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Chapter

Comparative Assessment of Shrimp Hydrolyzates as Alternative Organic Fertilizers for Legumes

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Abstract

The global annual production of shrimp is nearly 4 million metric tons generating almost half of this weight in waste. This study assessed the crop production of legumes fertilized with shrimp exoskeletons obtained by microwaves under greenhouse conditions. Plants were grown under the following fertilization regimes: (i) untreated shrimp waste, (ii) shrimp waste pellets, (iii) shrimp-based pellets having a hydrolysis degree of 42%, (iv) untreated cellulose pellets, (v) untreated soil, (vi) untreated cotton substrate, and (vii) two commercial fertilizers (CF1 and CF2). CF1 and CF2 showed the largest electric conductivity and ionic exchange capability, whereas the fertilizing pellets showed the lowest values. However, pH, densification and conductivity of soil were not affected by fertilization. Shrimp waste showed a high content of C, N, O, Ca and P mainly derived from chitin, proteins and minerals. All fertilizers showed typical type II isotherms, but the untreated soil and CF2 *per se* exhibited the largest water uptake. The soil microbiota increased during the growing cycle and then decreased as the reproductive phase started. Further, soil planted with *Phaseolus vulgaris* showed a larger microbial population than *Pisum sativum*. The best plant growth was achieved when treated with CF2, whereas the raw shrimp waste caused a beneficial plant growth and crop yield mainly in *Phaseolus vulgaris*.

Keywords: crop quality, fertilization, fertilizing pellets, legume development, organic fertilizers, shrimp waste

1. Introduction

The global annual production of shrimp is nearly 4 million tons generating almost half of this weight in waste. This waste in turn, is composed of chitin, which forms microfibrillar arrangements embedded in a protein matrix with $CaCO_3$. A green alternative for the use of this waste is to use it as an organic fertilizer in form of pellets or as a hydrolyzed material. The search for new organic fertilizers is important due to the limited availability of manure and compost in coast lines

resulting promising the use of shrimp waste as an alternative organic fertilizer for crops. Currently, there is no information regarding the organic cultivation of legumes fertilized with shrimp-based waste.

A rapid and efficient shrimp waste hydrolysis could be accomplished by microwaves, which are non-ionizing electromagnetic radiation having wavelengths from 1 mm to 1 m corresponding to frequencies from 300 GHz to 300 MHz, respectively. This radiation could provide the energy required to break the chemical bonds found in organic molecules such as C-C bonds (347 kJ/mol), and hydrogen bonds such those found in the lignocellulosic biomass of rice straw (3.9–10.1 kJ/mol) rendering a 5-fold increase in the yield of sugars [1].

Leguminous crops have been used for several centuries as a source of food for humans and animals [2]. These plants are originated from the Americas but they are now cultivated all over the world due to their high nutritional and culinary values. In fact, they contain high amounts of protein, vitamins (i.e., thiamine, pyridoxine, and folic acid), dietary fiber, complex carbohydrates (i.e., starch), and nutrients such as iron, potassium, phosphorous, selenium, molybdenum and calcium. They are highly desirable in the human diet since are low in sodium and calories [3]. Further, legumes are so important for human nutrition that ~12 million tons of *Phaseolus vulgaris* (PV) are consumed every year worldwide. Moreover, in 2014 the U.S. produced more than 86,700 metric tons of merely kidney beans. In fact, every day ~14% of the U.S. population eats dry edible beans. Legumes are a vibrant part of food security across the world, especially in many developing countries. Thus, ~400 million people in the tropics eat beans as part of their daily diet. Legumes also provide income for millions of farmers, typically in Latin America and Africa.

The growth and development of legumes would require appropriate quantities of nutrients for their optimal development; otherwise, physiological deficiency symptoms could occur [4]. Nowadays, the current trend is the use of organic fertilizers for optimal vegetable development. However, the heterogeneity of the physical and chemical characteristics of the different organic fertilizers may give rise to different crop yields. Interestingly, legumes are known to be nitrogen fixers as they take nitrogen from the air by demand and release it into the soil, fulfilling their own nitrogen needs. This implies the need for an organic fertilizer which provides low levels of nitrogen accordingly [5]. For this reason, the intense use of chemical fertilizers for plant development is not advisable since it causes depletion of beneficial soil microbiota and potential pollution of soil and water [6].

Nowadays, organic fertilizers derived from worm castings, peat, manure, and poultry guano have been used to obtain an efficient organic crop production of several plant species [7]. They increase the organic matter and microorganism activity, improve porosity, water retention, and ion exchange capabilities of the soil. They also prevent root burning or destruction of soil microflora since they contain amino acids, organic matter and a variety of micronutrients that replenish the nutrient level of the soil and feeding important soil microorganisms [8]. For instance, the application of vermicompost in soil decreases root rot of beans and produces vigorous plants [9].

The main objective of the current study was to compare the physical characteristics of several shrimp-based fertilizers and their microwave-assisted hydrolyzates on the development of leguminous plants treated with these fertilizers under greenhouse conditions following an organic production. Fertility and substrate management in organic greenhouse production is important in short-term, low fertility requiring crops. Developing organic fertilizers that slowly release nutrients could improve the crop management of legumes produced organically in container production systems.

2. Materials and methods

2.1 Production of shrimp-based fertilizers and experimental design

Dry shrimp exoskeletons were obtained from the pacific coast of Tumaco (Colombia), milled on a cutting mill (Model 3, Willey Arthur Thomas Co., Philadelphia, USA), and passed through a # 100 mesh sieve. This material was labeled as F0. In a separate experimental set, pellets were produced using microcrystalline cellulose (MCC) as a pelletization aid. Thus, pellets made of pure MCC were made by wetting ~20 g of MCC with 20 mL of distilled water and passed through a #16 mesh sieve (1190 μm size) with a force ≤11.2 N/cm^2 measured with a load cell (LCGD-10 K, Omega Engineering, Inc., Stamford, CT). The extruded thus obtained was put in the spheronizer chamber (Model 1LA70-4YA60, Siemens), which was operated at the spheronization rate of 15 Hz and spheronization time of 120 s producing beads, which were then oven-dried at 40°C for 24 h. These pellets were then labeled as FPC. In another experimental set, a 50:50 mixture of raw waste and MCC was wetted with 42.5 mL of water and submitted to spheronization under the same conditions as explained for the raw MCC. These pellets were labeled as FPE. On the other hand, a hydrolyzed shrimp waste was obtained using a focused microwave apparatus (Samsung, Model MW 630 WA). A 10% power was applied to ensure reproducibility. Approximately, 20 g of sample was dispersed in 200 mL of a 5% NaOH solution and submitted to a refluxing action keeping the temperature between 50 and 60°C. Radiation was continued for the selected exposure times of 0.85 h so a hydrolysis degree of 42% was obtained. The hydrolyzed product was then cooled down, neutralized with 1 N HCl, filtered and dried at 60°C for 24 h. Further, pellets of this material were made under the same conditions employed for FPE and labeled as FHPE.

The physicochemical and functional properties of these pellets were compared to those of the untreated soil substrate (SS), untreated cotton substrate (CS) and two commercial fertilizers named as CF1 and CF2. SS was obtained from a local farm and contained a mixture of virgin soil (fine loam) and rice husk at a 3:2 ratio. CF1 and CF2 (N-P-K of 13.2-1-0) corresponded to an organic and extruded synthetic fertilizer, respectively.

2.2 Treatments and cultural practices

The greenhouse study was conducted in a non-temperature controlled agricultural research station near Medellin (lat. 6.12° N, long. −75.54° E, altitude 2550 m) having a 4 × 4 m (width × length) greenhouse surrounded by a 10-mm light diffusive template glass. The growing condition in the greenhouse was a mean temperature of 23°C day/15°C night and from 65 to 85% RH as recorded during the growth season. No supplementary light or heating was applied in the greenhouse station.

The soil used in the study was a mixture of fine loam (taken from 0 to 30 cm of a virgin soil) and rice husk at a 3:2 ratio. The soil was put in 2 kg PVC pots (15 cm diameter). Healthy and mature legume seeds were obtained from a retail center of Medellin. Subsequently, one seed was sown in each pot randomly and irrigated uniformly with tap water. A plastic saucer was placed under each pot to prevent water loss by leaching. The plants were irrigated using one dripper per plant (at a discharging rate of 10 mL/h) and the total daily irrigation during the growing season ranged from 240 to 350 mL/plant. The irrigation volume ensured that soil was maintained wet in the growing medium.

After germination, only vigorous seedlings were selected for growth in each pot. Five replications of each treatment were arranged in a completely randomized design. The germinated seeds were then treated with ~4 g of the fertilizers in three amendments and these treatments were started on 1-week-old legume seedlings that emerged from direct seeding [12 d after direct seeding (DADS)]. Four and eight weeks after direct seeding, a second and third treatment was applied, whereas in the control treatments, no fertilizer was added (water only). The composition and physical properties of the fertilizers are listed in **Table 1**. Legume plants were trained to a single vertical pole around the main stem and fixed to a wooden stick having 1.5 m high from the ground to support the plant. There was no need to apply pesticides to control insects since plants were healthy and developed normally.

Plant height was evaluated on a monthly basis during the crop cycle. Harvesting started at 90 DADS and finished at 110 DADS. Legume plants were harvested twice a week when the pots reached maturity. Yield parameters that were measured for crop performance included pod length, pod mass, seed mass and pod number. The soil samples for chemical and microbiological analyses were collected from the surface layer (0–10 cm).

2.3 Nutrient content of fertilizers and soil samples

The pH of the 1% w/v fertilizer dispersion was measured with a handheld combo electrical conductivity (EC) and pH meter (EC600, Extech Instruments, Melrose, MA, USA). The moisture content of the materials was obtained by gravimetric methods, using a moisture balance analyzer (MB200, Ohaus, Parsippany, NJ, USA) equipped with a halogen lamp at 120°C. The sensitivity of the measurements was 0.01%. The total ash content was determined following the methodology described in the AOAC [10]. Briefly, samples were heated on a muffle oven (N31R, Mueller and Krempel, Nabertherm, Germany) at 546°C for 7 h. The amount of the cooled residue was taken as the total ash content. The content of sugars was determined by the phenol-sulfuric acid colorimetric method [11].

The elemental analysis was conducted by Energy Dispersive X-ray analysis (EDX) (JEOL 6490LV, Peabody, MA). About 0.2 g of the samples were spread evenly over an aluminum stub and sputter-coated on a vacuum chamber (Desk IV, Denton Vacuum, Moorestown, NJ USA) with a 30% gold coating for 5 min and operated at 15 kV. X-ray diffraction patterns were taken using an X-ray generator with CuKα radiation and the linear surface sample scanning was conducted for 300 s, 10 mm depth of field and 50 μm diffusion. A Malvern Nano-ZS90 Zetasizer equipped with a Zetasizer Software (vs 7.11, Malvern Instruments Ltd., UK) was employed to determine the particle charge at 25°C using the principle of Laser Doppler Velocimetry (LDV). The zeta potential (PZ) measurements were performed by adding 700 μL of the sample in a polystyrene cell. Samples were analyzed between 12 and 16 cycles with a voltage of 4 mV. The ionic exchange test was carried out by weighing from 0.5 to 1 g of sample and 10 mL of 6 N HCl was added and allowed to stand for 24 h followed by centrifugation for 20 min at 1550 rpm. Subsequently, it was submitted to washing with 1% saline solution twice and titrated with 0.8 N NaOH solution. All measurements were expressed on a dry weight basis.

2.4 Water sorption studies

Water sorption studies were conducted employing the static gravimetry method on chambers having several saturated salts rendering different relative humidities. Thus, K_2CO_3, NaBr, NaCl, KCl, KNO_3 and H_20 rendered constant relative

Sample	MC (%)	Sugars (mg/g)	pH	ε (%)	Prot (%)	Ash (%)	CHO (%)	ξ (mV)	BD (g/cm^3)	Soil con (μS/cm)	Soil pH	IE (meq/g)	Con (μS/cm)	Water sorption parameters (Young-Nelson model)				
														A (10^{-4})	B	E	ΔH (kJ/mol)	r^2
F0	*11.0 ± 0.3	*29.5 ± 0.1	*8.3 ± 0.2	*81.8 ± 0.0	*3.20 ± 0.16	*3.6 ± 0.37	90.3 ± 0.31	−15 ± 0.5	*0.32 ± 0.01	20.9 ± 3.4	6.7 ± 0.2	0.81 ± 0.14	*210 ± 4.4	1.63	0.20	16.41	−6.94	0.9668
FPC	2.5 ± 1.1	0.0 ± 0.0	*5.0 ± 0.1	*48 ± 0.0	0.0 ± 0.0	0.05 ± 0.01	*98 ± 1	−16.4 ± 0.5	0.89 ± 0.0	20.6 ± 2.6	7.1 ± 0.2	0.26 ± 0.03	53.8 ± 2.1	13.3	0.14	6.50	−4.64	0.9593
FPE	3.5 ± 1.2	*10.5 ± 0.1	*8.5 ± 0.1	62 ± 0.0	*1.2 ± 0.12	1.7 ± 0.3	*94 ± 1	−11.9 ± 1.6*	0.57 ± 0.02	*27.6 ± 2.5	7.0 ± 0.2	0.33 ± 0.03	*280.7 ± 1.6	99	0.11	5.36	−2.70	0.9800
FHPE	4.2 ± 1.3	0.002 ± 0.000	*8.5 ± 0.2	66 ± 0.0	0.0 ± 0.0	1.5 ± 0.4	90 ± 0.9	−9.4 ± 0.5*	0.51 ± 0.02	18.4 ± 2.7	7.1 ± 0.2	0.34 ± 0.01	*142.3 ± 0.6	4.9	0.02	0.90	0.27	0.9826
SS	*44 ± 1.3	0.002 ± 0.000	7.2 ± 0.4	*83.3 ± 0.0	*9.41 ± 0.43	*45.4 ± 1.3	*0.6 ± 0.1	−22.1 ± 0.3	*0.20 ± 0.02	16.4 ± 8.4	6.9 ± 0.1	0.67 ± 0.1	40.1 ± 3.1	3.11	0.61	46.09	−9.50	0.9582
CS	*8.5 ± 0.6	0.0 ± 0.0	7.1 ± 0.2	*89.8 ± 2.1	0.0 ± 0.0	0.05 ± 0.01	*98 ± 1	−21 ± 2.6	*0.15 ± 0.03	*35.3 ± 3.2	7.2 ± 0.1	*2.21 ± 0.51	*79.8 ± 2.2	0.423	0.34	24.13	−7.89	0.9888
CF1	*8.3 ± 0.5	0.002 ± 0.000	6.7 ± 0.2	58.3 ± 3	0.0 ± 0.0	*42.8 ± 0.40	*20.2 ± 1.2	−17.8 ± 4.6	*0.58 ± 0.02	10 ± 5.4	6.9 ± 0.2	0.41 ± 0.12	*459 ± 2.8	−0.81	0.28	10.69	−5.87	0.9941
CF2	5.8 ± 0.3	0.001 ± 0.000	6.7 ± 0.2	*70.3 ± 2.1	0.0 ± 0.0	*5.3 ± 0.3	*10 ± 2	−22.9 ± 5.2	*0.51 ± 0.02	12.5 ± 2.5	6.8 ± 0.2	*9.49 ± 3.1	*704 ± 3.7	−19	0.63	4.64	−3.81	0.9902
p-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	NA	NA	NA	NA	NA

MC, moisture content; Prot, proteins; CHO, carbohydrates; Con, conductivity; BD, bulk density; ε, porosity; ξ, zeta potential; FPC, cellulose pellets; FPE, exoskeleton pellets; FHPE, hydrolyzed exoskeleton pellets; SS, soil substrate; CS, cotton substrate; IE, ionic exchange; F0, raw waste; CF1 and CF2 correspond to the commercial fertilizers; A and B correspond to the fraction of adsorbed and absorbed water in the particle, respectively; E, equilibrium constant between the mono layer and liquid water; ΔH, heat of sorption. NA, not applicable; mean values with an asterisk within the column are significantly different according to the Tukey's test at $p < 0.05$.

Table 1.
Physical properties of shrimp-based fertilizers, substrates and commercial fertilizers.

humidities of 43, 58, 68, 75, 94 and 100%, respectively. The isotherms were built at 25°C and samples were allowed to reach equilibrium for 2 weeks when the difference between two consecutive measurements was not larger than 0.1%. Data were fitted to several sorption models, and only the one that presented the best fit was discussed in this study. The ability of the fertilizers for water sorption was studied by applying the Young and Nelson model which is expressed as:

$$m = m_m + m_c + m_i \tag{1}$$

$$m = \mathrm{A}(\theta + \beta) + \beta\psi \tag{2}$$

$$\theta = \frac{a_w}{a_w + (1 - a_w)E} \ldots \tag{3}$$

$$\psi = a_w\theta \tag{4}$$

$$E = e^{-(H_1 - H_l)/RT} \tag{5}$$

$$\beta = -\frac{Ea_w}{E - (E-1)a_w} + -\frac{E^2}{(E-1)}\mathrm{Ln}\frac{E - (E-1)a_w}{E} - (E+1)Ln(1 - a_w) \tag{6}$$

where m_m, m_c and m_i correspond to the tightly bound water, condensed external water and internally absorbed water, respectively. Further, m corresponds to the total moisture content, θ is the fraction of surface covered by a monomolecular layer, ψ is the fraction of surface covered by a water layer of two or more molecules thick, and β is the total amount of adsorbed moisture in the multilayer. Moreover, H_1, H_l, k and T, correspond to the heat of adsorption of water bound to the surface, heat of condensation, the gas constant and temperature, respectively. A and B are dimensionless constants related to the fraction of adsorbed and absorbed water in the particle, respectively, and E is the equilibrium constant between the monolayer and liquid water. The product Aθ is related to the amount of monolayer moisture, A (θ + β) is the externally absorbed moisture during the sorption phase, whereas Bψ corresponds to the amount of absorbed moisture during the sorption phase [12].

2.5 Total aerobic bacteria and fungi counts

These tests were conducted on samples without any previous treatment according to the National Technical Standard 4092 of microbiology. Briefly, 1 g of sample was dispersed in 10 mL of peptone water, making the pertinent dilution factors from 1×10^{-1} to 1×10^{-10}. Subsequently, 1 mL of the solution was poured onto a 20 mL culture plate (Merck). Samples were then incubated at 37°C between 24 and 48 h. The results were reported as colony forming units per gram of fertilizer (CFU/g).

2.6 Statistical analysis

The principal component analysis (PCA) was the type of multivariate analysis used to identify and compare the relationships and patterns among the physicochemical and functional properties of the fertilizers. The software Minitab® (v. 16 Minitab, Inc., State College, PA) was used for data processing. The relationship between the different crop characteristics was assessed by the Pearson's correlation coefficient at a significance level of $p < 0.05$. Additional *post hoc* assessment was performed using the Tukey's test ($p < 0.05$) when significant differences between means were observed. The condition of normality was checked using the Shapiro-Wilk test.

3. Results and discussion

3.1 Preparation and physical properties of the fertilizers

Microwave radiation accelerated the degradation of alkaline shrimp waste forming a product having a hydrolysis degree of 42%. Thus, hydroxyl radicals of the alkaline media along with microwave radiation contributed to molecular weight reduction of waste compounds such as carbohydrates and proteins and avoided the need for a time-consuming composting of the raw waste and thus, decreased the initial microbial population avoiding further release of putrescine and other nitrous volatile compounds. Shrimp waste possesses the striated type muscle arranged into muscle fibers that are bound together by a connective tissue where the prevalent amino acid is lysine. These muscle proteins are associated to chitin and minerals such as calcium phosphate. The protein and chitin availability are important since they will eventually turn into accessible nitrogen for legumes. The magnitude of the peptide and glycosidic bonds cleavage during microwave hydrolysis rendered an organic fertilizer having a moderate hydrolysis degree.

During the wet massing process MCC was essential as spheronization aid. Previous studies (data not shown) determined the need of at least 50% MCC as optimal in order to obtain a spherical pellet having good mechanical properties (FPE and FHPE). Thus, MCC fibers alone or combined with waste coalesced and formed larger particles which were then shaped once they passed through the screen orifices. These, in turn, were molded in the spheronizer which cut-off and rounded-off the sharply and roughly surfaces. The rotating plate operating at the 15 Hz rate and residence time of 120 s produced a denser and smoother pellet surface due to the combined action of the centrifugal force created by plate rotation, the vertical force formed by collision, and the gravitational force allowing for the formation of a toroidal or twisted rope motion having an spiral pattern. As a result, this high frequency and short residence time generated more frictional and rotational forces where the initial small, oblong and irregular particles experienced growth, folding and edge rounding which was subsequently shaped into dumb bells. These dumb bells were then twisted, broken, rounded and transformed into spherical or semispherical beads.

On the contrary, raw waste *per se* failed to produce pellets or aggregates due to the lack of plasticity needed for the spheronization process, this fact also occurred by employing a very short residence time resulting in pellets of a predominantly small size, oblong shape and rougher surface. The spheronization platform usually renders bead sizes of about 1000 μm. In this case, by using a #20 screen sieves the size of the resulting beads ranged from 1.2 to 3 mm. Particle size tends to increase with residence time and this variable was kept at 120 s avoiding loss of moisture and maintaining the required plasticity for pellet growth. This high spheronization rate guarantees the formation of beads with diameters larger than 1 mm. The spherical morphology and particle size played a major role on densification and porosity. This occurrence was reflected on the resulting porosity which in turn, decreased with pelletization. On the other hand, the degree of densification decreased by the spheronization process. This is explained by the highly regularly-shaped particles that are less likely to accommodate in the powder bed under the action of an external force as compared to the non-spheronized irregular particles. Flowability is the property that reflects the way in which gravity overcomes the cohesive forces and the interlocking structure of the particles. In general, the flowability of the pellets was high ranging from 13.4 to 16.4 g/s, independent of the average bead mass. A constant plate diameter of 30 cm was employed at spheronization rates of 15 Hz which is equivalent to 900 rpm and peripheral velocity of 1415 cm/s,

respectively. This rotational speed and short residence time (120 s) was suitable to obtain spherical beads.

3.2 Nutritional content of the fertilizers and plant development

The nutritional content of the shrimp-based fertilizers (SBF) is listed in **Table 1**. The hydrolyzed product retained much of the initial nutrients contained in the raw shrimp exoskeletons. The alkaline microwave hydrolysis disrupted the inter and intra-molecular hydrogen bond pattern of complex carbohydrates and proteins initially present in the material, disturbing the regularity of the 3D packing and stereochemistry between chains, especially of the most accessible amorphous regions. As a result, the alkaline hydrolysis of the non-crystalline fraction removed monomer blocks of repeated units, especially those located at the crystallite surface and hence, NaOH accessed the β-1,4N-acetyl and peptidic linkages, simultaneously. The net result was a reduction in the crystallinity of the shrimp fertilizer. In fact, the application of high intensity waves caused chemical and mechanical degradation in the waste particles, resulting in changes in the native shrimp protein and carbohydrate structure into a molten globule state.

The pH and moisture content of these fertilizers ranged from 5.0 to 8.5 and from 2.5 to 11%, respectively. Once the fertilizers were incorporated into the soil maintained a slightly neutral ambient (∼6.7–7.2) and the electrical conductivity ranged from ∼12 to 28 μS/cm. A neutral pH ensured a good availability of the nutrients to the leguminous plants. The high moisture content eased the transformation of macromolecular N into NH_4^+ and NO_3^- by bacteria action resulting in its mineralization and easy uptake by plants as reported previously [13]. The slightly alkaline pH of F0 is attributed to the presence of peptides, and elements such as Ca^{2+} and Mg^{2+}. Further, these divalent ions can then be adsorbed onto the surface of tiny clay particles of the soil which had a net negative charge. The magnesium level in the shrimp-based fertilizers (SBF) was lower than that of calcium so its effect on the soil structure was negligible. The negative surface charge of soil particles is believed to improve P availability in form of phosphates as present in shrimp waste. These phosphates along with the P_2O_5 of CF2 could be responsible for the large *PV* crop yield found in F0 and CF2, respectively. Conversely, K was virtually absent in most fertilizers and its synergistic effect on crop yield was not noticed.

The zeta potential indicates the average charge in the particles and gives a measurement of the ion activity of the fertilizers. All materials exhibited a net negative charge and CF2 had the largest ion exchange capability and electrical conductivity altogether. Conversely, FHPE exhibited the smallest value of electrical conductivity. Interestingly, CS showed a large ionic exchange capability, but a moderate electric conductivity due to the residual ionized functional groups present in this type of cellulose.

Table 2 lists the elemental composition of each type of SBF, substrates and commercial fertilizers. Alkaline microwave hydrolysis had a marked effect on the nutritional content of the shrimp waste. This had a large content of essentially C, N, Ca and P. On the other hand, Fe, Si, Al, Mg and Cl were present as the main microelements. The content of Mg, was larger in the F0 than SS, CS and pellets, whereas the K content was low in all cases except for CF1. The C/N ratio was slower than 10 for F0, FPE, FHPE, CF2, and FPC whereas CF1 (10.5) and SS (33.1) showed the largest C/N ratio due to their low content of N. Further, the SS and CF1 were poor in organic nitrogen, but rich in carbon, silicon and aluminum. On the other hand, CS had a poor content of most elements except for carbon and oxygen. The SS, FPC and CS presented low levels of essential elements such as N, P, and Ca as compared to F0, FPE and FHPE. Interestingly, CF1 and SS showed traces of other

Element	F0	FPC	FPE	FHPE	SS	CS	CF1	CF2	*p-value*
C	43 ± 3.3	33.1 ± 5	43	38.1	33.1 ± 1.1	*53.5 ± 4.5	41.0 ± 10.3	39.0 ± 3.63	0.00
O	33.3 ± 2.1	*42.5 ± 3	33.3	37.9	*42.5 ± 0.4	*46. ± 5.2	39 ± 6	37.0 ± 2.4	0.00
N	*15.9 ± 5.4	0 ± 0	*15.9	*7.95	0 ± 0	0 ± 0	0 ± 0	*15.9 ± 4.9	0.00
Ca	*5.8 ± 2.7	0 ± 0	*5.4	*2.8	0.2 ± 0.1	0 ± 0	0.9 ± 0.2	0.14 ± 0.14	0.00
P	*1.5 ± 1.4	0 ± 0	1.5	0.8	0 ± 0	0 ± 0	0 ± 0	1.2 ± 1.2	0.00
Si	0.2 ± 0.1	*18.2 ± 1	0 ± 0	0	*18.1 ± 1.6	0 ± 0	*11.4 ± 3.2	0.9 ± 0.4	0.00
Fe	1.5 ± 1.0	0 ± 0	0 ± 0	0.59	1.2 ± 0.8	0.0 ± 0.0	1.8 ± 1.8	5.4 ± 5.4	0.08
Al	0.1 ± 0.1	*4.1 ± 0.5	0 ± 0	*2.1	*4.1 ± 0.7	0.5 ± 0.5	*2.7 ± 0.5	0.0 ± 0.0	0.00
Mg	0.4 ± 0.1	0.21 ± 0.1	0.35	0.28	0.2 ± 0.1	0 ± 0	*0.6 ± 0.4	0.4 ± 0.3	0.05
Cl	*0.2 ± 0.1	0 ± 0	*0.23	0.12	0 ± 0	0 ± 0	*0.3 ± 0.1	0.03 ± 0.03	0.00
Na	0.2 ± 0.1	0.05 ± 0	0 ± 0	0.1	0.1 ± 0.1	0.0 ± 0.0	*0.8 ± 0.2	*0.47 ± 0.13	0.00
K	0 ± 0	0.13 ± 0	0 ± 0	0.1	0 ± 0	0 ± 0	*1.1 ± 0.2	0 ± 0	0.00
Ti	0 ± 0	0 ± 0	0 ± 0	0	0.5 ± 0.5	0. ± 0.0	*0.4 ± 0.4	0 ± 0	0.06
Mn	0 ± 0	0 ± 0	0 ± 0	*0.1	0 ± 0	0 ± 0	0.03 ± 0.03	0 ± 0	0.00
C/N	2.7	33.1	2.7	4.8	33.1	53.5	41	2.5	NA

F0, raw waste; FPC, cellulose pellets; FPE, exoskeleton pellets; FHPE, hydrolyzed exoskeleton pellets; SS, soil substrate; CS, cotton substrate CF1 and CF2 correspond to the commercial fertilizers; mean values with an asterisk within the column are significantly different according to the Tukey's test at $p < 0.05$.

Table 2.
Elemental analysis of the shrimp-based fertilizers, substrates and commercial organic fertilizers ($n = 3$).

microelements such as K, Ti, and essentially CF1 was the only fertilizer which contained traces of Mn. On the other hand, CF2 contained N from urea and P from P_2O_5 at a 13:1 ratio.

3.3 Water uptake

The water vapor sorption isotherm of a material describes the relationship between the relative vapor pressure or water activity, (a_w) and water content over a range of a_w values obtained at a given temperature [14]. The fitting water sorption parameters obtained from the Young-Nelson model revealed a good fitting to this model having an r^2 larger than 0.9582 as compared to other models not shown in this study.

Figure 1a shows that during the first sorption stage ($a_w < 0.45$), the isotherms exhibited a convex shape as the water molecules rapidly sorb onto the available sorption sites until a monolayer is formed. The shape of the isotherms during this first stage did not differ substantially among the different SBF, but was larger for SS and CF2. Thereafter, there was a gradual increase in water content with a_w up to ~0.80 where an abrupt increase of water content was observed possibly due to capillary condensation phenomena. Interestingly, most fertilizers showed a steady increase in monolayer and multilayer formation up to a_w of 0.45, afterwards the water molecules although still in vapor form, begin to diffuse within the particle core except for SS, FPE and FPC in which this process started at a very low a_w (**Figure 1b**). Therefore, in these materials isotherms proved that water did not form a continuous monolayer because the multilayer and particle water absorption occurred simultaneously. This phenomenon has been attributed to the tendency of water molecules to cluster around exchangeable cations found in different soils [14]. As a result, water molecules bind as succeeding layers of water molecules rather to empty sites on the surface of the particle. Thus, the formation of a second layer probably started at lower concentration than those corresponding to the monolayer formation. Clustering was expected to occur in most cases since the amount of water molecules on the particle was higher than the quantity that can be bound within the particle. Further, SS and CF2 *per se* had an innate ability to uptake and keep water within the particles and were able to preserve the wet environment for the optimal root and microbiota development.

CF2 at all a_w showed the lowest tendency for clustering, but the largest sorption within the particle core. The deconvoluted curves showed that the monolayer formation presented a type III Langmuir isotherm, whereas the curves for the multilayer sorption showed a type II isotherm. Interestingly, CF2 also showed the largest cation exchange capability and ionic conductivity. This agrees with previous studies that reported a relationship between the high water sorption and the ion exchange capability of the soil [15].

The raw soil substrate (SS) showed the largest E parameter and hence, presented the largest heat of endothermic sorption (ΔH). Further, SS and CF2 showed the largest intrinsic absorbed water (B parameter), whereas CS showed the largest adsorption ability forming multilayers. CF2 and SS showed the largest hygroscopicity, especially at a water activity larger than 0.4. Further, these two samples had the largest ability to absorb water intrinsically, whereas SS and CS *per se* were able to form large water multilayers around the particles. In addition, the water sorption behavior of SBF was comparable to that of CF1.

3.4 Soil microstructure and microbial activity

The ionic exchange capability of the SBF decreased upon hydrolysis as compared to F0 due to leakage of some ions such as calcium and phosphates. Further, the

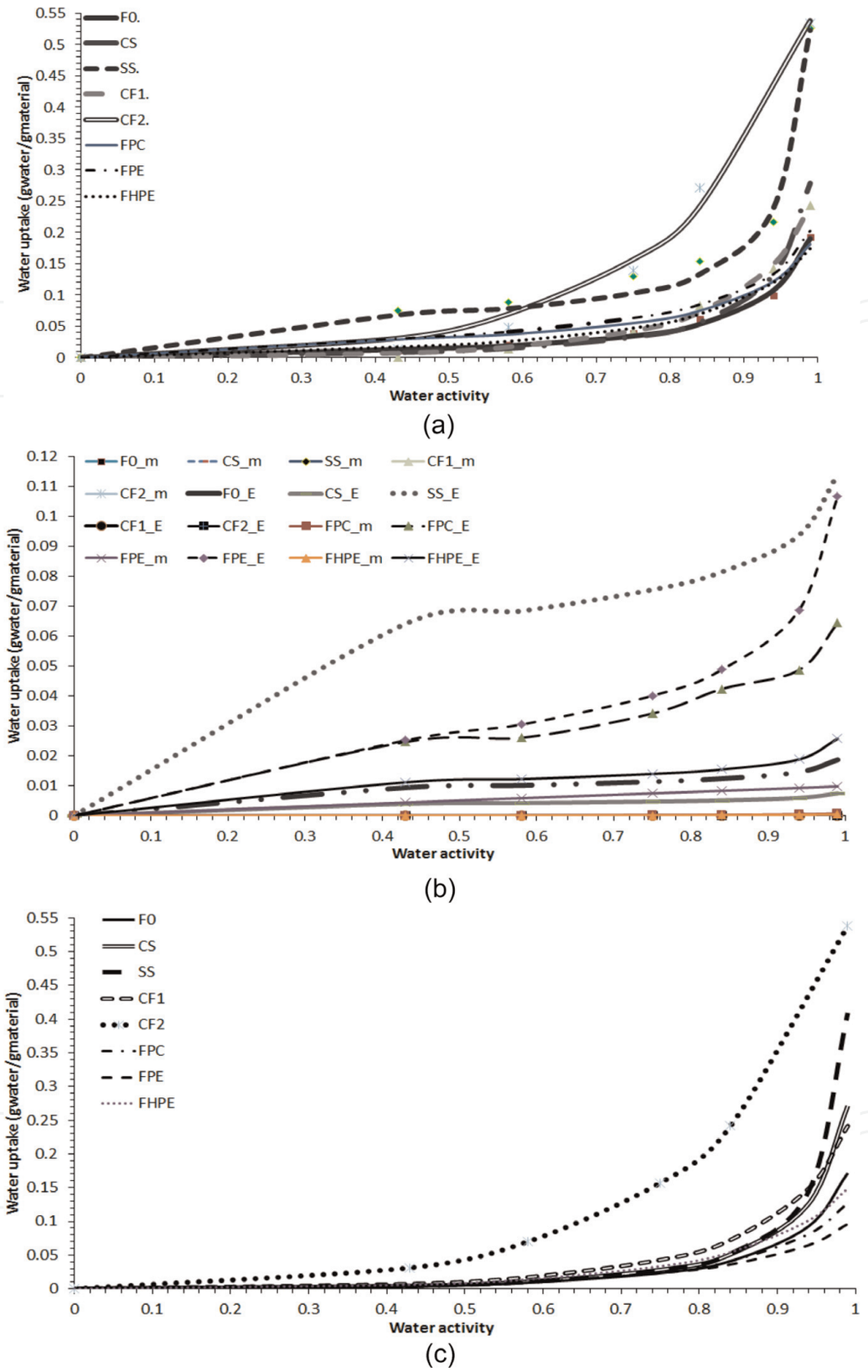


Figure 1.
Water sorption isotherms fitted to the Young-Nelson model. (a) Fitted isotherms, (b) deconvoluted sorption behavior for the monolayer and multilayers, and (c) deconvoluted sorption behavior for the intrinsic absorbed water (n = *3).*

incorporation of these fertilizers into the soil did not have a marked effect on the physicochemical properties of the topsoil due to a dilution effect. Thus, the electrical conductivity of the soil was low (10–28 μS/cm) as compared to the pure fertilizers, but outside the range recommended for other horticultural plants

(0.76–4.0 mS cm^{-1}) [16]. Further, the negative charge of the SBF is due to the residual amine groups of chitin and amino acids. The ash content of the SS (45.4%) and CF1 (42.8%) were larger than most fertilizers (<5.3%) mainly due to their high silicate and carbonate content. The content of carbohydrates of FPE and FHPE (90–94%) was lower than that of CS and FPC; whereas the content of proteins was relatively low and tended to disappear upon hydrolysis as happened for sugars. Moreover, densification (0.51–0.89 g/cm^3) and porosity (48–66%) increased upon pelletization, whereas CS and SS as expected showed the lowest bulk density, but the largest total porosity.

It was estimated that complex carbohydrates present in SBF such as chitin could act as a cementing agents bonding soil particles together improving soil structure and stability. Further, it is reported that calcium ions could act as a cementing agents, bonding soil particles into aggregates resulting in the formation of strong, water-stable aggregates [17]. However, the net postharvest bulk density of the soil did not vary significantly upon treatment with fertilizers probably due to the low applied rate, and density remained in the range generally considered suitable for the normal growth of crops. This low bulk density made root growth and penetration easier and improved the size and system of voids in the soil matrix enabling aeration and water movement. Moreover, the particle size of the powdered fertilizers ranged from 50 to 150 μm and that of the soil and pellets were about 300 and 2 mm, respectively being able to decompose slowly matching the particle size of the soil.

Figure 2 depicts legume growth as a function of time. The largest and fastest growing period of both legumes occurred within the first 2 months of the crop cycle. Both plants followed a sigmoid or S-shaped curve during the growing season corresponding to the period of rapid nutrient uptake. Further, both legumes showed the best growing phase upon fertilization with CF2. Conversely, a slow growth profile for both plants was observed once fertilized with FPE and FHPE. This phenomenon is explained by the reduction of essential nutrients different from C and O.

On the other hand, the pod length, pod mass, and seed mass of *PV* were outstanding when treated with CF2 and comparable to those of F0 (**Table 3**). Conversely, crop quality of *Pisum sativum* (*PS*) as described by these parameters was superior for SS and only FHPE showed good characteristics among the fertilizing pellets. Further, FPC had the worst crop quality an in this particular case plants were not able to render any kind of grain. Likewise, the fact of having a large pod number was not necessary translated into a large crop yield, but pod length, pod mass and seed mass were all good indicatives of crop yield for both legumes ($r > 0.859$).

The SBF were applied at a rate of 4 g/kg soil in three monthly amendments. SBF having 8–20% N had a variable effect on legume growth characteristics depending on the composition. As a result, they showed distinctive quantitative and qualitative

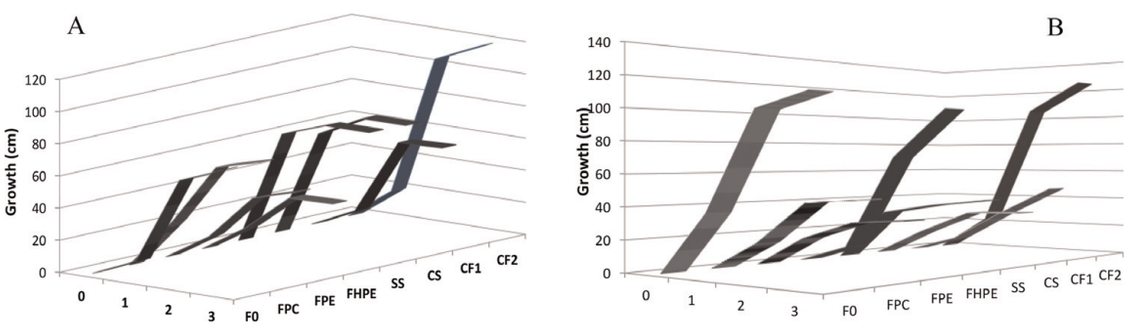


Figure 2.
Growth profiles given by shrimp-based fertilizers, substrates and commercial fertilizers: (A) Phaseolus vulgaris *and (B)* Pisum sativum.

Sample	*Phaseolus vulgaris*				*Pisum sativum*			
	Pod length (cm)	**Pod mass (g)**	**Seed mass (g)**	**Pod number**	**Pod length (cm)**	**Pod mass (g)**	**Seed mass (g)**	**Pod number**
F0	*10 ± 1.2	*4.56 ± 2.0	1.83 ± 0.3*	2 ± 0.3	5.5 ± 1.1	1.23 ± 0.8	*0.7 ± 0.1	*3 ± 1
FPC	7 ± 0	0.77	0.1 ± 0.0	1 ± 0.0	*0 ± 0	0 ± 0	*0 ± 0	*0 ± 0
FPE	4.1 ± 1.3	0.46 ± 0.1	0.22 ± 0.1	*4 ± 0.5	5 ± 0	0.6 ± 0	*0.28 ± 0	1 ± 0
FHPE	5 ± 1.6	0.82 ± 0.2	0.41 ± 0.1	1.5 ± 0.5	4.4 ± 0.5	0.4 ± 0.1	0.3 ± 0.1	*3 ± 0
SS	4.9 ± 1.3	0.62 ± 0.2	0.35 ± 0.1	1 ± 0.0	*6.5 ± 1.2	*3.9 ± 0.9	*1.43 ± 0.1	1 ± 0
CS	4.3 ± 1.3	0.26 ± 0.1	0.1 ± 0.0	2 ± 0.1	5 ± 0.5	1.1 ± 0.5	0.43 ± 0.1	2 ± 0.1
CF1	*7.7 ± 1.1	2.13 ± 0.8	*0.73 ± 0.1	*3 ± 1.0	6.0 ± 0.8	0.74 ± 0.1	0.46 ± 0.1	2 ± 1
CF2	*10.1 ± 0.1	*4.7 ± 0.1	*1.2 ± 0.1	1.1 ± 0.2	5.5 ± 0.6	1.5 ± 0.6	0.6 ± 0.2	2 ± 0.5
p-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

FPC, cellulose pellets; FPE, exoskeletons pellets; FHPE, hydrolyzed exoskeleton pellets; SS, soil substrate; CS, cotton substrate; CF, commercial fertilizers; mean values with an asterisk within the column are significantly different according to the Tukey's test at $p < 0.05$.

Table 3.
Effect of shrimp-based fertilizers, substrates and commercial fertilizers on plant development for Phaseolus vulgaris and Pisum sativum.

traits of grain yield of legumes, especially for *PV*. It has been reported that a large amendment of 20% organic fertilizer (vermicompost) was needed to get the highest pod weight, pod number, pod dry weight and pod length of legumes [5]. In this study, there was a remarkable mismatch between plant growth and plant yield. For instance, CF2 and F0 rendered plants with a good growth and crop yield especially for *PV*, whereas CP2 only led to a good plant growth rather than crop yield in *PS*. This is explained by the content of urea:P_2O_5 (N/P ratio of 13.2), which is recommended by the supplier for the rapid plant growth. In all cases, the N uptake and growth rate were prominent within 30 and 60 days after sowing. In other words, the growth rate progressively increased over time during the vegetative growth up to 4–8 weeks after which growth slowed down as the reproductive phase initiated. Legume growth was not significantly improved with most SBF despite of having a considerable content of available N due to the slow release of this element. However, macroelements such as N, C, P, and Ca were available 45 days after sowing for the appropriate blooming and protein development. Interestingly, the unfertilized CS showed a slow development and crop quality for both legumes, especially for *PS* and thus these plants were not very efficient as atmospheric N fixers to compensate for the lack of N in the CS. In this case, the branched root hair systems of the legumes were not sufficient to ease N mineralization during the growing phase and as a result, they showed the poorest crop yield.

The soil amended with the fertilizers had pH values between 6.7 and 7.1, which are considered optimum for the rapid development of most ubiquitous microorganisms. At this pH range N loss due to ammonia volatilization is prevented since this phenomenon only occurs at acid pH (<6.0) [18]. Soil porosity was ~83% and moisture at saturation was >40% and these levels were not affected by fertilization. The lower water content of SBF was attributed to the presence of insoluble carbohydrates, proteins and of calcium ions. The high moisture content in the soil near to field capacity was responsible for the high diversity of viable microbial during the legume developing phase. These in turn, promoted mineralization and increased available N. The high population of aerobic bacteria found during the whole crop cycle eased nitrogen fixation from the fertilizers and the atmosphere. Interestingly, *PV* was able to modify its own root environment to maximize nutrient uptake. Thus, the inherent absence of N of the unfertilized substrate forced the plant to increase the root pattern so the nitrogen demand could be obtained by microbial (especially fungi) N_2 fixation, as reported previously [19]. However, this N uptake was not sufficient to achieve an optimum plant growth of *PS* since the unfertilized substrates showed the poorest growth rate in the CS. Conversely, SS showed better crop quality than CS due to the higher content of Si, Mg, Fe and Ca which were absent in CS.

It is accepted that during the decomposition of an organic fertilizer the microbial population requires an optimal diet with a C:N ratio of ~15:1 to meet their needs for nutrients. Since the F0, FPE, FHPE and CF2 had a C:N ratio of less than 15:1 they had more N than the microflora require for their own growth in the initial crop cycle and are likely to provide significant plant available N leading to an increased mineral *N* levels through mineralization carried out by microbial metabolism (production of NH_4^+ and NO_3^-) [20]. This phenomenon was reflected on a large microbial population in the soil within the first month (>50,000 cfu/g of bacteria and > 100 cfu/g for fungi). Conversely, the SS, FPC, CS CF1 had a C:N ratio of more than 25:1 and thus, it is assumed a rapid immobilization of the scarce N by microorganisms in this growing phase [21]. Since those fertilizers had N content above 2.5%, they are expected to release nutrients once decomposed by the soil microbiota. The N, P and K ratio of the SBF, and SS were ~1–0.1–0.0, and 0–0–0.1, respectively. These ratios are different from other reported for fertilizers such as cow manure (0.97–0.69–1.66) and compost of raw straw (0.81–0.18–0.68).

Time (month)	F0		FPC		FPE		FHPE		SS		CS		CF1		CF2	
	PV	PS	PV	PS	PV	PS	PV	PS	PV	PS	PV	PS	PV	PS	PV	PS
Mesoaerobic bacteria (cfu × 10^4/g). Basal count: 5×10^4/g																
1	100	40	88	38	72	40	90	20	102	36	1	0.2	580	370	12	32
2	103	26	0.36	8.25	98	6	126	100	130	102	3	0.1	785	64	17	33
3	56	4.9	0.3	8.9	68	4.9	52	48	33	30	2	0.1	54	23	19	28
Fungi (cfu × 10^3/g). Basal count: 100/g																
1	0.1	2	2	3	14	2	20	36	10	23	0.25	0.5	7.8	6	18	48
2	1	6	0.1	1.7	1.1	1	1.2	1.4	14.5	15	1	0.5	2.5	4	27	37
3	10	12	0.6	0.1	0.2	5	1.6	0.81	23.6	28	1	0.5	1	3	18	47
Bacteria/fungi ratio																
1	10,000	200	440	127	51	200	45	6	102	16	40	4	744	617	7	7
2	1030	43	36	49	891	60	1050	714	90	68	30	2	3140	160	6	9
3	56	4	5	890	3400	10	325	593	14	11	20	2	540	77	11	6

FPC, cellulose pellets; FPE, exoskeleton pellets; FHPE, hydrolyzed exoeskeleton pellets; SS, soil substrate; CS: cotton substrate; CF, commercial fertilizers; PV, Phaseolus vulgaris; PS, Pisum sativum.

Table 4.
Total aerobic bacteria and fungi of the soil fertilized with the shrimp-based fertilizers and commercial fertilizers.

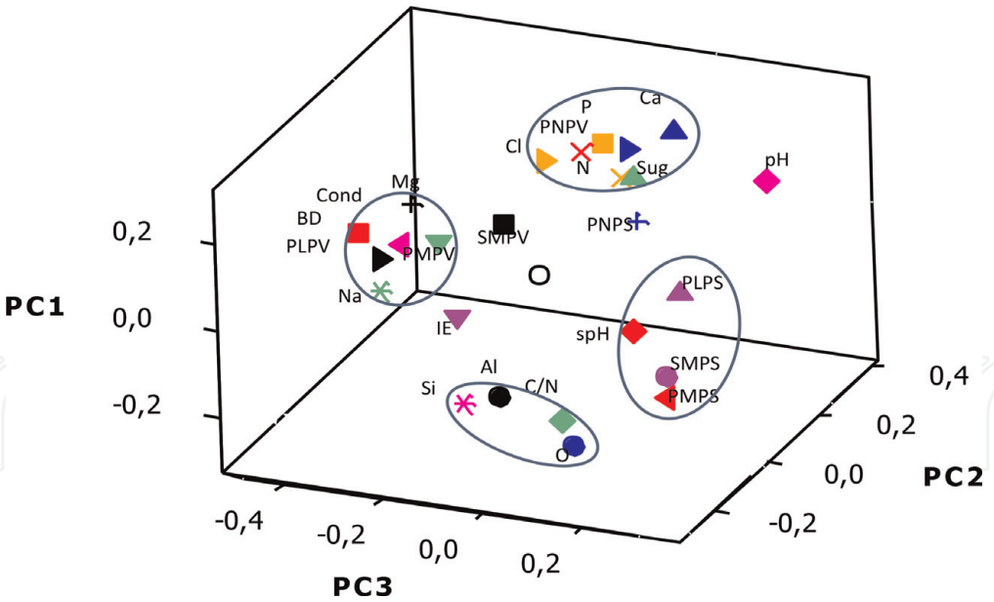


Figure 3.
Principal component plot for key properties of fertilizers.

The fertilizers once incorporated into the soil showed a variable microbial population which decreased over time, possibly due to depletion of soil nutrients that share with plants in a symbiotic way. In fact, the bacteria population was larger in soils containing *PV* than *PS* (**Table 4**). Conversely, the latter favored the proliferation of fungi in the soil. Further, fertilizer type also influenced the bacterial proliferation; for instance, CF1 rendered the largest bacterial population in the soil, whereas CF2 maintained a virtually constant bacterial count. On the contrary, the soil population of fungi tends to increase over time except for soils treated with CF1 and fertilizing pellets where tended to decrease. This fact was reflected on the bacteria to fungi ratio which decreased over time except for the fertilizing pellets and commercial products which increased and remained unchanged, respectively. The high microbial content of the fertilizers mingled with those of the soil microflora favoring the rapid development of bacteria and fungi, which in turn decreased during the crop cycle.

The multivariate analysis rendered interesting facts about this study. The first three components explained 73.3% of data variability (**Figure 3**). In the PCA plot four great clusters are observed apart from the center. The first one depicts the influence of Mg and Na on crop quality of *PV* and the second cluster relates the pod number of *PV* with the content of Na, P, N, and sugars. The third cluster is related to the crop quality of *PS* and soil pH; whereas the fourth cluster relates Si, Al with the C/N ratio. Moreover, a correlation analysis confirmed that fertilizers having a high content of Si also had high Al ($r > 0.920$). Likewise, fertilizers having a high level of N also showed low levels of O and C/N ($r > -0.874$). Further, high levels of Mg were correlated with those of Na ($r = 0.806$) and fertilizers having a high content of N also showed high levels of P ($r = 0.999$).

4. Conclusions

The raw waste rendered an optimal crop quality, especially for *PV*, but showed a lower growth as compared to CF2. Conversely, the pelletization of raw shrimp waste had a deleterious effect on the crop quality of both legumes. Further, the

absence of N, Ca and P in the unfertilized substrates limited legume growth, and microbial activity. This suggested that nutrient sufficiency ranges may require minor adjustment for plant development. Further, viable microorganism population increased in the beginning of the crop cycle and then declined possibly due to depletion of nutrients, but provided short-term fertility benefits for the legumes productivity. These fertilizers are considered more ecofriendly, more efficient, and accessible to marginal and small farmers located in the coast lines. Shrimp-based fertilizers were found to be an alternative soil amendment for legume crops grown using organic methods.

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Conflict of interest

The authors declare no conflict of interest.

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