Synergies of nutrient fractions for fertilization in agriculture

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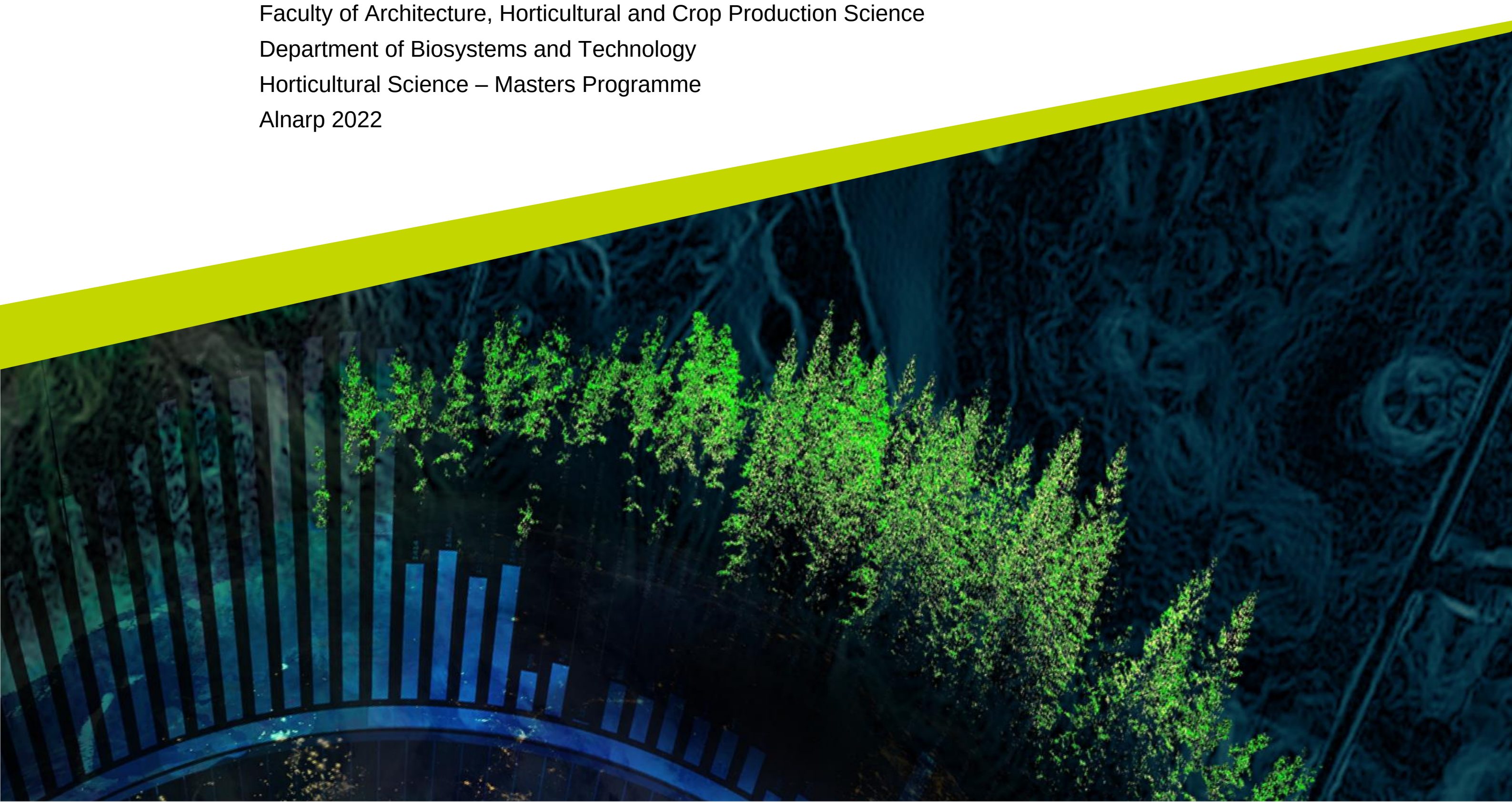
Horticultural Science – Masters Programme
Alnarp 2022



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Credits:	30 hp
Level:	Second cycle, A2E
Course title:	Independent Project in Horticultural Science
Course code:	EX0948
Programme/education:	Horticultural Science – Master’s Program
Course coordinating dept:	Department of Plant Breeding

Place of publication:	Alnarp
Year of publication:	2022
Cover picture:	Name of photographer (if any)

Keywords: Phosphorus, Wastewater, Polonite, molybdenum blue spectrophotometry, Fertilizer, Sunflower, Plant growth

Swedish University of Agricultural Sciences
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Department of Biosystems and Technology

Publishing and archiving

Abstract

A few countries are controlling the worlds phosphate reserves and this raw material is depleting at a faster rate. Most of the mined phosphate rock comes from Morocco and the Russia area which has unstable security. Studies shows that about 40% of potash export and 28% of nitrogen and phosphorus (P) fertilizer products comes from the Russia area. The current conflict in this region has increased shortage of these fertilizers impacting prices to increase sharply. The need for the development of chemical fertilizer substitutes especially for P is crucial for importing countries, the world's food security and the environment. For this reason, this study aimed to formulate a complete fertilizer product that included saturated Polonite which is a waste product of wastewater treatment with a proven record as a good substitute to chemical P fertilizer.

Polonite (Po), soil (S), peat (Pe), poultry manure (Pm) and Biofer N15 (B15) were the materials used for the formulation of the fertilizer product. To select the best formulated product, an initial molybdenum blue spectrophotometric P and pH analysis was carried out on base substrates (S, S+Po, S+Po+Pe) and incubated substrates. Incubated substrates were divided into six groups that included both fertilized (base substrates with either Pm or B15) and unfertilized (Pe, S, S+Po, S+Po+Pe) substrates with varying Po content between 10% and 20%. They were kept in a dark chamber at 20°C for a 0 and 7 days' period (week 0 to week 1). The final selected substrates were grouped into three according to their treatment namely; treatment O (S, S+Po, S+Po+Pe), treatment Pm (S+Pm, S+Po+Pm, S+Po+Pe+Pm) and treatment B15 (S+B15, S+Po+B15, S+Po+Pe+B15). The same method of P and pH analysis was conducted on the final selected substrates used for the pot experiment. For our study, calcium chloride extractable P ($_{\mathrm{CaCl2}}$P) and pH$_{\mathrm{CaCl2}}$ was measured whiles portions of the selected final substrates sent for lab analysis measured ammonium lactate extractable P (P-AL) and pH$_{\mathrm{water}}$. The pot experiment was performed in a climate chamber in the Biotron at SLU to test the effect of the final selected substrates on the growth of Pacino cola sunflower variety.

Results indicated that unfertilized substrates recorded low plant-available P as compared to fertilized substrates based on the results obtained from our study and lab analysis. Results from the lab analysis indicated that all substrate used for pot experiment obtained a pH$_{\mathrm{water}}$ of 5.8-6.7 suitable for sunflower growth. With the exception of Pe with low a pH$_{\mathrm{CaCl2}}$ of 2.73, all other substrates under initial, incubation experiment and final substrate analysis obtained a pH$_{\mathrm{CaCl2}}$ values within the range of 5.0-7.13. There were no significant changes in plant-available $_{\mathrm{CaCl2}}$P of incubated substrates from week 0 to week 1 but some changes in pH$_{\mathrm{CaCl2}}$ occurred in substrates S+Po and S+Po+Pe and S+Po+Pm. Results from the pot experiment found substrates fertilized with Pm and B15 had a significant impact on sunflower growth as compared to unfertilized substrates. However, the addition of Po had no significant effect on sunflower growth. Among the fertilized substrates, substrates fertilized with Pm performed better. Also, some deficiency symptoms on the plants were observed.

It can be concluded that saturated Polonite together with poultry manure may increase plant available-phosphorus, pH and nutrient efficiency use by plant that can lead to increase in plant growth and yield. However, large portions may be needed to meet plant nutrient needs per hectare and it is suggested this formulated product should be used together with chemical fertilizers. Also, the effectiveness of Po as an alternative P fertilizer need to be verified from another experiment by using materials with less P content together with saturated Polonite.

Keywords: Phosphorus, Wastewater, Polonite, Molybdenum blue spectrophotometry, Fertilizer, Sunflower, Plant growth

Preface

This work is dedicated to my parents and late brother

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Abbreviations

B15	Biofer N15
cm	Centimetre
F.C.	Field capacity
g	Grams
ha	Hectare
K	Potassium
kg	Kilogram
L	Litre
mg	Milligram
mL	Millilitre
N	Nitrogen
P	Phosphorus
Pe	Peat
Pm	Poultry manure
Po	Polonite
S	Soil
V	Volume
Vw	Volume weight

1. Introduction

The global population is expected to increase from 7.7 billion to 9.7 billion by 2050 and is likely to peak at about 11 billion around 2100 (UN, 2022). The need to meet the global food demand of the ever growing population has resulted in an increased demand for chemical fertilizers for higher crop yields worldwide (Lun *et al*., 2018). Nitrogen, phosphorus and potassium (NPK) are essential nutrients for plant growth. They are also the most extensively used chemical fertilizers for food production. Among all these essential plant nutrients, P plays a crucial role in optimum growth and development of plants (Noor *et al*., 2021). It plays a significant role in the maintenance of membrane structures, synthesis of biomolecules and formation of energy molecules (e.g. ATP, ADP). Phosphorus (P) stimulates seed germination, increase root, stem strength, flower and seed formation in plants and this in turn increases yield and food quality (Malhotra *et al*., 2018).

1.1. Problems associated with chemical fertilizer production and use

Despite the benefits derived from chemical fertilizers, the increased and continuous use has made sustainable agricultural production a major global issue (Khan *et al*., 2021). Large concentrations of applied P are fixed in soils in the form of aluminum, iron, calcium and magnesium phosphate making it unavailable to plants. Some portion of the accumulated P is lost to leaching and run-off leading to the eutrophication of both inland and coastal waters (Lun *et al*., 2018). Again, other fractions of P accumulated in surface water comes from the runoff from private sewers and municipal waste transport (Sarvajayakesavalu *et al*., 2018). Eutrophication which is evident in waters worldwide is a complex process that causes algae blooms which in turn have negative effects on biodiversity in water. Phosphate rock is the primary source of synthetic P fertilizer production. Most of these non-renewable mineral reserves occur in Morocco, China and the United States (Jama-Rodzenska et. al., 2021). The reserves in these three countries account for about 85% of phosphate rock worldwide (Kratz *et al*., 2019). Phosphate reserves in Morocco accounts for about 70%, followed by china with 6% (Jama-Rodzenska et. al., 2021). The mining of phosphate rock quadrupled from 46 Mt in 1961 to 198 Mt in 2011 to serve the growing demand (Lun *et al.*, 2018). Sarvajayakesavalu *et al.* (2018) stipulates that by 2100, 50% of the total reserve base will be depleted which may cause major food security issues. Also, the European commission in 2014 placed phosphate rock on the list of critical materials and added P in 2017. For this reason, most European countries such as Switzerland and Germany are continuously developing efficient ways to use renewable phosphate resources in the future (Kratz *et al.*, 2019). Unlike P, production of nitrogen fertilizer is infinite due to the conversion of nitrogen gas to ammonia by the Haber–Bosch industrial process. However, an enormous amount of energy is needed to convert the triple bond molecular to two molecules of ammonia (Theregowda *et al.*, 2019). According to Ghavam (2021), the Haber–Bosch industrial process emits high greenhouse gas of about 2.16 kgCO2-eq/kg NH_3 and uses an equivalent high amount of energy which spans over 30GJ/ton NH_3. In addition to the pollution associated with N production, the high energy prices further increase the cost of N fertilizers across the value chain. Chemical N fertilizer use can easily be ameliorated using nitrogen fixing crops and manures but mined P is subject to variability in price and physical availability on the global market as well as limitation of use of available P fertilizer alternatives.

1.2. Suggested solution and alternative P sources

Solution to phosphate reserve depletion, eutrophication, wastewater management and future food security issues lie in the concept of circular economy (Wali *et al.*, 2021). A circular economy is meant to prolong the life cycle and value of products within an economy while protecting the environment (EU, 2020). With this principle, life cycle of raw P material extracted from the environment can be extended through recycling. Farmyard manure and compost have long been used as a source of organic P fertilizer and soil ameliorants (Bloem et al., 2017). Also, sewage sludge, animal by-products and biogas digestates produced mainly from farmyard manures, plant materials and sewage sludge are used as P fertilizers (Bloem *et al.*, 2017). Sewage sludge is one of the organic waste with a high concentration of P (Bloem *et al.*, 2017). Aside from the high concentrations of P, it improves the chemical, physical and biochemical properties of the plant mediums (Bloem et al., 2017). In addition, European Union (EU) regulations allow for phosphate rocks, animal manures and a restricted list of P-containing recycled organic materials (e.g. treated household waste, bone and blood meal) for organic crop production (Løes *et al.* 2017). Among the organic sources, recovery and redistribution of P from wastewater to agricultural land is one mechanism for reconciling P wastage and scarcity. In terms of wastewater management, treatment using reactive filter media which is efficient in nutrient recovery is considered the best option (Cucarella *et al*., 2007).

The treatment of private and municipal wastewater which are common sources of pollution is now a common practice in most countries. Treatment systems that make use of media filters, natural systems (wetlands, greenhouse), aerobic treatment units (ATUs), waterless toilets (dry toilets) and disinfection systems (UV light, chlorination/dechlorination) are adopted for on-site wastewater treatments (Cucarella, 2009). In this system, media filters are prepackaged and normally placed after septic tanks to improve the quality of effluents before discharge. However, P removal efficiency is largely dependent on the properties (structure, particle size, porosity and pH) of the media filter used even though other factors come to play. A great number of reactive materials have been proposed as suitable filter media for P removal (Cucarella 2009). These materials are usually rich in Ca, Fe or Al compounds, which favor the interaction with P. According to Renman (2008), reactive materials can be classified into natural (opoka, limestone and shell sand), industrial (fly ash, red mud and blast furnace slag, ochre) and commercial products (Polonite and Light expanded clay aggregates). In Sweden, statistics since the 1970s show a steady decrease of about 90% in toxic metal concentrations in municipal sewage sludge (Kirchmann *at al*., 2016). This makes nutrient recycling from wastewater economically feasible in Sweden. According to Moges (2018), approximately 90% of nitrogen, 74% of phosphorous and 79% of potassium can be recovered from wastewater and organic food waste recycling. Reactive filter materials used for P removal in wastewater are also rich in calcium coupled with high pH values (Cucarella 2009). This waste product obtained after wastewater recycling has a potential benefit as a fertilizer in particular after the incorporation of other macro-and micro-nutrients (Cucarella 2009). Also, this innovation complements the United Nation (UN) adopted sustainable development goals (SDGs) of eradicating hunger and poverty through sustainable intensification of agricultural production by 2030 (Calabi-Floody *et al*., 2018).

1.2.1. Forms of P

Wastewater treatment technologies are mostly developed based on the understanding of the different forms, fractions and analysis of P (Carrillo *et al*., 2020). Potential availability of P to plants can be estimated using test P methods. P is a non-metal element that can bond to other elements as oxygen, carbon and nitrogen to form organic and inorganic phosphate forms (Carrillo *et al*., 2020). Organic compounds, orthophosphates and polyphosphates are the common forms of P present in wastewaters (Ruzhitskaya *et al*., 2017). Either orthophosphate or polyphosphate makes up to 70 to 90% of P in drain liquids and the rest occurs in the form of organic compounds (organophosphate). Organic P forms (poly and pyrophosphates) become available to plants after hydrolysis and mineralization into orthophosphate form. This conversion usually takes between a couple of hours, days, weeks or months depending on the biological (enzyme activity) and physico-chemical (adsorption/precipitation properties) conditions in the soil or media environment (Kratz *et al.*, 2019). In dissolved P form, orthophosphate (PO_4^{3-}) is the most common and generally occurs at a pH above 9 which can be detected in filtered (0.45 µm) wastewater samples (Rajabu 2019). P concentrations can be expressed as phosphate or orthophosphate or as phosphate phosphorus (PO_4^{3-}–P). PO_4^{3-} is a measure of the amount of orthophosphate molecules in a sample, while PO_4^{3-}–P is a measure of the amount of phosphorus ions in a sample (Carrillo *et al.*, 2020). PO_4^{3-} results combine the phosphorus and oxygen in the measurement, while PO_4^{3-}–P only considers the phosphorus in the measurement. The P analysis tests can be classified as total P test, dissolved P test and insoluble P test (EPA, 2012). The total P test analysis accounts for the sum of all different forms of P present in a sample. The dissolved P test measures the fraction of soluble P in a filtered sample. The Insoluble phosphorus is calculated by subtracting the dissolved P result from the total P result. In water, free phosphate ions (PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^{-}$) present can form blue molybdenum complex and are known as P-reactive while all other P organic and inorganic compounds are classified as non-reactive P (Carrillo *et al.*, 2020).

1.2.2. P measurement methods

Information on P speciation aids to understand the processes of improving soil conditions for plants after using by-products from recycled sewage sludge as a soil amendment (Li *et al.*, 2018). It aids in differentiating between organic and inorganic forms of P and also for easy selection of P speciation techniques (Doolette *et al.*, 2011). A chemical fractionation or sequential fractionation schemes is one of the widely used methods and it makes use of extractants of increasing strength to differentiate P forms and availability in soil (Luo *et al.*, 2017). Chemical fractionation schemes are suggested to classify inorganic phosphate into Al–P, Fe–P, Ca–P and Occluded-P (Jiang *et al.*, 1982; Luo *et al.*, 2017). Speciation methods can also be classified into operational and direct speciation (Li *et al.*, 2018). Operational speciation measure P forms according to their solubility in a variety of extractants for sequential fractionation. The limitation of this method is the provision of information only on P fractions available to plants and the environment in terms of P solubility in different extractants rather than the exact species of P. In terms of direct speciation, existing molecular forms (inorganic orthophosphate and organic orthophosphate monoester) and phases of P can be measured. ^{31}P nuclear magnetic resonance (NMR) and X-ray diffraction (XRD) techniques can be employed in measuring both operational and direct speciation in terms of the structure and chemical state of P (Li *et al.*, 2018). X-ray absorption near edge structure (XANES) spectroscopy is another method employed in direct P speciation in complex environmental samples (Eveborn *et al.*, 2009). Eveborn *et al.* (2009), employed this method in the determination of chemical forms of P embedded in filter media used for on-site treatment of wastewater. At ppm levels of P, methods such as colorimetry, atomic absorption spectroscopy, flow injection analysis, high-performance liquid chromatography and spectrophotometry have been identified as sensitive analytical methods of phosphate measurement (Ganesh *et al.*, 2012). In this study, the spectrophotometry method was selected for the analytical measurement of P. It is a method that measures sample solution P concentration by measuring the intensity of light absorbed by the solution (Libretexts 2020). The underlying principle is that each compound absorbs or transmits light over a certain range of wavelengths. This method can also be used to measure the amount of an unknown chemical substance. The absorbance measured by a spectrophotometer is a logarithmic measure of the amount of light that is absorbed by the sample and is directly proportional to concentration (Libretexts 2020). A Spectrophotometer is a common instrument used for P measurement in different samples. According to Manová *et al.* (2020), spectrophotometry remains the most multipurpose and cost-effective method that is applicable for the analysis of different objects.

1.3. The use of filter materials as an alternative P sources in agriculture

The recycling of filter materials as a P fertilizer source for plant cultivation is gradually gaining attention. Several studies have been conducted on testing the efficiency of saturated filter materials in increasing plant-available P. Dobbie *et al.* (2005) conducted a pot experiment with grass, barley, spruce and birch as well as a field experiment on acid grassland and barley using saturated ochre with available P of 0.94g/kg. With the pot experiment, soil was mixed with varying amounts of P-saturated ochre. The results showed that total P increased with increasing amounts of ochre. The P released from ochre during the start of the experiment were not significantly different from P available at end making saturated ochre a slow release fertilizer. Also, the pH of soil increased with increasing ochre content (from 5.5 to 8.7) of soil but both increase in P and pH had no adverse effects on crop production.

Tylstedt (2011) investigated the effect of phosphorus filters on barley growth and P uptake. Four filters namely Filtra P, Polonite, Filtralite and Hyttsand which had been used in the field for phosphorus separation for 16 months were added to soil for the experiment in a greenhouse. The addition of Filtra P and Filtralite increased production of shot biomass slightly as compared to Polonite and Hyttsand in joints that were not phosphorus fertilized and grown on the calcined soil. It was concluded that the addition of filter material to soil did not have any effect on the barley growth and phosphorus uptake of barley despite the very good dose application in some stages of the crop production.

Kvarnström *et al.* (2004) tested four filter substrates sampled from two constructed subsurface-flow wetlands (WL and WS) and from two infiltration basins (IB1 and IB2) to access the plant-availability phosphorus (P) accumulated on the substrates during wastewater treatment on rye grass in a greenhouse experiment. The use of filter substrates in wastewater treatment increased the content of total, inorganic and organic P for all the substrate except for IB1. Also, the plant yield did not increase significantly for any treatment compared with the control without P fertilization. They attributed this to the sufficient P in the soil used in the experiment. Total plant P uptake slightly increased after applying water soluble P and WL as compared to control, IB1, IB2 and WS treatments.

Bird *et al.* (2009) compared the efficacy of electric arc furnace slag P-loaded with dairy wastewater (1.09 mg total P removed g^{-1} slag) and triple superphosphate (TSP) fertiliser on the growth of *Medicago sativa*. At 10 weeks and at an application rate of approximately 3.8 mg P kg^{-1} soil, there were no significant differences compared to TSP fertiliser. At a high rate of approximately 22.9 mg P kg^{-1} soil electric arc furnace slag application produced 76% less biomass than TSP.

Cabeza *et al.* (2011) investigated magnesium–ammonium phosphates and calcium phosphate prepared from the by-products of three different sewage treatment plants. Other P products such as an alkali sinter phosphate, a heavy metal depleted sewage sludge ash, a cupola furnace slag, made from sewage sludge and a meat-and-bone meal ash prepared through thermal processes were tested for maize growth in a pot experiment over a period of 2 years. The growth patterns of the crop were compared with that of a commercial phosphate fertilizer and phosphate rock. In both acidic and in neutral soils, magnesium–ammonium phosphates and sintered-P products enhanced maize growth while calcium phosphate was only effective in the acid soil. It was concluded that magnesium–ammonium phosphates that are derived from these wastewaters and, to a certain extent, sintered-P could be used to enhance soil pH. It was also cited that direct use of P recovery, as ash, is of low value as P fertilizer in agriculture, but such ash products could be used as raw material for the fertilizer industries.

Cucarella (2009) found Polonite to be the most suitable substrate for the recycling after conducting a study on barley and ryegrass with different filter materials. Hylander *et al.* (2006), had a slight improvement in barley dry weight with an application of Polonite. Cucarella *et al.* (2009) conducted a study by applying saturated Polonite from an on-site wastewater treatment as a soil amendment to a mountain meadow. Polonite was found to significantly increase soil pH and decreased Al toxicity risks but showed no difference in yield and P uptake by meadow plants. Despite some positive outcomes from various studies, the potential of saturated filter materials especially Polonite being formulated into a commercial fertilizer product is still at the idea stage. Therefore, further studies on Polonite as a potential P fertilizer component is needed. Also, collaborations between major government and private stakeholders across the agricultural sector is crucial in bringing this innovative product to the limelight.

1.4. Aim and Objectives

- The aim is to formulate a complete, cost-effective and nutritionally attractive fertilizer product that includes saturated Polonite.
- The first objective is to determine the amount of potentially plant-available P nutrient after incubation of fertilizer mixtures where Polonite is included
- The second objective is to investigate the effects of the fertilizer product on sunflower growth.

2. Materials and Methods

2.1. Materials for fertilizer product formulation

The materials used in this study were soil (S), Polonite (Po), peat (Pe), poultry manure (Pm) and Biofer N15 (B15) (figure 1). These were selected because they were commercially available, environmentally friendly, economical and permitted for use in Scandinavia for crop production.

- Soil sample was obtained from Lönnstorps Research Station located in the southwest of Skåne. The soil was collected at a depth 0cm to 20cm from a 2m x 4m plot. The field-fresh soil was sieved to eliminate coarse rock and large plant material and thoroughly mixed to ensure uniformity. The soil collected was placed in plastic bags, stored outdoor and protected from rain and sunlight from mid-September to February.
- Polonite® is a registered commercial product derived from heating opoka at high temperatures up to 900^0C. Opoka is a sedimentary bedrock formed from remains of minute marine organisms (diatoms) which is found in Europe (Poland) and western Russia. It is rich in silica and calcium with significant amounts of aluminum and iron oxides (Cucarella 2009; Kolosov 2014). The high heating involved in opoka preparation converts most of the calcium carbonate ($CaCO_3$) into calcium oxide (CaO) making it more reactive in an aqueous solution. The product is grinded and sieved into particle sizes (2mm-6mm) for use in filter systems (Cucarella *et al.*, 2009). Po used in this study was obtained from Polonite Nordic AB (PNAB), a subsidiary of Alnarp Cleanwater. This Swedish company deals with phosphorus capture primarily from individual sewers using Polonite. Two bags (about 500kg) of air-dried saturated Polonite was obtained from the company and used to conduct the experiment.

- Poultry manure was obtained from Blomsterlandet in Sweden. The organic manure was in a pelleted form with an NPK ratio of 4:1.5:2 (table 1).

Table 1. The plant nutrient content in poultry manure (Blomsterlandet 2020)

Nutrient	Quantity (%)	Nutrient	Quantity(g/100g)
Total nitrogen	4.0	Phosphorus (P-AL)	1.1
NO_3^-N + NH_4^-N	0.3	Potassium (K-AL)	2.0
Water soluble N	0.3	Calcium (Ca-AL)	1.5
Phosphorus	1.3	Magnesium (Mg-AL)	0.4
Water soluble P	0.9	Iron	700 (mg/kg)
Citrate soluble P	0.8		
Potassium	2.0		
Water soluble K	2.0		
Magnesium	0.5		
Calcium	2.0		
Chlorine	<2		

- Biofer N15 is a special fertilizer for organic cultivation with a low P content produced by Gyllebo Gödning AB in Sweden (table 2). The KRAV-approved product was produced from animal by-products from the conventional and organic slaughterhouse. The KRAV label stands for food produced without artificial chemical pesticides, reduced climate impact, more biodiversity and better working conditions. The product is a good plant nutrient for fertilizing cereals, oilseeds, sugar beets and vegetables (Gyllebo Gödning 2020).

Table 2. The plant nutrient content in Biofer N15 fertilizer (Gyllebo Gödning 2020).

Nutrient	Quantity (%)	Nutrient	Quantity(mg/kg)
Nitrogen	14.5	Manganese	23
Phosphorus	0.30	Boron	22
Potassium	0.00	Iron	290
Sulfur	2.20	Lead	0.51
Magnesium	0.00	Cadmium	0.03
Sodium	0.70	Copper	<10
Calcium	0.40	Chromium	<10
		Quicksilver	0.02
		Nickel	1.4

	Zinc	230

- The brand of peat used to conduct the experiment was Naturtorv from Hasselfors Garden AB.

Figure 1. The materials used for fertilizer formulations

2.1.1. Reagents and standards for P analysis (after Murphy and Riley 1962)

All reagents used were purchased and they were all of analytical grade. Water used in standards and reagents preparation was ultra-pure collected from a Millipore water purification system. Prepared reagents were stored in glass and polypropylene bottles. Sulphuric acid (5N), ammonium molybdate, ascorbic acid (0.1M), potassium antimonyl tartrate (1mg Sb/ml) and calcium chloride ($CaCl_2$) were the reagents used. Sulphuric acid was prepared by diluting 70ml of concentrated sulphuric acid in 500ml pure water. In the case of ammonium molybdate, a standard ammonium heptamolybdate tetrahydrate was used. It was prepared by dissolving 20g ammonium heptamolybdate tetrahydrate in water and diluted to 500ml. The ascorbic acid solution was prepared fresh daily by dissolving 1.32g in 75ml of water. Potassium antimonyl tartrate was prepared by dissolving

0.2743g in distilled water and diluted to 100ml. Calcium chloride was prepared in two different concentrations (0.1M and 0.01M) according to the purpose for which it was used. Calcium chloride of 0.1M was prepared by dissolving 14.7g of calcium chloride dihydrate ($CaCl_2$ x $2H_2O$) in 1000ml ultra-pure water. This solution was used for the preparation of standard solutions. The 0.01M solution was prepared by dissolving 1.47g of $CaCl_2$ x $2H_2O$ in 1000ml ultra-pure water and this was used for extraction of P in samples. All calcium chloride solutions were prepared fresh daily. Aside from sulphuric acid and calcium chloride solutions, all other solutions were stored under stable room temperature conditions. A mixed reagent was prepared and used for molybdenum blue spectrophotometric P determination. The mixed reagent was prepared by mixing solutions of 125ml of sulphuric acid, 37.5ml of ammonium molybdate, 75ml of ascorbic acid solution and 12.5ml of potassium antimonyl tartrate. This reagent was prepared fresh daily when needed and used within 24 hours. For standard phosphate solution, 0.1757g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 1000ml of 0.1M calcium chloride dehydrate solution. This standard solution contained 40mg P (as phosphate)/L.

2.1.2. Instrumentation

- A Shimadzu UV-Visible spectrophotometer equipped with a 5-cm cuvette was used to measure the absorbance of P at 740nm to 1100nm wavelength.
- A VWR® Mega star 600R centrifuge was used to separate substance mixtures of different densities of samples.
- pH meter (pH- Power of hydrogen, is a measure of the acidity or basicity of an aqueous solution)

2.2. Measurement methods

2.2.1. Compacted bulk density of materials

Bulk density (B_D) is a measure of the weight of a material per unit volume. This measurement expresses the weight of a material (S, Po and Pe) in volume basis. After measuring the bulk density of the individual substrates or materials, the needed quantities calculated based on their bulk density are measured and mixed together. Then another compacted bulk density is performed on the mixed substrates to obtain the volume weights (Vw) of the mixed substrates for accuracy. All the quantities of S, Po, Pe, S+Po and S+Po+Pe was measured using the laboratory compacted bulk density method described in EN 13040:2007. The rigid test cylinder apparatus and an electronic scale was used for the measurement. The method involved weighing the empty test cylinder (mo), placing the collar and funnel in position followed by placing the screen above the funnel. Afterwards, a sufficient amount of the sample material was passed through the screen till the apparatus had overfilled. The screen was then removed and the excess material strike off with a straight edge and the plunger was placed on the material for 3 minutes. The plunger and collar were removed afterwards and the material was levelled once again. Finally, the material together with the cylinder was weighed and recorded as mx. This method was replicated 5 times for each material. The laboratory bulk density (B_D) were obtained using the equations in table 2. The same procedure was used in determining the Vw weight for all mixed substrates (S+Po and S+Po+Pe) used for incubation and pot experiment.

Table 3. Equations used for the calculation of laboratory bulk density of materials (EN 13040:2007).

Arithmetic mean	Laboratory bulk density
$m = \frac{\sum mx}{n}$	$B_D = \frac{m - mo}{V}$
m – arithmetic mean of sample and cylinder in g mx – mass of cylinder and sample in g ∑mx – sum of mass in g of n replicates n – number of replicates	B_D- laboratory bulk density in g/L m- arithmetic mean of sample and cylinder in g m_o- mass of empty test cylinder in grams V- Volume of cylinder in litres V= 0.881liter

2.2.2. Spectrophotometric molybdenum blue method for determination of phosphate

This method involves the formation of molybdophosphoric acid from orthophosphate and an excess of molybdate in acidic solution followed by reduction to give molybdenum blue (Pradhan *et al.*, 2013). The absorbance of produced molybdenum blue was measured spectrophotometrically at 880nm wavelength which gave maximum absorbance for samples. The intensity of the blue color was proportional to the amount of phosphate present in the sample solutions (figure 2). For this experiment, ascorbic acid was used as the reducing agent. Other reducing agents such as sodium sulphide and stannous chloride have been reported in the literature (Pradhan *et al.*, 2013).

2.2.3. Calibration and standards

Preparation of standards was performed by further dilution of the standard stock solution. A second stock solution of 20mg/L was prepared by placing 250ml of the aliquot into a measuring flask and diluted to 500ml with pure water. Standards for calibration curve were prepared from the latter by transferring 2.5ml, 5ml, 7.5ml, 10ml, 12.5, 15ml, 17.5ml, 20ml, 22.5ml and 25ml standards into a 100ml volumetric flasks followed by the addition of 8ml of mixed reagent and diluted to a volume of 50ml with pure water. The resulting solutions contained 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1mg/L concentrations of phosphate respectively. The absorbance was measured after 45 minutes at a 740nm to1100nm wavelength. The spectrophotometer was set at zero by using pure water as a blank. Calibration curve was necessary to establish a relation between the absorbance and the phosphate content from spectrophotometric analysis. The curve was obtained by plotting absorbance as a function of concentration of phosphate (mg/L).

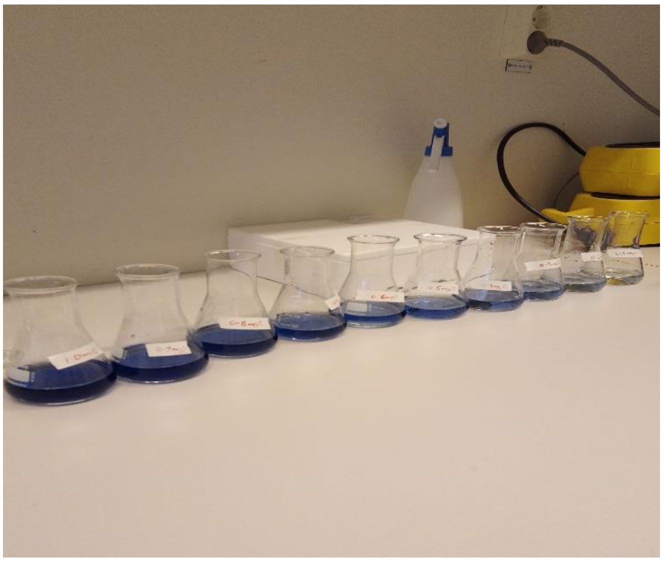


Figure 2.Colour development of standard solutions used for spectrophotometric phosphate analysis

2.2.4. Substrate samples extraction and analysis method

10g each of dried samples of substrates were weighed into 250ml plastic bottles for extraction using 100ml of 0.01M $CaCl_2$ solution. The extraction was done at a 1:10 weight to volume ratio. The samples were placed in an end-to-end mechanical shaker for 2 hours. Afterwards, the pH of the resulting suspension solutions was measured and 30ml of it was decanted into 50ml centrifuge tubes. They were placed in a centrifuge for 10mins at 1800g. 10ml of the resulting supernatant was transferred into test tubes. 0.1ml of 1M HCl (hydrochloric acid) was added to each test tube which was stored in a refrigerator at 4^oC overnight before phosphate analysis. For spectrophotometric phosphate analysis, 1.0ml of mixed reagent was mixed with 5ml of sample extracts and allowed to stand for 1 hour. The absorbance of each sample was then measured over 740mn to 1100mn wavelengths and the absorbance recorded. The spectrophotometer was set at zero by using 0.01M $CaCl_2$

solution as a blank. The unknown amounts of phosphate in the samples was deduced from the standard curve using the absorbance values. Since the extraction was done with 100ml of 0.01M $CaCl_2$ solution, resulting P and pH measured were termed as $_{CaCl2}$P (Calcium chloride extractable P) and pH_{CaCl2}.

2.3. Substrate formulations for incubation and pot experiment

All formulations were carried out using the base substrates S, S+Po and S+Po+Pe with three treatments grouped as; O (S, S+Po, S+Po+Pe), Pm (S+Pm, S+Po+Pm, S+Po+Pe+Pm) and B15 (S+B15, S+Po+B15, S+Po+Pe+B15). Treatment O represented unfertilized substrates (substrates without Pm or B15 amendment), treatment Pm represented fertilized substrates using poultry manure and treatment B15 represented fertilized substrates containing Biofer N15. Nitrogen sources (Pm or B15) used was applied at a rate of 600mgN/pot based on manufacture's recommendation and this was calculated based on NPK ratio of both Pm and B15. For Pm and B15, 15g/pot and 4.14g/pot was applied per each formulated substrate respectively for incubation. The same quantities of Pm and B15 were added to individual pots after mixing the substrate components S, Po and Pe together for the pot experiment. Two litre pots were used for the pot experiment.

2.3.1. Initial $_{CaCl2}$P and pH_{CaCl2} analysis on base substrates

This initial analysis was conducted on base substrates containing 20%Po. This analysis was done to probe the potential for plant-available P in the different combinations of materials before the commencement of the pot experiment. The combinations were made up to 100% volume (v) based on substrates Vw. Four replicates each of the substrate (table 4) were prepared, air-dried for 3 days and analyzed for plant-available P and pH using the sample extraction and orthophosphate molybdenum blue spectrophotometry methods.

Table 4. The percentage of quantities of materials used in the formulation of base substrates samples

Substrate	Quantities
S	100%S + 0%Po
S+Po	80% S + 20% Po
S+Po+Pe	60% S+ 20% Po + 20% Pe

2.3.2. Incubation experiment

Different formulations of substrates were made by varying the quantities of Po between 10%v and 20%v. The main purpose of incubation was to evaluate any changes in P and pH of substrates over time. In total, six groups with three sub-substrate formulations were made up to 1L each based on sample's Vw (table 5). For incubation, triplicate samples of selections under each group were dried at 40^{o}C till reached constant weight and bagged in labelled sealable plastic bags making total of 54 bags. The bags containing the substrates were totally sealed to prevent any air exchange. Afterwards, they were arranged randomly on trolleys at the same height in a dark chamber at 20^{o}C temperature and under 37% to 40% humidity. There was a limited air flow around the bags as they were kept in an air tight room. Due to a broken centrifuge instrument, incubation was done at weeks 0 and 1 only. All samples were analyzed for plant-available $_{CaCl2}$P and pH$_{CaCl2}$ using the sample extraction and orthophosphate molybdenum blue spectrophotometry methods.

Table 5. The percentages of quantities of materials used in the fertilizer formulations for incubation

Group 1, 10%Po	Quantity	Group 2, 20%Po	Quantity
Pe	100%v	S	100%v
S+Po	90%v +10%v	S+Po	80%v +20%v
S+Po+Pe	70%v +10%+20%	S+Po+Pe	60%v +20%+20%

Group 3, 10%Po	Quantity	Group 4, 10%Po	Quantity
S+B15	100%v	S+Pm	100%v
S+Po+B15	90%v +10%v	S+Po+Pm	90%v +10%v
S+Po+Pe+B15	70%v +10%+20%	S+Po+Pe+Pm	70%v +10%+20%

Group 5, 20%Po	Quantity	Group 6, 20%Po	Quantity
S+B15	100%v	S+Pm	100%v
S+Po+B15	80%v +20%v	S+Po+Pm	80%v +20%v
S+Po+Pe+B15	60%v +20%+20%	S+Po+Pe+Pm	60%v +20%+20%

2.3.3. Final substrate formulation

The final formulation was selected based on the best results obtained from (substrates with the highest extractable P) the initial formulation and incubated samples and used for the pot experiment. The pot experiment was performed using incubation fertilizer formulations under group 2, 5 and 6 (table 5). These groups contained 20%v of Polonite. Five replicates of two liters' samples under each group were prepared. Four replicates of each group was used in filling the pots for the pot experiment. Part of the remaining one replicate was sent to Eurofins Agro Testing

Sweden AB for sample analysis and the other part analyzed for the plant-available P and pH in the samples.

The atomic absorption spectrometry method was employed in the determination of P by Eurofins Agro Testing Sweden AB. The advantage of this method is the ability to perform multi-elemental analysis (Paraskova 2014). This method measures the different P fractions such as Fe-P and Al-P and other elements in the substrates. The technique involved breaking down of samples into atoms at very high temperatures. Air dried samples were first digested with nitric acid in an autoclave at 120°C and the resulting solutions used for P analysis. Final determination was performed by flameless atomic absorption spectrometry with electrothermal atomization (Swedish Standard 02 81 83). Atomic absorption spectrometer with graphite furnace and equipped with suitable lamp (hollow cathode lamp which is filled with inert gas) was the instrument used for nutrients measurement in samples. A suitable volume of samples was transferred to a graphite tube of the instrument and electrically heated. The temperature was gradually increased causing drying, ashing and atomization of the solution. Larger droplets were discarded and finer droplet transported to a gas stream on the outside of the graphite tube to the cathode for substance measurement (Paraskova 2014). The lamp emits a line spectrum of the substance in the cathode and when the light passes through the atomized sample, it is selectively absorbed by the atoms of this substance. The resulting absorbance of each element was recorded over a wavelength and resulting quantities obtained from a calibration solution curve obtained with known levels (Swedish Standard 02 81 83). The result was stated as the amount of P per litre of water respectively per kilogram of dry substrate.

pH of samples was measured using water in a 1:5 mass to volume ratio (1 part of substrate:5 part of water solution ratio). The resulting solution was placed in a shaking machine before measurement. Two buffer solutions at 20°C with pH of 4, 6.88 or 9.22 was used to calibrate the pH meter (DIN ISO 10390: 2005-12). pH of 4 was prepared by dissolving 10.21g of potassium hydrogen phthalate ($C_8H_5O_4K$) in 1000ml of water. pH of 6.88 was prepared by dissolving 3.39g of potassium dihydrogen phosphate(KH_2PO_4) and 3.53 disodium hydrogen phosphate (Na_2HPO_4) in 1000ml of water. pH of 9.22 was prepared by dissolving 3.8g of disodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$) in 1000ml of water. Water used for preparing all solutions had an electrical conductivity not higher than 0.2mS/m at 25°C and a pH greater than 5.6 (DIN ISO 10390: 2005-12).

2.3.4. Experimental site

This experiment was carried out at the Biotron in the Swedish University of Agricultural Sciences in Alnarp. The Biotron is a research facility with climate chambers with high regulation on factors like temperature, light, humidity and carbon dioxide. This experiment was conducted from February to April 2022. The plants were raised under temperatures between 15ºC to 20ºC, 70% humidity, 100% ventilation and a light intensity set at 500µ mol/m^2/s but was variable across the chamber. To determine the average light intensity across the chamber, an LI-COR light meter (model LI-189) was used to measure the quantum of light on the 9 trolleys on which plants were placed. This measurement was carried out on 7 April after harvesting and it produced an average light intensity of 415.6 ± 25.38µ mol/m^2/s. The chamber was programmed for a 16h photoperiod and the temperature was rotated between 15ºC (9hours) and 20ºC (15hours).

2.3.5. Selected crop

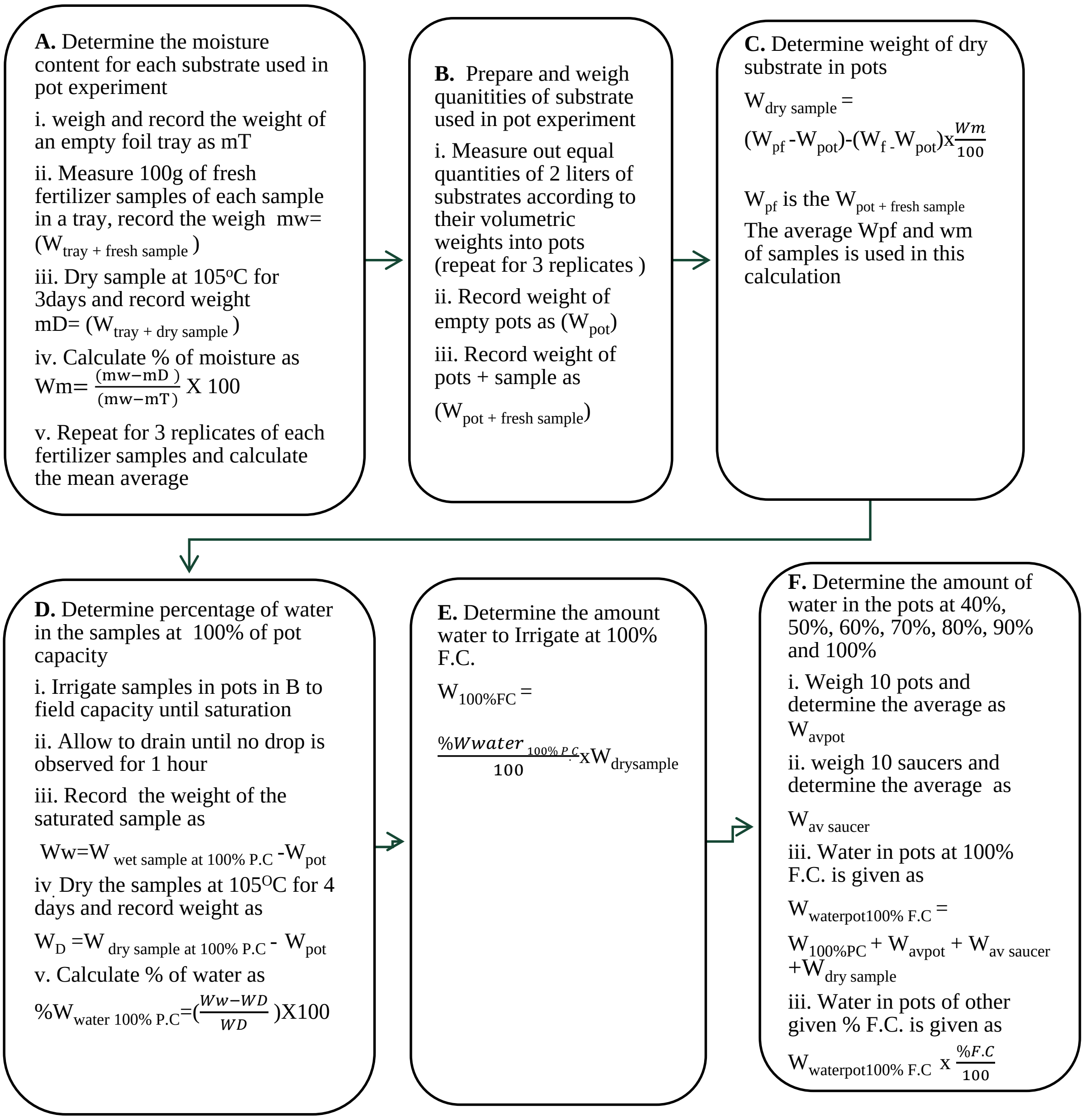
Sunflower (*Helianthus annuus L.*) is an important oilseed crop due to short duration and the ability to adapt wide range of climate and soil conditions providing a greater competitive advantage in relation to other crops such as soybean (Kandil *et. al*., 2017). Sunflower has large nutrient requirement making it prone to nutrient deficiencies which makes it a perfect choice for this experiment. The Pacino cola variety was used for the pot experiment. They are dwarf species of sunflower, producing masses of medium-large blooms on branched stems.

2.3.6. Experimental design and Conduct of the Study

To investigate the effects of the selected complete fertilizer products, an experiment was conducted in 2-liter plastic pots with sunflower in a climate chamber. The experiment lasted for 58 days from sowing to harvesting. The seeds of the sunflower were sown in a nursery tray with vermiculite in the greenhouse on 7 February and then transplanted on 15 February. The final formulated fertilizers had 9 different substrates with four replicates filled into the 2L plastic pots. A total of 36 pots with plants were used to conduct the study in the climate chamber for 48 days after transplanting. Pots were arranged on trolleys in the chamber according to a completely randomized design. The randomization was conducted by drawing random numbers assigned to treatments and replicates and then matched with their pot's numbers. All pots were arranged using their random numbers and placed on 9 trolleys forming a 3x3 matrix (figure 4) in the chamber. The trolleys were rotated around every 3 days to allow for equal distribution of climatic factors amongst the plants.

2.3.7. Irrigation

Irrigation was performed using deionized water. The applied water volume was measured using the pot's capacity, by determining the volume of water to irrigate at a given pot capacity (40%, 50%, 60%, 70%, 80%, 90% and 100%) for the different substrates (appendix 2). Plants were watered at two days' interval with 100ml of water for the first two weeks starting from the day of transplanting. For the rest of the 6 weeks, plants were watered daily according to 60% to 100% field capacity (F.C.) of growing media. The amount of irrigation at a particular percentage F.C of the plants were determined using the procedures described in figure 3. Unfertilized substrates were used in conducting this test.



A. Determine the moisture content for each substrate used in pot experiment

i. weigh and record the weight of an empty foil tray as mT

ii. Measure 100g of fresh fertilizer samples of each sample in a tray, record the weigh mw= ($W_{tray + fresh\ sample}$)

iii. Dry sample at 105°C for 3days and record weight mD= ($W_{tray + dry\ sample}$)

iv. Calculate % of moisture as $Wm= \frac{(mw-mD)}{(mw-mT)}$ X 100

v. Repeat for 3 replicates of each fertilizer samples and calculate the mean average

B. Prepare and weigh quanitities of substrate used in pot experiment

i. Measure out equal quantities of 2 liters of substrates according to their volumetric weights into pots (repeat for 3 replicates)

ii. Record weight of empty pots as (W_{pot})

iii. Record weight of pots + sample as

($W_{pot + fresh\ sample}$)

C. Determine weight of dry substrate in pots

$W_{dry\ sample}$ =

$(W_{pf} - W_{pot}) - (W_{f} - W_{pot}) x \frac{Wm}{100}$

W_{pf} is the $W_{pot + fresh\ sample}$

The average Wpf and wm of samples is used in this calculation

D. Determine percentage of water in the samples at 100% of pot capacity

i. Irrigate samples in pots in B to field capacity until saturation

ii. Allow to drain until no drop is observed for 1 hour

iii. Record the weight of the saturated sample as

$Ww = W_{wet\ sample\ at\ 100\%\ P.C} - W_{pot}$

iv. Dry the samples at 105°C for 4 days and record weight as

$W_D = W_{dry\ sample\ at\ 100\%\ P.C} - W_{pot}$

v. Calculate % of water as

$\%W_{water\ 100\%\ P.C} = (\frac{Ww-WD}{WD})X100$

E. Determine the amount water to Irrigate at 100% F.C.

$W_{100\%FC}$ =

$\frac{\%Wwater_{100\%\ P.C}}{100} x W_{drysample}$

F. Determine the amount of water in the pots at 40%, 50%, 60%, 70%, 80%, 90% and 100%

i. Weigh 10 pots and determine the average as W_{avpot}

ii. weigh 10 saucers and determine the average as

$W_{av\ saucer}$

iii. Water in pots at 100% F.C. is given as

$W_{waterpot100\%\ F.C}$ =

$W_{100\%PC} + W_{avpot} + W_{av\ saucer} + W_{dry\ sample}$

iii. Water in pots of other given % F.C. is given as

$W_{waterpot100\%\ F.C}\ x\ \frac{\%F.C}{100}$

Figure 3. Procedure for determining the volume of water for irrigation per pot at a given field capacity

2.3.8. Agronomic parameters

At the end of the experiment, plants were sampled at the vegetative stage (budding stage) for measuring growth traits of the plants including plant height, number of buds, diameter of big bud, and number of leaves. The plants were harvested on 5 March, 8 weeks from sowing. Afterwards, plants were divided as shoots (everything above the media) and roots (everything below the media). The roots were carefully washed using a sieve and the diameter of big bud measured in cm using a Vernier caliper. Plant height was measured in cm using a ruler (above the media to the top level). All roots and shoots were dried for 4 days at 65°C. Lastly, dried tissue samples were weighed, and records taken and analyzed. All dried root and shoot weights were measured in g. An assessment was also made on plants for purple colorization on braches and necrosis on the leaves that could indicate a lack of phosphorus. A further assessment was made to identify any the plants diseases like leaf spot.

Figure 4.Complete randomize arrangement of plants in the climate chamber (Picture by Yngve Svensson)

2.4. Statistical analysis

All statistical analyses were carried out using MINITAB 19 Statistical Software. All results were statistically analysed using the Analysis of variance (ANOVA) general linear model and Tukey pairwise comparisons with significance level of 5%. Raw data of results obtained for initial analysis, incubation, final analysis and agronomic parameters were first analysed by the general linear model and the means compared with Tukey pairwise comparisons test. One-way ANOVA and Tukey pairwise comparisons with significance level of 5% was used to determine the differences in the means of $_{CaCl2}P$ and pH_{CaCl2} of incubated substrates in week 0 and week 1.

3. Results

3.1. Amount of substrates used for conducting initial formulation, incubation and pot experiment

3.1.1. Bulk density and Volume weight of substrates

The calculation of the bulk density (B_D) of individual substrates and the Vw of their combinations determined by compacted bulk density (EN 13040:2007) is presented in appendix 1. For the individual substrates, Pe had the lowest B_D of 326g/L followed by Po at 963g/L and the highest B_D was recorded for S at 973g/L. In terms of combined substrates, S+Po had the highest Vw of 1002g/L followed by S+Po+Pe with the lowest Vw of 882g/L. Single substrates were measured outright from the mass while combined substrates were mixed together thoroughly according to preferred quantities before measuring the Vw of the combined materials (table 6). A liter of a material corresponded to the calculated Vw. This procedure was used in weighing out all substrates used for the initial formulation, incubation and pot experiment.

Table 6. The Measurement of the Vw of materials according to quantities. %Volume represent the amount of substrate deduced from the calculated Vw of the substrate. Vw defines the basis on which quantities of substrates are measured.

Substrates	%Volume (v)	Measured quantities per %volume (g/L)	One liter in Vw (g/L)
S	100	973	973
Pe	100	326	326
S+Po	90-10	875+ 96	1002
S+Po	80-20	778+ 193	1002
S+Po+Pe	60-20-20	584 + 193+ 65	882

3.2. Plant-available $_{CaCl2}$P and pH $_{CaCl2}$ of substrates

3.2.1. Calibration curve

The phosphate standards were analysed by the molybdenum blue spectrophotometry. The colour development was rapid but stable with no difference in absorbance being detectable when phosphate standards were kept for 2 hours. At the used wavelength (740nm-1100nm), the system obeyed Lambert–Beer's law in the concentration range of 0–1 mg/L of phosphate. Furthermore, the phosphate content of the samples was converted to the commonly used units of mg P/kg substrate by multiplying by a factor of 10. The dilution factor of 10 used was based on the extraction ratio of 10g substrate sample to 100mL of 0.01M $CaCl_2$ solution. It is reported that metal ions such as As^{5+} Si^{4+} Ca^{2+} and Cl^- associated with this method may not interfere with the phosphate concentrations due to their low concentrations in the small sample extracts (Houba *et al*., 2000). The P levels in all formulated substrates were deduced from the standard curve by dividing the absorbance value by 0.4297 which is the gradient of the line of equation (figure 5).

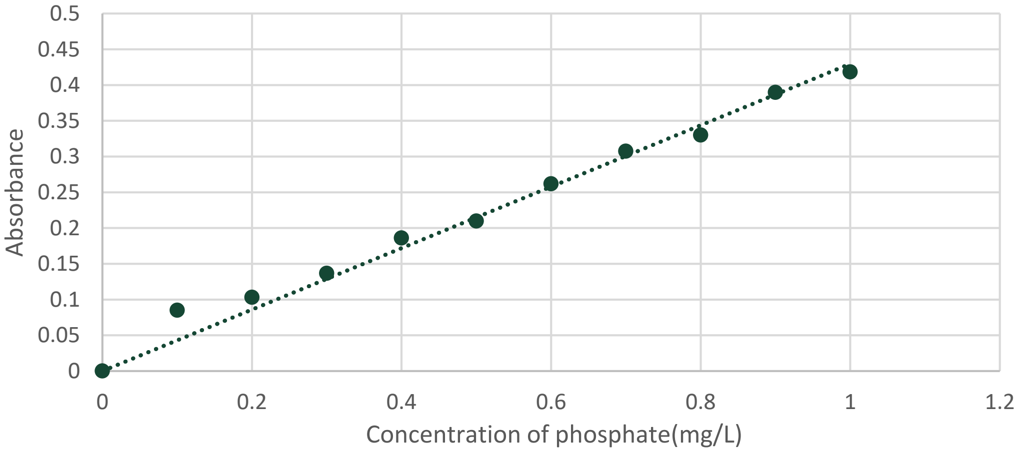


Figure 5. A standard curve for showing the relationship of absorbance and phosphate concentration. The equation of the linear regression line fitted to the data set (n=3) is: Y =0.4297x+0 and Correlation coefficient (R^2) = 0.9847

3.2.2. Initial $_{CaCl2}$P and pH $_{CaCl2}$ analysis on base substrates

Plant-available P of the base substrates (S, S+Po and S+Po+Pe) was significantly different ($p<0.05$). The addition of Po and Po+Pe together with S increased P content significantly (table 7). The substrate S+Po+Pe released the highest plant-available P at 5.13g/kg. The resulting pH of S was lower than the pH of combined substrates S+Po and S+Po+Pe.

Table 7. Mean values of pH and soluble plant-available P content of base substrates ± standard error, n=4. Letter a, b and c are used to describe differences in the means. Same letter signify means do not differ significantly and different letters indicate significant difference in the means

Substrate	$_{CaCl2}$P(mg/kg)	PH$_{CaCl2}$
S	2.39 ± 0.07^{a}	5.68 ± 0.06^{a}
S+Po	3.42 ± 0.13^{b}	6.97 ± 0.03^{b}
S+Po+Pe	5.13 ± 0.11^{c}	6.96 ± 0.04^{b}

3.2.3. $_{CaCl2}$P content and pH$_{CaCl2}$ of incubated substrates

The data sets under each group were analyzed separately. Group 1 and 2 were made up of unfertilized substrates while group 3, 4, 5 and 6 consisted of substrates fertilized with Pm or B15. Unfertilized substrate S+Po+Pe under group 1 and 2 obtained the highest $CaCl_2$-P of 4.5mg/kg-4.3mg/kg and 4.4mg/kg-4.9mg/kg in week 0 and 1 respectively (table 8). In terms of their pH values, substrate Pe in group 1 and substrate S in group 2 were found to have lowest pH values of 2.73 and 5.17 respectively in week 0 that were significantly different from the other substrates. The result obtained for group 3 followed similar pattern as group 1 except for P values under week 1. Plant-available P and pH recorded for substrates under group 4 in week 0 were not significantly different. The substrate S+Pm obtained P and pH values that were not significantly different under week 1. Fertilized substrates under group 5 recorded P values that differed significantly under week 0 and week 1. The substrate S+B15 under group 5 had pH values that differed significantly from the other substrates. Fertilized substrates under group 6 recorded P values that were not significantly different under week 0 and week 1. The pH values of substrates in week 0 differed significantly whiles substrate S+Pm recorded pH value that was significantly different from pH values obtained for other substrates in week 1. Overall, there were no significant changes in the mean values of plant-available P for incubated substrates between week 0 and week 1 (table 9). In terms of pH, substrates S+Po and S+Po+Pe under group 2 and S+Po+Pm under group 4 recorded significant changes. The pH of S+Po, S+Po+Pe S+Po+Pm significantly decreased by 4.76%, 5.35% and 4.11% respectively from week 0. All other substrates under each group had no significant changes in pH $_{CaCl2}$.

Table 8. Mean values of plant-available ${}_{CaCl2}$P and pH$_{CaCl2}$ obtained for incubated samples ± shows standard error, n=3. %Po represent the amount of Po used which is calculated from the Vw of Po. Same letter signify means do not differ significantly within each group

		Week 0		Week 1	
Group/ % Po	Substrate	Ca-P(mg/kg)	pH$_{CaCl2}$	Ca-P(mg/kg)	pH$_{CaCl2}$
1	Pe	3.35 ± 0.11^{a}	2.73 ± 0.06^{a}	3.67 ± 0.1^{b}	2.57 ± 0.03^{a}
10%	S+Po	3.20 ± 0.03^{a}	6.50 ± 0.13^{b}	3.06 ± 0.07^{a}	6.10 ± 0.05^{b}
	S+Po+Pe	4.51 ± 0.09^{b}	6.20 ± 0.1^{b}	4.25 ± 0.06^{c}	6.27 ± 0.15^{b}
2	S	2.04 ± 0.03^{f}	5.17 ± 0.06^{e}	2.12 ± 0.01^{f}	5.0 ± 0.05^{e}
20%	S+Po	3.23 ± 0.09^{e}	6.93 ± 0.06^{d}	3.06 ± 0.08^{e}	6.60 ± 0.05^{d}
	S+Po+Pe	4.44 ± 0.19^{d}	6.73 ± 0.03^{d}	4.88 ± 0.09^{d}	6.37 ± 0.03^{d}
3	S+B15	3.07 ± 0.35^{h}	5.20 ± 0.05^{h}	2.99 ± 0.11^{h}	5.07 ± 0.03^{h}
10%	S+Po+B15	3.22 ± 0.11^{h}	6.30 ± 0.05^{g}	3.79 ± 0.53^{g}	6.20 ± 0.05^{g}
	S+Po+Pe+B15	6.80 ± 0.1^{g}	6.13 ± 0.058^{g}	4.96 ± 0.31^{g}	6.13 ± 0.12^{g}
4	S+Pm	9.02 ± 1.17^{i}	6.07 ± 0.126^{i}	5.37 ± 0.44^{j}	5.87 ± 0.06^{j}
10%	S+Po+Pm	6.51 ± 0.43^{i}	6.57 ± 0.029^{i}	6.06 ± 1.22^{i}	6.30^{i}
	S+Po+Pe+Pm	8.28 ± 1.44^{i}	6.40 ± 0.132^{i}	9.45 ± 0.67^{i}	6.33 ± 0.03^{i}
5	S+B15	3.0 ± 0.17^{l}	5.17 ± 0.08^{l}	2.83 ± 0.15^{m}	5.30 ± 0.05^{l}
20%	S+Po+B15	4.81 ± 0.68^{kl}	6.57 ± 0.08^{k}	3.74 ± 0.01^{l}	6.73 ± 0.03^{k}
	S+Po+Pe+B15	6.41 ± 0.18^{k}	6.40 ± 0.05^{k}	6.09 ± 0.13^{k}	6.60 ± 0.05^{k}
6	S+Pm	7.41 ± 0.59^{n}	6.10 ± 0.05^{p}	4.28 ± 0.94^{n}	5.83 ± 0.19^{o}
20%	S+Po+Pm	6.72 ± 0.57^{n}	6.73 ± 0.03^{n}	6.52 ± 0.98^{n}	6.73 ± 0.06^{n}
	S+Po+Pe+Pm	8.44 ± 0.99^{n}	6.50 ± 0.05^{o}	7.84 ± 0.90^{n}	6.60 ± 0.05^{n}

*Table 9.p-Values from the Analysis of Variance showing changes in plant-available ${}_{CaCl2}$P and pH$_{CaCl2}$ of incubated substrates between week 0 and week 1. *Significant at P=0.05*

Group	Substrates	P	pH
1	Pe	0.130	0.089
	S+Po	0.214	0.070
	S+Po+Pe	0.102	0.768
2	S	0.097	0.067
	S+Po	0.280	0.019*
	S+Po+Pe	0.204	0.001*
3	S+B15	0.856	0.116
	S+Po+B15	0.408	0.288
	S+Po+Pe+B15	0.201	1.000
4	S+Pm	0.057	0.279
	S+Po+Pm	0.659	0.001*
	S+Po+Pe+Pm	0.557	0.692
5	S+B15	0.535	0.275
	S+Po+B15	0.248	0.152
	S+Po+Pe+B15	0.277	0.070
6	S+Pm	0.071	0.304
	S+Po+Pm	0.883	1.000
	S+Po+Pe+Pm	0.184	0.288

3.2.4. Plant-available $_{CaCl2}$P and pH $_{CaCl2}$ of substrates used for the pot experiment

The levels of plant-available P increased significantly in combined substrates as compared to single substrate S (table 10). Substrate S+Po+Pm recorded the highest increase in P release at 10.17mg/kg. Substrates S and S+B15 obtained the lowest pH as compared to all other substrates.

Table 10. Mean values of CaCl2-P and pH$_{CaCl2}$ obtained for fertilizers used for pot experiment ± means standard error

Substrate	Treatment type	P(mg/kg)	pH
S		1.97 ± 0.02^{a}	5.58 ± 0.005^{a}
S+Po	O	3.42 ± 1.56^{ab}	7.13^{f}
S+Po+Pe		4.57 ± 1.46^{abc}	6.72 ± 0.06^{c}
S+Pm		7.32 ± 0.36^{abc}	6.25 ± 0.03^{b}
S+Po+Pm	Pm	10.17 ± 1.48^{c}	7.10 ± 0.03^{ef}
S+Po+Pe+Pm		7.63 ± 0.47^{abc}	6.83 ± 0.013^{cd}
S+B15		8.42 ± 0.08^{bc}	5.75 ± 0.04^{a}
S+Po+B15	B15	5.81 ± 1.55^{abc}	6.94 ± 0.013^{de}
S+Po+Pe+B15		5.71 ± 0.24^{abc}	6.79 ± 0.02^{cd}

3.2.5. Results obtained from Eurofins Agro Testing Sweden AB on analysis of substrates for pot experiment samples

The P-AL represents the ammonium lactate extractable plant-available P in the substrates. The P-AL class indicates the high or low values of P. Class I meant low value while V indicated a very high P value. Amongst the classes, III is described as best P condition of substrate for plant growth (table 11). This class system also holds for ammonium lactate extractable plant-available potassium (K-AL) values. At values lower than class III, the phosphorus application should be increased and decreased at higher values (Eurofins 2019). Substrates without any Po amendment recorded the lowest P and pH$_{water}$ values (table 12). This low pH indicates substrates S and Pe were acidic.

Table 11. Ammonium lactate extractable phosphorus and potassium values with corresponding class range for mg/100g of fertilizer sample. Eurofins (2019).

P-AL number	P-AL class	K-AL number	K-AL class
<2.1	I	<4.1	I
2.1-4.0	II	4.1-8.0	II
4.1-8.0	III	8.1-16.0	III
8.1-12.0	IVA	16.1-32.0	IV
12.1-16.0	IVB	>32	V
>16.0	V		

Based on the class distribution, all substrates were found to contain a good amount of P and K. Magnesium content(Mg-AL) in fertilizers are high indicating high clay contents of S material. The limit for deficiency of Mg-AL is 4 - 10 mg / 100 g soil and based on this substrates S, S+Pm and S+B15 were deficient in magnesium. The potassium to magnesium ratio (K/Mg) are optimal in substrates with most values between 1 and 3 (Eurofins 2019). Ammonium lactate extractable plant-available Ca (Ca-AL) measured is more than what is available to the roots of plants but mostly dependent on the amount of clay (Eurofins 2019). The substrates without Po contained a low amount of calcium but all others substrates with Po had a good amount of calcium.

Table 12. Results obtained from Eurofins Agro Testing Sweden AB for analysis on fertilizers used for pot experiment n=2

Fertilizer	pH_{water}	P-AL mg/100g	P-AL class	K-AL mg/100g	K-AL class	Mg-AL mg/100g	K/Mg	Ca-AL mg/100g
S	5.9	5.5	III	10.5	III	10	1.05	230
S+Po	6.7	16.5	V	13	III	14	0.9	490
S+Po+Pe	6.3	19	V	14	III	18	0.8	525
S+Pm	6.0	5.9	III	15	III	10.5	1.45	215
S+Po+Pm	6.7	17.5	V	17	IV	14	1.2	485
S+Po+Pe+Pm	6.4	18	V	19.5	IV	17.5	1.1	495
S+B15	5.8	6.65	III	11	III	10	1.1	225
S+Po+B15	6.6	19.5	V	14	III	14	1	495
S+Po+Pe+B15	6.2	18.5	V	14	III	17.5	0.8	490

3.3. Sunflower growth

Various growth parameters of sunflower plants were used to analyse the effects of the different formulated fertilizers on plant growth.

*Table 13. p-Values from the Analysis of variance showing the effect and interaction of substrate and treatment type on sunflower growth parameters. Substrates included in analysis were S, S+Po, S+Po+Pe and treatment types included O (substrates without manure), Pm (substrates containing poultry manure) and B15 (substrates containing Biofer N15 manure). * significant at P≤0.05.*

	Plant height	Number of leaves	Number of buds	Diameter of big bud	Dry shoot weight	Dry root weight
Substrate	0.670	0.178	0.613	0.274	0.029*	0.170
Treatment type	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
Substrate⁎Treatment type	0.278	0.516	0.413	0.150	0.014*	0.150

3.3.1. Plant height (cm)

The height of plants varied between 18cm to 32cm. There was an increase in the height of plants cultivated with fertilized substrates S+Pm, S+Po+Pm, S+Po+Pe+Pm and S+B15, S+Po+B15, S+Po+Pe+b15 as compared to unfertilized substrates S, S+Po, S+Po+Pe with plant heights of less than 20cm. There was no significant difference in the mean values of unfertilized plants (figure 6). Similarly, fertilized plants recorded the highest plant height values but with no significant difference (p>0.05).

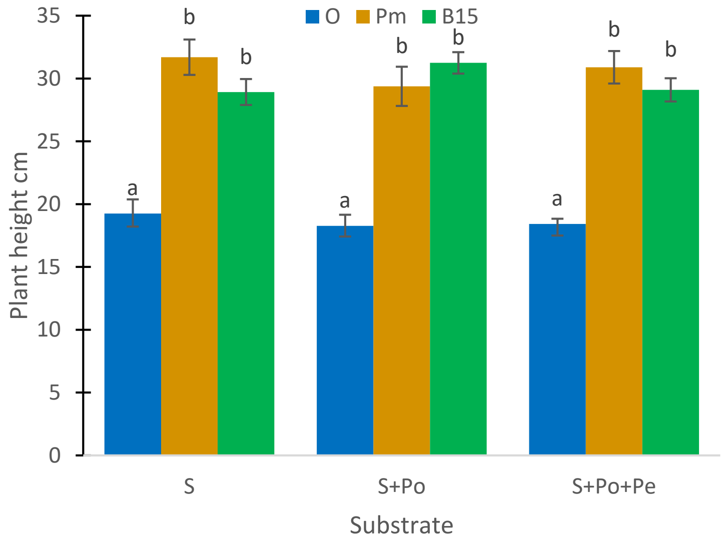


Figure 6. Mean values of plant heights obtained for different substrates. Soil (S), Soil+Polonite (S+Po) and Soil+Polonite+Peat (S+Po+Pe) represent substrates while O represent unfertilized substrates, Pm represent substrates containing poultry manure and B15 represent substrates treated with Biofer N15. This description is the same for all bar charts and n=4 is equal for all plant data except for S+Po+Pe+B15 substrate with n=3. Bar indicates standard error for mean values.

3.3.2. Number of leaves

Overall, the number of leaves per plant grown with unfertilized substrates was significantly different from plants cultivated with fertilized substrates (figure 7). The number of leaves of plants under all treatments ranged approximately from 21 to 27 leaves per plant (figure 8). There was no significant difference in the mean values recorded for unfertilized plants. The means of the number of leaves grown with S+Po+Pe+Pm and S+Po+Pe+B15 substrates recorded the lowest means for fertilized plants.

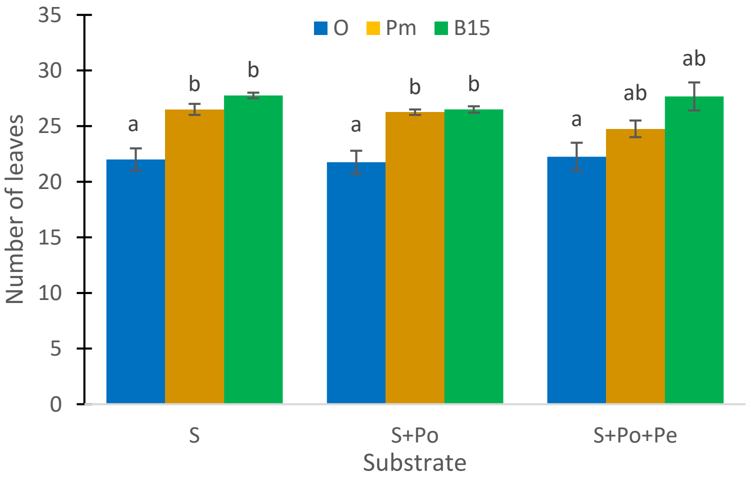


Figure 7.Mean values of number of leaves per treatment type .

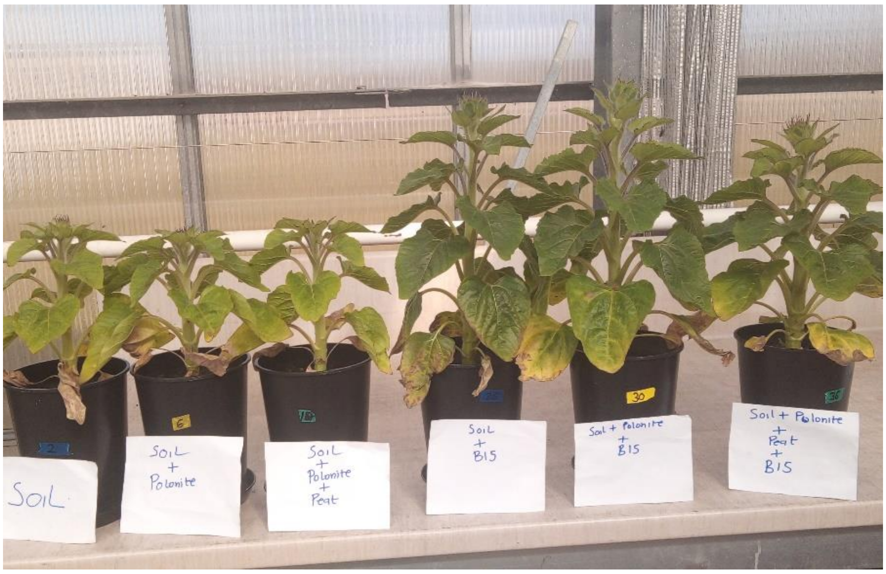


Figure 8. Sunflower plants showing variation in the number of leaves and plant height according to the substrate used.

3.3.3. Number of buds

Plants grown with unfertilized substrates developed one bud per plant and was the lowest recorded as compared to fertilized substrates (figure 9). The buds of plants cultivated with fertilizer Pm or B15 varied from 3 to 5 (figure 10). The number of buds for plants given Pm or B15 were not significantly different.

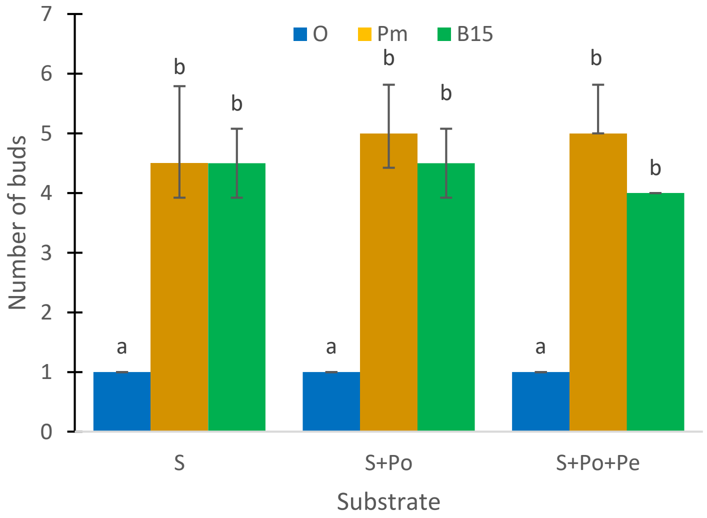


Figure 9. Mean values of the number of buds obtained per plant for different substrates

Figure 10. Picture showing number of buds per plant including the big bud

3.3.4. Diameter of big bud (cm)

Substrates with Pm or B15 component had a significant effect on diameters of big buds, recording highest bud diameter values ranging from 2.3cm to 2.8cm (figure 11). Unfertilized substrates recorded the lowest values in terms of bud diameter with values ranging between 1.2cm to 1.4cm.

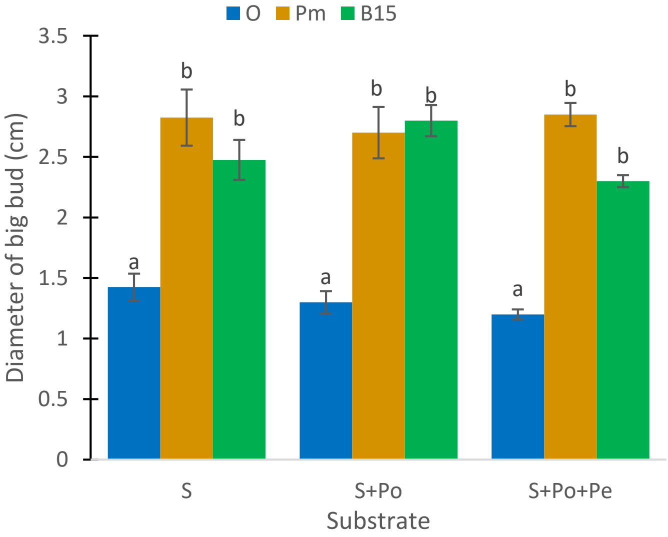


Figure 11. The mean values of the diameter of big bud per plant recorded for different substrates

3.3.5. Dry shoot weight (g)

Dry weight of plant biomass is considered to be the best and more reliable indicator for determination of plant growth. This is because the water present in plant cells depends on level of water present in the medium where it was grown. In terms of dry shoot weights, unfertilized plants recorded the lowest shoot weights that were significantly different from values obtained for plants fertilized with Pm or B15 (figure 12). Among the fertilized plants, those cultivated with S+Po+Pe+Pm substrate recorded the highest mean values while plants cultivated with S+B15 substrate recorded the lowest mean value. Overall, Pm fertilizers gave the best results in terms of plant shoot growth.

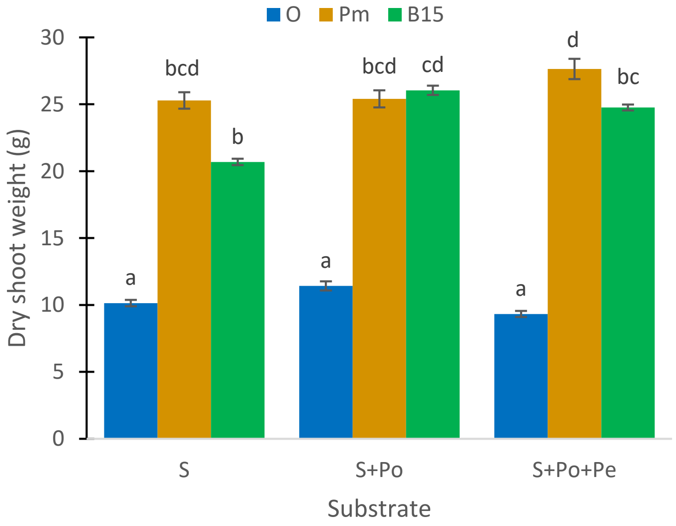


Figure 12. Mean values of dry shoot weights of plants measured for different substrates.

3.3.6. Dry root weight (g)

Following similar trends, addition of N (Pm or B15) increased dry root weights of plants significantly as compared to unfertilized plants. The plants sown in unfertilized substrates had the lowest dry root biomass. Amongst fertilized substrates, S+Po+Pe+B15 recorded the lowest dry root of about 25g (figure 13).

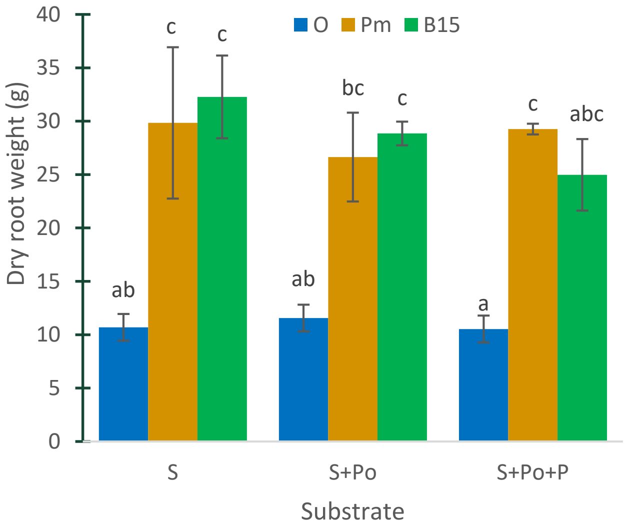


Figure 13. Mean values of dry root weights per substrate type

3.3.7. Other observed characteristics on plants

The upper and lower leaves of plants cultivated with substrates without any N amendments started yellowing from four weeks after transplanting. This phenomenon could be attributed to N deficiency. Around the same period, older leaves turned yellowish with dark brown spots indicating P deficiency. The occurrence indicating P deficiency appeared late on fertilized plants. At the time of harvesting, almost all leaves of unfertilized plants had turned yellowish and shedding their older brown leaves (figure 14). For fertilized plants, only their lower leaves depicted severe symptoms of P deficiency. One plant sown with S+Po+Pe+B15 substrate showed severe signs of fungal infection (leaf spot) which affected the growth of the plant. Chlorosis and necrosis observed on lower leaves of fertilized plants during the flowering stage could associated to K deficiency.

Figure 14.. Disease and nutrient deficient characteristics displayed by plants grown in different fertilizer types a. represents picture of plants grown with Pm fertilized substrate; b. refers to a plant cultivated with S+Po+Pe+B15 substrate and showing fungi attack; c. refers to plants grown with substrates fertilized with B15; d. refers to plants grown with unfertilized substrates.

4. Discussions

4.1. Phosphorus release capacity of substrates

P adsorption and desorption in a growing medium is important for plant fertilization and management of P into the environment. Adsorption indicates the clinging of compounds to a surface while desorption is the release of substances from a surface. Adsorption capacity of a growing medium can limit the availability of P to plants, while a good desorption characteristic can allow P to be accessible to plants. As earlier stated, about 80% of applied P becomes unavailable to plants due to adsorption process and other related factors (Asomaning 2020). This makes the need of formulating a growing medium with materials of good desorption properties a priority. Several studies conducted on Polonite found this material to retain a good amount phosphate (PO_4–P) when used for wastewater treatment. Nilsson *et. al*. (2013) found Polonite to retain 80% of phosphate as compared with Sorbulite which retained 75% when used in a column experiment. According to Renman *et al*. (2010), a compact bed filter containing 560 kg of Polonite and fed with 70 m^3 of wastewater from a single house removed an average PO_4 of 89% after 92 week of operation.

In the same fashion, the desorption rate of the accumulated phosphate from this reactive filter material makes it suitable for recycling into agriculture production. Using a batch experiment, 3.1mg/g orthophosphate desorption was obtained for Polonite (Kassa 2013). Another analysis showed that saturated Polonite contained 1.3mg P/g (Hylander *et al*., 2006). Even though P analysis was not performed on Po to ascertain its desorption value in this study, the addition of Po together with S resulted in an increase in P (table 7, 8, 10 and 12) thereby confirming some form of desorption of P from Polonite. The increase in P of S+Po substrate was observed from the results obtained from P analysis on substrates (table 7), incubation (table 8), final substrates (table 10) and Eurofins Agro Testing Sweden AB lab analysis (table 12). From table 7, P released increased from 2.4mg/kg to 3.4mg/kg after addition of Po to S. This showed that, Po coupled with increased in pH gave a desorption value of 1.03m/kg. From group 2 of incubated substrates (table 8), P increased from 2.04mg/kg to 3.2mg/kg in week 0 (1.2mg/kg of desorption from Po) and from 2.1mg/kg to 3.1mg/kg in week 1 (0.94mg/kg of desorption from Po). For substrates used for pot experiment, a desorption rate of 1.5mg/kg was observed given that P release increased from 1.97mg/kg for S to 3.4mg/kg for S+Po. From table 11, Lab results saw an increase in P-AL from 55mg/kg to 165mg/kg giving a value. 115mg/kg P desorption from Po The volume of Po was constant at 20%v for all substrates that gave these increase in P and the desorption values of Po. Furthermore, P release from combined substrates increased systematically looking at the results obtained from molybdenum blue spectrophotometry $_{CaCl2}$P analysis. For example, Substrate such as S+Po+Pe+Pm used for the pot experiment released an amount of plant-available $CaCl_2$-P that increased systematically from 1.97mg/kg to 10.2mg/kg (table 9). This trend was confirmed from the P release results obtained for substrate S+Po+Pe. The results obtained for S+Po+Pe incubated substrate (form 2.4mg/kg to 5.1mg/kg), final analysis on fertilizers (from 1.97mg/kg to 4.57mg/kg) and Eurofins Agro Testing Sweden AB lab analysis (from 55 mg/kg to 190mg/kg) could be verified from table 7,8,10 and 12. The systematic increase in P indicated that substrates (Pe and S) used in the formulation process had some level of P release capacity.

In addition, substrates fertilized with Pm and B15 were found to have the best orthophosphate P release capacity according to results obtained from the different analyses carried out. Fertilized substrates with Pm (S+Pm, S+Po+Pm and S+Po+Pe+Pm) used for the pot experiment displayed P release capacity of 73%, 66% and 40% more respectively, when compared to substrates S, S+Po and S+Po+B15. Similarly, substrates S+B15, S+Po+B15 and S+Po+Pe+B15 gave a 76.6%, 41% and 20% more when compared with the unfertilized substrates. The increase in the concentration of plant-available P in these fertilized substrate solutions could be attributed to the organic manure amendments (Pm or B15). Some studies have confirmed a positive effect of organic manure amendments on P release and uptake by plants (Grunes 1956; Ma *et al*., 2016). This positive outcome is in line with the results reported by Abbasi *et al*. (2015) after combining poultry manure with phosphate rock. The combination achieved about 80% P release as compared with phosphate rock only. The same study by Abbasi *et al*. (2015) indicated that 23% P utilization efficiency was obtained for plant grown with soil amended with Pm than for soil treated with single superphosphate amendment with a P utilization of 14% when applied to chilli plants. Also, Pm used in conducting this study contained an additional P-AL content of 1.1g/100g thereby increasing P levels of substrates formulated with Pm

Other factors such as clay content, calcium carbonate ($CaCO_3$) and soil organic carbon have been found to affect P release in soil (Prakash *et al*., 2017). Lab experiment by Prakash (2017) found Freundlich sorption constants increased with increase in clay and $CaCO_3$ content. However, increase in soil organic carbon and available P concentration in soils resorted in a substantial reduction in sorption constants. In the same study, phosphorus desorption results showed that the soils having high P sorption capacity had lower P desorption capacity. In our study, substrate S was detected to have a high clay content based on Mg-AL value (table 12) and this may have resulted in its low P release (figure 6) due high sorption of P by the substrate. Fertilized substrates with Po and Pm or B15 had a high P release capacity and this may be attributed to the increase in soil organic carbon by the manure and the high P release from the substrates (figure 7, 8, 10 and 12). In sequential extraction test, inorganic P fractions in samples are mostly bound to Ca, Al and Fe (Cabañas, 2017). In a study on the adsorption capacity on alum sludge by Hou *et al.* (2018), most of the P removed was found to be bound to Ca instead to Al and Fe. Also, the Ca-P was found to be more available to plants than Al-P or Fe-P. For this reason, the method of P extraction using $CaCl_2$ as an extractant as used in this study is highly recommended over the ammonium lactate method used by Eurofins Agro Testing Sweden AB. This is because the values obtained from the method can prove to be reliable when based on to select a fertilization scheme for crops. According to Cucarella *et. al.* (2009), the AL method used to estimate the plant available P in soils may not be appropriate for estimation for the mineral-based Polonite. It will be interesting to determine the total P of substrates using other reliable measurement such as X-ray absorption near edge structure (XANES) spectroscopy mentioned in this study. In terms of incubation, the anticipated results were not achieved due to broken equipment that shorten the duration and P analysis of incubated samples. No significant difference in P was observed for substrates in week 0 and week 1 under each group (figure 7 and 8). Abbasi *et al*. (2015) conducted a 60-day incubation on soil with 12 different treatments and found that P release of soil treated with poultry manure increased sharply from the beginning (from 10.4, 13.1, 18.8, 17.7, 20.2 and 9.6mg/kg) and decreased significantly at the end of the incubation period (0, 5, 15, 25, 35 and 60 days). Another experiment will be needed to investigate the changes in P release for the substrates used in this study.

4.2. Effects of pH on P release and plant growth

pH is another factor that greatly affects P absorption and desorption as well as nutrient availability for plant growth. Polonite is a known product with high pH which greatly reduces its bacteria content (Renman *et al*., 2004). Cucarella *et al.* (2009) recommended saturated Polonite as an alternative liming material after increasing soil pH significantly (5.2-5.8) when applied as soil amendment to a mountain meadow. The liming effect of Po was verified in this study from the results obtained after pH analysis on substrates (table 7, 8, 10 and 12). pH for final formulated substrates used for the pot experiment was measured in water (lab analysis) and 0.01M $CaC1_2$ (in our study). Minasny *et al*. (2011) suggested that soil pH in $CaCl_2$ is usually preferred as it is less affected by soil electrolyte concentration and provides a more consistent measurement. Based on this analogy, they developed a model that related the two methods of pH measurements by incorporating their electrical conductivity (EC) values. Since measurement of EC was not conducted in this study, pH_{water} was used to determine the effect on sunflower growth. Sunflowers grow well in soils with pH range that are slightly acidic to alkaline but not tolerant of acidic soils with a pH_{CaCl2} of 5.0 or below (GRDC 2017). Both unfertilized and fertilized substrates obtained pH values greater than 5 making them suitable for plant growth (table 12). Cucarella (2007) indicated that, P availability in soil may increase with increasing pH as high pH supports P dissolution of P bound to Al and Fe. The increase in P release in this study was attributed to both increase in pH and direct P release from materials used for formulation such as Po, Pe, Pm and B15 (table 7, 8, 10 and 12). However, the effect of pH on sunflower growth was inconclusive as different results in growth was recorded for plants cultivated with unfertilized soil S+Po and fertilized substrate S+Po+Pm with equal pH value of 6.7 (table 12). Except for substrates S+Po (from 6.93 to 6.60) and S+Po+Pe (from 6.73 to 6.37) under group 2 and S+Po+Pm (from 6.40 to 6.33) under group 4 that recorded significant changes in pH_{CaCl2} in week 0 and week 1, no changes in pH_{CaCl2} occurred in the other incubated substrates in week 0 and week 1 under each group. The results obtained by Abbasi *et al*. (2015) from a 60-day incubation experiment on soil with 12 different treatments found the pH of soil treated with poultry manure decreasing by 8% from day 0 (from 8.10 to 7.49).

4.3. Effect of fertilizers on sunflower growth

The study aimed to formulate a complete, cost-effective and nutritionally attractive fertilizer product that included saturated Polonite which is regarded as a waste product after wastewater treatment. Amongst the three groups of formulated substrates, fertilized substrates S+Pm, S+Po+Pm, S+Po+Pe+Pm and S+B15, S+Po+B15, S+Po+Pe+B15 proved to be effective towards sunflower growth. The positive results obtained in sunflower growth with fertilized substrates showed non-significant interactive effects between Po and the organic manure amendments except for the dry shoot biomass. The dry shoot biomass was significantly affected by the interaction between the substrates and fertilization with or without organic manure amendments Pm or B15 (table 13). However, most of the positive response of sunflower growth was not dependent on Polonite application even though P play a role in many cellular processes of the plants development. The addition of Polonite showed no significant effects on the growth of sunflower. Since the treatments mainly differed in the organic manure application, the results suggest that P in the substrates was sufficient for crop demand and the Polonite mimicked a slow release fertilizer. Cucarella *et al*., (2009) confirms that no difference in yield was obtained by meadow plants after addition of Po. This was evident in the differences in growth parameters measured (figure 8) for plants cultivated with organic manure amended substrates (treatment Pm and B15) to unfertilized substrates (treatment O). Plants cultivated with unfertilized substrates recorded the lowest biomass indicating the shortage of N nutrients content in the root zone of plants (figure 6-14). The height of plants fertilized with Pm or B15 obtained about 34%-39% increase as compared with unfertilized plants. Fertilized plants had broader leaves as well as 3 to 5 leaves more than unfertilized plants. Fertilized plants developed 4 to 5 more buds than unfertilized plants with just one bud each. Saturated Polonite (Po) and animal by-products (organic amendments such as Pm and B15) improves biochemical properties and structures of growing medium that may have had a positive effect on plant growth (Bloem et al., 2017). According to Ozer *et al*. (2004), nitrogen can contribute to the growth and development of both roots and vegetative parts through improving the carbon dioxide (CO_2) assimilation that causes increased photosynthesis and elongation of the vegetative parts. These results are in line with Jamal *et al*. (2018) who stated that the application of organic matter at different rates along with different sources of phosphorus could increase dry weight of plant roots significantly. In this study, the substrates had a good amount of P and K but displayed symptoms of P and K deficiencies during the flowering stage. This could be associated with slow release of nutrients from the substrates for plant use. This was an indication that sunflower plants required additional nutrients during the flowering stage. Some differences in the mean values of measured parameters were observed between fertilized substrates with Pm and B15. The discrepancies in the root biomass may be linked to losses of some of the root mass during the washing process to isolate the root mass from the medium. Also, values recorded for the diseased plant (figure 14) was not included in the data analysis and this may have affected the results of average values obtained for plants fertilized with B15. Regardless of these error factors, fertilized substrates with Pm gave the highest values for plant height, number of buds, diameter of buds and dry shoot weight (figure 6, 9, 11 and 12).

4.4. Fertilizer Application

Fertilizer application represents the amount of nutrients needed to replace the amount of nutrient removed by plants and it products. Achieving a good balance between plant nutrient needs and fertilizer application rates can be one of the methods in reducing eutrophication. Soil test approach for fertilizer application could help establish a good nutritional requirement for specific crop types while eliminating deficiencies. Fertilization of sunflower differ among varieties, purpose of use and also dependent on NPK nutrient levels in soil or growing medium (Süzer 2010). Sunflowers are classified into two categories as oil type (used for oil production) and confectionary sunflowers (used for food production) (Al-Hameedi *et al*., 2020). Süzer (2010) obtained 50kgN/ha for first dwarf sunflower variety (DW-1) and 80kgN/ha for both second dwarf variety (DW-2) and Trakya-80 after a field experiment taking into consideration marginal economic analysis. Highest seed yield for the dwarf varieties was obtained with plant spacing of 15cm × 70 cm which corresponded to 95,230 plants/ha. Another experiment saw best results in the growth of sunflower plants and harvest yield using 150-75-50 kgNPK/ha (AIP Conference 2019). Reddy *et al*. (1996) during the winter season in sandy clay loam soil observed a significantly higher dry matter production (2251 kg/ha) by the application of 90 kg N/ha as compared to 60 kg N/ha (1977 kg/ha). Due to higher dry matter production, significantly higher seed yield per plant of sunflower (19.6 g/plant) was obtained with application of 90 kg N/ha as compared to application of 60 kg N/ha (15.7 g/plant). Maragatham *et al*. (2000) reported that the application of 120, 60 and 50 kg N, P and sulphur (S) per ha, respectively increased the seed yield and oil yield in sunflower.

In most EU countries, the application of both chemical and organic fertilizers are regulated. Some of these regulation is listed in legislation concerns implementations of the Nitrates Directive (91/676/EEC) in terms of National Action Plans, the Water Framework Directive (2000/60/IEC) in terms of River Basin Management Plans and the Industrial Emissions Directive (2010/75/EU) (Amery *et al*., 2014). Amery *et al*. (2014) study on agricultural P legislation in Sweden reported that livestock manure or other organic fertilisers may not be applied in quantities above 22 kg P/ha/year. They indicated that this quantity was calculated as an average for the holding's entire application area per year during the last 5 years. They concluded that the type of fertilisation restrictions varied widely among Member States and that of phosphorus legislation in European countries and regions varied from no direct regulation to strict maximum phosphorus application rates that was dependent upon fertiliser type, crop type and soil P content. Also, Tylstedt (2011) reported that the Swedish Environmental Protection Agency regulation require that 35kg/ha and 22kg/ha of total phosphorus content in terms of sewage sludge could be spread yearly on arable land of I-II and III-V AL-P class soils respectively. Eriksson et al., (2010), reported that 34% of arable soils in Sweden have a good or surplus P content, 37% have satisfactory content and 29% have low or very low content. These statistics on arable soils may have changed after the continuous use of land and losses through natural factors such as runoff, leaching etc. during this period. Based on the recommended NPK fertilizer needed per ha for sunflower, large quantities of the formulated organic fertilizer product will be needed to make up for the plant nutrients requirement per ha. For this reason, it is suggested that the formulated fertilizer product should be used together with inorganic fertilizer to limit the quantities of chemical fertilizers use.

5. Conclusion

The results obtained from the spectrophotometric molybdenum blue phosphate analysis on base substrates indicates that the addition of Po and Pe together with S increased plant-available ${}_{CaCl2}$P by 53.4%. However, the use of Pm and B15 in combination with base substrates released higher amounts of ${}_{CaCl2}$P and P-AL. Results from the P analysis on pot experiment substrates indicates substrate S+Po+Pe+Pm gave the highest ${}_{CaCl2}$P release value at 10.2mg/kg. In the case of lab analysis results, S+Po+B15 released a maximum of 195mgP-AL/kg. Calcium-rich substrates such as Polonite, have proven to be appropriate for P recycling from wastewater to agriculture since they have a moderate to high P sorption and desorption capacity. In this study, Polonite increased the pH and calcium content of all the substrates containing Po. When the formulated substrates were used to grow sunflower, substrates fertilized with Pm exhibited the highest plant growth. Even though the addition of Polonite had no significant effect on sunflower growth, it can be concluded that Polonite together with poultry manure (Po+Pm) may be a good alternative organic fertilizer to chemical fertilizers such as phosphorus, nitrogen, potassium, calcium and magnesium for application to improve the growth and yield of sunflower crop. However, a field experiment will be needed to confirm the economic, social and ecological feasibility of the application of this particular fertilizer product. Also, another experiment using materials with lower P content will be needed to evaluate the effectiveness of Polonite as a P fertilizer substitute.

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Acknowledgements

Research funding was received from Alnarp Cleanwater headed by Yngve Svensson. A special thanks to my supervisors Siri Caspersen and Yngve Svensson for their immense contribution. We would like to thank Jan-Eric Englund for his assistance with the statistical analyses. This thesis has been produced during my scholarship period at the Swedish University of Agricultural Sciences, which was funded by the Swedish Institute.

Appendix 1

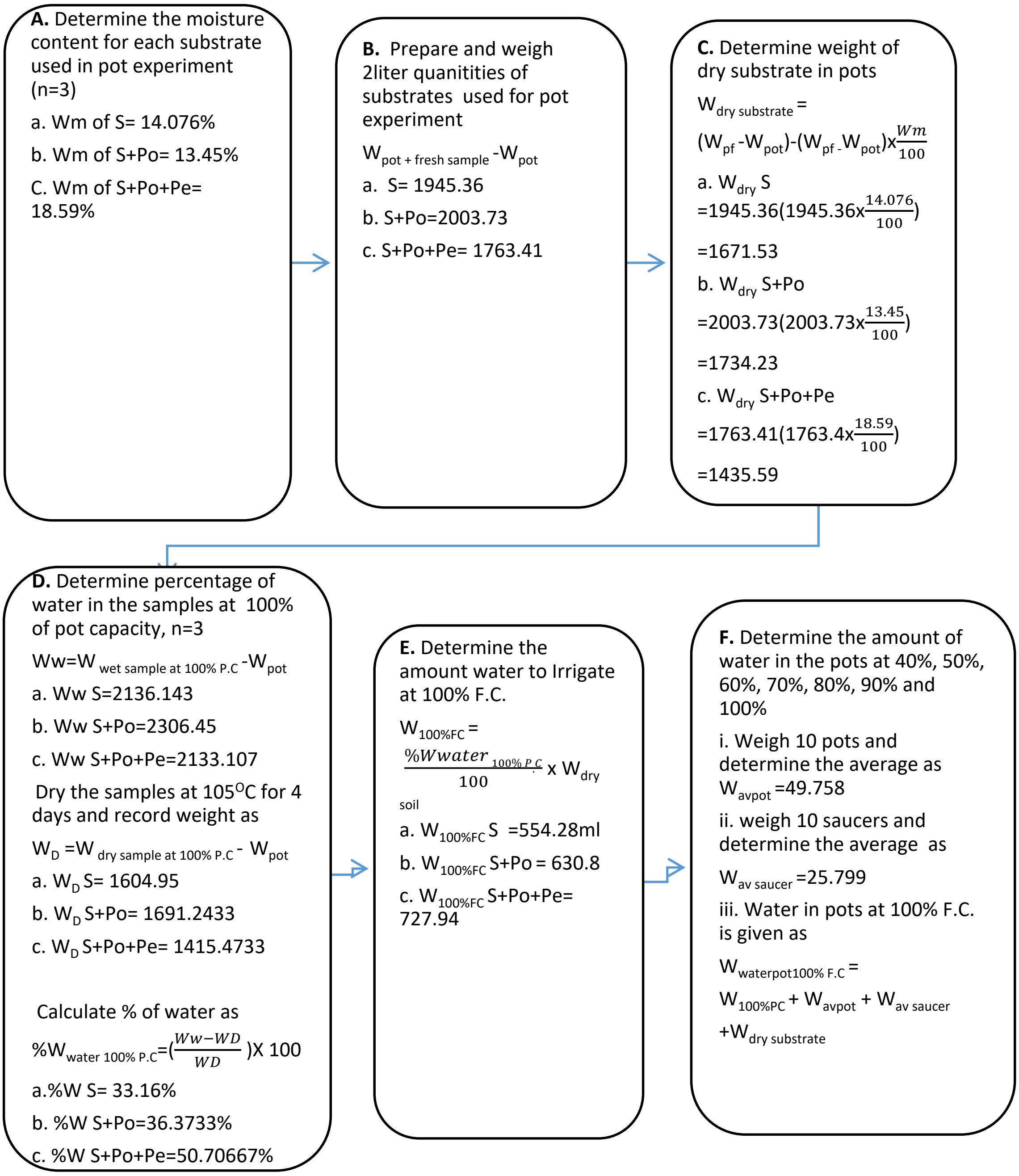
Calculation of BD and Vw of substrates based on compacted bulk density, n=5 for mx and mo

BD of S	BD of Po	BD of Pe	Vw of S+Po	Vw of S+Po+Pe
mL=$\frac{mx}{n}=\frac{11.5377}{5}$ mL=2.307534kg mo=$\frac{7.25301}{5}$ mo=1.450602kg BD=$\frac{\text{mL}-\text{mo}}{V}$ =$\frac{2.307534-1.450602}{0.881}$ BD=0.97268kg/L Vw=972.68g/L	Ml=$\frac{11.4949}{5}$ Ml=2.298984kg Mo=$\frac{7.25236}{5}$ Mo=1.450472kg BD=$\frac{2.29898-1.450472}{0.881}$ BD=0.96312kg/L Vw=963.12g/L	Ml=$\frac{8.69081}{5}$ Ml=1.738162kg Mo=$\frac{7.25365}{5}$ Mo=1.45073 BD =$\frac{1.7381-1.45073}{0.881}$ BD=0.326256kg/L Vw=326.26g/L	Ml=$\frac{11.6641}{5}$ Ml=2.33282kg Mo=1.450178 Vw =$\frac{2.33282-1.450178}{0.881}$ Vw =1.0018637kg/L Vw=1001.86g/L	Ml=$\frac{11.1135}{5}$ Ml=2.227002kg Mo=1.450222 Vw =$\frac{2.227002-1.450222}{0.881}$ Vw =0.8817026kg/L Vw=881.70g/L

BD- bulk density, Vw- volumet weight, S-soil, S+Po- soil+Polonite, S+Po+Pe- soil+Polonite+peat

Appendix 2

Calculation of the amount of irrigation according to the pot’s capacity at 40%, 50%, 60%, 70%, 80%, 90% and 100% field capacity



A. Determine the moisture content for each substrate used in pot experiment (n=3)

a. Wm of S= 14.076%

b. Wm of S+Po= 13.45%

C. Wm of S+Po+Pe= 18.59%

B. Prepare and weigh 2liter quanitities of substrates used for pot experiment

$W_{\text{pot + fresh sample}} - W_{\text{pot}}$

a. S= 1945.36

b. S+Po=2003.73

c. S+Po+Pe= 1763.41

C. Determine weight of dry substrate in pots

$W_{\text{dry substrate}} =$

$(W_{pf} - W_{pot}) - (W_{pf} - W_{pot}) x \frac{Wm}{100}$

a. W_{dry} S

$=1945.36(1945.36x\frac{14.076}{100})$

=1671.53

b. W_{dry} S+Po

$=2003.73(2003.73x\frac{13.45}{100})$

=1734.23

c. W_{dry} S+Po+Pe

$=1763.41(1763.4x\frac{18.59}{100})$

=1435.59

D. Determine percentage of water in the samples at 100% of pot capacity, n=3

$Ww = W_{\text{wet sample at 100\% P.C}} - W_{\text{pot}}$

a. Ww S=2136.143

b. Ww S+Po=2306.45

c. Ww S+Po+Pe=2133.107

Dry the samples at 105^{O}C for 4 days and record weight as

$W_D = W_{\text{dry sample at 100\% P.C}} - W_{\text{pot}}$

a. W_D S= 1604.95

b. W_D S+Po= 1691.2433

c. W_D S+Po+Pe= 1415.4733

Calculate % of water as

$\%W_{\text{water 100\% P.C}} = (\frac{Ww-WD}{WD}) X\ 100$

a.%W S= 33.16%

b. %W S+Po=36.3733%

c. %W S+Po+Pe=50.70667%

E. Determine the amount water to Irrigate at 100% F.C.

$W_{100\%FC} = \frac{\%Wwater_{100\%\ P.C}}{100} x\ W_{\text{dry soil}}$

a. $W_{100\%FC}$ S =554.28ml

b. $W_{100\%FC}$ S+Po = 630.8

c. $W_{100\%FC}$ S+Po+Pe= 727.94

F. Determine the amount of water in the pots at 40%, 50%, 60%, 70%, 80%, 90% and 100%

i. Weigh 10 pots and determine the average as W_{avpot} =49.758

ii. weigh 10 saucers and determine the average as

$W_{\text{av saucer}}$ =25.799

iii. Water in pots at 100% F.C. is given as

$W_{\text{waterpot100\% F.C}} =$

$W_{100\%PC} + W_{avpot} + W_{\text{av saucer}}$

$+W_{\text{dry substrate}}$

F. The amount of water in the pots at 40%, 50%, 60%, 70%, 80%, 90% and 100% F.C.

Substrate	40%	50%	60%	70%	80%	90%	100%
S	1968.79	2024.23	2079.66	2135.08	2190.51	2245.94	2301.34
S+Po	2062.11	2125.19	2188.27	2251.35	2314.43	2377.51	2440.59
So +Po + Pe	1802.33	1875.12	1947.91	2020.71	2093.5	2166.3	2239.09

S-soil, S+Po- soil+Polonite, S+Po+Pe- soil+Polonite+peat, F.C.-field capacity