively influenced above-ground biomass, shoot P concentration and uptake by the plants under (-) PO conditions but did not affect P uptake and P acquisition efficiency (%) under (+) PO conditions. Other nutrients, including potassium, sodium and magnesium, were significantly influenced by AMF inoculation. Saturated polonite addition significantly and positively influenced soil P concentration, plant above-ground biomass, shoot P concentration and uptake. Saturated polonite also significantly increased soil pH compared to control but negatively affected ammonium-N in the soil. Root colonization was positively influenced by AMF inoculation, but there was no correlation observed between root colonization and P uptake. Ammonium sulfate at 100% before incubation and 150% after incubation reduced soil pH compared to control. Nitrogen (N) addition overall affected shoot concentration and uptake of Na, S and Mn. Ammonium sulfate and nitrate positively influenced soil N availability, but did not affect soil P availability and negatively influenced shoot P concentration and uptake compared to control treatment. Ammonium sulfate supplied at 150% (600 mg/pot) reduced soil pH compared to control after incubation. When applied to PO treatments, ammonium sulfate (100%) positively influenced shoot fresh and dry weight compared to ammonium nitrate.

The study concluded that AMF inoculation may increase plant growth (above-ground biomass), shoot P concentrations and uptake, but this depends on whether soil P is enough due to P addition or deficiency in the soil. In addition, saturated polonite as a P fertilizer alternative can potentially increase plant growth (above-ground biomass), shoot P concentration and uptake.

**Keywords:** Phosphorus, Arbuscular Mycorrhiza fungi, Saturated polonite, Nitrogen, *Allium porrum*, Sustainability, circular economy.

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## Abbreviations

AN	Ammonium Nitrate
AS	Ammonium Sulfate
P	Phosphorus
AMF	Arbuscular Mycorrhizal Fungi
S	Soil
cm	Centimetre
PO	Polonite (Saturated)
PAE	Phosphorus Acquisition Efficiency
Mg	Magnesium
Ca	Calcium
B	Boron
N	Nitrogen
Na	Sodium
Mn	Manganese
S	Sulfur
L	Liters
F.C	Field capacity
ATCP	Amorphous tricalcium phosphate

# Introduction

The global population keeps rising, with the expectation of population growth to reach 9.3 billion by 2050 (Lee, 2011), which is expected to reach 10.2 to 11.1 billion in 2100 (Lee, 2011; Roser & Rodés-Guirao, 2020). This means a rise in food demand needs to be met with a maximized yield, one of the primary reasons for farmers' increase in fertilizer inputs dependency, which also has negative consequences if used excessively and in disproportionate quantities (Bisht & Singh Chauhan, 2021; Dawson & Hilton, 2011). Moreover, the production and cost of fertilizers largely depend on natural gas. However, following Russia's recent invasion of Ukraine, there has been a global increase in mineral fertilizer and energy crisis, which now negatively affects global food security and prices. As a result, on 9th November 2022, the European Commission presented a Communication on "Ensuring Availability and Affordability of Fertilizers", through which actions and guidance on tackling challenges faced by farmers and industries in the EU and developing countries were addressed. Among actions presented to maintain a sustainable EU fertilizer production and reduce dependencies included; sustainable farming practices and training, substituting mineral fertilizers with organic and alternative fertilizer products through an integrated nutrient management action plan and transition to greener fertilizers (European Commission, 2022).

The European Commission also presented an ambitious package of measures within the Biodiversity Strategy 2030. Amongst the measures is the Farm to Fork strategy to address challenges farmers and industries face in the EU and developing countries. Farm to Fork's strategy aims to reduce the use of chemical pesticides by 50% and decrease nutrient losses by at least 50% by 2030 (Huygens et al., 2019). The strategy also aims to reduce fertilizer use by 20% and increase the area of organically farmed agricultural land to 25% (Bindraban et al., 2020).

Among selected macronutrients, phosphorus (P), after nitrogen (N), is an essential plant nutrient farmers depend on as an input. Phosphorus in plants promotes cell division, enzyme activation or inactivation (Razaq et al., 2017) and the development of the growing tip of especially young plants and seedlings. Increased phosphorus supply has been shown to enhance shoot and root growth, leaf area, and biomass accumulation (Kim & Li, 2016; Salama et al., 2019). Phosphorus deficiency can lead to crop productivity and yield loss while adequate

phosphorus levels are required for optimum growth and reproduction (Ikhajiagbe et al., 2020).

In addition, the maintenance of membrane structures, biomolecule synthesis and formation of high-energy molecules indicates phosphorus's presence and function in living cells (Day & Ludeke, 1993; Malhotra et al., 2018). Phosphorus in soil exists in a variety of organic and inorganic compounds whose biological availability in the soil environment varies considerably. Inorganic P includes apatitic minerals, phosphate ions such as  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$ , and  $\text{PO}_4^{3-}$  (Yadav et al., 2012), and formed compounds primarily dominated by hydrous sesquioxides, amorphous, and crystalline aluminum (Al), iron (Fe), and calcium (Ca) forms majorly influenced by pH of soil. Aluminum and iron form compounds with inorganic P in acidic, non-calcareous soils, while Ca form compounds with inorganic P in alkaline, calcareous soils (Ngatia & Taylor, 2019; Sharpley, 1995). Organic P forms exist as inositol phosphates, phosphonates, orthophosphate monoesters and organic polyphosphates (e.g., adenosine triphosphate) (Ngatia & Taylor, 2019; Turner, 2005).

## 1.1 Issues Regarding Phosphorus Availability in Soil, Phosphate Rock Extraction and Eutrophication.

Phosphorus plays a significant role in the plant life cycle from the vegetative to the reproductive stage. However, P remains a limiting plant nutrient in agroecosystems due to its characteristic slow diffusion and high soil fixation (Asomaning, 2020). Phosphorus in soil has strong sorption with soil particles making it relatively less mobile and soluble, coupled with low acquisition efficiency by plants rendering it a less plant-available nutrient. Significant factors influencing soil P availability to plants include organic matter availability, the concentration of P in solution, the amount of free oxides of iron and aluminum and an essential factor for this study, pH (Yadav et al., 2012). As mentioned earlier, too high pH ( $> 7.5$ ) and too low pH ( $< 5.5$ ) negatively influence P availability and uptake (Penn & Camberato, 2019) due to accelerated reactions with Ca as well as Fe and Al, respectively.

Phosphorus fixation resulting in decreased availability, usually calls for large amounts of P fertilizer application on most crop-cultivated lands (Chan et al., 2007). However, crops such as vegetables rarely use more than 40-50 per cent of the amount applied (Williams, 1950). Vegetables and most plants often use only an estimated 10-25 per cent of the phosphate applied (Syers et al., 2008; Williams, 1950). Continued fertilization in excess of P leads to accumulation in soil which further leaches or washes into waterbodies via increasing external interferences such as non-point source runoff/erosion from agricultural land to surface water, further resulting in freshwater eutrophication (Schindler, 2012). In addition, another

major contributor to P eutrophication aside from non-point erosion from P-accumulated farmlands include sewage waste and discharges from municipalities and industries (Ngatia & Taylor, 2019). Globally, phosphorus eutrophication continues to harm the environment as its over-enrichment of aquatic ecosystems causes an exponential rise of algae blooms or water plants, anoxic events, changes in biomass and species composition, and the death of aquatic inhabitants such as fish (Ngatia & Taylor, 2019). Consequences of which include monetary losses (water purification cost), risks to public health and the destruction of recreational facilities (Carpenter et al., 1998; Wilson & Carpenter, 1999).

Mineral phosphate fertilizers are among the most relied-on agricultural inputs. Phosphate fertilizers are synthetic sources of P manufactured from non-renewable phosphate rock (EC, 2017) deposits obtained from reserves with locations around the globe. China, Morocco, the United States and Russia are the major phosphate-producing countries (Geissler et al., 2015; Jama-Rodzeńska et al., 2021), with total reserves accounting for about 85% of global phosphate rock available. Sustainable agriculture production and food security are at risk due to the rise in P fertilizer input dependence in agriculture production systems, depletion of these phosphate reserves (Amann et al., 2018; Sarvajayakesavalu et al., 2018) and rise in political unrest in phosphate rock mining countries including Morocco (Arieff, 2011) and Russia (Ozili, 2022).

Following the actions addressed by the European Commission's Communication (2022) on the fertilizer production crisis "substituting mineral fertilizers with organic and alternative fertilizer products", a circular economy is needed, especially for importing countries, where P is recovered, recycled and used as a fertilizer substitute. This reduces demand on phosphate from reserves, reduces negative environmental impact due to P eutrophication and mining and enhances P- use efficiency while promoting sustainable agriculture production and food security.

## 1.2 Phosphorus Recovery and Recycling

Several studies have suggested ways to reduce P eutrophication, such as controlling nutrient loads and ecosystem restoration (Ngatia & Taylor, 2019) and recovery of P from wastewater treatment and sewage sludge from municipalities using different technologies, with further subsequent study reviews (Cieślik & Konieczka, 2017; Cornel & Schaum, 2009; Egle et al., 2015). Egle et al. (2015) review of fifty (50) identified technologies ranging from simple precipitation of dissolved P to complex multi-step approaches saw only a few technologies displaying potential for full-scale implementation.

Capturing phosphorus (P) using filter materials from reactive substances has also been investigated and proven to reduce Phosphorus concentration in wastewater significantly. Polonite® remains one of the selected and studied reactive materials

which captures phosphorus with its high P-sorption capacity and as a potential P fertilizer substitute (Cucarella et al., 2007; Gustafsson et al., 2008; Renman & Renman, 2010). Polonite<sup>®</sup> is a filter media manufactured via heating (900 °C), crushing and sieving of a siliceous sedimentary mineral called Opoka rock mined in Poland (Brogowski & Renman, 2004). Polonite contains a large amount of calcium oxide that accounts for its high reactivity and effective phosphorus sorption capacity characteristic, which is over 100g P sorbed/kg substrate (Nelin & Renman, 2008). The study by Gustafsson et al. (2008) observed soluble amorphous tricalcium phosphate (ATCP) formed in the mineral-based sorbents (Filtrate and Polonite<sup>®</sup>) with 18% of the accumulated PO<sub>4</sub> readily dissolved in the experiments indicating a crystallization of some parts of the accumulated phosphorus in slightly less soluble phases. This suggests that saturated filter material such as polonite can gradually release phosphorus through dissolution to plants when applied as a P fertilizer source and promote sustainable crop production. In addition, polonite is known to be high in pH and contain biofilm, which reduces bacterial count (Hylander et al., 2006).

Cucarella et al. (2007), in a pot experimental study with barley, also tested the fertilizer potential of three P-saturated reactive filter media (which included polonite, wollastonite and Filtrate P) with a high affinity for P and the ability to remove P from wastewater. After saturation, relatively higher P content was found in polonite compared to Filtrate P and wollastonite. Compared with the control treatment, improved yield was seen amongst all three materials, with the highest yield per unit of amendment from saturated polonite-treated plants due to its higher P content.

The alkaline property of saturated polonite also allows it to contribute positively to significantly reducing Al toxicity risk but not to improve P uptake by plants upon application (Cucarella et al., 2009). A study by Cucarella et al. (2009) involved the application of saturated polonite obtained from on-site wastewater treatment as a soil amendment to mountain meadow cultivation. Results from the study showed increased soil pH and decreased Al toxicity risk (hydrolytic acidity). However, no difference was observed in yield and P uptake by mountain meadow plants compared to controls. Saturated polonite contains high amounts of P after being used as a P-filter material. However, due to its elevated pH, P remains bonded to calcium. Hence, P is relatively inaccessible for plant uptake (Barrow, 2017). Saturated polonite also has no nitrogen (N) content. Therefore, efficient application on farmlands as a P fertilizer substitute would be enhanced by lowering pH and incorporating N and other nutrients.

### 1.3 Lowering pH to boost P Availability

Soil acidification processes in agricultural land can be accelerated in several ways: via acid rain (Singh & Agrawal, 2007), removal of agricultural produce from the land, the application of acidifying soil amendments (Brownrigg et al., 2022), ammonium-based fertilizers and urea (Goulding, 2016).

Several studies have been performed to reduce soil pH (Fageria et al., 2010; Pierre & Pierre, 1928), while others further focused on enhancing P availability (Brownrigg et al., 2022; Khorsandi, 1994) in calcareous soils. An example is a study which used acidifying amendments such as sulfuric acid, which increased soil acidity, salinity, DTPA-extractable Fe, available P (NaHCO<sub>3</sub>-extractable), and crop yield upon application to sorghum grown in two calcareous soils (Khorsandi, 1994). Brownrigg et al. (2022) found that reducing pH in three calcium carbonate content (CaCO<sub>3</sub>) rich calcareous soils with acidifying amendments (Oxalic acid dihydrate, sulfuric acid, ammonium sulphate, elemental sulphur, and glucose) both with and without mono-ammonium phosphate (MAP) as P source improved P solubility and diffusion from mono-ammonium phosphate after a 14-day (52 days for S<sup>0</sup>). However, P fertilizer uptake in wheat crops grown in these highly calcareous soils did not improve.

Another primary driver of soil pH changes in agricultural systems is the form of N fertilizer applied. Nitrogen fertilizer forms that influence soil pH include urea (CO(NH<sub>2</sub>)<sub>2</sub>), monoammonium phosphate (NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>), diammonium phosphate ((NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>), ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), calcium ammonium nitrate (CaCO<sub>3</sub>+NH<sub>4</sub>(NO<sub>3</sub>)) and ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>). Soil pH is therefore influenced by N as cation ammonium (NH<sub>4</sub><sup>+</sup>), anion nitrate (NO<sub>3</sub><sup>-</sup>) or uncharged urea molecule ([CO(NH<sub>2</sub>)<sub>2</sub>]0). Ammonia-based fertilizers lower soil pH during nitrification due to two H<sup>+</sup> ions generated per ammonium molecule nitrified compared to nitrate-based fertilizers or urea (Pierre & Pierre, 1928; Wang et al., 2020).

A comparison of the effects of urea and ammonium sulphate on grain yield, soil pH, calcium, magnesium (Mg), base saturation, aluminum, and acidity (H + Al) saturation in a lowland rice production study was conducted by Fageria et al. (2010). Results showed a higher linear decrease in soil pH with the application of N by ammonium sulphate than by urea fertilizer. In addition, Ca and Mg saturation decreased while Al and acidity saturation increased when both fertilizers were applied at higher N rates than low N rates.

In addition, Pierre & Pierre (1928) studied the effects of nitrogen fertilizers: sodium nitrate (NaNO<sub>3</sub>), ammonium sulphate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>), urea, ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), ammonium phosphate, calcium cyanamid (CaCN<sub>2</sub>), Leuna saltpetre and calcium nitrate (Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O) on reaction of different soils. The soils were treated in two-gallon pots with equivalent N amounts in the fertilizers and used for crop cultivation in succession. Hydrogen-ion concentration and the



exchangeable hydrogen of the soils were then studied to determine the residual effects of the fertilizers on soil reaction. According to the H-ion concentration data, a significant increase in H-ion concentration was caused by ammonium sulphate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>), with ammonium phosphate, Leuna salpetre, ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), and urea, respectively, following in order. However, sodium nitrate, calcium nitrate, and calcium cyanamid decreased the H-ion concentration.

## 1.4 Arbuscular Mycorrhizal Fungi (AMF) 's Influence on Nutrient Mobility and Uptake

Reducing pH to make P "available" in soluble forms does not automatically mean improved plant nutrient uptake (Barrow, 2017). Concerning enhancing P uptake and utilization efficiency, several studies have suggested using, for example, alternative production systems (Wells et al., 2000), relying on functions of microorganisms (Richardson et al., 2011; Shrivastava et al., 2018) or using crops having genotypes with internally improved P-utilization efficiency traits (J. Chen et al., 2009), grown on P-deficient soils. After being studied and proven by many scholars, plant-arbuscular mycorrhizal fungi (AMF) symbiosis is a potential method for enhancing available P uptake by plants and, by extension, improving sustainable agriculture. Arbuscular Mycorrhiza Fungi is therefore gaining attention regarding sustainable agriculture (Devi et al., 2021).

Through its symbiotic relations with most terrestrial plants, the primary function of AMF is microbial biofertilization (Dasgupta et al., 2021). It acts as an extension of plant roots by producing large underground extra-radical mycelium (ERM), which extends from the roots to the surrounding rhizosphere (Dasgupta et al., 2021; Novais et al., 2020). AMF are obligate biotrophs and cannot grow without the host plant. Thus, carbon or plant-derived photosynthate (Smith & Read, 2010) are received from host plants in exchange for ecological benefits such as nutrient uptake stress tolerance, soil health and fertility (M. Chen et al., 2018) that AMF provides. Therefore, when available phosphate is present in the soil, plants can pull it up through their roots using phosphate transporters (Młodzińska & Zbońska, 2016). However, when available P (H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) concentrations are deficient in the soil, AMF with increased affinity for H<sub>2</sub>PO<sub>4</sub><sup>-</sup> facilitate phosphorus uptake by the host plant (Cress et al., 1979). Several factors, aside from phosphorus and nitrogen levels (Koide, 1991; Sanders & Tinker, 1973; Sylvia & Neal, 1990), influence AM fungi's effectiveness in supporting allium growth. Such factors include the pH of the soil (Graw, 1979), available water (Nelsen & Safir, 1982), temperature conditions (Hayman, 1974) and other factors such as aeration and organic matter content (Saif, 1983; St. John et al., 1983)

Many studies have also been done and reviewed regarding the ability of arbuscular mycorrhizal fungi to enhance P and N uptake by plants (Abdullahi & Sheriff, 2013; Ames et al., 1983; Atul-Nayyar et al., 2009; George et al., 1995; Miransari, 2011; Perner et al., 2006; Roy-Bolduc & Hijri, 2011; Xu et al., 2014)

Ames et al. (1983) studied cultivated Celery plants treated with or without mycorrhiza in pots treated with either an organic (ground plant tissue) or inorganic  $[(\text{NH}_4)_2\text{SO}_4]$  as sources of  $^{15}\text{N}$ . The Mycorrhiza inoculated plants and control were fertilized with inorganic  $^{15}\text{N}$  over a 30-day period and an 88-day period for organic  $^{15}\text{N}$ . The roots were separated from the soil in a side chamber, and data taken on mycorrhizal fungal colonization, number of hyphal crossings through the mesh into the area of  $^{15}\text{N}$  placement, length of hyphae per gram of soil, dry and fresh weight of both shoot and roots. Though no significant difference was observed regarding shoot dry weight or shoot P content between mycorrhizal and control plants, significantly ( $P= 0.01$ ), more  $^{15}\text{N}$  was derived by mycorrhizal plants, from both N sources, than by control plants. Furthermore, there was a significant ( $P = 0.001$ ) and positive correlation between  $^{15}\text{N}$  in mycorrhizal plants and percent mycorrhizal fungal colonization ( $r= 0.58$ ), the number of hyphal crossings ( $\pm 10 \mu$  diameter) through the mesh into the area of  $^{15}\text{N}$  placement ( $r= 0.76$ ), the total length of hyphae per gram of soil ( $r = 0.74$ ), and length of hyphae of  $5 \mu$  diameter in the soil ( $r = 0.77$ ).

Xu et al. (2014) investigated the effects of AMF inoculation and six levels of soil Olsen-P (10.4, 17.1, 30.9, 40.0, 62.1, and 95.5  $\text{mg kg}^{-1}$  treatments) on root colonization, soil spore density, and the growth and P uptake of asparagus. Results from the study showed the highest root colonization (76%) and soil spore density (26.3 spores  $\text{g}^{-1}$  soil) in the 17.1  $\text{mg kg}^{-1}$  treatment. Significant reduction ( $P < 0.05$ ) of mycorrhizal dependency occurred with increasing soil Olsen-P. Furthermore, Xu et al. (2014) observed a significant correlation ( $P < 0.01$ ) between mycorrhizal P uptake and root colonization which shows a positive influence of AMF on improved P uptake and subsequent plant growth. In addition, results showed a decrease (14.5% from 67.9 to 59.3  $\text{mg Olsen-P kg}^{-1}$ ) in the P concentration of soil required for maximum plant growth. The study, therefore, suggested that through the addition of AMF to the suitable P fertilization, P efficiency was improved by AMF via increased phosphorus uptake and optimal growth.

Improving P utilization efficiency using saturated polonite as a P fertilizer substitute to create a circular economy, reduce eutrophication and promote sustainable agriculture remains in the study phase. Therefore, this study will investigate the synergy between AMF, saturated polonite and nitrogen fertilization in fostering growth and nutrient uptake by host plants (leek).

## 1.5 Aim

The study will investigate the effect of mycorrhizal inoculation on growth, N and P uptake by host plant (leek) fertilized with saturated polonite combined with either ammonium sulphate or nitrate.

### 1.5.1 Research Questions

The study aim is further divided into the following research questions.

- a. Is there an influence of ammonium sulphate and ammonium nitrate on the availability of P in soil?
- b. Is there an influence of mycorrhizal inoculation on growth as well as N and P uptake by host plant fertilized with ammonium sulphate and ammonium nitrate combined with saturated polonite?

### 1.5.2 Contributions of Study to Achieving the Sustainable Development Goals (SDGs) and Circular Economy

#### *Sustainable development Goals*

The present study on the effect of mycorrhizal inoculation and saturated polonite on *Allium porrum* growth and nutrient uptake has the potential to make significant contributions to sustainable development goals. It could promote environmentally friendly agricultural practices, improve food security, and advance the principles of the circular economy, which are crucial for a sustainable and resilient future. The study may contribute to achieving four SDGs, including Zero Hunger (SDG 2), Responsible Consumption and Production (SDG 12), Climate Action (SDG 13) and Life on Land (SDG 15).

**SDG 2. Zero Hunger:** this goal aims to end hunger, achieve food security, improve nutrition, and promote sustainable agriculture by the year 2030 (Schneider & Tarawali, 2021). By studying the impact of mycorrhizal inoculation and specific inorganic nitrogen sources combined with phosphorus-saturated polonite on the growth and nutrient uptake of *Allium porrum*, this research may lead to more efficient and sustainable agricultural practices such as reducing fertilizer inputs. This can potentially increase crop yields and improve the nutritional value of the produce, helping to address food security and reducing hunger.

**SDG 12. Responsible Consumption and Production:** it is focused on promoting sustainable patterns of consumption and production by the year 2030 (Seyedsayamdost, 2020). The research will explore different fertilization methods and their impact on crop growth and nutrient uptake. Suppose the findings show

that mycorrhizal inoculation and saturated polonite fertilization can enhance nutrient uptake in leeks. In that case, it may promote the responsible use of fertilizers and reduce the overuse of chemical phosphorus fertilizers which can lead to minimizing environmental pollution and degradation caused by mining and overuse of rock phosphate (EC, 2017; Ngatia & Taylor, 2019).

SDG 13. Climate Action: also aims to take urgent and effective measures to combat climate change and its impacts by the year 2030 (Osborn et al., 2015). Sustainable agricultural practices that enhance nutrient uptake in plants and promote efficient use of resources, such as the combination of mycorrhizal inoculation and saturated polonite, can contribute to climate action by reducing greenhouse gas emissions associated with traditional P fertilizer production and usage (Walling & Vaneeckhaute, 2020).

SDG 15. Life on Land: aims to protect, restore, and sustainably manage terrestrial ecosystems, forests, and biodiversity by the year 2030 (Osborn et al., 2015). Mycorrhizal inoculation and saturated polonite may promote soil health and fertility, as mycorrhizal fungi can improve soil structure and nutrient cycling. This can lead to better land use practices, prevent soil erosion, and help preserve biodiversity and ecosystems on land and in water bodies, thereby promoting life on land and water.

### *Circular Economy*

Regarding circular economy, this study has the potential to align with the circular economy principles, including reuse, reduce, recycle, recover and redesign. The present study has the potential to achieve this by closing the nutrient loop, nutrient recycling, and reducing waste and pollution.

Through the combined application of mycorrhizal inoculation and saturated polonite, the research can promote a redesign of the nutrient system such that phosphorus from municipal wastewater treatment is recovered and reused as a P fertilizer source, taken up by plants and returned to the soil again through crop residues and organic matter which is reported to be a much faster way of cycling P than through rocks and sediments (Schipanski & Bennett, 2021), completing the cycle and reducing the reliance on one-way nutrient flows.

Mycorrhizal fungi help facilitate nutrient cycling and enhance the nutrient uptake efficiency of plants through biofertilization (Dasgupta et al., 2021), reducing the need for continuous external inputs. Using organic phosphorus sources like saturated polonite from opoka can contribute to recycling phosphorus, a finite and essential resource, from wastewater rather than relying solely on mined phosphorus fertilizers. Additionally, by facilitating the transfer of nutrients, especially phosphorus, from the soil (primarily from saturated polonite) to the plant roots, Mycorrhizal fungi reduce the need for additional synthetic fertilizers. This

contributes to a circular economy by recycling nutrients already present in the ecosystem and from wastewater rather than relying solely on external inputs.

Overuse of synthetic phosphorus fertilizers often leads to nutrient runoff and environmental pollution (Ngatia & Taylor, 2019). By enhancing nutrient uptake efficiency through mycorrhizal inoculation and using saturated polonite, the study can potentially reduce nutrient losses to the environment, minimizing waste and pollution.

# Materials and Methods

## 2.1 Soil source

The soil was collected from Lönstorps Research Station in southwest Skåne at 0 to 20 cm depth from different portions of a 2m x 4m plot. The soil type acquired was loam with about 15 % clay and 3 % organic material. The soil was prepared via sieving and uniform mixing and stored outdoors but out of direct sunlight and rain.

## 2.2 Nitrogen Fertilizer Sources

Nitrogen fertilizers used in the study included ammonium sulfate and ammonium nitrate. Ammonium sulfate has a relatively more significant acidifying action on soil than other nitrogen fertilizers due to its ability to release acidic ammonium ions ( $\text{NH}_4^+$ ) and sulfate ions ( $\text{SO}_4^{2-}$ ) when dissolved in water, capable of lowering soil pH (Chien et al., 2011; Finch et al., 2023). This is one reason why it was selected to test its effect on pH and P availability from saturated polonite. For comparison, ammonium nitrate was also obtained and used to determine the effect of different sources of N that may influence soil pH, P and N availability in soil.

## 2.3 Source of Phosphorus

Saturated Polonite® was used as a source of P. Polonite is extracted commercially by crushing, sieving, and heat treatment (up to 900 °C) of Opoka to increase its pH and reactivity or phosphorus binding capacity. Opoka is a natural sedimentary rock rich in silica and calcium carbonate. It is used for water treatment due to its characteristics (Brogowski & Renman, 2004) and is found in Poland, western Ukraine, and other parts of south-eastern Europe.

Polonite is produced and marketed by a Swedish company called Polonite Nordic AB (PNAB), a subsidiary of Alnarp Cleanwater AB, for preliminary individual sewer treatment systems while capturing phosphorus. The filtration process usually uses 500kg of polonite for one family household. The polonite

typically lasts three years before becoming saturated and needing a filter exchange. The saturated polonite is, therefore, a valuable phosphorous source that can be used for agricultural purposes by spreading on farmlands.

## 2.4 Test Plant

The test plant was Leek (*Allium porrum*). Leek is a tall, hardy, biennial vegetable which shares similar characteristics as onion and garlic but has a lesser tendency to form bulbs. Like onion and garlic, leeks belong to the genus *Allium* of the family *Alliaceae* (Swamy & Veere Gowda, 2006). One of the physiological characteristics of leeks is that their basal leaf sheaths can be 15-25 cm long and 5 cm in diameter. (Swamy & Veere Gowda, 2006). Leek is a relatively long-season crop that takes approximately 120 days from seeding to harvest. It is more cold-tolerant compared to the onion in its early stages, but frost damage can occur during harvest (Swiader et al., 1992).

Although leeks can be grown in a variety of soil types, deep topsoil is preferred for vigorous plant growth and above-average yields (Swamy & Veere Gowda, 2006). Leek's requirement for manure and fertilizer may be high compared to onions due to its larger mass. Leek's phosphorus requirements and applications of 50-100 kg P<sub>2</sub>O<sub>5</sub> per hectare are sufficient. Potash requirements are also low, with 150-200 kg K<sub>2</sub>O per hectare as potash sulfate sufficing (Swamy & Veere Gowda, 2006). According to Kaniszewski et al. (1989), In dry years, the highest yield of leeks was obtained with a pre-planting application of 200 kg of N/ha under irrigated and non-irrigated conditions. A split application of 600 kg of N produced the highest yields in wet years.

Leek was selected for the study due to its medium to high demand for nutrients (Booij et al., 1996; Boyhan et al., 2009; Greenwood et al., 1980; Karic et al., 2005) and its ability to form associations with arbuscular mycorrhiza fungi (Koide & Schreiner, 1992; Perner et al., 2006; Rolini et al., 2001). Växtpass-certified leek seeds (Purjolök Zermatt variety) with a 90% germination rate, and sizes ranging from 2.0-2.25 mm, were purchased from Olssons Frö AB and used as test plants.

## 2.5 Mycorrhiza Inoculum (AMF)

The arbuscular mycorrhizal fungus and its respective diatomaceous carrier material were obtained from Symbiosis and Plant-Microbe Association Research Laboratory (SYMPLANTA®). The research-grade AM fungal inoculum *Rhizophagus irregularis* isolate obtained is composed of mainly fungal spores as propagules, produced aseptically, in vitro and supplied in diatom earth ('diatomite')

powder with 50% humidity content. The carrier material is also sterilized under gamma-irradiation but re-packed and sealed under non-sterile conditions.

## 2.6 Incubation Experiment

### 2.6.1 Treatments and experimental design

The incubation experiment took place in an incubation chamber of the Department of Biosystems and Technology at the Swedish University of Agricultural Sciences (SLU). The main purpose for conducting the incubation experiment was to determine P availability and pH changes in each treatment over a period of time.

The experiment followed a two-way factorial design with treatments including control, ammonium sulphate (at 50, 100 and 150% of 140 mg NL<sup>-1</sup>), ammonium nitrate (at 150% of 140 mg NL<sup>-1</sup>) and saturated polonite, with two replications each (n = 2) due to inadequate soil quantity. Each pot was filled with 609 g of soil with a volume of 0.6 L. Concentration levels of ammonium sulphate used included 200 mg/pot (50%), 400 mg/pot (100%) and 600 mg/pot (150%). The ammonium nitrate concentration level used was 370.06 mg/pot (150%).

Before choosing a suitable quantity of saturated polonite for the incubation experiment, a pH (water) analysis was done using soil mixed with different levels of polonite (0, 4, 20, and 40 g/L soil) in water at a mass to volume of ratio of 1:5 (i.e., 1 part of soil: 5 part of water). The different levels of saturated polonite were mixed with soil after being crushed and sieved through a 2.0 mm sieve mesh. Solutions formed were thoroughly mixed for one hour using a piece of shaking equipment to ensure a uniform mixture. The pH of the samples was tested using a pH meter, and 15 g/L (9 g saturated polonite/0.6 L) was selected as the P fertilizer rate according to results obtained from the pH test. Two samples of grounded saturated polonite were also taken to Eurofins Environment Testing Sweden AB to determine P using the aqua regia extraction method.

Aqua regia extraction of phosphorus involves using aqua regia (a mixture of nitric acid and hydrochloric acid) as a leaching agent to dissolve P from various sources such as soil, spent auto catalysts, and metallurgical grade silicon (MG-Si) (Hasani et al., 2017; Huang et al., 2016; Melo & Guedes, 2020). Aqua regia is effective in extracting P due to its strong oxidizing properties and ability to dissolve many materials (Baghalha et al., 2009). The extraction process is influenced by temperature, acid concentrations, stirring speed, particle size, and liquid/solid ratio (Istiqomah et al., 2019).

The pots were covered to prevent light incidence, randomly arranged on trolleys at the same height and kept in an incubation chamber at 18 °C night temperature and 20 °C Day temperature as well as 22 °C Day and 20 °C night ventilation temperatures under 37% to 40% humidity for 95 days from 9<sup>th</sup> February to 15<sup>th</sup> May



2023. To determine available P in the treatments before and after incubation, separate samples were prepared and sent for analysis at LMI AB Helsingborg according to methods used by Spurway (1949).

The acetic acid extraction method by Spurway (1949) estimates plant-available phosphorus in the soil or soilless growing media in greenhouses (Elliott et al., 1994; Markus, 1986). To estimate plant-available P in soil, a mild concentration of acetic acid, typically 1M acetic acid (CH<sub>3</sub>COOH), is used for the extraction process. The mild acetic acid, therefore, generates a slightly acidic environment that aids in releasing P into the solution for analysis (Markus, 1986). After the extraction, the mixture is filtered to separate the liquid phase from the soil particles. The filtrate containing the dissolved P is then analyzed using a suitable method, such as colorimetry or spectrophotometry. These techniques measure the concentration of P in the filtrate, providing an estimate of plant-available P in the soil.

## 2.7 Cultivation Experiment

### 2.7.1 Experimental Design and Conduct of Study

The cultivation experiment was carried out in the greenhouse of SLU-Alnarp with a primary aim to investigate the effect of mycorrhizal inoculation on growth and P uptake by host plant not fertilized (control), fertilized with ammonium sulfate only (AS), ammonium nitrate only (AN), saturated polonite only (PO), AS+PO and AN+PO. Each pot was filled with 610 g of soil with a volume of 0.6 L.

The experiment was done using a three-way (2 x 2 x 3) factorial completely randomized design with factors which included mycorrhizal inoculation (AMF), saturated polonite and two N-sources (ammonium sulfate and ammonium nitrate). Nitrogen was applied to N-receiving plants in four stages; before planting in week 1, two times in week four and once in week 6. From the beginning of the cultivation experiment, concentration levels of ammonium sulphate and nitrate used were determined using the same formulas used in the incubation experiment for determining fertilizer quantity and applied at 400 mg/pot and 247.06 mg/pot per standardized rate of N application (140 mg N/L soil). Nitrogen was further supplied to N-receiving plants in the form of ammonium nitrate applied at 3.5 mg, two times in the fourth week and once in the sixth week. The crushed saturated polonite was applied once before planting. Two grams (2 g) of mycorrhizal inoculum were supplied to each AMF-receiving pot, while each nonmycorrhizal-receiving pot had 1 g of carrier material. The treatments were then replicated five times to a total of sixty pots.

### 2.7.2 Agronomic Practices

Cultivation lasted over 56 days after planting to harvest from 3<sup>rd</sup> March to 28<sup>th</sup> April 2023. Plant growth parameters analyzed included biomass (shoot fresh and dry weight, root fresh weight) and plant height. Dry root weight was not analyzed, as the roots were prepared for the estimation of root infection and colonization by AMF.

Agronomic practices applied after sowing included filling in, irrigation and weed control. Filling in involved planting extra seeds to cover for germination losses in some pots. Weeds were controlled by hand picking. Regarding irrigation, all plants were supplied with deionized water, free from heavy metals and chemicals that may alter the study results. Irrigation of the plants was done at two-day intervals throughout the cultivation period. The quantity of deionized water used was measured using the pot media's capacity to hold water (from 40 to 100%) to avoid the leaching of nutrients. This was predetermined through a simple water-holding capacity test.

To determine the pot water-holding capacity (PC), soil only (control) and saturated polonite fertilized (9 g/pot) soil were filled in three pots each. The pots were watered to saturation and freely drained for 24 hours while avoiding evaporation. The net weight of wet soil in the pot (at 100% of PC) was determined after drainage. The treatments were then oven-dried at 105 °C for three days and weighed. Soil water content at 100% of PC as a percentage of soil dry weight was calculated, and the dry weight (105°C) of the soil in the pots was used to calculate the amount of water in the pot at 100% of PC. Weights of water at 40, 50, 60, 70 and 80% of PC were then calculated to determine how much water would be needed for irrigating plants during cultivation.

### 2.7.3 Harvesting and Analysis

After six weeks of vegetative growth, the plant shoots and roots were harvested for analysis. A separation was made between shoots and roots. Both parts were then carefully separated from the soil and organic matter. The base of each shoot was cleaned with deionized water and dried with tissue paper, after which shoot biomass (i.e., number of plants per pot and shoot fresh and dry weights) was recorded. Roots were also cleaned, and root biomass (i.e., root fresh) was recorded. One-third of the roots were then weighed and prepared for oven drying at 65<sup>o</sup> C for two days to determine root dry weight. The rest were kept in ethanol for mycorrhiza colonization determination.

### 2.7.4 Assessing Nutrient Content in Shoot

A leaf mineral analysis was conducted using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) to determine the nutrient content in the shoots

(Charles & Fredeen, 1997; Fassel & Kniseley, 1974). This procedure was done after the dried shoots had been subjected to Rapid Nitric Acid Digestion (Huang et al., 2004; Pequerul et al., 1993). Rapid Nitric Acid Digestion is a common method for assessing plant nutrient contents via plant material extraction and digestion for subsequent elemental analysis (Huang et al., 2004). Nitric acid ( $\text{HNO}_3$ ) is a potent oxidizing agent used in this procedure to break down organic matter and convert elements into their ionic forms, making them more accessible for analysis using either ISP-OES, Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) or Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Beauchemin, 2008; Bings et al., 2006; Todolí & Mermet, 2006).

In brief, dried leaves of the test plant (leek) were collected from each treatment with four replicates, ground via a laboratory mixer mill (MM 400) to increase the surface area and ensure uniformity in subsequent digestion and further oven dried (at 60 °C) for three hours. Concentrated nitric acid (10 ml) was added to the digestion vessels containing the samples. The samples were then digested using Microwave Accelerated Reaction System (MARS 5) with a temperature control sensor (Huang et al., 2004) at 185 °C and high pressure as increased temperature and pressure accelerate digestion by promoting the reaction between the nitric acid and the organic matter present in the samples. There was, therefore, oxidation of organic compounds, decomposition of complex materials, and conversion of the elements into soluble ionic forms (Huang et al., 2004; Pequerul et al., 1993). After digestion, the nitric acid was diluted to 100 mL. The resulting digested sample solution, containing the dissolved elements in ionic form, was then sent to LMI Helsingborg for nutrient assessment via ICP-OES. Assessing N concentration in the shoots was not conducted due to inadequate dry shoots.

The ICP-OES technique is used to determine the concentration of specific elements in a sample (K. F. Khan, 2019). When atoms and ions are excited by an external source of plasma energy, the energy is absorbed to move electrons from the ground state to an excited state, and this is the fundamental principle at work here. When excited atoms return to their ground state, they emit emission rays, the wavelength of which is measured by a spectrometer. The intensity of the photon rays is used to calculate the component of each element, while their location determines the element type (K. F. Khan, 2019). The sophisticated instrumentation of ICP-OES allows for the precise simultaneous detection of two to seventy elements. In addition, it is versatile enough to process samples types such as liquids (both organic and inorganic), solids, and aqueous. Furthermore, food analysis, geological studies, drug/metabolite analysis, agricultural investigations and environmental and forensic sciences can all benefit from ICP-OES (S. R. Khan et al., 2022).

### 2.7.5 Estimation of Root Colonization

Root samples (two-thirds out of the whole) were collected from each plant from the soil only (control) and saturated polonite treatments and stained according to methods described by Phillips & Hayman (1970) to estimate the amount of mycorrhizal infection on roots. The collected roots were washed with deionized water and fully soaked in plastic bottles containing 50% ethanol. To remove the host cytoplasm and most of the nuclei to make the roots very clear to allow distinct visibility of the vascular cylinder in each root, the roots were heated at 90 °C in 10% KOH. Afterwards, the roots were rinsed in water and acidified with dilute 1% HCl. To stain the roots, they were simmered for 5 minutes in 0.05% trypan blue in lactophenol. The roots were then randomly selected and placed on a glass slide making three rows of five roots lying horizontally parallel to each other. Using a total of 200 crossing points, the parameters estimated included the presence or absence and number of vesicles, hyphae and arbuscles.

### 2.7.6 Estimation of P Acquisition Efficiency

Phosphorus acquisition efficiency refers to the ability of plants to acquire phosphorus from the soil. It can be estimated by measuring the amount of phosphorus acquired by the roots under different levels of phosphorus availability (Simpson et al., 2021). One way to improve phosphorus acquisition efficiency is through the use of mycorrhizal fungi, which form symbiotic associations with plants and enhance their phosphorus uptake (Campos et al., 2018). Another approach is breeding for phosphorus-efficient cultivars, which can be achieved by improving phosphorus use efficiency and/or phosphorus acquisition efficiency (McLachlan et al., 2021). Phosphorus acquisition efficiency by the leek plants was calculated using the formula below to determine whether or not AMF influenced the ability of the leek plants to take up phosphorus in the PO-treated plants.

$$\text{Phosphorus acquisition efficiency (\%)} = \frac{\text{Total P in the leek plant (mg)}}{\text{P added (mg)}} \times 100$$

## 2.8 Statistical analysis

Data from the incubation experiment were subjected to a two-way analysis of variance (ANOVA) general linear model with the N-treatments and saturated polonite treatments as experimental factors. Data from the cultivation experiment were analyzed using a three-way ANOVA with mycorrhizal inoculation (AMF), saturated polonite and N-source as factors (n = 5). Tukey pairwise test with a

significance level ( $P < 0.05$ ) was carried out to determine the influences of AMF, saturated polonite and N-source on root colonization, plant growth, and plant N and P concentration and uptake. The statistical software used for data analysis was MINITAB 19 (Alin, 2010; Lesik, 2018) software.

# Results

## 3.1 Soil pH, available P and N

Soil pH was significantly influenced by saturated polonite and by the source of nitrogen (Table 1). The highest mean pH was recorded in PO-fertilized soil, while pH was significantly lower for AS than control. Available P was significantly influenced by saturated polonite but not the nitrogen source. All treatments that received PO recorded higher mean available P compared to treatments which did not. Regarding the mean nitrogen concentration, significant differences were observed in N treatments only. The highest mean nitrogen concentration was recorded in treatments with AN.

Nitrate-N and ammonium-N were each significantly influenced by the nitrogen source but not saturated polonite. Nitrate-N was significantly higher in AN-treated soils compared to AS and control. Ammonium-N was significantly higher in AS-treated soils compared to AN and control. Interaction between saturated polonite and N-source did not significantly influence pH, N, nitrate-N, ammonium-N and P availability before incubation.

Table 1. Effect of saturated polonite (PO at 9000 mg/0.6l pot), ammonium sulfate (AS at 400 mg/0.6l pot) and ammonium nitrate (AN at 247 mg/0.6l) on pH, nitrate-N, ammonium-N, P and N availability before incubation (n=2). Letters (ns) indicate no significant effect per each p-value. The symbols (\*), (\*\*) and (\*\*\*) indicates a significant effect at ( $p < 0.05$ ), ( $p < 0.01$ ) and ( $p < 0.001$ ), respectively. Superscripted letters (a), (b) and (c) assigned to means indicate significant differences. Means that do not share a letter are significantly different. Means assigned (a) are higher than those assigned (b) and (c). This description is the same for all tables.

Treatment	pH	Phosphorus (mg/L)	Nitrogen (mg/L)	Nitrate-N (mg/L)	Ammonium-N (mg/L)
Saturated Polonite					
p-value	<b>0.006**</b>	<b>0.012*</b>	<b>0.321 ns</b>	<b>0.090 ns</b>	<b>0.646 ns</b>
(+) PO	5.5 <sup>a</sup>	10.50 <sup>a</sup>	160.00 <sup>a</sup>	125.83 <sup>a</sup>	25.00 <sup>a</sup>
(-) PO	5.3 <sup>b</sup>	7.67 <sup>b</sup>	151.17 <sup>a</sup>	138.50 <sup>a</sup>	23.33 <sup>a</sup>
N- source					
p-value	<b>0.035*</b>	<b>0.329 ns</b>	<b>&lt; 0.001***</b>	<b>&lt;0.001***</b>	<b>&lt;0.001***</b>
control	5.5 <sup>a</sup>	10.00 <sup>a</sup>	96.75 <sup>c</sup>	96.25 <sup>b</sup>	1.25 <sup>c</sup>
AS 100%	5.3 <sup>b</sup>	8.50 <sup>a</sup>	160.00 <sup>b</sup>	115.00 <sup>b</sup>	47.25 <sup>a</sup>
AN 100%	5.4 <sup>ab</sup>	8.75 <sup>a</sup>	210.00 <sup>a</sup>	185.25 <sup>a</sup>	24.00 <sup>b</sup>
Polonite x N-source					
P- value	<b>0.850 ns</b>	<b>0.596 ns</b>	<b>0.619 ns</b>	<b>0.686 ns</b>	<b>0.906 ns</b>

### 3.1.1 Soil pH, available Nitrate-N, Ammonium-N, P and N after incubation

Table 2 shows results from the incubation experiment indicating the effect of PO, AS and AN on pH, nitrate-N, ammonium-N, N and P availability. The mean pH ranged from 5.3 to 5.6. There were significant pH differences influenced by PO and by N- sources and levels (Table 2). Treatments with the addition of PO were less acidic compared to treatments without PO. Regarding N sources, a significant difference in pH was observed between AS (50%) and AS (150%). The mean pH was lower in AS (150%) compared to AS (50%).

Available phosphorus content was significantly influenced by PO addition (table 2). Nitrogen fertilizers, however, showed no significant influence on P availability. There was also no combined effect of PO and nitrogen fertilizers, as shown by their non-significant interaction. Nitrogen availability was significantly influenced by the N-source and rate of application but neither by PO nor by the interaction between PO and N-source.

Nitrate-N was significantly affected by N-source but not by PO. Nitrate-N was significantly higher in AN (150%) compared to the control and the rest of the treatments. Ammonium-N was significantly influenced by PO but not by N-source.

Changes in pH, nitrate-N, ammonium-N, P and N concentrations after the 95-day incubation period was observed between the control, PO, AS and AN (at 100%) as there was no initial analysis of treatments such as AS and AN (at 50 and 150%),

AS + PO (at 50 and 150%) and AN + PO (at 50, and 150%) to compare with due to inadequate soil. Meanwhile, after incubation, the mean values for extractable phosphorus in AS (100%) and PO + AS (100%) treatments were increased by 20% and 16%, respectively.

*Table 2. Main effects and interactions of saturated polonite (PO at 9000 mg/0.6l pot), ammonium sulfate (AS at levels 200,400 and 600 mg/0.6l pot) and ammonium nitrate (AN at 370 mg/0.6l) on pH, nitrate-N, ammonium-N, P and N availability after a 95-day incubation period. n=2*

Treatment	pH	Phosphorus (mg/L)	Nitrogen (mg/L)	Nitrate-N (mg/L)	Ammonium -N (mg/L)
<b>Saturated Polonite</b>					
p-value	<b>&lt; 0.001***</b>	<b>&lt; 0.001***</b>	<b>0.746 ns</b>	<b>0.664 ns</b>	<b>&lt; 0.001***</b>
(+) Polonite	5.5 <sup>a</sup>	11.50 <sup>a</sup>	123.0 <sup>a</sup>	121.0 <sup>a</sup>	1.0 <sup>b</sup>
(-) Polonite	5.4 <sup>b</sup>	8.90 <sup>b</sup>	124.0 <sup>a</sup>	122.0 <sup>a</sup>	2.4 <sup>a</sup>
<b>N- source</b>					
p-value	<b>&lt; 0.001***</b>	<b>0.310 ns</b>	<b>0.009**</b>	<b>0.001**</b>	<b>0.298 ns</b>
control	5.6 <sup>a</sup>	10.75 <sup>a</sup>	110.0 <sup>b</sup>	107.0 <sup>c</sup>	2.25 <sup>a</sup>
AS 50%	5.5 <sup>ab</sup>	10.25 <sup>a</sup>	122.5 <sup>ab</sup>	120.0 <sup>b</sup>	1.50 <sup>a</sup>
AS 100%	5.5 <sup>abc</sup>	10.00 <sup>a</sup>	125.0 <sup>ab</sup>	122.5 <sup>ab</sup>	1.50 <sup>a</sup>
AS 150%	5.3 <sup>c</sup>	9.75 <sup>a</sup>	127.5 <sup>a</sup>	125.0 <sup>ab</sup>	1.50 <sup>a</sup>
AN 150%	5.4 <sup>abc</sup>	10.25 <sup>a</sup>	132.5 <sup>a</sup>	132.5 <sup>a</sup>	1.75 <sup>a</sup>
<b>Polonite x N-source</b>					
p-value	<b>0.415 ns</b>	<b>0.171 ns</b>	<b>0.274 ns</b>	<b>0.092 ns</b>	<b>0.298 ns</b>



## 3.2 Plant Growth Response

Table 3. Main effects and interactions of AMF, N and saturated polonite on growth of leek plant. *n*=5

Treatment	Plant height (cm)	Fresh Shoot weight (g)	Dry Shoot weight (g)	Fresh Root weight (g)	Dry Root weight (g)
AMF					
p-value	<b>0.044*</b>	<b>0.072 ns</b>	<b>0.342 ns</b>	<b>0.380 ns</b>	<b>0.606 ns</b>
(+) AMF	25.113 <sup>a</sup>	4.905 <sup>a</sup>	0.582 <sup>a</sup>	3.272 <sup>a</sup>	0.067 <sup>a</sup>
(-) AMF	23.377 <sup>b</sup>	4.307 <sup>a</sup>	0.549 <sup>a</sup>	3.504 <sup>a</sup>	0.080 <sup>a</sup>
Saturated Polonite					
p-value	<b>0.090 ns</b>	<b>0.047*</b>	<b>0.011*</b>	<b>0.147 ns</b>	<b>0.684 ns</b>
(+) PO	24.970 <sup>a</sup>	4.938 <sup>a</sup>	0.611 <sup>a</sup>	3.581 <sup>a</sup>	0.069 <sup>a</sup>
(-) PO	23.520 <sup>a</sup>	4.275 <sup>b</sup>	0.520 <sup>b</sup>	3.194 <sup>a</sup>	0.079 <sup>a</sup>
N- source					
p-value	<b>0.468 ns</b>	<b>0.021*</b>	<b>0.015*</b>	<b>0.054 ns</b>	<b>0.606 ns</b>
Control	24.695 <sup>a</sup>	5.044 <sup>a</sup>	0.593 <sup>ab</sup>	3.770 <sup>a</sup>	0.090 <sup>a</sup>
AS	24.525 <sup>a</sup>	4.821 <sup>ab</sup>	0.612 <sup>a</sup>	3.385 <sup>ab</sup>	0.070 <sup>a</sup>
AN	23.515 <sup>a</sup>	3.953 <sup>b</sup>	0.492 <sup>b</sup>	2.989 <sup>b</sup>	0.061 <sup>a</sup>
Interactions (p-values)					
AMF x PO	0.085 ns	0.008**	0.007**	0.099 ns	0.976 ns
AMF x N-source	0.588 ns	0.173 ns	0.086 ns	0.996 ns	0.369 ns
PO x N-source	0.860 ns	0.004**	0.010*	0.040*	0.395 ns
AMF x PO x N-source	0.313 ns	0.643 ns	0.792 ns	0.608 ns	0.672 ns

### 3.2.1 Dry Shoot Weight

As indicated in Table 3, AMF did not have a significant main effect on the dry shoot weight of the leek plants. Instead, a significant effect was observed due to interactions between AMF and PO (Tables 3 and 4). For PO non-receiving treatments (i.e., control, AS only and AN only), higher mean dry shoot weight was observed in plants that received AMF inoculation compared to treatments that did not (table 4).

PO and N-source also significantly affected dry shoot weight (Table 3, Figure 1). Higher mean shoot dry weight was recorded from treatments that received PO compared to treatments that did not. In addition, higher mean dry shoot weight was observed in all AS-treated pots compared to AN.

Regarding interactive effects, significantly higher mean dry shoot weight was observed in treatments which had PO without AMF compared to PO with AMF (tables 3 and 4). Furthermore, significantly higher mean dry shoot weight was also observed in plants that received a combination of AS and PO.

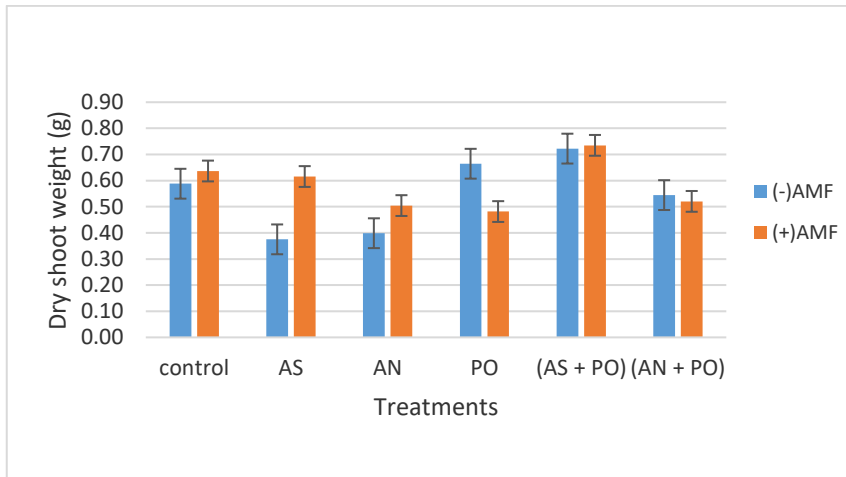


Figure 1. Mean values of plant shoot dry weight obtained for different treatments. Control (soil only), ammonium sulfate (AS), ammonium nitrate (AN), saturated polonite (PO). (+) and (-) AMF represent treatments with and without mycorrhiza inoculation, respectively. This description is the same for all bar charts. Bars indicate standard errors of mean values.  $n = 5$

### 3.2.2 Fresh Shoot weight

Overall, AMF inoculation did not have a significant main effect on fresh shoot weight but was influenced by PO and source of N (Table 3, Figure 2). The highest mean shoot fresh weight was observed in treatments that received PO compared to treatments which did not. In addition, the means shoot fresh weight of control treatments was higher compared to AN-treated plants (Table 3). No significant difference was found between AN and AS or control and AS.

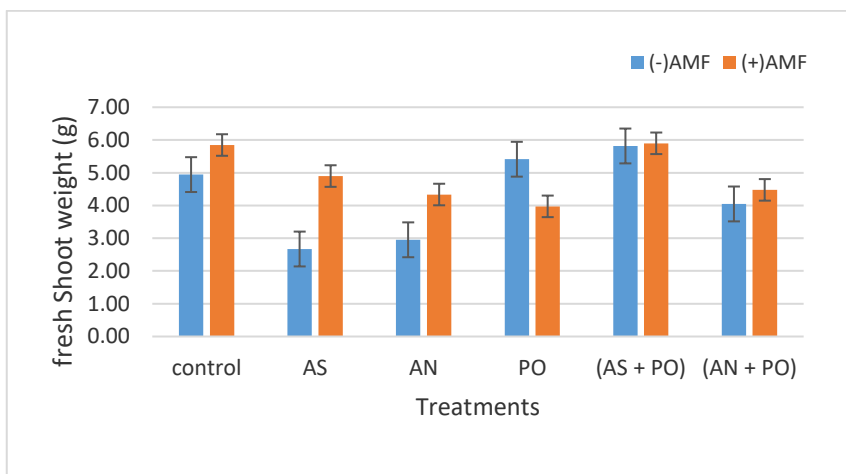


Figure 2. Mean values of plant shoot fresh weight obtained for different treatments. Control (soil only), ammonium sulfate (AS), ammonium nitrate (AN), saturated polonite (PO).  $n=5$

Regarding interactions, mean shoot fresh weight was significantly influenced by interactions between PO and AMF and PO and N-source (Table 3). A significant effect of AMF was observed in plants treated without PO (Table 4, Figure 2). AMF-inoculated plants with no PO fertilization recorded higher mean fresh shoot weight than non-AMF plants (Table 4). In addition, AS+PO treatments recorded higher mean shoot fresh weight compared to AN+PO (Table 4). Without PO addition, there was no significant difference between AS and AN (Table 4).

Table 4. Effect of AMF and nitrogen source on plant growth with or without saturated polonite. *n*=5

<b>Treatment</b>	<b>Plant height (cm)</b>	<b>Fresh Shoot weight (g)</b>	<b>Dry Shoot weight (g)</b>	<b>Fresh Root Weight (g)</b>	<b>Dry Root Weight (g)</b>
With PO addition					
<b>AMF</b>					
P-Value	<b>0.793 ns</b>	<b>0.539 ns</b>	<b>0.234 ns</b>	<b>0.091 ns</b>	<b>0.208 ns</b>
(-) AMF	24.84 <sup>a</sup>	5.092 <sup>a</sup>	0.644 <sup>a</sup>	3.918 <sup>a</sup>	0.062 <sup>a</sup>
(+) AMF	25.10 <sup>a</sup>	4.783 <sup>a</sup>	0.579 <sup>a</sup>	3.244 <sup>a</sup>	0.075 <sup>a</sup>
<b>N-source</b>					
P-Value	<b>0.728 ns</b>	<b>0.040*</b>	<b>0.015*</b>	<b>0.181 ns</b>	<b>0.402 ns</b>
Control	25.46 <sup>a</sup>	4.693 <sup>ab</sup>	0.573 <sup>ab</sup>	3.591 <sup>a</sup>	0.062 <sup>a</sup>
AS	24.95 <sup>a</sup>	5.858 <sup>a</sup>	0.729 <sup>a</sup>	4.025 <sup>a</sup>	0.078 <sup>a</sup>
AN	24.50 <sup>a</sup>	4.262 <sup>b</sup>	0.533 <sup>b</sup>	3.127 <sup>a</sup>	0.065 <sup>a</sup>
AMF x N-source					
P-Value	<b>0.775 ns</b>	<b>0.282 ns</b>	<b>0.298 ns</b>	<b>0.763 ns</b>	<b>0.517 ns</b>
Without PO addition					
<b>AMF</b>					
P-Value	<b>0.027*</b>	<b>0.001**</b>	<b>0.007**</b>	<b>0.506 ns</b>	<b>0.265 ns</b>
(+) AMF	25.13 <sup>a</sup>	5.027 <sup>a</sup>	0.586 <sup>a</sup>	3.299 <sup>a</sup>	0.869 <sup>a</sup>
(-) AMF	21.91 <sup>b</sup>	3.522 <sup>b</sup>	0.454 <sup>b</sup>	3.060 <sup>a</sup>	0.735 <sup>a</sup>
<b>N-source</b>					
P-Value	<b>0.594 ns</b>	<b>0.004**</b>	<b>0.021*</b>	<b>0.019*</b>	<b>0.120 ns</b>
Control	23.93 <sup>a</sup>	5.395 <sup>a</sup>	0.612 <sup>a</sup>	3.943 <sup>a</sup>	0.969 <sup>a</sup>
AS	24.10 <sup>a</sup>	3.785 <sup>b</sup>	0.495 <sup>ab</sup>	2.745 <sup>b</sup>	0.724 <sup>a</sup>
AN	22.53 <sup>a</sup>	3.644 <sup>b</sup>	0.452 <sup>b</sup>	2.850 <sup>b</sup>	0.713 <sup>a</sup>
AMF x N-source					
P-Value	<b>0.326 ns</b>	<b>0.439 ns</b>	<b>0.224 ns</b>	<b>0.837 ns</b>	<b>0.286 ns</b>

### 3.2.3 Fresh Root Weight

Fresh root weight was not overall significantly influenced by AMF, PO or N-source (Table 3, Figure 3). Regarding interactions, only PO and N-source interaction had a significant effect on mean fresh root weight (Table 3). The treatments without PO experienced a significant influence on root fresh weight by N-source (Table 4). Mean root fresh weight was highest in control treatments compared to AS and AN (Table 4)

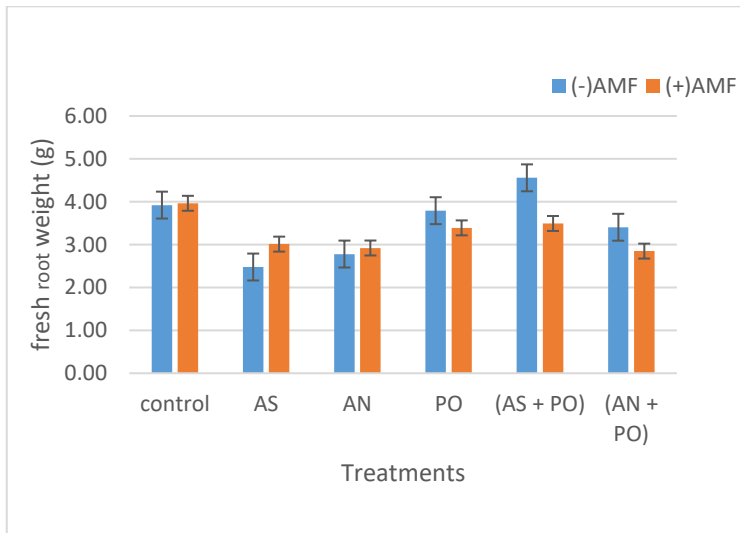


Figure 3. Mean values of fresh root weight obtained for different treatments: soil (control), ammonium sulfate (AS), ammonium nitrate (AN), saturated polonite (PO) and their interactions.  $n=5$

### 3.2.4 Dry Root Weight

AMF, PO and N-source did not significantly affect the dry root weight of the leek plants (Table 3, Figure 4). Regarding interactions, no significant effect of interactions between the treatments was recorded.

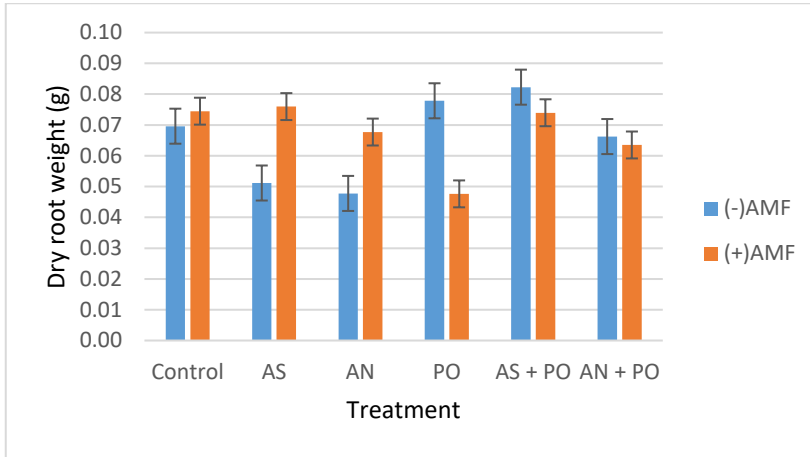


Figure 4. Mean values of dry root weight obtained for different treatments: soil (control), ammonium sulfate (AS), ammonium nitrate (AN) and saturated polonite (PO).  $n = 5$

### 3.2.5 Plant Height

Overall, plant height was not significantly affected by saturated polonite or source of N (Table 3, Figure 5). There was also no significant effect due to treatment interactions (Table 3). A significant effect was, however, seen due to AMF inoculation. Mean plant height was higher in AMF-inoculated plants than in non-AMF plants (Table 3, Figure 5).

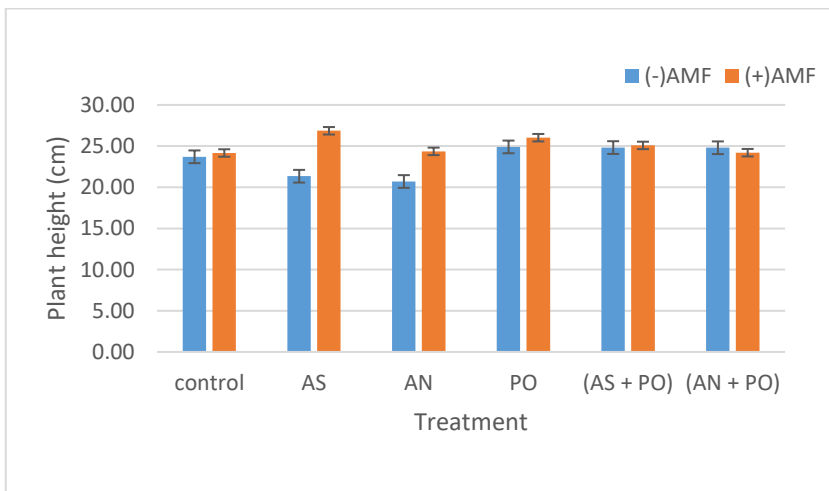


Figure 5. Mean values of plant height obtained for different treatments: Soil (control), ammonium sulfate (AS), ammonium nitrate (AN) and saturated polonite (PO).  $n = 5$

### 3.3 Shoot Nutrient Concentration and Uptake

AMF significantly (at  $p < 0.001$ ) influenced the concentration of macronutrients, including P and K, in the leek shoots (Table 5). PO significantly (at  $p < 0.001$ ) influenced the concentration of P (Table 5) and Mn (Table 6) in the shoots of the leek plants. Furthermore, N application had a significant (at  $p < 0.001$ ) effect on nutrients such as P, Na, S (Table 5) and Mn (Table 6). PO and N had a significant (at  $p < 0.001$ ) effect on concentrations of Na and Mn (Table 5 and Table 6).

Regarding nutrient uptake, AMF significantly (at  $p < 0.05$ ) influenced the uptake of macronutrients, such as P and Mg (Table 7) and micronutrients, including Mn and Na (Table 8), in the leek shoots. Furthermore, PO significantly (at  $p < 0.001$ ) influenced the uptake of P, Ca, Mg and Mn in the shoots of the leek plants. Additionally, N application had a significant ( $p < 0.001$ ) effect on nutrients such as P, Na, S and Mn. Uptake of Na, S, Mg and Mn in the leek plants were significantly influenced (at  $p < 0.001$ ) by PO and N interactions (Table 7 and Table 8).

Table 5. Main effect and interactions of AMF, PO and N on macronutrient concentrations in the shoot of leek plants  $n = 4$

Treatment	P (%)	K (%)	Na (%)	S (%)	Ca (%)	Mg (%)
AMF	< 0.001 ***	< 0.001 ***	0.015*	0.816 ns	0.772 ns	0.004**
Saturated Polonite	< 0.001 ***	0.093 ns	0.005**	0.935 ns	0.036*	0.037*
N	< 0.001 ***	0.184	< 0.001 ***	< 0.001 ***	0.037*	0.896 ns
AMF x PO	0.040*	0.040*	0.910 ns	0.770 ns	0.357 ns	0.010*
AMF x N	0.149 ns	0.404 ns	0.196 ns	0.542 ns	0.144 ns	0.043*
PO x N	0.299 ns	0.769 ns	< 0.001 ***	0.018*	0.067*	0.002**
AMF x PO x N	0.862 ns	0.695 ns	0.981 ns	0.380 ns	0.312 ns	0.525 ns

Table 6. Main effect and interactions of AMF, PO and N on micronutrient concentrations in the shoot of leek plants.  $n = 4$

Treatment	Fe (mg.kg <sup>-1</sup> )	Al (mg.kg <sup>-1</sup> )	Zn (mg.kg <sup>-1</sup> )	Mn (mg.kg <sup>-1</sup> )	B (mg.kg <sup>-1</sup> )	Mo (mg.kg <sup>-1</sup> )
AMF	0.755 ns	0.247 ns	0.384 ns	0.916 ns	0.168 ns	0.141 ns
Saturated Polonite	0.299 ns	0.850 ns	0.037*	< 0.001 ***	0.403 ns	0.107 ns
N	0.020*	0.315 ns	0.013*	< 0.001 ***	0.372 ns	0.021*
AMF x PO	0.742 ns	0.931 ns	0.651 ns	0.909 ns	0.644 ns	0.881 ns
AMF x N	0.600 ns	0.588 ns	0.274 ns	0.079 ns	0.281 ns	0.643 ns
PO x N	0.835 ns	0.823 ns	0.968 ns	< 0.001 ***	0.851 ns	0.005**
AMF x PO x N	0.796 ns	0.937 ns	0.821 ns	0.831 ns	0.702 ns	0.335 ns

Table 7. Main effect and interactions of AMF, PO and N on macronutrient uptake in the shoot of the leek plants.  $n = 4$

Treatment	P (mg)	K (mg)	Na (mg)	S (mg)	Ca (mg)	Mg (mg)	Fe (mg)
AMF	0.005**	0.073 ns	0.009**	0.096 ns	0.169 ns	0.003**	0.435 ns
PO	< 0.001 ***	0.028*	0.001**	0.007**	< 0.001 ***	< 0.001 ***	0.941 ns
N	< 0.001 ***	0.029*	< 0.001 ***	< 0.001 ***	0.017*	0.042*	0.019*
AMF x PO	0.001**	0.016*	0.041*	0.019*	< 0.001 ***	< 0.001 ***	0.439 ns
AMF x N	0.019*	0.042*	0.069 ns	0.041*	0.031*	0.014*	0.344 ns
PO x N-Source	0.396 ns	0.013*	< 0.001 ***	< 0.001 ***	0.001**	< 0.001 ***	0.534 ns
AMF x PO x N	0.181 ns	0.815 ns	0.289 ns	0.088 ns	0.080 ns	0.147 ns	0.918 ns

Table 8. Main effect and interactions of AMF, PO and N on micro nutrient uptake in the shoot of the leek plants.  $n = 4$

Treatment	Al (mg)	Zn (mg)	Mn (mg)	B (mg)	Mo (mg)
AMF	0.208 ns	0.154 ns	0.001**	0.050*	0.154 ns
PO	0.606 ns	0.002**	< 0.001 ***	0.008**	0.887 ns
N	0.225 ns	0.006**	< 0.001 ***	0.141 ns	0.003**
AMF x PO	0.897 ns	0.007**	< 0.001 ***	0.005**	0.186 ns
AMF x N-Source	0.564 ns	0.230 ns	0.154 ns	0.045*	0.069 ns
PO x N-Source	0.572 ns	0.105 ns	< 0.001 ***	0.070 ns	0.075 ns
AMF x PO x N-Source	0.951 ns	0.743 ns	0.010*	0.262 ns	0.145 ns



### 3.3.1 Phosphorus Concentration in the shoot

The mean percentage P concentration in the shoot of each plant was significantly influenced by AMF, PO and N-source (Table 9 and Figure 6). No significant difference existed between P concentrations in AS and AN-treated plants (Table 9). Regarding the interactive effect, AMF inoculation and PO fertilization significantly affected P concentrations in the leek plants. A significant effect on P concentration influenced by AMF inoculation and N-source was observed in plants which did not receive PO (see Table 10).

*Table 9. Main effect and interactions of AMF, N-source and saturated polonite on leek plant's shoot P concentration (%). n=4*

Treatment	Shoot P concentration (%)
AMF	
p-value	0.003**
(+) AMF	0.254 <sup>a</sup>
(-) AMF	0.214 <sup>b</sup>
Saturated Polonite	
p-value	< 0.001***
(+) PO	0.270 <sup>a</sup>
(-) PO	0.198 <sup>b</sup>
N- source	
p-value	< 0.001***
Control	0.283 <sup>a</sup>
AS	0.202 <sup>b</sup>
AN	0.217 <sup>b</sup>
Interactions (p-values)	
AMF x PO	0.007**
AMF x N-source	0.116 ns
PO x N-source	0.350 ns
AMF x PO x N-source	0.588 ns

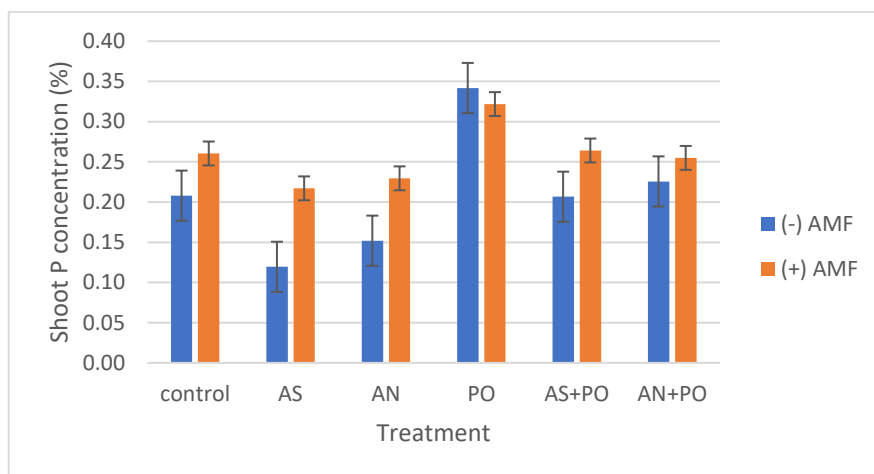


Figure 6. Mean values of percentage P concentration obtained for different treatments: Soil (control), ammonium sulfate (AS), ammonium nitrate (AN) and saturated polonite (PO).  $n=4$ .

Table 10. Main effect and interactions of AMF and nitrogen source on shoot P concentration (%) with or without saturated polonite.  $n=4$

Treatment	Shoot P concentration (%)
With saturated polonite	
AMF	
P-Value	0.882 ns
(+) AMF	0.272 <sup>a</sup>
(-) AMF	0.269 <sup>a</sup>
N-source	
P-Value	0.005**
Control	0.332 <sup>a</sup>
AS	0.235 <sup>b</sup>
AN	0.244 <sup>b</sup>
AMF x N-source	
P-Value	0.276 ns
Without saturated polonite	
AMF	
P-Value	< 0.001***
(+) AMF	0.236 <sup>a</sup>
(-) AMF	0.160 <sup>b</sup>
N-source	
P-Value	< 0.001***
Control	0.234 <sup>a</sup>
AS	0.191 <sup>b</sup>
AN	0.168 <sup>b</sup>
AMF x N-source	
P-Value	0.247 ns

### 3.3.2 Shoot P Uptake

Phosphorus uptake by the leek plants was significantly influenced by AMF, PO, and N-Source (Table 11 and Figure 7). The highest P uptake was recorded in (+) AMF, (+) PO and non-N fertilized treatments (control). Regarding the interactive effect (Table 11), the uptake of P was significantly influenced by AMF and PO, as well as AMF and N-source interactions. AMF inoculation significantly increased shoot P uptake in (-) PO treatments (Table 12) while under (+) PO conditions shoot P uptake was high in control treatments without AMF compared to AN treatments without AMF. Regarding AMF's interaction with N-source, high shoot P uptake was recorded in AS-treated plants which had AMF inoculation (Table 13).

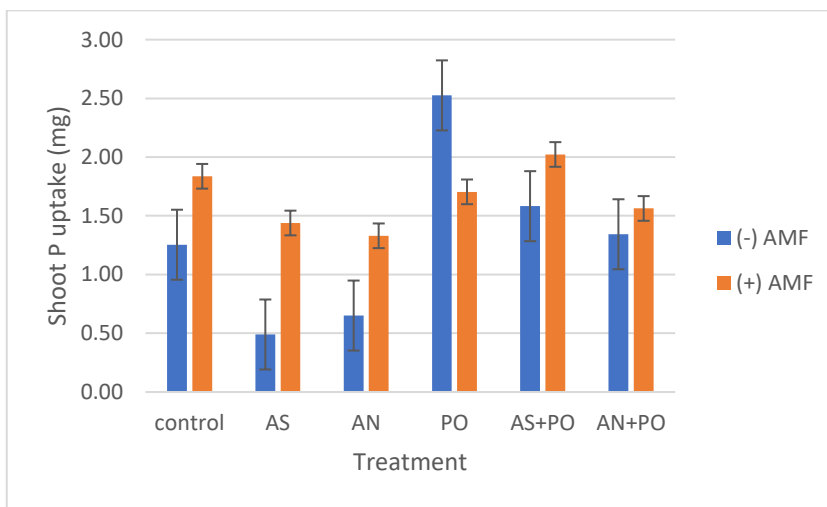


Figure 7. Mean values of P uptake (mg) were obtained for different treatments: soil (control), ammonium sulfate (AS), ammonium nitrate (AN) and saturated polonite (PO).  $n=4$

Table 11. Main effect and interactions of AMF, PO and nitrogen source on shoot P uptake (mg).  
n=4

Treatment	Shoot P uptake (mg)
AMF	
p-value	0.005**
(+) AMF	1.650 <sup>a</sup>
(-) AMF	1.131 <sup>b</sup>
Saturated Polonite	
p-value	< 0.001***
(+) PO	1.790 <sup>a</sup>
(-) PO	1.667 <sup>b</sup>
N- source	
p-value	< 0.001***
Control	1.830 <sup>a</sup>
AS	1.383 <sup>b</sup>
AN	1.222 <sup>b</sup>
Interactions (p-values)	
AMF x PO	0.001**
AMF x N-source	0.019*
PO x N-source	0.396 ns
AMF x PO x N-source	0.181 ns

Table 12. Main effects and interactions of AMF and N-source on shoot P uptake with or without saturated polonite. n=4

Treatment	Shoot P uptake (mg)
With saturated polonite	
AMF	
p-value	0.799 ns
(+) AMF	1.817 <sup>a</sup>
(-) AMF	1.763 <sup>a</sup>
N-source	
p-value	0.056 ns
control	2.115 <sup>a</sup>
AS	1.802 <sup>a</sup>
AN	1.453 <sup>a</sup>
Interactions (significant differences only)	
AMF x N-source	0.051 ns
(-) AMF x control	2.526 <sup>a</sup>
(-) AMF x AN	1.343 <sup>b</sup>
Without saturated polonite	
AMF	
p-value	< 0.001***
(+) AMF	1.535 <sup>a</sup>
(-) AMF	0.798 <sup>b</sup>
N-source	
p-value	< 0.001***
control	1.545 <sup>a</sup>
AS	0.964 <sup>b</sup>
AN	0.990 <sup>b</sup>
Interactions	
AMF x N-source	0.303 ns

Table 13. Effect of AMF interactions with each N-source treatment on shoot P uptake.  $n = 4$

Treatment	Shoot P uptake (mg)
With Control	
AMF	
p-value	0.689 ns
(+) AMF	1.770 <sup>a</sup>
(-) AMF	1.890 <sup>a</sup>
With AS	
AMF	
p-value	< 0.031*
(+) AMF	1.731 <sup>a</sup>
(-) AMF	1.036 <sup>b</sup>
With AN	
AMF	
p-value	0.96 ns
(+) AMF	1.446 <sup>a</sup>
(-) AMF	0.997 <sup>a</sup>

### 3.4 Phosphorus Acquisition Efficiency (PAE)

Under (+) PO conditions, AMF and N-source did not have a significant main effect on PAE (%). Regarding interactions between AMF and N-source under (+) PO conditions, plants in control treatments without AMF inoculation recorded higher PAE (%) compared those in AN treatments without AMF inoculation (Table 14).

Table 14. Main effect and interaction of AMF and N-source on phosphorus acquisition efficiency (%) under (+) PO conditions. (n = 4)

Treatment	PAE (%)
<b>AMF</b>	
p-value	0.799 ns
(+) AMF	2.860 <sup>a</sup>
(-) AMF	2.947 <sup>a</sup>
<b>N-source</b>	
p-value	0.056 ns
control	3.430 <sup>a</sup>
AS	2.924 <sup>ab</sup>
AN	2.357 <sup>b</sup>
<b>Interactions (significant differences only)</b>	
AMF x N-source	0.051 ns
(-) AMF x control	4.097 <sup>a</sup>
(-) AMF x AN	2.178 <sup>b</sup>

### 3.5 Root Colonization by AMF

Percentage root colonization was significantly affected by AMF inoculation but not PO (Table 15). There was also no significant effect on the percentage of root colonization by the interaction between AMF and PO.

Table 15. Main effect and interaction of AMF and PO on root colonization n = 4

<b>Treatment</b>	<b>Plant root colonization</b>
<b>AMF</b>	
P value	< 0.001***
(+) AMF	38.500 <sup>a</sup>
(-) AMF	8.875 <sup>b</sup>
<b>PO</b>	
P value	0.265 ns
(+) PO	26.625 <sup>a</sup>
(-) PO	20.750 <sup>a</sup>
<b>Interaction</b>	
AMF x PO	0.285 ns

### 3.5.1 Relationship between AMF Root Colonization and Shoot P Uptake

#### *(-) AMF and Shoot P uptake*

No significant relationship was recorded between (-) AMF colonization and P uptake by the leek plants (Table 16).

*Table 16. Correlation coefficient (r) and P value indicating the relationship between (-) AMF root colonization and shoot P uptake n = 4*

Relationship	Correlation coefficient (r)	p-value
(-) AMF root colonization x Shoot P uptake	-0.207	0.622 ns

#### *(+) AMF and Shoot P uptake*

No significant relationship was observed between (+) AMF root colonization of plant roots and P uptake by the leek plants (Table 17).

*Table 17. Correlation coefficient (r) and P value indicating the relationship between (+) AMF root colonization and shoot P uptake n = 4*

Relationship	Correlation coefficient (r)	p-value
(+) AMF root colonization x P uptake	-0.282	0.423 ns



# Discussion

## 4.1 Effect of pH on Phosphorus Availability

As discussed earlier in the introduction, phosphorus release and availability through desorption from soil particles or dissolution from phosphorus-containing minerals depend on several factors such as the soil pH (Kanabo & Gilkes, 1987), organic matter availability, the concentration of P in the soil solution, and the number of free oxides of iron and aluminum (Yadav et al., 2012). Therefore, too high (> 7.5) or low (< 5.5) pH, for example, will negatively affect soil P availability and uptake by plants (Penn & Camberato, 2019) as this is influenced by rapid reactions with Ca as well as Fe and Al respectively (Asomaning, 2020). Various biological, chemical, and physical processes can influence soil properties and nutrient dynamics during a soil incubation period. Soil incubation facilitates nutrient transformations, including converting organic nutrients into inorganic forms that are more readily available to plants. For instance, organic nitrogen compounds may be converted into ammonium ( $\text{NH}_4^+$ ) through ammonification and, subsequently, into nitrate ( $\text{NO}_3^-$ ) through nitrification (Beeckman et al., 2018). Phosphorus may undergo mineralization, dissolution, and precipitation reactions, determining its availability (Thomas Sims & Pierzynski, 2018). Nitrification can be influenced by several factors, including moisture content (Sahrawat, 2008). Adequate soil moisture is necessary for nitrification to occur. Excessive waterlogging can limit oxygen availability and inhibit nitrification, while arid conditions can also slow the process (Sahrawat, 2008).

In the present study, pH and P addition are seen to be the significant factors influencing soil P availability in the soil. The addition of AS, AN, and PO influenced the treatments' pH and P concentration (see Table 1). Saturated polonite can increase soil pH due to its inherent alkaline nature while inhabiting 80% of phosphate ( $\text{PO}_4\text{-P}$ ) as a potential P supply to plants (Nilsson et al., 2013). Even though soil pH was higher in PO treatments compared to non-PO treated soils (see Table 1 and Table 2), the mean pH recorded in (+) PO soils was between 7 and 5.5 (Table 1 and Table 2), which has been reported to be a good pH range for maximum phosphorus availability in soil solution (Penn & Camberato, 2019). It is, therefore, likely that the recorded mean pH in the PO-treated soils (see Table 1 and Table 2)

may have positively influenced P dissolution from PO, leading to higher plant available P concentration in soil (Table 1 and Table 2). At this point, it could be concluded that saturated polonite can increase soil pH and influence P availability depending on the soil's initial pH. The adsorption capacity of PO was not analyzed, but analysis of PO from Eurofins AB to determine the inherent concentration of P showed a mean P of 6850 mg/kg of PO. Thus, 9g of PO used as P fertilizer in the present study had 61.65 mg of P, which may have influenced P availability due to P addition (Table 2). Thus, it confirms that when a low pH soil is treated with PO, soil pH increases, and depending on the new pH, P becomes available due to increased P dissolution (Gustafsson et al., 2008) for plant uptake. This result agrees with studies on polonite as a phosphorus retention filter material with high pH, its phosphorous desorbing capacity and its suitability for recycling into agriculture production (Gustafsson et al., 2008; Hylander et al., 2006; Kassa, 2013). It also agrees with reports on the effect of pH on the availability of P (Price, 2006). A mean available P of 11.5 (Table 2) was recorded in the PO-treated soil after 95 days of incubation. However, after analysis, 9 g of PO contained approximately 61.65 mg of P. This may indicate that not all P in PO was released during the period, and therefore, a possible explanation may be that P release from PO is a gradual process and take time. The desorption capacity of polonite was evaluated by Nelin & Renman (2008), and it was found that saturated polonite had a lower desorption capacity compared to another reactive material called Sorbulite.

The source and rate of nitrogen fertilizer application also influence pH dynamics. Results from the incubation experiment indicated that AS addition at 150% significantly reduced soil pH compared to control (see Table 2). These results agree with previous studies (Pierre & Pierre, 1928; Wang et al., 2020). The soil used in this study had about 15% clay, 0.6 mg/L of Fe and 2.6 mg/L of Al. Clay minerals and oxides of Fe and Al are known to have increased surface areas providing many sites for the adsorption of soil P depending on soil type (Freese et al., 1992). Soil testing and analysis to measure the soil's P adsorption capacity was not conducted to determine the specific threshold for "too high" concentrations of free oxides of Fe and Al in the soil. This would have provided supporting information on why P was low in the low pH treatments (see Table 1 and Table 2), i.e., the soil's ability to retain or release P in the treatment pots. Results from the incubation experiment may not come with a higher degree of confidence due to fewer replications (n = 2). However, they agree with studies on the negative effect of low pH on plant-available P (Fageria et al., 2010; Penn & Camberato, 2019; Sims & Pierzynski, 2005). Results from the incubation experiment may have also been affected due to dryness observed in all treatments, which may have slowed down nitrification during the incubation period (Sahrawat, 2008). There might also have been a possibility of a calculation error regarding the dose of AN (100%) applied, as AN treatments had an increased N content compared to AS (100%) (Table 1).

Additionally, P availability may have been affected by soil dryness. The activities of phosphate-solubilizing microorganisms are one of the factors that affect P availability in the soil (Rawat et al., 2021). Conditions, such as water content, temperature, available organic material, pH, and their interactions, control these soil microbial communities (Fierer, 2017; Wu et al., 2015; Xue et al., 2018). Thus, droughts and floods can easily affect the diversity and structure of soil microbial communities, affecting P availability.

The nitrogen source significantly influenced ammonium-N and nitrate-N (Table 1). This indicates that the type of nitrogen fertilization affects ammonium-N and nitrate-N levels in the soil. Ammonium nitrate fertilization leads to higher levels of  $\text{N-NO}_3^-$  in the soil compared to urea fertilization (Powlson & Dawson, 2022). Additionally, the amount of  $\text{N-NH}_4^+$  nitrogen in the soil depends on the fertilizer form, with significantly more  $\text{N-NH}_4^+$  nitrogen present in urea-fertilized soil (Dromantienè et al., 2020). The migration of  $\text{N-NH}_4^+$  into deeper soil layers is negligible (Dal Molin et al., 2020). Nitrogen fertilizers can also affect soil acidification, with N fertilizers potentially acidifying the soil after nitrification and nitrate leaching (Liu et al., 2021). However, the specific effects of different nitrogen forms on soil nitrifiers are not well known (Verma & Sagar, 2020). Overall, the type of nitrogen fertilization can significantly impact ammonium-N and nitrate-N levels in the soil, with different forms of nitrogen fertilizers leading to different levels of these compounds.

PO also significantly influenced ammonium-N after incubation (Table 2), indicating that P availability in the soil might negatively influence ammonium-N levels in the soil. Long-term P addition in agricultural soils can enhance gross N mineralization rates and increase overall N availability for crops in P-deficient soils (Mehnaz et al., 2019). However, the response of ammonia oxidizers to P addition is not well understood. One study found that P addition did not affect the abundances and community structures of ammonia-oxidizing archaea (AOA) and bacteria (AOB) in high P agricultural soil (Cheng et al., 2018). Another study showed that P addition reduced  $^{15}\text{N}$  in microbes without water stress, possibly by directly stimulating nitrification and denitrification (Liu & Zhang, 2018). Overall, the effect of P availability on ammonium-N levels in the soil may depend on various factors such as soil type, nutrient management practices, and water availability.

## 4.2 Effect of AMF Inoculation on Plant Growth

Soil phosphorus level is among the critical factors influencing the formation and effectiveness of AMF (Sanders & Tinker, 1973) to support plant growth. Based on results obtained from analyses of above-ground biomass, AMF inoculation did not have a significant main effect on shoot fresh and dry weights (Table 3). However, inoculation with AMF increased plant shoot fresh and dry weight in treatments that did not receive saturated polonite (Table 4). This may be attributed to the difference in the availability of phosphorus. Results from the Spurway soil analysis showed higher P in all treatments that received saturated polonite than those that did not (Table 1). These results agree with studies on the positive growth response to AMF inoculation in soils with low to deficient phosphorus content (Abdullahi & Sheriff, 2013; Mosse, 1973; Nouri et al., 2014; Rhodes, 1980; Stribley et al., 1980).

Mosse (1973) compared the growth of mycorrhizal and nonmycorrhizal onion plants in several soils which received increasing amounts of phosphorus as  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ . While mycorrhizal plants grew better at all levels of applied phosphate, mycorrhizal plants grew worst in soils where more than 0.2 g  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  was added per kg of soil; as such soils had a rapid build-up of high phosphorus concentrations in the plants. Abdullahi & Sheriff (2013) also conducted a study to determine if AMF could reduce the excessive amount of chemical fertilizer used for cultivating onion. Varying levels of N and P fertilizer used were 00-00, 40-20, 60-30, 80-40, 100-50 and 120-60 kg ha<sup>-1</sup> N and P, respectively. Observations from this study saw a reduction in plant growth response and nutrient concentration of AMF inoculated plants as fertilizer application increased from 80-40 to 100-50 and 120-60 kg ha<sup>-1</sup> of N and P. Additionally, a meta-analysis by Nouri et al. (2014) compiled data from multiple studies to evaluate the impact of P fertilization on the plant growth response to AM fungi. The analysis revealed that AM fungi generally had a more significant positive effect on plant growth under low-P conditions compared to high-P conditions. The study also highlighted that the interaction between P fertilization and AM fungi is complex and can vary depending on plant species, soil characteristics, and experimental conditions. Inoculation with AM fungi could reduce the excessive use of fertilizer. In contrast to the results of this study, Plenchette et al. (1983) reported increased plant growth to inoculation at all levels of phosphorus (from 4 to 144 mg P per week per 18 kg of calcined montmorillonite clay growth medium) supplied. Such growth enhancement was seen to vary with host species (leek, marigold and apple) as well as inoculum (i.e., two species of *Glomus* tested per host species). This is also in line with the results from Mosse (1973).

### 4.3 Phosphorus Concentration and Uptake in Leek Shoots

Mycorrhizal inoculation and the soil's phosphorus content can significantly impact the P concentration in plants, particularly in terms of mycorrhizal symbiosis and nutrient availability. AMF's mutualistic relationship has been observed to enhance nutrient uptake and growth in alliums such as onions (El-Sherbeny et al., 2022). In the present study, AMF inoculation significantly influenced shoot concentrations of macronutrients such as P, K, Na and Mg (Table 5). Significantly higher P concentrations (Table 5 and Table 9) recorded in shoots of the leek plants, which were inoculated compared to non-inoculated ones, indicate that mycorrhizal inoculation can enhance the percentage P concentration in plants by increasing P uptake (Table 11). These results support results from Xu et al. (2014), which recorded higher shoot and root P concentrations in (+) AMF-treated asparagus plants. (Guo et al., 2006) also observed that mycorrhizal colonization resulted in increased shoot dry weight, shoot-to-root ratio, shoot length, sheath diameter, and phosphorus concentrations of spring onion.

Regarding AMF interaction with N-source, results from Table 13 suggest that AMF inoculation in AS-treated leek plants may improve shoot P uptake. Ammonium sulfate reduced pH (Table 1) below the recommended pH, supporting soil P availability (Penn & Camberato, 2019). Therefore, results from Table 13 may indicate that P availability may have been less in the AS-treated soils and, therefore, AMF's ability to positively influence shoot P uptake when inoculated may have manifested. This agrees with study reports on AMF's ability to positively influence the uptake of P in soils with less available P (Abdullahi & Sheriff, 2013; Cress et al., 1979; Xu et al., 2014).

Saturated polonite influenced concentrations and uptake of P, Ca, Na, S, Mg, Mn, Zn and B (Table 5, Table 6, Table 7 and Table 8). Even though mean P concentrations in mycorrhizal plants treated with PO were relatively higher compared to mycorrhizal non-PO treated plants (Table 10), inoculation in non-PO treatments significantly increased P concentration compared to PO-treated mycorrhizal plants. This shows that when soil P levels are low (as seen in control, AS and AN only in Table 1), plants with mycorrhizal associations exhibit increased P uptake compared to nonmycorrhizal plants (Cress et al., 1979; Xu et al., 2014).

Mycorrhizal fungi can access and release bound or insoluble P forms through various mechanisms, including the recruitment of bacteria that produce a mineralization soil enzyme called alkaline phosphatase, associated with the breakdown of organic P compounds contributing to its availability (Fall et al., 2022). Suppose the soil contains an adequate supply of available P. In that case, mycorrhizal colonization may not significantly increase the plant's P concentration, as the plant can acquire sufficient P directly. However, mycorrhizal inoculation can

substantially enhance the plant's P uptake in P-deficient soils leading to higher P concentrations in plant tissues (Abdullahi & Sheriff, 2013; Xu et al., 2014).

Nitrogen fertilization can lead to increased leek plant growth, yield and yield components (Lencha et al., 2016). McCollum & Simmonds (1976) reported that nitrogen fertilization increases the diameter and length of bulbs of leeks. From the results, the N source significantly influenced not just the concentration of K, Na, Mn ( $p < 0.001$ ) and Ca, Fe, Zn ( $p < 0.05$ ) but P concentration and uptake by the leek plants as seen from (Tables 5,7, 9, 10, 11 and 12). The highest P concentration and uptake recorded in only control treatments compared to AS and AN (see Table 9 and Table 11) support that nitrogen, amongst other mechanisms such as depletion of P, soil barriers, transactional limitations, parent materials with low P, P sinks, and anthropogenic forcings increase the limitation of phosphorus and plants' demand for growth (Vitousek et al., 2010). Additionally, depending on the type of nitrogen fertilizer, the rate of application used and its interactions with soil, it can cause acidification or alkalization of the soil. Nitrogen fertilizers, particularly ammonium-based fertilizers, can lower the soil pH over time (Fageria et al., 2010; Wang et al., 2020).

Results from the present study showed that soil pH was significantly reduced due to AS 100% application (Table 1) and AS 150% (Table 2) compared to control, and these AS 100%-fertilized treatments recorded significantly low shoot P uptake and concentrations (Table 11 and Table 9) compared to control treatments. These results suggest that pH changes, influenced by AS application, can affect the availability and uptake of nutrients, including phosphorus (Penn & Camberato, 2019) in leek plants. Leek plants prefer a slightly acidic to neutral pH range (6.5–7.0) for optimal nutrient uptake (Swamy & Veere Gowda, 2006). If nitrogen fertilization alters the soil pH unfavourably, it may negatively affect phosphorus availability and subsequently impact P concentrations in leek plants.

#### 4.4 Phosphorus Acquisition Efficiency (PAE)

The present study showed that under (+) PO and (-) AMF conditions PAE (%) was high in control treatments compared to AN treatments (Table 14). This result is similar to results in Table 12, where shoot P uptake was high in control treatments compared to AN treatments under (+) PO and (-) AMF conditions. The results indicate that under (+) PO and (-) AMF conditions without increased N application via AN, P uptake and PAE (%) by leek plants is increased. High PAE (%) may have been positively influenced due to P addition through PO fertilization or possibly less N application (control) as this may have contributed to increased pH and PAE (%)

Soil P availability could therefore be a crucial factor, as (+) PO conditions may increase soil P levels which could lead to decreased mycorrhizal responsiveness and less reliance on the leek plant's mycorrhizal pathway for P uptake (Guang-Ming Huang et al., 2021). Increased pH in ((+) PO x control) treatments compared to ((+) PO x AN) treatments may have also contributed to increased shoot P uptake and PAE (%).

## 4.5 AMF Root Colonization and P Uptake

The results summarized in Table 11 and Table 15 indicate that AMF inoculation can positively influence shoot P uptake and leek root colonization. While the effect of AMF inoculation on shoot P uptake is significant (Table 12), the non-significant p-value in Table 16 and Table 17 indicate no relationship between AMF colonization and shoot P uptake.

In contrast, Xu et al. (2014) observed a significant positive relationship ( $p < 0.01$ ,  $n = 6$ ) between AMF-P and mycorrhizal colonization of asparagus roots. Mycorrhizal colonization also increased shoot dry weight, shoot-to-root ratio, shoot length, sheath diameter, and phosphorus concentrations in spring onion shoots (Guo et al., 2006). AMF alpha diversity, root colonization, hyphal density, and expression of phosphate transporter genes were also positively correlated with shoot phosphorus concentration and uptake (Lang et al., 2022). However, in a study with rye plants, despite being colonized by mycorrhizae, the phosphorus uptake from secondary phosphorus fertilizers was lower in AM plants compared to non-mycorrhizal plants. On the other hand, a meta-analysis of various studies showed that mycorrhizal plants significantly increased phosphorus uptake compared to non-mycorrhizal plants (Schwalb et al., 2021).

## 4.6 Agroecology and Sustainable Leek Production through AMF Inoculation and Saturated Polonite Fertilization

Agroecology involves an integrated approach with diversified crops and animal husbandry practices, addressing food security, climate resilience, and socioeconomic well-being (Yadav et al., 2021). It is a process based on ecological principles that aim to manage agroecosystems effectively, promote soil management, and achieve sustainable yields as well as environmental sustainability (Yadav et al., 2021). Agroecology involves implementing ecological principles and practices to improve soil quality and efficiency in agriculture on a long-term basis (Pagliarino et al., 2020). Sustainable cropping systems based on agroecological

principles are essential for addressing the challenges of increasing crop production while minimizing negative environmental impacts (Altieri, 2004; Reijntjes et al., 1992). These systems rely on the appropriate use of inputs, soil improvement, and the active engagement of farmers in managing resources and innovation.

From the results of the present study, AMF inoculation significantly and positively influenced above-ground biomass (Table 4), shoot P concentration (Table 9 and Table 10) and uptake by the plants (Table 11 and Table 12) in the under (-) PO conditions as the AMF was able to enhance uptake of the relatively low P in the soil (control). These results suggest that using AMF inoculation in leek cultivation may enable the responsible use of adequate amounts of P fertilizer inputs by the farmer for maximum plant growth (Xu et al., 2014) and sustainable production. This would further contribute to reducing the negative environmental impact associated with excessive phosphorus application in crop production (Ngatia & Taylor, 2019). One principle of agroecology is enhancing beneficial biological interactions and synergism among biodiversity components in an agroecosystem, promoting key ecological processes and services (Reijntjes et al., 1992). Inoculation of leek plants with AMF would go a long way to promote this principle through the plant-fungi relationship, which benefits both the plant and fungi.

Saturated polonite addition significantly and positively influenced soil P concentration (Table 1 and Table 2), shoot P concentration and uptake (Table 9 and Table 11) and above-ground biomass (Table 3). Saturated polonite also significantly increased soil pH (Table 1 and Table 2) but negatively affected ammonium-N in the soil. Results from Eurofins AB analysis of P in the saturated polonite together with Spurway soil analysis for P in PO treatments saturated polonite may release P slowly over a period of time, indicating a promising potential of saturated polonite if used as a P fertilizer source for sustainable leek production. Participatory research involving field experiments and farm trials where a selection of farmers are allowed to use the saturated polonite and afterwards, interviewed on how the use of the product affected cultivation and yield, farmers' environment, finances, feedback from customers and relationship with neighbouring farmers who do not use saturated product as their source of P. compared to regular application of chemical phosphate fertilizers. This would be needed to determine the economic, social and ecological feasibility of applying saturated polonite. This may contribute to the assessment of the interest of farmers toward its full adoption and application on farm fields as an alternative source of P fertilizer.



## Conclusion

The present study observed the effect of AMF inoculation on the growth and nutrient uptake of leek plants fertilized with saturated polonite, ammonium sulfate and ammonium nitrate. Nitrogen application overall affected shoot concentration and uptake of Na, S and Mn. Low P concentration and uptake was recorded in AS and AN treatments compared to control. Ammonium sulfate supplied at 150% (600 mg/pot) and 100% (400 mg/pot) reduced soil pH compared to control but no significant difference was observed between control and AS regarding P availability as the pH in control and AS treatments were below the requirement for enhancing P availability. When supplied at 100 % in the cultivation experiment, it positively influenced shoot fresh and dry weight compared to AN when applied to PO-treated plants. Reduced soil pH depending on the original soil pH may have positive or negative influence on P availability in the soil. In addition, results from the study suggest that AMF inoculation with leek plants can enhance above-ground biomass, concentration and uptake of P, amongst other nutrients, including K, Na and Mg by leek plants. However, AMF's capacity to support leek plants fertilized with P using saturated polonite may depend on the amount of P in the soil and the amount of P fertilizer input supplied to the plant. Under (-) PO conditions, AMF improved leek shoot growth, P concentration and uptake by the leek plants. AMF's interaction with AS also improved P uptake compared to AN and control. Under (+) PO conditions, leek shoot growth, P concentration, uptake and PAE (%) were not influenced by AMF inoculation. Control treatments without AMF inoculation had higher shoot P uptake and PAE (%) compared to AN which may have occurred due to increased pH in control treatments fertilized with PO compared to AN. There was also no relationship between root colonization and shoot P uptake. Saturated polonite positively influenced soil P concentration, plant above-ground biomass, shoot P concentration and uptake by the leek plants. It can be concluded that AMF inoculation has the potential to reduce P input dependence while supporting leek growth (above-ground biomass), shoot P concentration and uptake. Saturated polonite has the potential to contribute to soil pH increase, leek shoot growth, and P uptake, making it a good P fertilizer alternative to support sustainable crop production and circular economy. However, their interactive effect on shoot growth, shoot P concentration and uptake may depend on how much P is already available in the soil. The study used a single addition of saturated polonite but not

different levels. Another study where different levels of saturated polonite indicating increasing levels of added P would further verify AMF's ability to reduce P application and determine how much PO would be enough to combine with AMF inoculation to support plant growth and nutrient uptake.

## Popular science summary

This scientific study addresses the pressing issue of increasing global food demand and the challenges associated with meeting it sustainably. With a growing population, there's a higher need for food production, which, in turn, requires more phosphorus-based fertilizers. The problem is that much of this phosphorus comes from non-renewable phosphate rocks in politically unstable regions, which can threaten global trade and supply.

To mitigate these challenges, the study explores the potential of arbuscular mycorrhizal fungi (AMF) to enhance plant phosphorus uptake, reducing dependence on fertilizers. The circular economy concept is also emphasized, suggesting that recycled phosphorus fertilizers should be used responsibly in crop cultivation.

The study conducted experiments involving soil incubation and plant cultivation, examining the effects of AMF inoculation and different types of nitrogen fertilizers on soil pH, phosphorus availability, and plant growth, particularly leeks. Results showed that AMF inoculation positively influenced plant growth and phosphorus uptake when soil phosphorus levels were low, but not when they were sufficient. Saturated polonite, a potential alternative phosphorus fertilizer, also demonstrated positive effects on plant growth and phosphorus concentration.

In conclusion, the study highlights that AMF inoculation and the use of saturated polonite can enhance plant growth and phosphorus uptake, but their effectiveness depends on soil phosphorus availability. These findings have implications for sustainable food production, food security, and environmental protection.

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# Appendix 1

## Improving Crop Growth and Nutrient Efficiency with Mycorrhizal Inoculation and Sustainable Fertilization

*Fact Sheet for Farmers*

### Introduction

As a farmer, you are constantly seeking ways to enhance your crop yields while minimizing environmental impact. This study explores a sustainable approach to achieve these goals.

### Key Findings

#### Benefit of Mycorrhizal Inoculation

- Mycorrhizal fungi is a type of symbiotic fungi that form mutualistic relationships with the roots of most terrestrial plants.
- It can significantly boost crop growth and nutrient uptake. By fostering a symbiotic relationship with plant roots, they enhance nutrient absorption.
- Mycorrhizal inoculation has been shown to increase shoot growth and improve the concentration and uptake of essential nutrients, including phosphorus (P), potassium (K), sodium (Na), and magnesium (Mg) in leek plants.

### Saturated Polonite as a Sustainable Fertilizer

- Saturated polonite is obtained as a result of filtering municipal waste water using Polonite filter material. It absorbs phosphorus in the filtration process and becomes saturated after use.
- It is a promising sustainable fertilizer which positively influences soil nutrient concentrations and plant growth, particularly in phosphorus-scarce soils.
- It has the potential to contribute to soil health and fertility while increasing crop productivity.

### Balancing Nitrogen Application:

- The choice of nitrogen fertilizer (ammonium sulfate or ammonium nitrate) can affect soil pH and nutrient availability. Careful selection is essential based on your specific soil conditions and crop requirements.
- Sustainable nitrogen use can improve crop growth and nutrient uptake while minimizing environmental impacts.

## Implications for Farmers

By adopting mycorrhizal inoculation and utilizing saturated polonite, you can:

- Enhance crop yields, particularly in phosphorus-deficient soils.
- Reduce dependence on synthetic fertilizers.
- Improve health of the soil, which contributes to long-term sustainability and productivity.

## Practical Application

- Consider incorporating mycorrhizal inoculants into your soil preparation process to facilitate the beneficial relationship between mycorrhizal fungi and plant roots
- Evaluate the nutrient needs of your crops and experiment with saturated polonite as a sustainable phosphorus source.



Polonite

Source: Polonite Nordic

## Conclusion

- Sustainable agricultural practices are not only good for your crop yields but also for the environment. Mycorrhizal inoculation and saturated polonite can play a crucial role in achieving this balance.

## Next Steps

- Consult with local agricultural experts and extension services to determine the most suitable mycorrhizal inoculants and saturated polonite sources for your region.
- Implement small-scale trials on your farm to assess the effectiveness of these sustainable practices.
- Share your experiences and findings with fellow farmers and contribute to the adoption of environmentally friendly and economically viable agriculture.

## Reference

For full list of references, please see reference list in:

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## Appendix 2

### Analysis of Variance PAE

Source	DF	Adj SS	Adj MS	F-Value	P-Value
AMF	1	3.551	3.5510	6.75	0.041
Error	6	3.154	0.5257		
Total	7	6.705			

### Analysis of Variance Root Colonization

Source	DF	Adj SS	Adj MS	F-Value	P-Value
AMF	1	3600.0	3600.00	39.80	0.000
PO	1	121.0	121.00	1.34	0.270
AMF*PO	1	144.0	144.00	1.59	0.231
Error	12	1085.5	90.46		
Total	15	4950.5			

### Analysis of Variance shoot dry weight

Source	DF	Adj SS	Adj MS	F-Value	P-Value
AMF	1	0.01677	0.016767	0.92	0.342
Polonite	1	0.12593	0.125932	6.92	0.011
N Source	2	0.16618	0.083090	4.56	0.015
AMF*Polonite	1	0.14481	0.144806	7.95	0.007
AMF*N Source	2	0.09418	0.047092	2.59	0.086
Polonite*N Source	2	0.18668	0.093338	5.13	0.010
AMF*Polonite*N Source	2	0.00833	0.004164	0.23	0.796
Error	48	0.87375	0.018203		
Total	59	1.61663			

**Analysis of Variance Shoot fresh weight**

<b>Source</b>	<b>DF</b>	<b>Adj SS</b>	<b>Adj MS</b>	<b>F-Value</b>	<b>P-Value</b>
AMF	1	5.359	5.3587	3.38	0.072
Polonite	1	6.593	6.5928	4.16	0.047
N Source	2	13.297	6.6483	4.19	0.021
AMF*Polonite	1	12.338	12.3380	7.78	0.008
AMF*N Source	2	5.771	2.8854	1.82	0.173
Polonite*N Source	2	19.274	9.6369	6.08	0.004
AMF*Polonite*N Source	2	1.414	0.7069	0.45	0.643
Error	48	76.114	1.5857		
Total	59	140.158			

**Analysis of Variance Root Fresh weight**

<b>Source</b>	<b>DF</b>	<b>Adj SS</b>	<b>Adj MS</b>	<b>F-Value</b>	<b>P-Value</b>
AMF	1	0.8093	0.80929	0.78	0.380
Polonite	1	2.2426	2.24263	2.18	0.147
N Source	2	6.4117	3.20586	3.11	0.054
AMF*Polonite	1	2.9227	2.92273	2.83	0.099
AMF*N Source	2	0.0088	0.00439	0.00	0.996
Polonite*N Source	2	7.1257	3.56284	3.46	0.040
AMF*Polonite*N Source	2	1.0356	0.51780	0.50	0.608
Error	48	49.4923	1.03109		
Total	59	70.0487			

**Analysis of Variance plant height**

<b>Source</b>	<b>DF</b>	<b>Adj SS</b>	<b>Adj MS</b>	<b>F-Value</b>	<b>P-Value</b>
AMF	1	45.240	45.240	4.29	0.044
Polonite	1	31.538	31.538	2.99	0.090
N Source	2	16.276	8.138	0.77	0.468
AMF*Polonite	1	32.708	32.708	3.10	0.085
AMF*N Source	2	11.345	5.673	0.54	0.588
Polonite*N Source	2	3.184	1.592	0.15	0.860
AMF*Polonite*N Source	2	25.105	12.553	1.19	0.313
Error	48	506.532	10.553		
Total	59	671.929			

**Analysis of Variance Root Dry Weight**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
AMF	1	0.001591	0.001591	0.27	0.606
Polonite	1	0.000991	0.000991	0.17	0.684
N Source	2	0.005972	0.002986	0.51	0.606
AMF*Polonite	1	0.000006	0.000006	0.00	0.976
AMF*N Source	2	0.011213	0.005606	0.96	0.396
Polonite*N Source	2	0.011236	0.005618	0.96	0.395
AMF*Polonite*N Source	2	0.004716	0.002358	0.40	0.672
Error	29	0.169931	0.005860		
Total	40	0.214715			

**Analysis of Variance pH Before Incubation**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Polonite	1	0.083333	0.083333	16.67	0.006
N-source	2	0.061667	0.030833	6.17	0.035
Polonite*N-source	2	0.001667	0.000833	0.17	0.850
Error	6	0.030000	0.005000		
Total	11	0.176667			

**Analysis of Variance P before incubation**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Polonite	1	24.083	24.083	12.57	0.012
N-source	2	5.167	2.583	1.35	0.329
Polonite*N-source	2	2.167	1.083	0.57	0.596
Error	6	11.500	1.917		
Total	11	42.917			

**Analysis of Variance N before incubation**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Polonite	1	234.1	234.1	1.17	0.321
N-source	2	25768.2	12884.1	64.39	0.000
Polonite*N-source	2	208.2	104.1	0.52	0.619
Error	6	1200.5	200.1		
Total	11	27410.9			

**Analysis of Variance plant available P in the soil after Incubation**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
polonite	1	33.800	33.8000	84.50	0.000
N- sources	4	2.200	0.5500	1.37	0.310
polonite*N- sources	4	3.200	0.8000	2.00	0.171
Error	10	4.000	0.4000		
Total	19	43.200			

**Analysis of Variance pH after incubation**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
polonite	1	0.16200	0.162000	54.00	0.000
N- sources	4	0.20300	0.050750	16.92	0.000
polonite*N- sources	4	0.01300	0.003250	1.08	0.415
Error	10	0.03000	0.003000		
Total	19	0.40800			

**Analysis of Variance N after incubation**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
polonite	1	5.00	5.000	0.11	0.746
N- sources	4	1130.00	282.500	6.28	0.009
polonite*N- sources	4	270.00	67.500	1.50	0.274
Error	10	450.00	45.000		
Total	19	1855.00			

**Analysis of Variance Shoot P concentration**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
AMF	1	0.028911	0.028911	15.23	0.000
PO	1	0.061230	0.061230	32.25	0.000
N-Source	2	0.060464	0.030232	15.93	0.000
AMF*PO	1	0.008617	0.008617	4.54	0.040
AMF*N-Source	2	0.007628	0.003814	2.01	0.149
PO*N-Source	2	0.004738	0.002369	1.25	0.299
AMF*PO*N-Source	2	0.000566	0.000283	0.15	0.862
Error	36	0.068340	0.001898		
Total	47	0.240496			



**Analysis of Variance Shoot P uptake**

<b>Source</b>	<b>DF</b>	<b>Adj SS</b>	<b>Adj MS</b>	<b>F-Value</b>	<b>P-Value</b>
AMF	1	1.4005	1.4005	8.89	0.005
PO	1	4.6636	4.6636	29.61	0.000
N-Source	2	3.1789	1.5895	10.09	0.000
AMF*PO	1	1.8759	1.8759	11.91	0.001
AMF*N-Source	2	1.3960	0.6980	4.43	0.019
PO*N-Source	2	0.2996	0.1498	0.95	0.396
AMF*PO*N-Source	2	0.5653	0.2827	1.79	0.181
Error	36	5.6698	0.1575		
Total	47	19.0496			

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