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La granjA:
Revista de
Ciencias de la Vida

Editorial

Dear reader:

The sage Rumi once said that to be a lamp or a ladder to help others to be seen was the goal of the soul. Following this objective, we are pleased to present new articles from our selection.

The first article comes from Mexico, in which Yasmín Reyes and her team of researchers from the Institute of Nuclear Sciences of the UNAM, the University Center of the Coast and the Autonomous University of the State of Morelios, present a fascinating literature review about Chirality and its characteristics within living systems. Likewise, Paco Noriega and his team of researchers from the Salesian Polytechnic University of Ecuador and the Polytechnic School of Chimborazo, present their study on the anti-inflammatory activity on living organisms of Cannabis (*Cannabis sativa*) and Chilca (*Baccharis latifolia*), with interesting pharmaceutical potential.

On the other hand, we know that mining activity is a sensitive and current issue with risks associated in each phase. As a strategy to minimize them, Raúl Andrés Moreno Farfán, from the Complutense University of Madrid, shows a study of geomorphological restoration of degraded spaces, which can minimize the environmental impact, reducing possible social conflicts associated with this activity.

In the framework of agricultural sciences, there are proposals for treating pests. In this case, Marco Vinicius Sinche Serra and his team from the Nuclear Sciences Department of the Polytechnic National School, show economic proposals of gamma-irradiated baits, which can control the population of fruit flies in Ecuador. Likewise, from the University of Cuenca of Ecuador, Patricio CastroQuezada and his team show how different types of Solanaceae tolerate the nematode presence of the root knot *Meloidogyne incognita* to propose management strategies of these pests depending on the type of crop.

Ana Francisca González Pedraza and her team of researchers from the University of Pamplona also worked on agricultural sciences, showing the effect of the use of different organic fertilizers on pea crops, one of the many crops that support a large amount of agrochemicals. In this regard, Daniel Trigoso-Becerril and his team of researchers from the National Agrarian University of the Jungle of Peru present the effects of these compounds on different soils of rice crops.

On the other hand, in the area of sustainable development, Patricio Pacheco-Peña and his team of researchers from Universidad de Investigación de Tecnologia Experimental Yachay, International University of Ecuador, Pontificia Universidad Católica del Ecuador, show co-management ideas between community and public actors about the valuable water resource, as a tool to adapt to contemporary scenarios of Global Climate Change.

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Regarding the same topic, Christian Orozco, researcher at the Central University, analyzes contractual agriculture and its interactions with large corporations from different currents of thought such as French, Anglo-Saxon and Latin American.

From the veterinary sciences, Víctor Carhuapoma and his team of researchers from the National University of Huancavelica and the Universidad Nacional Mayor de Huancavelica San Marcos of Peru, show the characteristics of the bacteria that cause pneumonia in alpacas, using post-mortem bodies of neonates of high Andean communities of Huancavelica.

We hope these articles will be interesting and will have a great impact on your research.

Sincerely,

Ignacio de los Ríos Carmedano Ph.D
Universidad Politécnica de Madrid
Editor in Chief

Sheila Serrano Vincenti Ph.D
Universidad Politécnica Salesiana
Editor in Chief

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Scientific paper/ Artículo científico
Chemical sciences

Chirality in natural sciences: an approach at different scales

Quiralidad en las ciencias naturales: un acercamiento a distintas escalas

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Abstract

The terms right and left are applicable beyond everyday life and living beings. The fingers on the right hand are arranged in relation to the center of the hand, which is not identical or superimposable to that of the left hand. Both variants are symmetrical versions, but not identical. The spatial arrangement can be observed not only in objects, but also in trajectories. For example, in the bats' flight trajectories. In this article some examples of the chirality condition at different organization levels are defined and some of the recent advances on the subject are mentioned. Understanding the origin of chiral asymmetry found in particles, molecules, and macromolecules, allows us to infer current questions such as chemical evolution, the origin of life, and aspects related to evolution and the development of living beings, among others.

Keywords: Chirality, symmetry, enantiomers, optical activity, biomolecules.

Resumen

Los términos derecha e izquierda son aplicables más allá de la cotidianidad humana y los seres vivos. Los dedos de la mano derecha tienen una disposición respecto al centro de la mano, que no es idéntica o superponible a aquella de la mano izquierda. Ambas variantes son versiones simétricas, pero no idénticas. El arreglo espacial puede observarse no solo en objetos, sino también en trayectorias. Por ejemplo, en las trayectorias del vuelo de los murciélagos. En el presente artículo se definen algunos ejemplos de la condición de quiralidad en distintos niveles de organización y se mencionan algunos de los recientes avances en el tema. Entender el origen de la asimetría quiral encontrada en partículas, moléculas, y macromoléculas, permite inferir preguntas vigentes como la evolución química, el origen de la vida, y aspectos relacionados con la evolución y el desarrollo de los seres vivos, entre otras.

Palabras clave: Quiralidad, simetría, enantiómeros, actividad óptica, biomoléculas.

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1 Introduction

Chirality is a little explored topic at different levels. Therefore, a content was devised to suit, condense and correctly reflect the information reported by science. The topics discussed below are little known in different communities, and it is believed that they can be useful to delve into this subject, especially because chirality is a complex and dynamic system in both science and art. This vision made it possible to analyze the phenomenon at different scales through the trajectory of other works, thus forming a historical and current study. Apparently, the existing information on chirality at different levels is scarce, hence it is important to have a better understanding of the subject.

2 Historical background

In 1815 Jean Baptiste Biot put polarized light into solutions of different organic compounds, noting their hand-style deflection, i.e., a leftward deflection and a rightward deflection, calling them 'optical activity'. Then, Louis Pasteur's experiments in 1848 with single crystals of tartaric acid (Figure 1) were decisive because a set of chiral crystals was held in his hand (Suh et al., 1997). In the 1950s the weak forces were found to be one of the main forces of our universe, bringing with it an answer that is also a question: this fundamental force also shows asymmetry, favoring interaction with left-handed particles (StackExchange, 2020). The possible origin of this behavior is that particles, molecules and everything in our reality behave like helices (which are almost synonymous of chirality) by arranging themselves in space in this way. Hence, a helix is the most reliable representation of chirality. This is not known, but the possible interaction of magnetic fields at the atomic level may be the answer. Shown below is a molecule of tartaric acid (Figure 1), a chemical compound used by Louis Pasteur and his wife Marie Laurent where chiral symmetry was first seen. This was the beginning of specular structural characterization in chemistry.

3 In Physics

In the entropy of the second law of thermodynamics, physics indicates that left and right are nearly equal in their properties, but cosmic rays offer data that may contradict the parity between left and right configurations in the universe. Cosmic rays are believed to originate from the Sun, stars or black holes (Kohler, 2020) and can ionize organic molecules causing chemical reactivity that are not always useful for living systems. When a particle is charged and spins around its own axis and around a nucleus, it produces a magnetic field. So, the magnetic field is a behavior due to the motion of a charged particle. If this motion stops, the magnetic field also vanishes. Maxwell's equations bring together the concepts of electricity and magnetism as two sides of the same coin (Beléndez, 2008). Talking about cosmic radiation is important because when this radiation interacts with some atoms, it forms muons and electrons that interact with molecules of a certain chirality, favoring their synthesis. It would explain the loss of parity in these molecules, although it does not explain why living systems do not respect this parity molecularly (Figure 2).

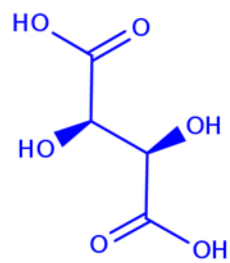

Figure 1. *L* Version with CAS number 87-69-4 and condensed structure $[CH(OH)COOH]_2$ of tartaric acid (C4H6O6 and molecular weight 150,088). D Version has a CAS number 147-71-7 and a condensed structure $HOC(O)CH(OH)CH(OH)CH(OH)C(O)OH$.

The second law of thermodynamics does not respect symmetry either, because it indicates that systems tend to stabilize or condition themselves to the characteristics of the medium which are the conditions of the universe where all reality exists. The ultimate end of everything is disorder and surrender to the conditions of our universe. Weak forces also tend to favor asymmetry. Historically, the polarization of light has been identified for centuries in many cultures. This phenomenon is called 'polarization', because when light passes through a single crystal of Iceland spar, which is calcite, a calcium carbonate generates two points or 'poles' (Craig and Thirunamachandran, 1999). Trilobites had eyes composed of this calcium carbonate (Farace and Aznar, 2011). A preferential coupling to one of the poles by an atom or a part of the molecule could be the answer (Craig and Thirunamachandran, 1999). This answer about the coupling of fundamental particles with radiations and the possible molecular selection in the same molecular evolution of the universe could bring answers, other couplings (Torres-Silva, 2008) and asymmetric molecular selection in the most complex phenomenon that our universe has seen: life. Wu's experiment where parity is violated by weak forces (Wu et al., 1957) is the equivalent to the one we belong to as chiral complex machines.

Figure 2. *Nyctinomops laticaudatus* bat colony in flight. The bats leave the caves in a helical, counterclockwise flight, resulting in a 'social convention' (Gardner, 1985). (Image by M. Zozaya Naturalist, under CC BY NC permission).

4 In biological systems

4.1 Microorganisms

Since approximately 3000 million years ago, prokaryotes have lived in bacterial biofilms (Nazar, 2007). This ability to form cooperative collectives serves an important survival strategy when bacteria are exposed to variable and adverse conditions (Finkelshtein et al., 2017); likewise, they present great advantages to protect against environmental perturbations, e.g., humidity, pH, and temperature (Nazar, 2007), and 1000- to 1500-fold resistance to antibiotics (Gohil et al., 2018).

When biofilms are formed, bacteria can use coordinated chemical signals to communicate with each other. This interrelationship works by means of messengers: small molecules that allow bacteria to sense the existing population and respond to different changing environmental conditions. This communication mechanism is called quorum sensing. Gram-negative bacteria use acyl-homoserine-lactone molecules, while in Gram-positive bacteria modified oligopeptides prevail (Nazar, 2007).

The formation of elaborate and convoluted colonies with chiral and/or fractal geometries can be seen in different species, such as *Bacillus subtilis*, which uses flagella (Ingham and Jacob, 2008) and *Paenibacillus vortex*, which also possesses advanced social motility employing chemotactic signaling. *P. vortex* forms swarms with thousands of bacteria with leading groups, called vortices, which cooperatively rotate, thus, the vortex expands in size and moves outward, leaving a trail and originating convoluted colonies with chiral or fractal shapes (Finkelshtein et al., 2017), although the biological basis of the formation is currently not well understood (Ingham and Jacob, 2008).

4.2 Macroorganisms

Chirality in biological systems is a quality typical to all of them. Chirality can be present not only in microscopic systems (since most molecules in living beings are chiral), but also macroscopically, such as in the case of butterfly wings (Figure 3) or the helical shells of different mollusks (Nieto-Ortega, 2014). The same happens in the case of climbing plants that wind in the form of helices on different types of plants, such as trees or shrubs, as well as on various materials such as stakes; the way of doing so is dextrorotatory (to the right), as in the case of the day dondiego, although there are also those that do so by means of a levorotatory twist (to the left), as is the case of the honeysuckle (Pérez Benítez and Arroyo Carmona, 2018). Citing the example of humans, which are structurally chiral, the liver is on the right and the heart on the left, thus showing functional chirality (Hegstrom and Kondepudi, 1990).

Although there is no reason to say that one mirror image is better than the other, there is some preference for one type of chirality over another (Hegstrom and Kondepudi, 1990). For example, an organism prefers functional chirality to use one of its limbs more than the other; an example of this would be that humans use one hand more than the other to perform various activities (Barrera-Calva et al., 2012).

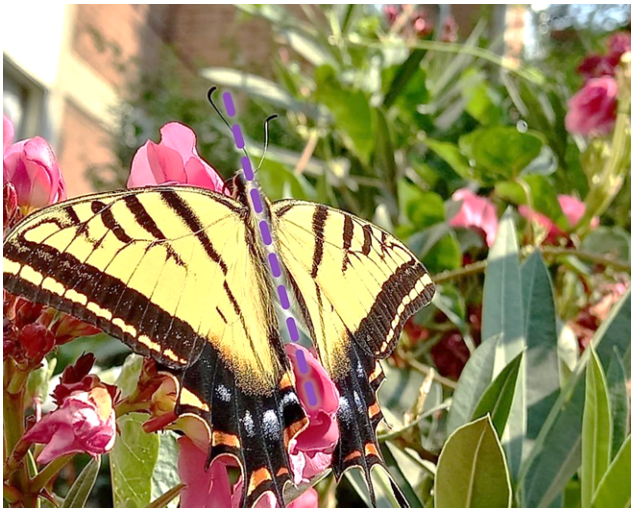

Figure 3. Butterfly 'Xochiquetzal Comet Butterfly'; the plane of bilateral symmetry is pointed out (Image by N. Pacheco-Coronel Naturalista, under CC BY NC permission). Chirality occurs when there are one or more chiral elements, i.e., a chiral axis, plane or center.

Many species use the right hemisphere of the brain to react quickly to new conditions; on the other hand, they use the left hemisphere to control responses that have other alternatives in response to them, thus categorizing their possibilities where ordinarily hemispheric functions have a functional complementarity participation (Barrera-Calva et al., 2012). In humans, the left hemisphere is the responsible of communicative vocalization (including the movement of the tongue, jaw, tongue and lip muscles). However, this is not exclusive to humans; crickets and birds have also been found to possess this chiral property (Barrera-Calva et al., 2012).

A classic example of chirality can be observed in Gastropods due to the direction in which they coil: dextral (clockwise or right-handed) and sinistral (left-handed) (Schilthuizen and Davison, 2005), also known as levorotatory. This chirality originates in early development (Vargas and Zardoya, 2015).

In the third division of larval development when the embryo grows from four to eight cells, mollusks undergo chiral segmentation, i.e., the larval planes are oriented obliquely to the polar axis of the oocyst. When the early division patterns and the direction in which they are oriented are defined, the consequent body asymmetries are determined, i.e., if the division is clockwise, it is a dextral organism; on the other hand, if the case is counterclockwise then the organism will be levorotatory or sinistral (Vargas and Zardoya, 2015).

In the internal anatomy, the internal organs differ in levorotatory and dextrorotatory, since the internal organs are reduced on the side towards which the turn occurs (Vargas and Zardoya, 2015). In certain species these body asymmetries can prevent copulation between the same species, because the genitalia are also inverted with respect to individuals with the opposite chirality (Schilthuizen and Davison, 2005). Therefore, organisms that have an opposite chirality to the majority of those living in a population will have problems finding mates (Schilthuizen and Davison, 2005).

Among organisms dominated by dextrorotatory chirality, levorotatory individuals seem to exist only as a result of mutations, with very low frequencies (from one in hundreds to one in thousands), although this may vary with the species, even in species where levorotatory and dextrorotatory individuals show a similar proportion, as in the case of the snail *Liguus poeyanus* (Hegstrom and Kondepudi, 1990).

Additionally, genes that are expressed exclusively on one side of the body and that determine some of the asymmetries of gastropods have been described. In a dexotrophic organism, the right side of the body of the organism would express chirality-dependent genes, which would be related to the formation of shell-producing cells, with a higher rate on the right side, or on the left side in the case of levorotrophs. If the asymmetric action of these genes is cancelled, completely symmetrical shells are obtained (Vargas and Zardoya, 2015). Genes that regulate the development of body asymmetries (levorotatory and dextrorotatory) also act in other deuterostomes and vertebrates, therefore, it would seem possible that this cascade of genes was present in a common ancestor of mollusks and deuterostomes, being this the one that established such body asymmetries (Vargas and Zardoya, 2015).

In the few species where chirality has been characterized, it is determined by a single genetic locus with delayed inheritance, meaning that the inheritance factor controlling orientation is maternal (Schilthuizen and Davison, 2005).

5 Cellular Structures

5.1 Membrane

Biological membranes are composed of a double layer or bilayer of lipids (generally phospholipids), the latter containing four sections: two fatty acid chains linked to a glycerol molecule and this in turn to a phosphate; at the other end of the phosphate, molecules such as choline, serine, inosine, ethanolamine, among others, can be found (Figure 4b). The structure of the membrane is a characteristic that continues to explain the diversity of living beings. In 1990, the microbiologists Otto Kandler and Mark Wheelis supported a classification system of living organisms into three domains (Archaea, Bacteria and Eukarya), using membrane composition as one of the main bases (Woese et al., 1990). Although Woese had already developed the use of sequences to classify living organisms, since the 1970s more context was needed to convince the community with his classification system. In the 1990 description, three features are considered to differentiate the domains: cell structure (prokaryote, eukaryote), fatty acid to glycerol binding type (ether, ester) and rRNA type (18S rRNA, 16S rRNA) (Woese et al., 1990).

Homochirality is not apparent in the membrane, but up close it is observed that glycerol presents two configurations: glycerol-1-phosphate (G1P) in Archaea and glycerol-3-phosphate (G3P) in Bacteria and Eukarya, where G1P is an enantiomer (levorotatory) of G3P (dextrorotatory) (Figure 4b). Both molecules are synthesized by dehydrogenases and even though they have equivalent function and localization due to their polarity and molecules bound by both ends, G1DPH and G3DPH dehydrogenases do not share an evolutionary history (Akanuma, 2019). With this background, a dilemma known as 'the lipid split' persisted, i.e., how and when did homochirality originate? In this regard, it is accepted that the origin was heterochiral (a mixture of G1P and G3P), i.e. the Last Universal Common Ancestor (LUCA) had a heterochiral membrane and it has recently been shown, thanks to molecular phylogenetics, that homochirality is modern (Figure 4a). This analysis with bacterial homologs of G1DPH and archaeal homologs of G3DPH concluded that the ancestor of Bacteria did not contain G1DPH, therefore, the G1P molecule in Archaea was selected after its origin and its origin was heterochiral as in LUCA (Akanuma, 2019; Lombard et al., 2012). The latter allows explaining the uniqueness of eukaryotes in presenting a membrane with 3PG when they originated by a process of endosymbiosis, where the archaeon (currently homochiral 1PG) acts as a host for a bacterium (13PG) if this archaeon had a heterochiral membrane.

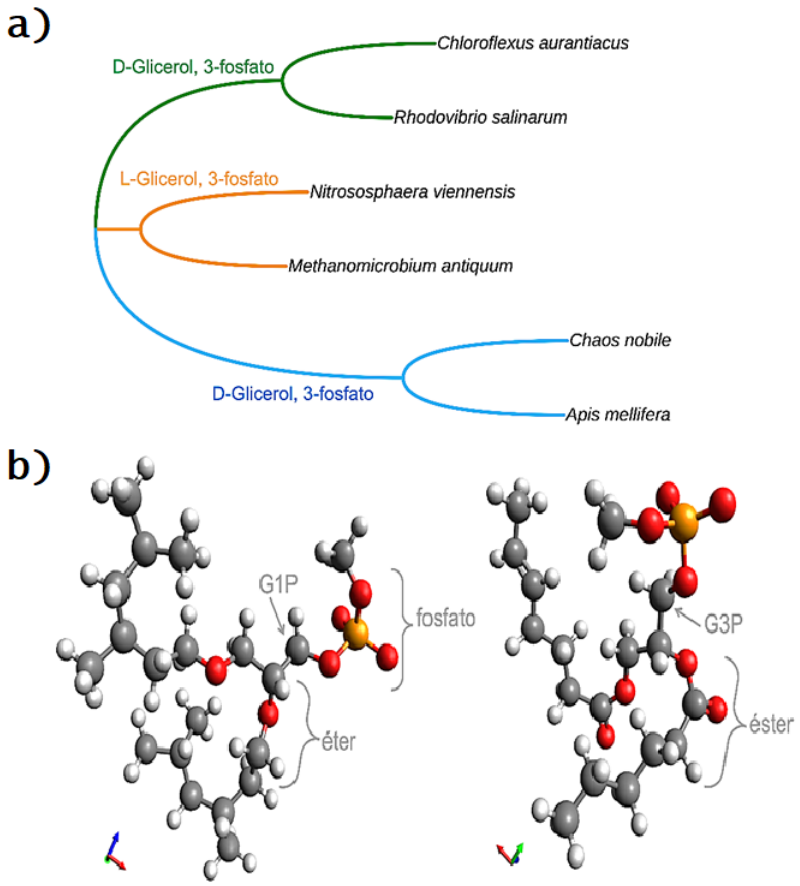

Figure 4. (a) Phylogeny performed with sequence alignment of the three domains (Bacteria in green, Archaea in orange and Eukarya in blue) performed with 2120 positions of the 16S rrna gene, the maximum likelihood method and the GTR evolutionary model in the MEGA X program (Kumar et al., 2018). Enantiomers in each domain are indicated. (b) Chemical structure of phospholipids, G1P (ether in Archaea) and G3P (ester in Bacteria, Eukarya) enantiomers are indicated.

5.2 Cell wall

Most prokaryotes of the Bacteria domain are around either of the two Gram types (positive or negative). In them the arrangement of the cover is quite different, but they coincide in containing a murein cell wall (from Latin *murus* 'wall'), chemically named peptidoglycan. The murein has two sections: several fibers of a polysaccharide alternating N-acetylglucosamine (NAG) with N-acetylmuramic acid (NAM) linked together by a peptide. Some of the amino acids are known as non-canonical because they are not in the list of the 20 universal amino acids in living beings (canonical), some examples are: L-ornithine, L-homoserine and L-hydroxylysine and others that stand out for being dextrorotatory, such as: D-isoglutamate, D-ornithine, D-alanine and D-serine (Vollmer et al., 2008). D-amino acids are also found in some molecules of peptidoglycan-integrated molecules, e.g., teichoic acid (from Greek τειχοζ, teïkhos, 'wall') How and why do these isomeric versions originate? It must be considered that these heterochiral peptides are not synthesized in the ribosome but by enzymes, among them transpeptidases, and the interconversion of L- and D- amino acids is effected by epimerases and racemases enzymes, which belong to the enzyme group of isomerases with the enzyme classification E.C. 5.x.x.x.x. Apparently, the lack of D-amino acids in murein and teichoic acids renders Gram-positive and Gram-negative organisms vulnerable to antimicrobials (Radkov and Moe, 2014).

5.3 Protein complexes

It is common for cells to have extracellular (pili and flagella) and intracellular (cytoskeleton and periplasmic flagella in spirochetes) filamentous structures, or biological particles such as viruses. Some of these fibrillar structures are organized in a helix, thus with an intrinsically chiral conformation. The structure, like the assembly of helical molecules, is the result of selection so homochirality is present in the cell, cell type, or an entire lineage (Satir, 2016). Structures linked to motility are clear examples of a helical polymer: the flagellum and cytoskeleton fibers. Even though the composition and structure of the flagellum are different among the three domains, the common denominator also contains helical fibers. The bacterial flagellum has been by far the most studied and is composed of a tandem flagellin protein attached to the cell body by a hook and ends in a basal body. A rotational feature in the basal body of the flagellum confers motility to the bacterium. Rotation of the flagellum in a basal or minimum energy condition occurs counterclockwise and is reversed as a result of signal transduction, e.g., chemical (toxicants, nutrients) or physical (photons, temperature).

Other helical fibrous structures are the proteins that constitute the cytoskeleton, being actin and tubulin the most widespread among living organisms. It was assumed that the microfilaments of the cytoskeleton were found only in complex cell types (eukaryotes), but proteins homologous to these two proteins have also been found in bacteria and archaea (e.g. MreB, Ftsz and Crenactin). Actin and tubulin are linked to motility, and in eukaryotes they are also related with cell division and motility. An example of the latter is spermatozoa: the microfilaments are helically packed and provide propulsion and allow a twisting motion that can change direction, facilitating crossing in the zona pellucida (Satir, 2016).

Likewise, in prokaryotes the twist of the helical protein MreB is conserved, which implies that the direction of twist of the helical protein is defined since cell division. Also, it has been found that the reference for this twist in MreB is the cell wall, which is opposite to the reticulum that forms the peptidoglycan. It is an opposition that has been found to be conserved even in phylogenetically distant lineages (e.g. phyla as distinct as Proteobacteria and Firmicutes) and is crucial for the fate of the cell shape during division (Wang et al., 2012), recalling that the wall provides cell support. This MreB-peptidoglycan arrangement does not carry over to eukaryotes, because the microfibril helices (actin as homolog of MreB) in eukaryotes are cytoplasmic and radial, without wrapping the bacillus or cocci on the inside (periplasm), as occurs in prokaryotes. The operon for MreB also encodes MreC and MreD, which spatially place MreB with peptidoglycan synthesis proteins (e.g. transglycosylases, transpeptidases) during cell elongation.

6 Macromolecules

6.1 The DNA

DNA (deoxyribonucleic acid) is the main molecule of life, and is the one that carries encoded genetic information typical of different living beings. This molecule is made up of a double strand with two strands composed of successive bonds of the sugar deoxyribose and phosphates (Martínez-Frías, 2010). In general, there are three main families of DNA helices (Figure 5): A-DNA, which can easily form only within certain purine stretches, B-DNA, which is favored by a mixture of sequences (although the exact conformation depends on the particular nucleotide sequence), and Z-DNA, which is favored by alternating purine-pyrimidine steps (Ussery, 2002).

Like proteins, DNA exists under a single chirality. Only the D enantiomer of the sugar is present in nucleic acids, i.e. ,the orientation of DNA and RNA is right-handed, so their most stable conformation is a right-handed helix (Globus and Blandford, 2020). This is extremely important, as living organisms are governed by this preference. However, the helix of the Z-DNA family has a left-handed orientation (Ussery, 2002), which implies that there are different conformations of the DNA helix and therefore different biological functions.

In terms of structural features, B-DNA has double strands running in opposite directions and an asymmetric structure with alternating major and minor grooves, the number of base pairs per helical turn is 10.5 and the width of its helix is $\sim$ 2nm. On the other hand, A-DNA is favored in many solutions that are relatively devoid of water. Unlike B-DNA, its helix is wider ($\sim$ 2,3 nm), the number of base pairs per helical turn is 11 and the plane of the base pairs is tilted about 20° with respect to the helix axis (Lehninger et al., 2008).

The structure of Z-DNA changes radically in the first two, and the most obvious distinction will be a helical rotation to the left. The width of its helix is 1.8 nm, it has 12 base pairs per helical turn, and the structure appears thinner and more elongated, taking on a zigzag appearance. This form of Z-DNA is difficult to observe, as it is highly unstable (Lehninger et al., 2008).

Regarding biological functions, A-DNA plays an important role in transcription and Long Terminal Repeat (LTR) sequences such as the sequence found in human immunodeficiency virus (HIV). Short stretches of purines have been found to exist in genomes that probably form A-DNA conformations in much greater abundance than would be expected for mononucleotide composition, ranging from about a quarter of the genome in bacteria to almost half of the DNA in eukaryotes (Ussery, 2002). B-DNA is sequence-dependent, and rigid (or flexible) sequences can serve as sites for protein binding and the formation of specific complexes, whereas Z-DNA may play an important role as an enhancer in transcription and terminal differentiation. It can be found in prokaryotes, eukaryotes and viruses. In some eukaryote genomes, 10% or more regions with the Z-DNA configuration may be present (Ussery, 2002). In living cells, most DNA is found in a mixture of A-DNA and B-DNA, with some small regions capable of forming Z-DNA (Ussery, 2002).

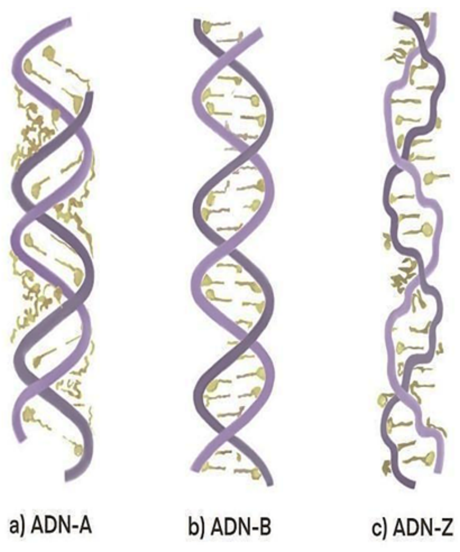

Figure 5. Different DNA helix configurations: (a) A-DNA, (b) B-DNA and (c) Z-DNA are presented with a longitudinal view.

6.2 The RNA

The importance of chirality in RNA lies in the fact that prebiotic chemistry was essentially racemic. The chiral element in RNA is a sugar that undergoes particularly rapid racemization under the warm, humid conditions in which prebiotic chemistry presumably took place (Sandars, 2005). Its replication is only successful if homochiral nucleotide monomers are used. In the presence of racemic monomers, replication is inhibited, thus homochirality preceded the RNA world by a pre-RNA world in which selection operated using some other type of genetic material without the chiral constraints of RNA (Bailey, 2000).

However, enantiomeric cross-inhibition by RNA stops the polymerization process, preventing the formation of long polymers. Thus, Sandars (2005) has shown that under certain circumstances enantiomeric cross-inhibition could be the driving force for a chiral bifurcation leading to homochiral polymers.

It should be noted that the polymer likes to adopt a helical shape for structural reasons in both cases (PNA and RNA). In the case of PNA, the choice of orientation is random or triggered by an external influence. In contrast, the RNA polymer is forced to adopt an orientation determined by that of the constituent monomers (Sandars, 2005).
. Although this may well not be the system that nature used, it represents one possible way in which homochirality could have evolved.

6.3 Chlorophylls and bacteriochlorophylls

Chlorophylls and bacteriochlorophylls are optically active molecules with several chiral centers, necessary for their natural biological function and the assembly of their supramolecular complexes (Senge et al., 2014). They are cyclic tetrapyrroles with a central Mg, a five-part isocyclic ring and a long-chain esterifying alcohol at C17 (Patty et al., 2017).

The central magnesium atoms of chlorophylls (Chls) and bacteriochlorophylls (BChls) are in most cases penta-coordinated, resulting in the formation of a new stereochemical center and the possibility of two different types of chlorophyll-ligand interaction (Senge et al., 2014).

Bacteriochlorophylls do not normally occur as 'isolated' pigments in monomeric and/or free form. In their functional state, they are often bound to apoprotein side chains. This is facilitated by axial ligands, which allow important interactions with the apoprotein environment, aiding the organization of chlorophyll proteins and modulating their electronic properties (Senge et al., 2014). Like most amino acids and proteins, any tetrapyrrole complex containing a protein, even achiral, becomes optically active.

7 Biomolecules

Life on Earth exists because biomolecules (sugars and amino acids) are mainly in their predominant homochiral form (Breslow, 2011). The two mirror images of a chiral molecule are called enantiomers. The enantiomers of amino acids and sugars are called L- or D- (Cava et al., 2011). Living beings have an exclusive affinity for L-amino acids in ribosomal protein synthesis, and for D-sugars (D-ribose) in nucleotides (Sasabe and Suzuki, 2018). If proteins with L-amino acids had random D- enantiomers, they would have variable conformations. However, this is not something that occurs in actual biology because specific enzymes produce the L-amino acids, and the same is true for sugars (Breslow, 2011).

7.1 Sugars

Sugars have a generic formula of $C_nH_{2n}O_n$. Most sugars are optically active because they have asymmetrically substituted carbons. The relative position of the first asymmetric carbon in the sugar chain determines whether the sugar is D- or L-. Sugars such as ribose and glucose have more than one chiral center and are classified based on the configuration of the chiral center farthest from the carbonyl group (Breslow, 2011).

L- enantiomers of sugars are known as rare sugars because they are not found very often in nature. However, these rare sugars have great potential in the food and pharmaceutical industries (Chen et al., 2020). For example, they are used as antiviral drugs for treating serious viral diseases, such as HIV or hepatitis (Helanto et al., 2009), and although L-sugars have not been found in biological processes, experiments have been done to determine whether some L-sugars contribute to total energy metabolism (Livesey and Brown, 1995).

7.2 Amino acids

Proteins are formed from 20 essential amino acids, which are α-amino acids. The general structure of an amino acid consists of a carboxyl group ($-COOH$) and an amino group ($-NH_2$) attached to the α carbon, which in turn is attached to a hydrogen ($-H$) and a side chain (R group) (Genchi, 2017). Amino acids generally have a single chiral center (Breslow, 2011), which is precisely the carbon (Figure 6). Enantiomers of amino acids are called L- or D- depending on the optical activity of the glyceraldehyde isomer on which the amino acid can be superimposed. Both enantiomers are produced by chemical synthesis, but most enzymes exhibit marked selectivity and, because of this, many biochemical processes use and produce particular enantiomers (Cava et al., 2011).

Although L-amino acids are the most common in living organisms, D-amino acids have been found to play a role in some biological processes, such as D-aspartate, which is an important regulator of adult neurogenesis, and D-serine which acts as a co-agonist of N-methyl D-aspartate-type glutamate receptors in the brain, and which are involved in learning, memory, and behavior in mammals. Also, D-amino acids are known to have been used as nutrients to support bacterial growth, regulate bacterial spore germination, and are components of the bacterial cell wall (Cava et al., 2011).

There are three theories to try to explain the presence of D-amino acids in the proteins of living beings. The first is that it may result from the direct incorporation of a D-amino acid into the peptide chain (produced, for example, by an amino acid racemase). The second is that it may be due to non-enzymatic racemization associated with aging or disease. And the third is that it may be due to a post-translational enzymatic modification (Genchi, 2017).

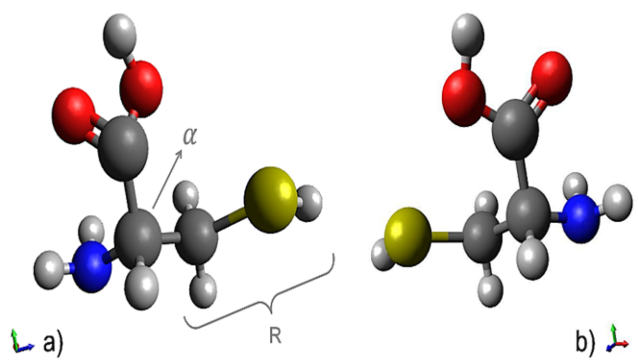

Figure 6. Cysteine amino acid in its enantiomeric versions (mirror images): dextrorotatory (a) and levorotatory (b).

7.2.1 Amino acid racemization

Racemization is a reversible first order reaction that can be described by Equation 1 (Bada, 1985).

$$\text{L-amino acidD-amino acid} \qquad (1)$$

It was more than a century ago when it was first observed that amino acids underwent racemization when heated in highly acidic and basic solutions. In the early 20th century, racemization of amino acids into peptides and proteins was first observed in alkaline solutions at elevated temperatures. Racemization can also occur at neutral pH and at rates that are comparable to those under acidic pH and in dilute bases. In addition, racemization is detected in proteins from both fossils and living organisms (e.g., mammals), so it has even been suggested that racemization could be the basis of a useful dating method for determining the age of organisms (Bada, 1985).

Amino acid racemases and D-amino acid oxidases (DAO) are the enzymes responsible for the synthesis and degradation of D-amino acids. As L-amino acids are the predominant amino acids in living organisms, they act as the substrate for the generation of D-amino acids and what racemases do is to change the stereochemistry of the chiral α-carbon in amino acids to convert L-amino acids into D-amino acids (Genchi, 2017).

7.2.2 Origin of homochirality in sugars and amino acids

Achiral compounds can crystallize in chiral structures. Enantiomorphic minerals such as quartz are optically active. These types of minerals, whether from space or Earth, could have served as prebiotic chiral nurseries due to surface-mediated chirality transfer, providing an excess of L-amino acids or D-sugars in the prebiotic Earth (Evans et al., 2012).

Generally, any chemical reaction that forms a product with a chiral center will produce equal amounts of L and D enantiomers, known as a racemic mixture, but this is not always the case: Frank's autocatalytic kinetic model for an asymmetric synthesis suggests that a slight excess of one enantiomer can influence and favor the synthesis of that enantiomer over its optical antithesis (Evans et al., 2012). At present, it is still uncertain what processes led to the change from a racemic world to the homochiral world in which we live (Sasabe and Suzuki, 2018).

Organic compounds, including some amino acids, have been found in some meteorites that have fallen to Earth. In the meteorite that fell near Murchison, Australia in 1969, five α-methyl amino acids were found, and all had a small but significant excess of what were described as L- enantiomers (or S enantiomers). One idea to explain this is that racemic mixtures of α-methyl amino acids formed in the Kuiper belt that were then selectively broken down by unshielded right-handed circularly polarized light, and the α-methyl amino acids with an excess of L- enantiomers reached Earth via chondrites (Breslow, 2011).

7.3 Other low molecular weight biomolecules

Thalidomide, antibiotics and amphetamines are examples of drugs with one or more chiral centers that are indicated by the prefixes R and S. The chiral versions of thalidomide have different effects on the human body: the S version is teratogenic, and the R version has tranquilizing properties (Elder et al., 2021). Examples of antibiotics are ofloxacin and its R-version, levofloxacin (Maia et al., 2018). Another example is chloramphenicol, which presents two chiral centers (there are S,S and R,R versions) and inhibits protein synthesis, therefore, it is a bacteriostatic antibiotic. This antibiotic is used only in developing countries for some meningitis and conjunctivitis; however, its use is restricted due to its toxic effects and the development of antimicrobial resistance.

Some enterobacteria can degrade R,R-(-)-chloramphenicol as a microbial resistance strategy and through metabolic pathways not applicable for S,S-(+)-chloramphenicol. Recently, a form of resistance to the S,S version has been found through its racemization to the metabolizable R,R form (Elder et al., 2021).

8 Conclusions

Chirality is an essentially geometrical property that comes from molecules and has physicochemical relevance. In living systems, other unicellular organisms such as eukaryotes and metazoans to social behavior have this symmetry. Especially in living systems, it is relevant that mirror symmetry gives distinct physical and chemical properties. In this respect, the origin of chirality in biology is a necessary question to answer.

This review has tried to solve and approach this question from thermodynamic and emergent properties, where chirality does have relatively measurable changes in our simulations and some of our experiments. The fact that L-amino acids and D-sugars are the main compounds of the living evolutionary machinery allows to assume molecular evolutionary mechanisms and molecular natural selection. This approach from molecular biophysics is giving some answers, and therefore we continue looking for the relevance of chirality in living systems through our research.

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NATURAL PRODUCTS

In-vivo ANTI-INFLAMMATORY ACTIVITY OF A TOPICAL FORMULATION WITH ACTIVE PRINCIPLES ON ESSENTIAL OILS OF *Cannabis sativa* L. (CANNABIS) AND *Baccharis latifolia* (RUIZ & PAV) PER. (CHILCA)

ACTIVIDAD ANTIINFLAMATORIA *in-vivo* DE UNA FORMULACIÓN TÓPICA CON PRINCIPIOS ACTIVOS DE ACEITES ESENCIALES DE *Cannabis sativa* L. (CÁÑAMO) Y *Baccharis latifolia* (RUIZ & PAV) PER. (CHILCA)

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Abstract

Essential oils of *Cannabis sativa* (cannabis) and *Baccharis latifolia* (chilca), were used as anti-inflammatory ingredients in an ointment for topical application. Different formulations designed based on these two essential oils were evaluated in-vivo to measure efficacy, using the feet swelling induction method in rats, and an over-the-counter formulation with 1% diclofenac active ingredient was used as a positive control. The chemical evaluation of the two oils yielded the following main components, for chilca oil: liguloxide 14.02%, andro encecalinol 9.84%, kesane 7.53%, limonene 5.6% and Z-cadin-4-en-7-ol with 5.03%; while for cannabis essential oil: E-caryophillene 27.91%, myrcene 21.19%, α-pinene 8.05%, α-humulene 8.03%, limonene 7.18%, terpinolene 7.12% and β-pinene 4.68%. The results of the research indicate that those formulas that combined two essential oils in the formulation at 1%, are the ones with the highest anti-inflammatory activity. Statistically, the significance is high in relation to the positive control in those whose oil composition is essential oil of cannabis 75% and chilca oil 25%; and essential oil of cannabis 50% and chilca oil 50%. The other formulas have activity, but this is similar to the commercial formula used as control. Based on the results, it is possible to propose both natural products as anti-inflammatories, and to foresee the design and commercialization of topical pharmaceutical drugs using these two essential oils.

Keywords: *Cannabis sativa*, *Baccharis latifolia*, essential oils, anti-inflammatory activity, anti-inflammatory synergy.

Resumen

Dos productos naturales, aceites esenciales de *Cannabis sativa* (cannabis) y *Baccharis latifolia* (chilca), fueron empleados como ingredientes antinflamatorios en un ungüento de aplicación tópica. Para medir la eficacia, las diversas fórmulas diseñadas a base de estos dos aceites esenciales fueron evaluadas in vivo, mediante el método de inducción del edema subplantar en ratas, como control positivo se empleó una formulación de venta libre con ingrediente activo diclofenaco al 1%. La evaluación química de los dos aceites presentó para el aceite de chilca los siguientes componentes principales: ligulóxido 14.02%, andro encecalinol 9.84%, kesano 7.53%, limoneno 5.6% y Z-cadin-4-en-7-ol con el 5.03%; mientras que en el aceite esencial de cannabis las moléculas más abundantes fueron: E-cariofileno 27.91%, mirceno 21,19%, α-pineno 8.05%, α-humuleno 8.03%, limoneno 7.18%, terpinoleno 7.12% y β-pineno 4.68%. Los resultados de la investigación señalan que aquellas fórmulas con mezclas de los dos aceites esenciales en la formulación al 1%, son las que poseen la mayor actividad antiinflamatoria, desde el punto de vista estadístico la significancia es alta en relación al control positivo en aquellas cuya composición de aceites es la siguiente: aceite esencial de cannabis 75% y aceite de chilca 25%, y aceite esencial de cannabis 50% y aceite de chilca 50%. El resto de las formulaciones presentan actividad, pero esta es similar a la de la fórmula comercial usada como control. De los resultados encontrados se puede proponer a ambos productos naturales como antinflamatorios, y prever el diseño y comercialización de medicamentos farmacéuticos tópicos usando a estos dos aceites esenciales.

Palabras clave: *Cannabis sativa*, *Baccharis latifolia*, aceites esenciales, actividad antiinflamatoria, sinergia antiinflamatoria.

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1 Introduction

Inflammatory processes are related to pathologies such as: autoimmune diseases (Urakov and Urakova, 2021; Lochhead et al., 2021; Murata, 2018), various infections (Shah, 2019; Cervilla et al., 2002); cardiovascular diseases (Golia et al., 2014; Van Eeden et al., 2012) and traumas (Brown et al., 2021; Mortaz et al., 2018). According to the technical bulletin (INEC, 2020), polytrauma is the second cause of hospital morbidity, only below gastrointestinal infections, causing a high consumption of nonsteroidal anti-inflammatory drugs (NSAIDs). The healthy inflammatory response is temporarily beneficial, but has a precarious balance that can be altered causing unintentional tissue damage and generating abnormal or chronic inflammation. This imbalance generates an uncontrolled "proinflammatory" state capable of causing disease as a result of oxidative stress, which is the result of damage caused by reactive oxygen species (ROS), its basis being inflammation (Schewe, 1995).

Many medicinal plants are used for their anti-inflammatory properties as part of the ancestral knowledge of peoples around the world; additionally, there are *in-vitro* and *in-vivo* assays in several of these species that prove the anti-inflammatory potential of the active principles they contain (Oguntibeju, 2018; Nunes et al., 2020; Tasneem et al., 2019; Yatoo et al., 2018). Essential oils are among the extracts used for their anti-inflammatory activity, many of these biological matrices have shown secondary metabolites with high activity (Miguel, 2010; Pérez et al., 2011; Grassmann et al., 2000). Among the promising species, either for their traditional use or for their scientific evidence, there are essential oil of *C. sativa* (cannabis) (Orlando et al., 2021; Di Sotto et al., 2022) and *B. latifolia* (chilca) (Abad and Bermejo, 2007; Sequeda-Castañeda et al., 2015).

The essential oils from the two medicinal plants were analyzed in this research and were used as active ingredients in a topical formulation, whose effectiveness was studied in vivo in an animal model and compared with an over-the-counter commercial drug widely used in the environment. In this way, an alternative formulation is proposed, which uses natural active ingredients with a high efficacy to mitigate inflammations resulting from trauma or rheumatoid pathologies.

2 Materials and Methods

2.1 Extraction of essential oils

The essential oil of cannabis was bought at Eden Garden Essentials, in San Clemente, USA. The product comes with its quality data sheet. *B. latifolia* leaves (HUPS-as-011 voucher herbarium of the Laboratories of Life Sciences-UPS), were collected in the city of Riobamba, in the province of Chimborazo at the following coordinates: 1°40′26″ S, longitude: 78°38′37″ W, altitude: 2752 m.a.s.l. The fresh material was processed in a stainless steel distiller with a capacity of 64 liters that operates with the system known as water and steam in the Life Sciences laboratories of the Universidad Politécnica Salesiana, Quito.

2.2 Chemical composition of essential oils

The technical data sheet of the essential oil of cannabis details its chemical composition. The identification of compounds of the essential oil of B. latifolia was carried out through gas chromatography coupled to mass spectrometry, using a Trace 1310 gas chromatograph coupled to a Thermo Fisher Scientific ISQ 7000 mass spectrometer with a Termo Scientific TR-5MS chromatographic column, 30 m long, 0.25 mm thick and with a film thickness of 0.25 μm. The sample was prepared by diluting 10 μL of *B. latifolia* essential oil in 990 μL of dichloromethane; the injection volume was 1 μL. The carrier gas was 99.9999 % pure helium at a flow rate of 1 1 $mL\ min^{-1}$, and a split-ratio of 1:40. The injector temperature was 250°C.

The initial temperature in the column was 60°C for 5 minutes, until reaching 100°C at a rate of 2°C/min; then it increased to 150°C, at a rate of 3°C/min, reaching 200°C at 5 °C/min, and finally it reached 230°C maintaining this temperature for 5 minutes, with a total analysis time of 60 minutes.

The mass spectrometer conditions were: ionization energy: 70 eV; emission current: 10 μAmp; scanning range: 1 scan/s; mass range: 40-350 Da; trap temperature: 230°C; transfer line temperature: 200°C.

Identification of the molecules was performed using the NIST 2001 mass spectral database. In addition, the arithmetic retention index (RI), of each compound was calculated comparing with a series of C8-C30 alkanes and finally contrasting the theoretical retention indices from Adams (2012) database.

2.3 Preparation of anti-inflammatory ointments

The type of formulation was O/W (oil in water), the formula is described in Table 1. Five formulations were prepared with different proportions of the essential oils and a control formulation without oils, where the percentage of water was 64 %, the percentages of each formulation are described in Table 2.

Table 1. Formula of the anti-inflammatory ointments.

Phases	Compound	Quantity in percentage
Oily phase	Mineral oil	12
	Stearic acid	10
	Cocoa butter	5
	Cetyl alcohol	5
	Beeswax	3
	Essential oil/s	1
Aqueous phase	Water	63
	Tea	1

2.4 *In-vivo* anti-inflammatory activity

The subplantar edema method induced by carrageenan injection proposed by Winter et al. (1962), was used, using rats weighing between 180 and 220 grams, aged 4 to 8 weeks, under controlled feeding conditions. The laboratory animals were housed for 2 weeks in groups of 5 for each treatment: 5 concentrations, a control formulation and a positive control. A commercial formulation containing 1% diclofenac was used as a positive control. Each animal was injected with a 0.3 % carrageenan solution in propylene glycol, with a waiting time of 30 minutes to generate edema. A total of 5 values were taken for each individual: T before carrageenan application, To at 30 minutes, T1 at 1 hour, T2 at 3 hours and T3 at 5 hours after application. The values were determined in volume units using a plethysmometer, Figure 1 shows the experiment graphically.

Table 2. Proportions of essential oils in each formulation.

Formulation with base cream	Proportion of essential oils
Formulation A	Cannabis oil 25 % *B. latifolia* oil 75 %
Formulation B	Cannabis oil 50 % *B. latifolia* oil 50 %
Formulation C	Cannabis oil 75 % *B. latifolia* oil 25 %
Formulation D	Cannabis oil 100 %
Formulation E	*B. latifolia* oil 100 %
Control formula	Essential oils 0 %

2.5 Statistics

Since there were seven groups of data at different times, including both positive and control groups, the statistical study was carried out in 4 stages. Using the R program version 2021, the data were analyzed, considering as the main parameter the proportion of inflammation caused at the beginning and the level of deflation in the three times in which the measurements were taken; these parameters were expressed in net value of inflammation, having as value the volume of the inflamed paw minus the volume of the normal paw. First, it was determined if the values presented normality by means of the Kolmogorov-Smisnov test with the Lilliefors adjustment, taking into account the contrast by treatment applied and by time elapsed after the application of the treatment. Homoscedasticity analysis was then performed using Levene's test in all the inflammation measurement times to determine that the variances are homogeneous, i.e., that there is not a very large variability between the study groups and that a Tukey test can be applied later.

The main analysis was the Anova test. Previously, a covariance analysis was performed to rule out the possibility of an interference of results with a variable that is not considered, in this case a possible interaction with the volume of the rat's paw in the initial state; the analysis was conduc-

Figure 1. Graphical scheme of the carrageenan-induced subplantar edema method and measurement in a manual plethysmometer.

ted using the Ancovac test. The Anova analysis was performed in a one-way model to determine a significant difference between the means. The differences between each of the groups compared to others were observed in the three measurement times after the treatment was applied to determine which treatment is the most effective against inflammation, considering the control and positive control groups (formulation with diclofenac at 1%). Subsequently, an Anova study was performed in a linear model to see the behavior of each of the treatments as a factor within a linear regression equation, contrasting each one against the control.

3 Results and Discussion

3.1 Chemical composition of essential oils

The cannabis oil purchased at Eden Garden Essentials has a certificate of chemical composition, which is detailed in Table 3.

Table 3. Chemical composition of *C. sativa* essential oil, provided by Eden Garden Essentials.

No.	Compound	% RDA	Theorical ratio[a]
1	hexanol	0.05	863
2	Not identified	0.08	-
3	α-tujene	0.07	924
4	α-pinene	8.05	932
5	camphene	0.08	946
6	sabinene	0.95	969

No.	Compound	% RDA	Theorical ratio[a]
7	β-pinene	4.68	974
8	Not identified	0.04	-
9	3-p-menthene	0.06	984
10	myrcene	21.19	988
11	mesythilene	0.03	994
12	Not identified	0.24	-
13	α-phellandrene	0.63	1002
14	δ-3-carene	0.69	1008
15	α-terpinene	0.11	1014
16	p-cymene	0.22	1020
17	limonene	7.18	1024
18	β-phellandrene	0.32	1025
19	1,8-cineole	0.29	1026
20	Z-β-ocimene	0.24	1032
21	E-β-ocimene	2.65	1044
22	γ-terpinene	0.1	1054
23	1-octanol	0.04	1063
24	E-sabinene hydrate	0.05	1098
25	terpinolene	7.12	1086
26	fenchone	0.15	1083
27	linalool	2.25	1095
28	α-fenchol	0.7	1114
29	Not identified	0.06	-
30	borneol	0.13	1165
31	p-cimen-8-ol	0.08	1179
32	α-terpineol	0.33	1186
33	citronelol	0.16	1223
34	α-copaene	0.05	1374
35	Hexyl hexanoate	0.04	1382
36	Z-caryophyllene	0.14	1408
37	Not identified	0.04	-
38	Z-α-bergamotene	0.05	1411
39	E-caryophyllene	27.91	1417
40	α-elemene	0.04	1434
41	E-bergamotene	0.42	1432
42	Not identified	0.72	-
43	α-humulene	8.03	1452
44	Not identified	0.25	-
45	Not identified	0.1	-
46	β-selinene	0.29	1489
47	γ-amorphene	0.04	1495
48	α-selinene	0.21	1498
49	(E,E)-α-farnesene	0.08	1505
50	β-bisabolene	0.03	1505
51	Not identified	0.05	-
52	Not identified	0.05	-
53	Not identified	0.11	-
54	Not identified	0.14	-
55	selina-3,7(11)-dieno	0.18	1545
56	germacrene B	0.1	1559

No.	Compound	% RDA	Theorical ratio[a]
57	Cariophyllene oxide	0.9	1582
58	Humulene hepoxide II	0.18	1608
59	Not identified	0.62	-
60	Not identified	0.22	-
Total unidentified		2.71	
Total identified		97.29	

[a]Theoretical retention rates retrieved from Adams (2012) database.

The most important compounds are: E-caryophyllene 27.91 %, myrcene 21.19 %, α-pinene 8.05 %, α-humulene 8.03 %, limonene 7.18 %, terpinolene 7.12 % and β-pinene 4.68 %. The chemical composition of *B. latifolia* essential oil is shown in Table 4. The main compounds are: ligulooxide 14.02 %, andro encecalinol 9.84 %, kesane 7.53 %, limonene 5.6 % and Z-cadin-4-en-7-ol with 5.03 %.

Table 4. Chemical composition of the essential oil of *B. latifolia*.

No.	Name	% RDA	Theorical RI[a]	Exp. RI[b]
1	α-tujene	4.77	924	925
2	α-pinene	4.27	932	933
3	camphene	0.68	946	950
4	tuuja-2,4(10)-dieno	2.39	953	974
5	verbenone	2.49	961	980
6	β-pinene	1.1	974	992
7	3-carene	3.4	1008	1011
8	limonene	5.6	1024	1032
9	β-ocimene	0.85	1032	1048
10	terpinolen	0.66	1086	1088
11	gurjunene	0.68	1409	1406
12	caryophyllene	1.3	1417	1420
13	humulene	1.27	1454	1457
14	γ-curcumene	2.91	1475	1479
15	α-curcumene	1.97	1483	1484
16	himachalene	0.26	1481	1486
17	E-muurola-4(14),5-dieno	3.41	1493	1498
18	β-curcumene	2.19	1514	1512
19	α-7-epi-selinene	1.98	1520	1523
20	zoranene	1.01	1529	1529
21	kesane	7.53	1530	1535
22	γ-vetivevene	0.53	1531	1537
23	liguloxide	14.02	1534	1544
24	spatulenol	2.39	1578	1585
25	caryophyllene oxide	0.25	1582	1589
26	-10 epi-eudesmol	1.23	1622	1614
27	muurola-4,10(14)-dien-1-ol	0.99	1630	1633
28	epi-cadinol	1.06	1638	1638
29	Z-cadin-4-en-7-ol	5.03	1635	1647
30	α-cadinol	1.46	1652	1655
31	valerianol	0.78	1658	1668
32	andro encecalinol	9.84	1677	1679
33	α-bisabolol	1.71	1685	1698
34	ciperotundone	2.1	1695	1706

No.	Name	% RDA	Theorical RI[a]	Exp. RI[b]
35	Not identified	1.65		1751
36	Not identified	2.82		1764
37	Not identified	1.84		1769
38	Not identified	1.57		1792
Total identified		92.11		
Total unidentified		7.89		

[a]Theoretical retention rates retrieved from Adams (2012) database.

[b]xperimental retention rates compared to a homologous series of C8-C30 hydrocarbons.

3.2 *In vivo* anti-inflammatory evaluation

After homogenizing the treatments by applying the normality and homogeneity tests, the statistical analysis Anova test was performed relating the anti-inflammatory efficacy in volume for each of the treatments including the control formula (T) without active ingredients, positive control (PC) with diclofenac at 1% and the 5 formulations with different proportions of essential oils at 1% which are: A (100% E.O. cannabis), B (75% E.O. B. latifolia and 25% E.O. cannabis), C (50% E.O. B. latifolia and 50% E.O. cannabis), D (25% E.O. *B. latifolia* and 75% E.O. cannabis), E (100% E.O. *B. latifolia*). Measurements were taken 3 times as described in the trial, i.e. 1, 3 and 5 hours after product application. Figure 2 shows the results of the 3 trials.

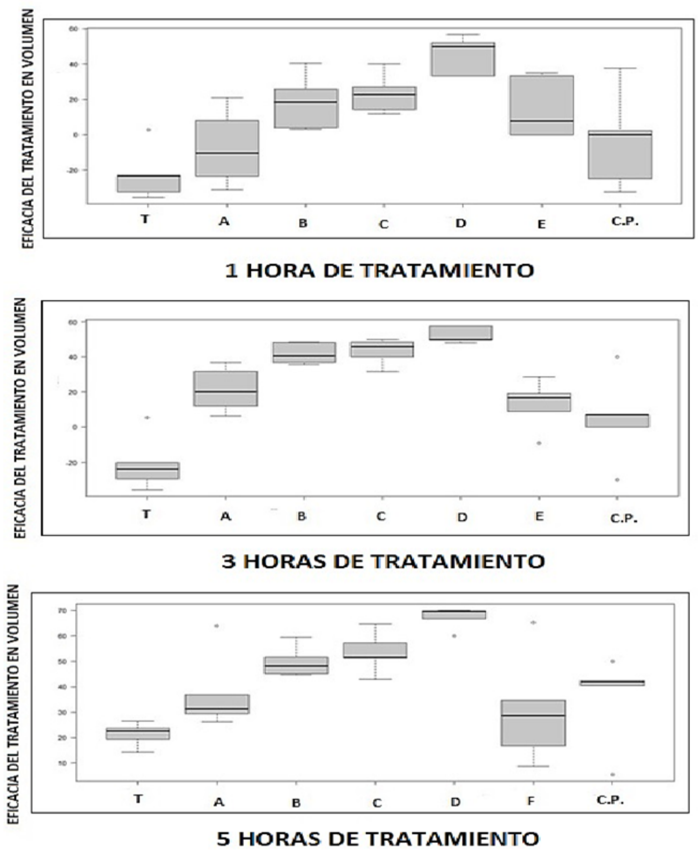

Figure 2. Treatments after 1 hour, 3 hours, 5 hours: T (control sample), A (100% E.O cannabis), B (75% E.O. *B. latifolia* and 25% E.O cannabis), C (50% E.O *B. latifolia* and 50% E.O cannabis), D (25% E.O *B. latifolia* and 75% E.O cannabis), E (100% E.O *B. latifolia*), P.C. (positive control).

Figure 3 shows a comparative analysis between the formulations with the essential oils and the positive control with diclofenac.

4 Conclusions

The chemical composition of cannabis essential oil provided in the certificate of the company Eden Garden Essentials contains as majority molecules caryophyllene, myrcene, humulene, limonene and pinenes, similar to what is stated in the literature (Malingre et al., 1975; Novak et al., 2001). For both caryophyllene(Dahham et al., 2015; Bakır et al., 2008) and myrcene (Surendran et al., 2021), there are studies confirming its anti-inflammatory activity in various assays.

Unlike the essential oil of cannabis, *B. latifolia* has few studies, which do not allow to have enough literature to compare. The study conducted by Valarezo et al. (2013), shows similarities and differences in the chemical composition of the oil, which could be due to the ecological variables of the places where the plant is harvested. Regarding the most abundant components in our research: liguloxide, andro encecalinol and kesane, there are no bioactivity studies, leaving open the possibility of isolating these molecules and verifying their medicinal properties, including anti-inflammatory properties.

Basically, all the formulations containing individual essential oils or in mixtures at 1% in the formulation show activity if considering their comparison with the positive control (commercial formulation with diclofenac at 1%). However, those formulas of essential oils whose positive activity from the point of view of statistical significance present a better anti-inflammatory bioactivity with respect to the positive control and pure essential oils, are noteworthy. The two most effective formulations are those with 25% *B. latifolia* oil (250 mg in 100 grams of ointment) and 75% cannabis oil (750 mg in 100 grams of ointment) and the one containing 50% B. latifolia oil (500 mg in 100 grams of ointment) and 50% cannabis oil (500 mg in 100 grams of ointment), practically in all tests (1, 3 and 5 hours). The statistics reveal their significance compared to the positive control, which would enable to propose them as possible formulas in the market of natural products.

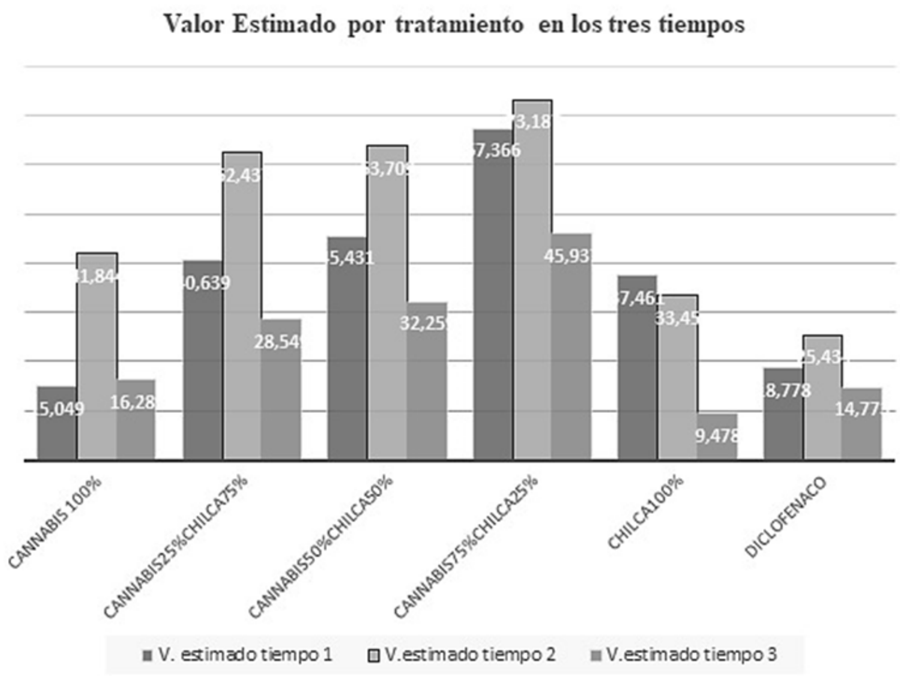

Figure 3. Comparative evaluation of the treatments and the positive control (diclofenac 1% formulation), in the 3 study times, time 1 (1 hour), time 2 (3 hours) and time 3 (5 hours).

The amounts of essential oils used are small, which would result in a commercially competitive product. Additionally, *B. latifolia* is abundant in the Andes of Ecuador and the country is beginning to produce medical cannabis, where essential oils could be one of the metabolites beyond cannabinols.

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BIOTECHNOLOGY

HOST STATUS OF THREE SOLANACEAE SPECIES FROM LASIOCARPA SECTION TO ROOT-KNOT NEMATODE *Meloidogyne Incognita*

CAPACIDAD HOSPEDANTE DE TRES ESPECIES DE SOLANÁCEAS DE LA SECCIÓN LASIOCARPA AL NEMATODO AGALLADOR DE LA RAÍZ *Meloidogyne Incognita*

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Abstract

Root knot nematode *M. incognita* is one of the most dangerous and common species affecting Solanaceae family, including the naranjilla crop (*S. quitoense*). The objective of this work was to evaluate three species of Solanaceas as hosts (*S. sessiliflorum*, *S. hirtum* and *S. quitoense*) for an isolated of *M. incognita* in greenhouse. Plants of three species were planted in pots and each plant was inoculated with 2500 eggs and second stage juveniles (J2). Host suitability was assessed 80 days after inoculation. Initial inoculum was obtained from infested roots of *S. quitoense* plants collected in commercial naranjilla orchards. A completely randomized experimental design was used. The variables evaluated at 80 days after inoculation were: gall index (GI), nematode reproduction factor (RF), dry weight of the foliar area, plant height and stem diameter. All species were galled, but *S. sessiliflorum* and *S. hirtum* showed the least number of root knots with values of 33.73 and 34.73. Both were classified as resistant / hypersensitive with reproduction factors of 0.94 and 0.85 (RF> 1) respectively, while *S. quitoense* was susceptible with a value of 1.56. In terms of foliage yield (dry weight), plant height and stem diameter, *S. sessiliflorum* and *S. hirtum* showed a tolerance response in relation to *S. quitoense*.

Keywords: Host range, hypersensitive, root-knot nematode, *Solanum hirtum*, *Solanum sessiliflorum*.

Resumen

El nematodo del nudo de la raíz *Meloidogyne incognita* es una de las especies más peligrosas y comunes que afectan a las solanáceas, entre ellas la naranjilla *Solanum quitoense*. El objetivo de este trabajo fue evaluar el potencial reproductivo de un aislamiento de *M. incognita* en tres especies de Solanaceas en invernadero: *Solanum sessiliflorum*, *Solanum hirtum* (reportada anteriormente como resistente) y *S. quitoense* (susceptible). Plantas de las tres especies fueron sembradas en maceta y a las cuatro semanas fueron inoculadas con 2500 huevos más juveniles en estado 2 (J2). El inóculo inicial se obtuvo de raíces infestadas de plantas de *S. quitoense* recolectadas en huertos comerciales de naranjilla. Se utilizó un diseño experimental completamente aleatorizado. Las variables evaluadas a los 80 días después de la inoculación fueron: índice de agallas (GI), factor de reproducción de nematodos (RF), peso seco del área foliar, altura de la planta y diámetro del tallo. Se encontró que las tres especies mostraron agallamiento, pero *S. sessiliflorum* y *S. hirtum* mostraron el menor número de nudos de raíz con valores de 33,73 y 34,73. Además, *S. sessiliflorum* y *S. hirtum* presentaron una categoría de resistente/hipersensitivo con factores de reproducción de 0,94 y 0,85 (RF $>$ 1) respectivamente, mientras que *S. quitoense* fue susceptible con un valor de 1,56. En términos de rendimiento de follaje (peso seco), altura de la planta y diámetro del tallo se observó una respuesta de tolerancia en *S. sessiliflorum* y *S. hirtum* en relación a *S. quitoense.*

Palabras clave: Capacidad hospedante, hipersensibilidad, nematodo agallador, *Solanum hirtum*, *Solanum sessiliflorum*.

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1 Introduction

Naranjilla (*S. quitoense*), known as "lulo" in Colombia, is an important fruit crop in Ecuador. It is a perennial herb plant belonging to the Solanaceae family. It is considered the "golden fruit" of the Andes by its color, whose flavor is acid and exotic (Ramírez et al., 2018). According to Whalen and Caruso (1983) solanaceae belonging to the Lasiocarpa family is mainly found in northeast South America, and it is said that naranjilla tres are native from Colombia and Ecuador (Morton, 1987; Council, 1989). The main cultivated variety is *S. quitoense* Var. *Quitoense* without thorns. Naranjilla is a commonly underused crop for manufacturing juices, ice cream, jellies and other sweets. However, this crop has economic potential (Heiser, 1993).

One of the greatest challenges for producers is the susceptibility of naranjilla to the root knot nematode *M. incognita* and the fungus *Fusarium oxysporum* (Mosquera-Espinosa, 2016). Nematodes are biotrophic parasites capable of infecting more than 2000 plant species. These parasites infect plant roots and induce the formation of giant feeding cells that reduce plant nutrition and water absorption. As a result, plants may show several symptoms such as wilting, stunting, and significantly reduced yields (Barbary et al., 2015; Ralmi et al., 2016; El Sappah et al., 2019).

To fight againts *M. incognita*, farmers in Ecuador crop naranjilla in recently dismantled areas, free of nematodes or use nematicides directly to the soil (Ramírez et al., 2018). However, these products can generate resistance, affect human health and the environment. In addition, many countries, especially within the European Union, have now banned the use of chemicals such as carbofuran for controlling nematodes (Caromel et al., 2005; Barbary et al., 2015).

The use of resistant cultivars may be the cheapest and best environmental method for controlling root knot nematodes (Barbary et al., 2016). In the case of naranjilla, hybridization or the use of grafts of other nematode-resistant, compatible solanaceous species could be the key to improving plant performance (Anderson et al., 2005; Heiser, 1985). There are several wild species related to naranjilla such as: *Solanum tequilense*, *Solanum vestissirmum*, *Solanum lasiocarpum*, *Solanum arboretum*, *S. sessiliflorum* and *S. hirtum* (Ramírez et al., 2018). Among these, *S. hirtum* and *S. arboretum* have been used as patterns for the Baeza lulo variety (*S. quitoense* var. *quitoense*) developed by the National Autonomous Institute of Agricultural Research (INIAP) (Sowell and Shively, 2012; Clements et al., 2017).

In some cases, hybridization between *S. quitoense* and wild relatives has resulted in viable plants such as *S. quitoense* × *S. hirtum* (Heiser, 1972). These hybrids have resulted in segregation plants with some resistance to root knot nematodes (Heiser, 1993; Ramírez et al., 2018). However, fruits of these hybrids have a bad taste (Heiser, 1972; Ramírez et al., 2018) and roots are infected by the nematode present in the development of nodules caused by the penetration of the nematode, indicating absence of immunity (Navarrete et al., 2018). Additionally, the fact of classifying *S. hirtum* as resistant to its host capacity includes the reproductive factor and excludes the galled index, as proposed by Canto-Saenz modified scheme (De Almeida et al., 1997; Sasser et al., 1984).

Regarding *S. sessiliflorum* in Ecuador, Puyo and INIAP Palora hybrids have been cropped. The commercial hybrid of Puyo results from a cross between "coccona" (*S. sessiliflorum*) × naranjilla var., Agria (*S. quitoense* Var. *quitoense*). The hybrid INIAP Palora results from the crossing between naranjilla var. Baeza (*S. quitoense* Var. *quitoense*), used as male progenitor and "coccona" (*S. sessiliflorum*) as female progenitor (Revelo et al., 2010). However, there are no studies on host capacity or reproductive potential of *S. sessiliflorum* species for the galled nematode. Thus, the aim of this study was to determine the host capacity of three species of Solanaceae belonging to the Lasiocarpa section for an isolate of *M. incognita*.

2 Materials and methods

The host capacity of the species *S. sessiliflorum*, *S. hirtum* for *S. quitoense* para *M. incognita* was evaluated in this research. This study was carried out in a greenhouse of the Faculty of Agricultural Sciences of University of Cuenca, province of Azuay, at an altitude of 2567 meters above sea level, at the coordinates UTM 2°55′12.564″, 79°1′30.6122″. The average greenhouse temperature during the experiment varied between 12 to 32°C and the relative humidity between 60 and 80%.

The propagation of plants of the three species was made from seeds collected in the province of Morona Santiago (eastern Ecuador). Seeds were planted in germination trays with sterile substrate (peat). After two months, seedlings of the three species were planted in 2 kg pots with sterile substrate composed of loamy soil (Vertisol order), black hill soil and bocashi (4:4:2).

The inoculum of *M. incognita* was obtained from naranjilla plants collected in the province of Morona Santiago. The specific MI-F initiators or primers GTGAGGATTCAGCTCCCG and MI-R ACGAGATACTTCTCCGTCC (Martínez-Gallardo et al., 2019) were used to identify the nematode species. Amplification reactions were performed on an Eppendorf Mastercycler NexusGSX1 thermal cycler, at a final volume of 25 μl which contained 2.5 μl buffer (10X) (Invitrogen), 0.5 μl of each dNTP (10 mM), 1.5 μl of $MgCl_2$ (50 mM), 2.5 μl of each primer (100 μm), 0.2 μl of Taq polymerase (Invitrogen, 5 or μl^{-1}) and 1 μl of DNA (10 ng/μl). For PCR, an initial denaturation was performed at 94°C for 2 min, followed by 35 cycles of 94°C for 30s, 64°C for 30s and 68°C for 1 min, followed by a final extension at 72°C for 5 min. Samples were analyzed in 1% of agarose gel with ethidium bromide (0.25 μg ml^{-1}) and visualized in a BioRad UV transilluminator. A molecular marker of 1 kpb (Invitrogen) was used as a molecular standard.

The population of *M. incognita* was multiplied in tomato plants var. Sheila in greenhouse conditions in pots of 2 kg with sterile substrate and was kept in the greenhouse of the Department of Phytopathology of the University of Cuenca. Tomato seedlings were inoculated with 20 mL of the extract obtained from naranjilla infected roots. Inoculation was made in four 3 cm deep holes in the tomato seedling. After 60 days, infected roots were processed according to Hussey and Baker's extraction methodology (Hussey, 1973) using 0.5% sodium hypochlorite (NaClO). For the extraction of eggs, an optical stereoscope was used to identify *M. incognita* eggs and larvae embedded in the oval masses. The masses were separated from the plant tissue with sieves of 150 and 25 μm pores and placed in Petri dishes that remained for 72 hours at a temperature of 28°C, to allow the hatching of nematode eggs and emergence of juvenile nematodes in the J2 state.

This inoculum was used to infest 4-week seedlings of the species *S. sessiliflorum*, *S. hirtum* and *S. quitoense*. Each plant was inoculated with 2500 younger eggs in state 2 (J2). Inoculation was done by placing 2 mL of the inoculum suspension in four 3-cm deep holes around the base of each plant. Then holes were filled with soil. Sterile water was placed in the control plants instead of the inoculum.

After 80 days of inoculation, the roots of the three species were washed individually with running water. Excess water was removed with paper towels and stained with Floxine B to facilitate the counting of egg masses (Taylor and Sasser, 1978). The root gall index (GI) was obtained using a rating scale of 0-5 (0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = > 100 galls) (Taylor and Sasser, 1978). Eggs were extracted from each root with 0.5% of sodium hypochlorite (NaClO) (Hussey, 1973) and were counted to determine the final population density (Pf) (Oostenbrink, 1966). The density of the final population of nematodes (Pf) was estimated as the total number of J2 and eggs extracted from the roots of each plant and the reproduction factor RF= Pf/π was calculated (Bybd et al., 1983). The state of the host was determined by the gall index (GI) and the reproductive factor according to the modified Canto-Saenz scheme (De Almeida et al., 1997; Sasser et al., 1984). Plants with $GI > 2$ are defined as susceptible ($RF > 1$) or resistant/hypersensitive ($RF \geq 1$); plants with $GI \leq 2$ are defined as resistant ($RF \geq 1$) or tolerant ($RF > 1$) (Sasser et al., 1984; De Almeida and De A Santos, 2002; Maleita et al., 2011).

These variables were also registered: number of knots per root, dry weight of the leaf area (g), plant height (cm) and stem diameter (cm). For the variable dry weight of the foliar area for each seedling, it was placed on paper covers and dried in a Nabertherm furnace at 60°C for 72 hours. It was then weighed with a Boeco BWL 61 digital scale.

A completely randomized experimental design was used. The normal distribution of observations for each species was determined by the modified Shapiro Wilk test with P significance levels $< 0{,}05$

and the homogeneity of variances by the Levene test. The data were statistically analyzed by analysis of variance with means separated by Duncan's multiple-range test at 5 % significance level with the Infostat statistical package (Di Rienzo et al., 2008).

3 Results

The results of the experiment showed that the species *S. sessiliflorum* and *S. hirtum* varied in their host capacity in relation to *S. quitoense* for *M. incognita*. The *M. incognita* species used as an inoculum was verified by PCR, with specific initiators for the species. A visible band of approximately 1000 bp was visualized, which was expected for the 28S rNRA region of the nematode in the three species (Hu et al., 2011; Martínez-Gallardo et al., 2019).

The three infected species allowed the development of the nematode. However, a different degree of gall and reproduction was presented for the three species analyzed. For the reproduction factor (RF), significant differences between *S. sessiliflorum* and *S. hirtum* were found in relation to the control species *S. quitoense* (Table 1). A difference in reproduction was observed for *S. quitoense* with 1.56 ($RF > 1$), while RF values varied between 0.85 for *S. hirtum* and 0.94 for *S. sessiliflorum*. Even though *S. sessiliflorum* favored the multiplication of nematodes, it was not statistically different from the species reported as resistant *S. hirtum* (Navarrete et al., 2018).

Table 1. Host status of three Solanaceae species for *M. incognita* evaluated 80 days after inoculation with 2500 eggs + juvenile (J2) per plant.

Species	GI[1]	Pf[2]	Average number of galls	RF[3]	Hosting capacity[4]
S. sessiliflorum	3.4	1888.5 ± 259.1 a	33.73 ± 13.89 a	0.94	R^H
S. hirtum	3.4	1702.3 ± 252.9 a	34.73 ± 18.09 a	0.85	R^H
S. quitoense	4.9	3125.9 ± 259.1 b	167.87 ± 34.68 b	1.56	S

[1] GI = Gall index (0-5): 0= without galls or egg masses; 1= 1 to 2 galls or egg masses; 2= 3 to 10 galls or egg masses; 3= 11 to 30 galls or egg masses; 4= 31 to 100 galls or egg masses; 5= >100 galls or egg masses per root.
[2]Pf= final population density (J2+eggs). Pf data are averages of three replicates ± standard deviation (mean values followed by the same letter do not differ according to Duncan's 5 % probability test).
[3]RF Reproduction Factor.
[4]Host Status Categories R^H = Resistant/Hypersensitive (RF≤1 and GI>2) and S=susceptibleSensitive (RF>1 and GI>2).

In terms of the gall index (GI) for the three species, a lower level of gall was also observed for *S. sessiliflorum* and *S. hirtum* compared to *S. quitoense*. The gall index values had a value of 3.4 for the species *S. sessiliflorum* (galls=33.73) and *S. hirtum* (galls=34.73), with no statistical difference; whereas *S. quitoense* used as control had a value of 4.9 (galls= 167.87), confirming the viability of the inoculum. *S. sessiliflorum* and *S. hirtum* were categorized as resistant/hypersensitive according to the classification proposed by Canto-Saenz (De Almeida et al., 1997; Sasser et al., 1984). For the dry weight variables of the leaf area, plant height and stem diameter (Table 2), tolerance to the nematodes was observed for *S. sessiliflorum* and *S. hirtum*, with respect to *S. quitoense*, in which $p - values$ showed significant differences at the level of 1 % between inoculated plants and non-inoculated plants. Thus, for the variable dry weight of the leaf area, a value of 8.44 vr 10.57 was obtained. For plant height 16.33 vr 18.20 and stem diameter 0.91 vr 1.08, respectively.

Table 2. Response for the variables dry weight of leaf area, stem diameter and plant height in three Solanaceae species inoculated with *M. incognita*.

Species	Inoculated Plants	Non inoculated Plants	Response
Dry weight of the leaf area (g)			
S. sessiliflorum	6.12 ± 0.58 a	5.73 ± 0.12 a	Tolerant
S. hirtum	8.18 ± 0.52 a	8.30 ± 0.56 a	Tolerant
S. quitoense	8.44 ± 0.46 a	10.53 ± 0.25 b	Non tolerant
Height of the plant (cm)			
S. sessiliflorum	8.15 ± 0.74 a	9.33 ± 0.58 a	Tolerant
S. hirtum	15.92 ± 1.13 a	15.33 ± 1.04 a	Tolerant.
S. quitoense	16.33 ± 0.94 a	18.20 ± 0.56 b	Non tolerant
Stem diameter (cm)			
S. sessiliflorum	0.89 ± 0.035 a	0.91 ± 0.03 a	Tolerant
S. hirtum	0.87 ± 0.052 a	0.81 ± 0.051 a	Tolerant
S. quitoense	0.91 ± 0.051 a	1.08 ± 0.045 b	Non tolerant

Means with different letters between inoculated plants and non-inoculated plants indicate highly significant differences ($P<0,001$).
The values in the table correspond to the averages and their standard deviation.

4 Discussion

In Ecuador, naranjilla or lulo has a high incidence of the galled nematode. *M. incognita* is a pathogen of many crops, including Solanaceae in 5 continents. The nematode causes damage to the plant at the root level, reducing yield and even leading to plant death over time (Ramírez et al., 2018). Within the Lasiocarpa section, different species related to naranjilla have been reported that could present resistance to the galled root nematode (Heiser, 1972, 1993; Ramírez et al., 2018).

The host capacity of three species is reported to *M. incognita*: *S. sessiliflorum*, *S. hirtum* and *S. quitoense*. The nematode species was verified by PCR amplification with specific initiators. The BLAST analysis (Basic Local Alignment Search Tool) of the MF and MR initiator sequences showed that they are specific to *M. incognita*, with 100 % coincidences to the sequences available in the National Center for Biotechnology Information (NCBI). These results indicate that the initiators are sensitive to the studied plants (Hu et al., 2011; Martínez-Gallardo et al., 2019); and that PCR conditions are ideal for the rapid and reliable detection of the pathogen in galls for different solanaceae species, as shown by Navarrete et al. (2018).

Resistance to the galled nematode is defined as the capacity of a plant to suppress the development or reproduction (Sasser et al., 1984; Roberts, 2002; Dong et al., 2007) or the ability of a host to suppress the disease (Sasser et al., 1984; Roberts, 2002). In general, nematologists and geneticists have evaluated resistance to the galled nematode in different cultures based on the root gall index and/or mass production of eggs (Holbrook et al., 2000; Timper et al., 2000) or egg counts (Choi et al., 1999). Other authors have also used the gall count to evaluate resistance to the gall nematode in plants (Harris et al., 2003; Navarrete et al., 2018; Correia et al., 2019). The number of galls and the galling degree can be used to reflect a plant's ability to decrease or overcome the attack of the galled nematode (Dong et al., 2007).

Regarding naranjilla, there are not many studies on resistance to *M. incognita*. Navarrete et al. (2018) assessed resistance in different solanaceae but based on reproductive factor. This investigation points *S. quitoense* as sensitive and *S. hirtum* as resistant to *M. incognita* under greenhouse conditions. Thus, *S. hirtum* did not present immunity to the galled nematode by allowing the penetration of the nematodes and the presence of nodules or galls in the root. However, it does have an impact on the reproductive capacity of the pathogen. In this study, *S. hirtum* and *S. quitoense* were evaluated, but *S. sessiliflorum* was not included to assess the resistance level of the galled nematode.

In the present study, the results corroborate that *S. quitoense* was considered sensitive (RF close to 5.0) and a good host ($RF = 1.1$-5.0) according to De Almeida and De A Santos (2002) and Ibrahim et al. (1993), respectively, as previously stated by Navarrete et al. (2018). For the reproduction factor, *S. quitoense* exceeded the initial population (3125 eggs plus J2). In contrast, according to the results of the present study, *S. hirtum* and *S. sessiliflorum* were considered resistant/hypersensitive (if RF and GI are considered according to Canto Saenz´s classification) or poor hosts ($RF < 1$) (Maleita et al., 2011).

The estimated RF for the final population variable of nematodes indicates that there was a hypersensitive resistance response in *S. sessiliflorum* and *S. hirtum*, because the average number of eggs plus J2 (1888 and 1702, respectively) was lower than the initial population of the nematode (2500 eggs plus J2) and the reproduction factor was less than 1. Both *S. hirtum* and *S. sessiliflorum* showed no significant statistical differences in the resistance reaction. It has been stated that *S. hirtum* could be used as a pattern of *S. quitoense*, sensitive to nematodes (Navarrete et al., 2018). The results of this study suggest that the species *S. sessiliflorum*, having the same level of hypersensitive resistance as *S. hirtum*, could also be considered to be used as a pattern or in a selection program for naranjilla versus nematode.

GI of *M. incognita* attributed to the species under evaluation showed a relationship with the RF of the nematode. In the experiment for *S. sessiliflorum* and *S. hirtum*, GI presented intermediate values. Low or intermediate GI values demonstrate the difficulty of nematodes in establishing parasitism in the roots of these species or cultivars, as obtained from lettuce cultivars (Correia et al., 2019).

In terms of the variables dry weight of the leaf area, height of the plant and stem diameter, significant differences were found between *S. sessiliflorum* and *S. hirtum* in relation to *S. quitoense*. Navarrete et al. (2018) found no significant difference for the dry weight variable of the foliar area of *S. hirtum* between inoculated and non-inoculated plants, classifying it as tolerant according to Cook (1974), while *S. quitoense* was classified as non-tolerant because there were differences between inoculated plants and non-inoculated plants. In this paper, differences in plant height and stem diameter have also been found between the species *S. sessiliflorum* and *S. hirtum* in relation to *S. quitoense*, variables that are possibly related to tolerance response. However, additional research will be needed to observe these results in terms of field performance, as proposed by Navarrete et al. (2018).

From the point of view of improvement or use as patterns, it would be interesting to have a resistance response similar to immunity, which is characterized by the total absence of galls in the roots and absence of giant cells in the vascular cylinder. These are induced by the attack of nematodes that restrict water flow and adequate nutrient absorption in sensitive plants (Correia et al., 2019). In the case of naranjilla, no species has been found to prevent the penetration of the nematode or the development of galls in the roots of plants, even in wild species with no commercial value (Heiser, 1972, 1993; Navarrete et al., 2018; Ramírez et al., 2018). However, despite showing galling between *S. sessiliflorum* and *S. hirtum*, they could be used as patterns for naranjilla growing. The variability observed in PF and RF values for these species may reflect an influence of plant genetic background that could be used in *S. quitoense* breeding programs against root knot nematodes.

5 Conclusion

Knowing the host capacity of *S. sessiliflorum* and *S. hirtum* based on RF and GI is useful for continuing a genetic improvement plan in naranjilla and observing the behavior of species used as patterns in areas heavily infested by galled nematode. *S. sessiliflorum* and *S. hirtum* had different host capacity compared to *S. quitoense* for the *M. incognita* nematode and both species could be used as an alternative in an integrated pest management program, since repeated exposure of *S. quitoense* in the field could lead to a selection of virulent nematode isolates.

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Sustainable Development

Water Co-management between public and community actors as a tool for adaptation to Global Climate Change: the case of Santa Clara de San Millán Commune, DM Quito

Cogestión del agua entre actores públicos y comunitarios como herramienta de adaptación al Cambio Climático Global: el caso de la Comuna Santa Clara de San Millán, DM Quito

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Abstract

Water management in rural, urban, and peri-urban communities is a priority topic for human development. In this article, ideas related to the adequate co-management of this resource (between community and public actors) are specified as a tool for adaptation to contemporary scenarios of Global Climate Change. The study area corresponds to a peri-urban zone of Quito. The information was ordered and processed using ATLAS.ti software and a geographic information system; current regulations were ranked through a Kelsen Pyramid. The results generated are divided into three sections that conceptualize the bases for adequate water management, considering management models with a territorial approach and adaptation measures to Climate Change made by co-management actors. This action model generates a consistent tool for managing water resources, as well as other natural resources.

Keywords: Water Co-management, Quito, Santa Clara de San Millán Commune, Climate Change adaptation, legal regulations, territorial management.

Resumen

La gestión del agua en comunidades rurales, urbanas y periurbanas es un tema prioritario para el desarrollo del ser humano; en el presente artículo se puntualizan ideas relacionadas con la cogestión adecuada de este recurso (entre actores comunitarios y públicos) como una herramienta de adaptación a escenarios contemporáneos de Cambio Climático Global. El área de estudio corresponde a una zona periurbana de Quito. Se ordenó y procesó la información con el software ATLAS.ti y un sistema de información geográfica; la normativa vigente fue jerarquizada a través de una Pirámide de Kelsen. Los resultados generados se dividen en tres apartados que conceptualizan las bases para un adecuado manejo del agua, considerando modelos de gestión con enfoque territorial y medidas de adaptación al Cambio Climático ejecutadas a través de la cogestión entre actores. Este modelo de acción constituye una herramienta consistente para el manejo del recurso agua, así como de otros recursos naturales.

Palabras clave: Cogestión del agua, Quito, Comuna Santa Clara de San Millán, adaptación al Cambio Climático, normativa legal, gestión territorial.

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1 Introduction

Since ancient times, water has been considered a source of life and a sacred symbol for the communities, nationalities, and indigenous peoples of the Andes; however, historically they have been deprived of several rights, including the right to access this resource in conditions of adequate quality and quantity, required for proper human health (Boelens, 2011). If revising history, water has always been an element that has generated confrontations, not only because of the quality of the resource, but also because of the initiatives to establish norms for its use; in Latin America, since colonial times, the most influential groups have been favored. In the republican period, very few or no changes were made; water continued to be monopolized by power groups and influential socio-political people (Granda et al., 2004); even today, water sources continue to be highly coveted by the large companies that provide service in the cities (Gómez, 2009).

In response to these realities, initiatives for co-management of this resource have originated in the Andean zone of Ecuador; one of them the Pesillo Regional Drinking Water Project in Imbabura, in cooperation with the community, state and international actors involved, in which the elements for the co-management of water are: access, availability, project design, financing, implementation and administration (Perugachi and Cachipuendo, 2020); these being the main elements that enable adequate management of the resource.

The main weaknesses of community water systems, generally managed by water and sanitation boards, revolve around the lack of economic resources and technical designs to make their catchment, transportation, purification, and service distribution systems more efficient (Molina et al., 2018).

For Pinos (2020), good water management is part of a healthy urban and rural metabolism, and usually depends on the governance of people involved. Sandoval and Günther (2013) mention that the integral management of water is usually composed of public, social and private actors; while the competence of water regulation is in charge of the environmental control entity, in this country: the Ministry of Environment, Water and Ecological Transition of Ecuador (MAAE).

There are few ancestral communities in the Andean zone of Ecuador that have managed water sources by their own, promoting their management and conservation; one of them, adjacent to the city of Quito, is Santa Clara de San Millán commune (hereinafter referred to simply as Commune or CSCSM) (Pacheco, 2019). It was originally separated from the city and with the passage of time and the consequent population growth of the city, it has been gradually integrated as a peri-urban element.

Changes in land use have caused this Commune to give part of its territory and resources, transforming them into spaces with urban characteristics (Jácome, 2018; Pacheco, 2019). It is worth mentioning that the struggle for water has not been an alien process for the people of this commune; for example, history shows the intervention of organizations outside it, which triggered conflicts over the management of the resource: the first, with Mariana de Jesús citadel in 1936 and the second, with Central University of Ecuador in 1955 (Jácome, 2018).

The Commune currently has an internal water management system for human consumption, which is managed by a group known as the "Water Board of the Santa Clara de San Millán Commune" (Pacheco, 2019). This system and the operation of the Board is based on the Regulations to the Organic Law on Water Resources, Uses and Development of Water (LORHUyA) (Presidencia de la República del Ecuador, 2014), which establishes that the Drinking Water and Sanitation Management Boards (JAAPS) are non-profit community groups whose purpose is to provide public drinking water service in rural or peri-urban areas, where the municipal Autonomous Decentralized Governments (GAD) have not been able to achieve it through their public companies. It should be mentioned that these organizations have administrative, financial, and managerial autonomy to fulfill their functions, which usually include an effective and efficient service for their beneficiaries (Martínez and Abril, 2020).

Likewise, it is worth mentioning that water management is also related to changes in land use that modify the local environment and contribute to increase variability related to Climate Change (Yánez et al., 2011, 2012), one of its negative effects is seen in groundwater that does not reach the usual recharge time; therefore, groundwater and nearby surface flows decrease, significantly affecting the availability of the resource (UNESCO, 2015).

In this sense, institutions such as the Intergovernmental Panel on Climate Change (IPCC) promote guidelines to face this change, considering different environmental factors. According to the IPCC (2019), it is essential to maintain a maximum temperature increase below 2 $°C$ with respect to pre-industrial levels until 2050. With the current level of greenhouse gas emissions, it is believed that an increase of 1.5 $°C$ will be reached in the period 2030-2052; not exceeding this limit must be one of society's short-term objectives. Failure to achieve this goal would generate a scenario that could endanger life as we know it on the planet.

Because of this, it is important to create strategies at the local level that enable adaptation and at the same time contribute to mitigating the effects of climate change, through responsible water use, in which the environmental, economic, and social components are combined (Gallo and Jiménez, 2019; MAE, 2017). Water co-management involves the participation of all stakeholders and requires a multi-criteria analysis to maintain a sustainable and efficient system (Rivera, 2016); in addition, it should be considered that water is a main element for food sovereignty and economic development.

The aim of this research is to propose a management alternative in which ideas are harmonized around the appropriate co-management of water (between community and public actors) as a tool to adapt to contemporary scenarios of Global Climate Change.

2 Materials and Methods

2.1 Area of study

Gómez (2009) mentions that Santa Clara de San Millán Commune can be considered an ancestral commune, with direct descendants of the "Quitu-Cara" indigenous ethnic group. However, it was barely recognized as a social organization with territorial autonomy in 1911; currently, the Municipality of the Metropolitan District of Quito (Consejo Metropolitano de Quito, 2014) establishes that it is recognized as a Commune with its respective legal and government autonomy, known as Cabildo.

Geographically, the Commune is located to the west of Quito, Ecuador (Figure 1), on one of the slopes of the Pichincha volcano, in an altitudinal range between 2800 to 3250 masl, with an average temperature of 12 $°C$ and an average annual precipitation of 1488.2 mm (INAMHI, 2017).

The ecosystems in the surroundings of the Commune include a eucalyptus forest (*Eucalyptus globulus* Labill.) and shrubby paramo in higher areas (with vegetation corresponding to the genera: *Baccharis, Monnina, Chuquiraga, Puya, Cortaderia, Oreopanax, Gynoxis, Diplostephium, Monticalia*, among others) and paramo brush (mainly *Calamagrostis, Festuca, Castilleja, Azorella, Hypochaeris, Valeriana, Gentiana, Stellaria, Bartsia*), in which two water sources are located, which are managed and used for human consumption by the Commune Board (Pacheco, 2019).

By 2010, this commune had a population of 10287 inhabitants (INEC, 2010); its main economic activity constitutes commerce, especially in the lower zone; the economic-productive dynamics changes in the upper zone, observing the development of agricultural activities mainly destined for self-consumption (Pacheco, 2019).

2.2 Methodology

The field information and mainly the bibliographic information was organized in ATLAS.ti version 7 (https://atlasti.com/). This program made it possible to generate semantic networks for each subtopic of interest. In the case of maps and other geographic information, ArcGis ArcMap version 10.4.1 was used (https://desktop.arcgis.com/es/arcmap/). For the characterization of the Commune and its JAAPS, the information proposed by Pacheco (2019) was used, who mentions a technical-participatory methodological model, in which most of the information is collected by applying a Community Participatory Diagnosis, using techniques such as: talking maps, surveys and interviews, construction of historical timelines, SWOT matrix.

Figure 1. Aerial photography and referential maps of the location of Santa Clara de San Millán Commune.

Regarding the construction of the legal content, it was generated from the analysis of the legal regulations in force in Ecuador regarding water management, including the competencies of each key actor; the order determined by the Kelsen Pyramid (Muñóz, 2017) was applied as a legal hierarchy instrument. The issue of adaptation to Climate Change was addressed under the ecosystemic approach proposed by Yánez et al. (2012) for the Metropolitan District of Quito.

To generate the water co-management proposal, the recommendations of the Fund for Water Protection (FONAG, 2020) were followed, which include, among other actions: describing the current state of the resource and establishing short-term goals based on lines of action such as the generation of hydroclimatic and water quality data, characterization of participation, strengthening governance, restoration of degraded areas, management of conservation areas, training activities and environmental education.

The proposals of Perugachi and Cachipuendo (2020) were also considered, who mention that to manage water it is important to establish a commonwealth, i.e., cooperation between public administration and communities, and not only attend to a technocratic and centralist view. Therefore, community participation in the co-management of water resources, the creation and/or strengthening of grassroots social organizations, the analysis of organizational capacity, the description of endogenous and exogenous factors that could weaken community management, the combination of technical and community knowledge, as well as the increase of governance and governability were considered.

3 Results and Discussion

3.1 Legal basis as an instrument for proper water management in Ecuador

The management and handling of water resources has always been a controversial issue; in Ecuador, the first approach that proposed access to drinking water as a right for people's health occurred in the Political Constitution of Ecuador in 1998 (Martínez and Abril, 2020). The Constituent Assembly of Montecristi (2007-2008) began with a discourse of rights, not only of people but also of the environment, establishing the protection and treatment of water resources as a significant element; this environmental approach was confronted with the classic discourse of exploitation (oriented primarily to the present needs) without guidelines for real sustainability. Despite this, Ecuadorians approved the 2008 Constitution of the Republic of Ecuador, establishing the bases to prevent the privatization of water and to promote its adequate management with a multidisciplinary approach of various actors (Acosta and Martínez, 2010).

In the first place, the Constitution establishes that access to water is a fundamental human right, especially to drinking water; it prohibits water hoarding and privatization; it refers that the State is responsible for providing drinking water services in the country and mentions that the authority in charge of water management will plan, regulate, and control its use (Asamblea Constituyente, 2008).

The LORHUyA, which is the main regulatory body regarding water resources, establishes two unique forms of administration, public and community, and establishes the different levels of management (State, single water authority, decentralized autonomous governments "GAD's", public companies and JAAPS), related among themselves (Figure 2). It regulates and controls the management, authorization of use, conservation, restoration, and exploitation, and ensures the collective rights of the Communes, Communities, Peoples and Nationalities in the active participation with respect to water (Presidencia de la República del Ecuador, 2014).

Additionally, the organic codes, in this case the Organic Code of Territorial Organization, Autonomy and Decentralization (COOTAD, 2010, updated to 2019) and the Organic Environmental Code (COA, 2017). The COOTAD generates the relevant competencies at each of the government levels from the general to the specific, including the provincial, cantonal and parish; the water resource is mentioned in this Code, which establishes that the GADs are responsible for the proper integrated management of watersheds and the provision of drinking water as an essential service for the life of the inhabitants of urban, peri-urban and rural areas, including the active and coordinated participation of community organizations, represented by the JAAPS. The COA, for its part, emphasizes the processes of evaluation, control and monitoring of the quality and quantity of water in the water bodies, in addition to establishing the environmental obligations and responsibilities of the GADs with respect to the treatment and recovery of the resource.

Finally, Ministerial Agreements 0031 and 0194 (Secretaría Nacional del Agua, 2017, 2018), establish the independence of community organizations such as the JAAPS in the management of water, through the image of legal personality to which they can access through a process with the single water authority. Once generated, the social organizations are free to have an internal regulation that does not need to be approved by the governing body, likewise, their authorities will be chosen internally without the influence of the MAAE and it is complemented with an instruction for the strengthening of the processes of the community organizations to the water authority. Figure 2 shows in a summarized and hierarchical way the main actors related to water management in Ecuador.

3.2 Water management in different spatial approaches: an approach to urban, rural, and peri-urban areas

Water management for domestic use has different models, depending on the spatial and territorial reality. In urban and peri-urban areas, the participation of public companies or their concessions is much more common; in rural areas, it is generally the JAAPS who intervenes, normally regulated by community action/opinion and other actors such as local action Non-Governmental Organizations; however, they are rarely advised by a public company. Urban areas, due to the constant increase of their population, need a large amount of vital liquid to satisfy their basic needs, thus requiring greater economic and technical efforts in the management and distribution of water. To address each of these realities, different cases are presented to demonstrate their structure and operation.

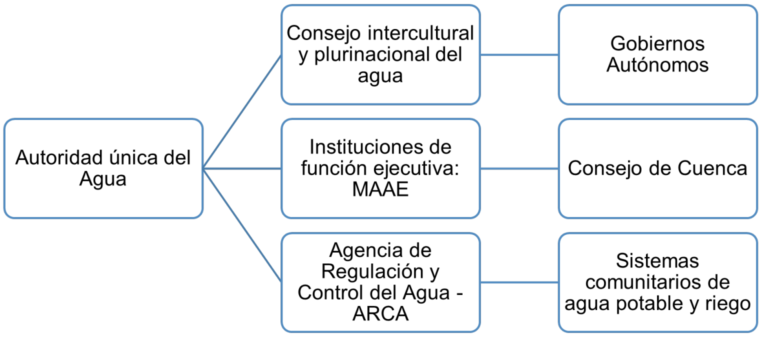

Figure 2. Hierarchy of actors engaged in water management
Source: LORHUyA

To refer to **urban areas**, it is necessary to establish the meaning of urban: according to the RAE (2021) it is defined as "belonging or related to the city". Capel (1975) in a classic idea mentions that the following elements should be included to characterize this concept: population size and density, the appearance of the urban core, including the dynamics of buildings and infrastructure, economic activity, and way of life, preferably based on a non-agricultural economy; likewise, the human population should be mentioned as an important factor, including its heterogeneity, the "urban culture" and the degree of social interaction.

The general rule in terms of water collection in the Andean zone of Ecuador is to do it in watersheds located at a considerable altitude, where water quality facilitates its treatment, since pollution sources are less common in these areas with less anthropic influence; however, it is worth mentioning that agricultural activities, especially burning and grazing, are gaining more space in Ecuador's paramo (Chuncho and Chuncho, 2019). In short, the activities that prevail in the drinking water service in public enterprises are: collection, conduction, storage of raw water, treatment, and conduction of treated water to the distribution center. Drainage and wastewater treatment are part of this management process, forming a cycle. According to Peña et al. (2016) the urban water cycle involves all these actions, including those aimed at preserving the resource.

Cities like Quito, such as Bogotá, Colombia's most populated center, are also supplied by main systems that get water from micro-watersheds. There, *Empresa de Acueducto y Alcantarillado de Bogotá* "EAB" is in charge of providing the service to the community (Peña et al., 2016).

Regarding **rural areas**, the RAE (2021) defines them as "pertaining or related to the life of the countryside and its labors"; among other authors, Larrubia-Vargas (1998) mentions that times the word "rural" in ancient was completely associated with the agrarian. Nowadays, it would be difficult to mention that only activities associated with agriculture are the only ones that take place in rural areas. Something striking in the rural area is that the natural component predominates over the artificial structural, likewise the population density has relatively low values and the economic flow is considered to be small, which can cause a greater number of social inequalities and a lifestyle that can be considered simple.

Water management in rural areas of Ecuador is mainly carried out through the model of community organizations, whose work is generally not sufficiently visible; local actors share interests and a vision more rooted in nature. Water Boards or JAAPS are created to manage the resource; this type of organization has less complex water systems than urban ones that consist of water collection, raw water conduction, raw water treatment, mainly by chlorination, and distribution to home networks (Pacheco, 2019). The predominant difference between public and community systems is the treatment of raw water, being more technical in public networks, where methods such as screening, coagulation-flocculation, sedimentation, and filtration are used (Chulluncuy, 2011).

A negative aspect in community networks is the conduction process of raw and treated water, which is done through ditches or canals, a situation that leads to a decrease in water quality due to cross-contamination by not having a total cover in the circulation of water to its partial and final destination (Pacheco, 2019).

According to Perugachi and Cachipuendo (2020), the responsible entity is *Empresa Pública de Agua Potable Pesillo-Imbabura* with the support of community participation, which is structured by 5 actors: 2 corresponding to the GADs, 2 from the JAAPS and a president of the association. The community and people disagree with it since they had the idea of autonomously managing the resource.

Finally, the term **periurban** is not coined by the RAE. However, the word can refer to the processes of territorial organization, whether orderly or unorderly in large cities (Mansilla, 2018). The peri-urban area is often related with the periphery and with poverty; for Vieyra et al. (2018), this social condition has a multidimensional and multifaceted approach; in this way, the peri-urban area is understood as an area of accelerated transformations of the territory, which cause a physical expansion of the city, generating economic and socio-environmental impacts, and a gap between the city center and the periphery. There is another similar term to characterize these zones, which is rururban space, defined by Cardoso and Fritschy (2012) as a hybrid between urban and rural dynamics; De Mattos (2002) has called it part of the "metamorphosis" of cities.

Water management in a peri-urban area such as the one addressed in this study (Santa Clara de San Millán) is represented by the Commune, its rural socioeconomic characteristics, but also by the fact that it is adjacent to the north-central area of the city of Quito. This community manages the water resource in its territory, through a water system for human consumption, which consists of catching water, ditches, and PVC pipes for transporting raw water, pressure breaker chambers at different points, raw water purification area and PVC pipes for transporting treated water to houses (Figures 3 and 4).

In addition, JAAPS works in the Commune, which is annually assigned by the Cabildo to conduct this work. The Board consists of a committee chosen annually by the partners to provide the service, which has a single cost for each beneficiary; it is important to mention that several partners benefit from the service provided by the Board and by the EPMAPS (Empresa Pública Metropolitana de Agua Potable y Saneamiento de Quito).

Among the main strengths observed in the local water management are the participation and teamwork of the committee and the members, the cooperation activities that are carried out from time to time for the maintenance and cleaning of the system. On the other hand, weaknesses correspond to some technical and economic aspects that need to be characterized and improved, such as: inadequate dosage of calcium hypochlorite in the chlorination of raw water, pipes in risk areas due to potential mass movements, leaking pipes, debts of the beneficiaries of the service, universal single charge without taking into account the value of actual consumption per beneficiary, among others, aspects that should be improved to promote the long-term sustainability of the system (Pacheco, 2019).

3.3 Adaptation to Climate Change and water co-management and the natural landscape at the community level.

It is estimated that South America, among other regions, would have devastating consequences in an accelerated climate change; one of the risks is the melting of glaciers in the Andes Mountains and changes in high Andean ecosystems (Yánez, 2009; Yánez et al., 2011), which would decrease the amount of drinking water by people in the region; the acceleration in glacier melting dates back to 1976 when Ecuador had 60 km^2 of glaciers in the mountain range (Francou et al., 2013); according to Cáceres (2010) the loss of them has been 38 % in approximately 30 years.

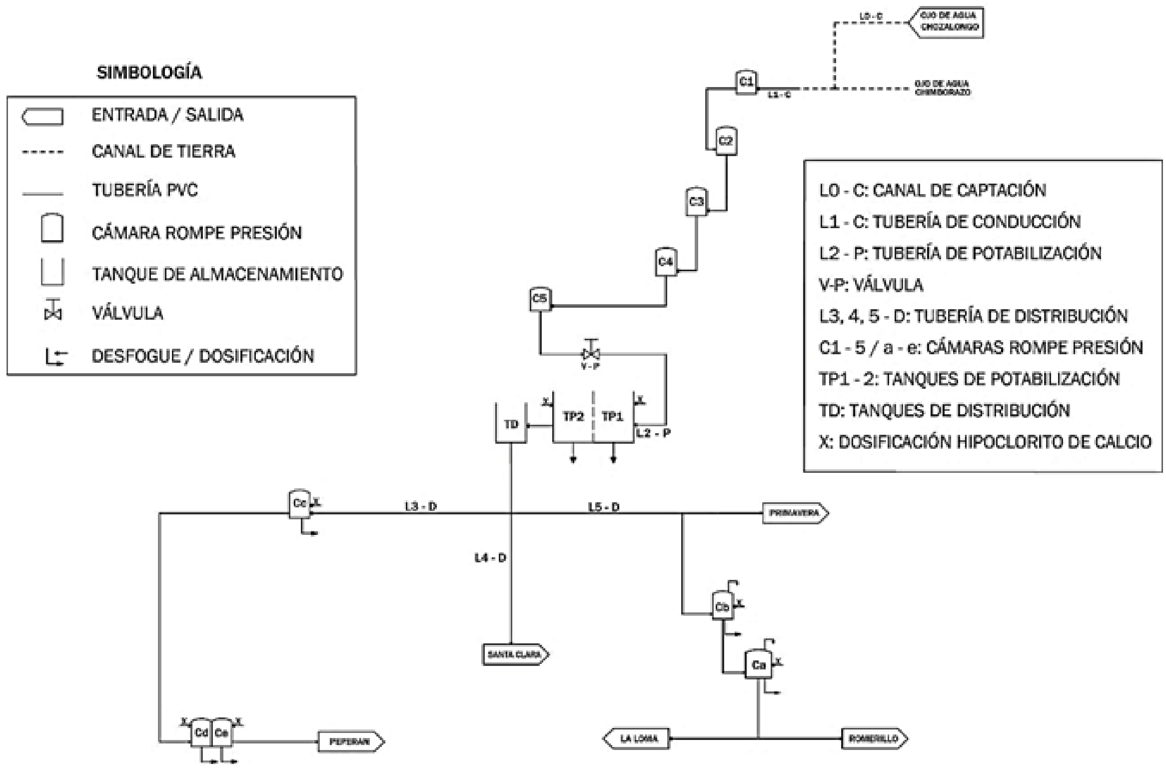

Figure 3. Diagram of the water system at Santa Clara de San Millán commune.

Other negative effects of climate change must also be considered: decrease in precipitation, sea level rise, loss of biodiversity, droughts, extreme heat waves, floods, among others (ONU, 2015). In this scenario, the efforts made by governments in the formulation of development plans are important, as they present mitigation and adaptation tools. Other ways to address the phenomenon are international agreements, especially those involving emissions control and the generation of compensations to those harmed (UNESCO, 2018).

Therefore, adaptation to climate change requires management tools (methodologies and regulations) based on an ecosystemic approach, with active community participation. According to Magrin (2015), one of the most complex options for climate change adaptation is that based on ecosystems, as it requires cooperation of different organizations and actors. In this study, it is more feasible since the inhabitants of the Commune are willing to preserve the natural ecosystems in the area.

In this sense, Delgado et al. (2015) and Gómez-Ruiz and Lindig-Cisneros (2017) state that such management and preservation instruments should enable communities and organizations to focus on meeting their needs and maintaining ecosystem services, using, among other tools, ecological restoration in the areas when appropriate.

Therefore, in the area of this research, it is recommended the gradual replacement of the eucalyptus forest by a forest with native species that would generate greater ecosystem benefits (including water) than monospecific forests with introduced species (Cordero Rivera, 2011; Anchaluisa and Suárez, 2013; Faries and Ríos, 2019), it is especially necessary in resilient peri-urban areas. Therefore, we consider that the forest of the commune requires landscape restructuring to improve its current conditions.

In this scenario, the concept of adaptation through co-management is paramount, and the prioritization of actions is essential for the development of communities and participatory management, especially in terms of water and forest/moorland resources.

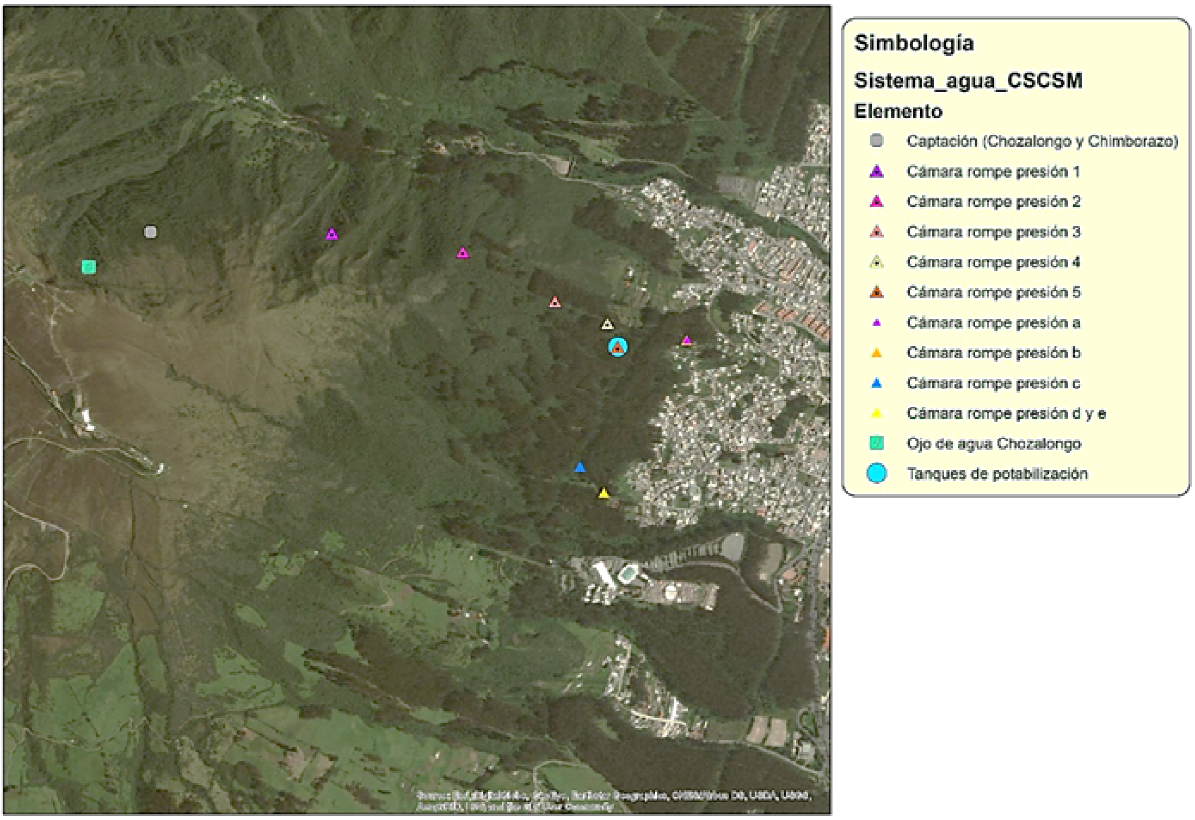

Figure 4. Location map of the water system at Santa Clara de San Millán commune. Adapted from Google Earth, 2020.

There are interesting models that can be applied in the commune, like the one proposed by Barazorda and Pérez (2014) and Saborío et al. (2019) in similar environments in a context of climate change, who combine landscape management activities and reasonable and rational water management.

The water co-management model to be applied in the commune must be holistic, enabling an adequate interaction between human rights and water management, and cannot be discriminatory; therefore, it must include different age and gender groups as key management actors. Dialogue and cooperation must be generated to move from a potential conflict in the management and use the resource to an agreed solution. Similarly, joint work between JAAPS and EPMAPS should be balanced in each of the management competencies, including environmental sanitation. The strengthening of internal capacities should be based on shared work, including the ancestral and territorial knowledge of the people in the commune, in addition to the experience and technological knowledge of the members of the public company (FONAG, 2020; Perugachi and Cachipuendo, 2020).

Another axis of management should be the participatory governance of the resource, that would evaluate a fair price for the service, including expenses related to planning, mitigation of climate change effects in the area, monitoring, provision of water-related services, system maintenance, effective internal communication, and administrative costs (WWAP, 2019). Two cross-cutting elements to consider in all phases would have to do with avoiding inefficient management actions and corruption events, elements that can lead to an increase in the system's internal vulnerabilities and inadequate water management.

Finally, regarding environmental sanitation, water purification, when necessary, can appeal to three options: the use of technology through Wastewater Treatment Plants; the application of Oxidation Lagoons; solutions based on the dynamics of water in the ecosystem and its natural purification in the corresponding micro-watershed (García, 2021; Laforteza et al., 2018; Scott et al., 2016). In rural and peri-urban communities, the second and third options are the most successful due to the economic-operational capacities of the JAAPS.

4 Conclusions

Proper water management by human beings at all levels of government, from local to regional, is one of the main tools that will enable proper adaptation to climate change.

Currently, the most common management models are based on a poorly connected work between key actors in a location, whether urban, rural or peri-urban. Regarding the Santa Clara de San Millán commune and other similar ones, it is noteworthy that the proposal generated enables articulating, under legal, technical and communication aspects, the necessary processes for a participatory management of the water resource present in the area. The strengthening model from grassroots organizations, in this case the Board, would allow the creation and improvement of the capacities of people involved.

In this context, we consider that adaptation to Climate Change scenarios based on ecosystems and communities is the best way to establish and develop measures that allow achieving a real adaptation to this change, significantly reducing the vulnerability of the population in this and other similar communities.

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Sustainable Development

Large agribusiness corporations and contract farming: Theoretical approaches

Las grandes corporaciones agroindustriales y la agricultura contractual: Aproximaciones teóricas

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Abstract

Contract farming constitutes a long-term productive and commercial modality in time and space; for this reason, it is essential to approach the different interpretations of its determinants. In this sense, this article analyzes the theoretical and interpretative contributions that the main currents and schools of economic thought have raised about the factors that have decisively contributed to the development of the productive and commercial modality of the contract in agriculture, especially from the perspective of the large agro-industrial corporations. The methodology applied in this research is the literature review. The investigated currents are the French, the Anglo-Saxon, and the Latin American. In the end, it is concluded that there is a re-functionalization of the agrarian economy through contract farming; contracts are yet another business strategy for sourcing agricultural supplies; and the sources that large agro-industrial corporations must promote this productive and commercial modality are market, contractual-informal and technological.

Keywords: Contract farming, vertical integration, large agribusiness corporations, family farming.

Resumen

La agricultura contractual constituye una modalidad productiva y comercial de largo recorrido en el tiempo y el espacio; por esta razón, se considera esencial aproximarse a las distintas interpretaciones de sus determinantes. En este sentido, el presente artículo analiza las aportaciones teóricas e interpretativas que desde las principales corrientes y escuelas de pensamiento económico se han planteado en torno a los factores que han contribuido decisivamente en el desarrollo de la modalidad productiva y comercial del contrato en la agricultura, especialmente desde la perspectiva de las grandes corporaciones agroindustriales. La metodología aplicada en esta investigación es la revisión de literatura. Las corrientes investigadas son la francesa, la anglosajona y la latinoamericana. Al final, se concluye que a través de la agricultura contractual se produce una refuncionalización de la economía agraria; los contratos constituyen una estrategia empresarial para abastecerse de suministros agrícolas; y los resortes con los que cuentan las grandes corporaciones agroindustriales para fomentar esta modalidad productiva y comercial son de mercado, contractual-informales y tecnológicos.

Palabras clave: Agricultura contractual, integración vertical, grandes corporaciones agroindustriales, agricultura familiar.

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1 Introduction

Initially, before presenting and developing the theoretical perspectives that study contract farming (CF), it is necessary to mention some concepts that will improve the analysis. In the first place, small and medium agricultural production (the contracted or integrated part) is specifically made up of peasant producers, whose production is based on the ownership and use of a relatively small or medium-sized area of arable land, and on family labor (Óman and Rama, 1986).

Secondly, a large agro-industry company (LAC) (the contracting, integrating or anchor party of the agricultural contract) is a company of national, foreign or mixed capital that holds a dominance position in the market in which it operates, generally, although not always in a context where monopsonistic or oligopsonistic forms of competition predominate, specifically in the agri-food sector, engage in one or more of the following activities: production, industrialization and commercialization of agricultural, livestock, forestry, fishery goods, among others related to biological natural resources. Also, following Barker (1972, cited in Soria, Rodríguez, and Langreo, 1988, p. 225), CF is understood as:

> a system for the production and supply of agricultural commodities under futures contracts, the essence of such a system being an agreement to supply an agricultural commodity of specified characteristics at the time and in the quantity required by a known buyer. Such a system often includes a transfer of some business responsibilities and some transfer degree of commercial risk. (p. 225).

2 Interpretative aspects of contract farming

Thus, with these concepts in mind, the structure of this section is based on the contributions regarding the role of the LAC in the growth of CF based on the initially mentioned currents, namely: the French current, with certain Marxist influences; the Anglo-Saxon current; and the Latin American current, strongly influenced by the theorizations of the first school, i.e., the French school (Posada, 1999).

2.1 The French current

The analysis of large agro-industrial companies (LACs) and their business strategies is based on the so-called "agroindustrialization theory", which, according to Fenollar (1978), can be interpreted as follows:

> the one which reveals the "economic law" of agricultural development within a process of industrialization and urbanization, as well as to show the "specificity" of agriculture/industry relations and the profound insertion of the rural world into global society, leading to the consolidation of a system of production, transformation and distribution of agricultural products (S.A.I) [agro-industrial system] whose objective is to provide nutritional needs, and which is characterized by a decreasing contribution of agriculture in the formation of the final food product, by a growth of the large agro-food firms, by the development of contractual relations with the more "industrialized" branches of the agricultural sector and by the articulation of family farming in this system (...) The theory of agroindustrialization indicates that the capitalist characteristic of agriculture is accentuated not as a function of the specific development of the CMP [capitalist mode of production] within agriculture (...), but as a function of the interrelations between agriculture and the capitalist system as a whole (pp. 173-174).

Therefore, it is a theory that tries to understand the integration processes of rural economy in the development of capitalism in general, and agrarian capitalism in particular (defined as a mode of production based on the private ownership of most of the means used to produce, the mercantile character of production and the private appropriation of production and profit, where "the social relations that give origin and purpose to the economic process are those established between the owners of the enterprises and the wage earners who lack the means to produce" in the agrarian world (Palazuelos, 2017, p. 123)). In this sense CF is understood as one more instrument (within a wide variety of business strategies) to subsume and subjugate the rural sector in the development of capitalism (adopting the own characteristics of the corporate food regime since the 1980s (Porter and Phillips-Howard, 1995)).

From this perspective, and in line with the classical Marxist approach Lenin (1981) and Mandel

(1969, cited in Lebossé and Ouisse, 1979), the development of agrarian capitalism has been theorized using the same analytical frameworks coming from the study of industrial capitalism, i.e., those used to study the passage from formal subsumption to real subsumption of the labor process in the capital (Marx, 1975), giving rise to a practically absolute proletarianization process (McMichael, 2013). Then, from this point on, it is considered that the rural sector, whose central axis is small rural family production, continues to exist as such because agriculture, for a series of reasons that will gradually disappear, maintains a certain temporary delay in its industrialization process, and all that this implies (Posada, 1999).

In this sense, authors such as Malassis (1979) have considered that the development of agrarian capitalism (understood as the capitalization of agriculture) can follow two different paths. The first led by the industrial sector, which would control and invest in agrarian property, i.e., the so-called vertical integration. The second would be an indirect way of investment and control by the industrial sector over the agrarian sector through CF. Both ways of development of agrarian capitalism have in common the fact that the proactive actor in the process is the industrial sector, or, more specifically, the industrial bourgeoisie (especially international, but also local) through its companies.

Therefore, it is understood that this second way has more advantages over the first (and would explain to a large extent the growing role of CF in recent decades) insofar as it allows industrial capital to circumvent the axial problem of land control; CF is economically more lucrative than vertical integration, since peasants are generally very reluctant to sell their land, resulting in expensive investments and lower profit rates (Posada, 1999). Thus, this business strategy led by LACs can be included in the accumulation concept by dispossession (Harvey, 1984, cited in Bernstein, 2016, p. 626), an axial element of the corporate agri-food regime. Likewise, Lebossé and Ouisse (1979) argue that CF:

> integrated into a food complex and controlled by the capitalist State is the one that best serves the essential interests of the capitalists... In all agricultural domains the maintenance of a transformed artisan allows, under present conditions, a greater exploitation to the benefit of the capitalist sector i.e., a greater transfer of value than the direct exploitation of a wage labor force in a capitalist agriculture (p. 203).

Hence, capitalist enterprises prefer CF as long as the maintenance of pre-capitalist elements embedded in the dominant mode of production "within the food sphere" ensures a higher level of exploitation towards the farmer, a greater transfer of value when this farmer is a peasant (when his subsistence depends not only on the income provided by his participation in CF projects, but also on agricultural production for self-consumption and, in addition, has the labor support of his family) than when he is purely a salaried agricultural worker for the company. Thus, according to Lebossé and Ouisse (1979) it is understood that the maintenance of class agrarian structures based on small and medium agricultural producers "artisanal agriculture", is better than the development of agrarian capitalism (a scheme based on a mass of free wage laborers on the one hand, and capitalist entrepreneurs who own the land and the means of agricultural production on the other), only when the first condition is fulfilled, i.e., when there is a greater extraction of value.

Specifically, this thesis is based on two postulates. The first, quasi-integration (in the form of CF) ensures a significant transfer of the integrated surplus (Etges, 1991; Da Costa, 2003). Thus:

> It is therefore interesting for the integrated company to perform a quasi-integration, since it thus allocates a part of the agricultural surplus, without having to bear additional investments. Total integration, on the contrary, would make it possible to have *all* the agricultural surplus labor, but would imply a heavy burden of supplementary investments. *It would then be the capitalist group as a whole which would obtain lower profit rates than "normal"* (Lebossé and Ouisse, 1979, p. 210).

But how is the transfer of the surplus? it is argued from this theoretical perspective that the selling price of the final product is lower than the "capitalist cost of production". Hence, entrepreneurs who can pay lower wages use this quasi-integration process. Lebossé and Ouisse (1979) argue that the lack of profitability of small and medium farmers integrated into the CF does not depend on their "technical incapacity", nor on the small size of their plots, but on the fact that integrating LAC "confiscate the productivity gains of their farms", even though they are the ones who supply the labor force, the land, and in most of the cases the equipment. In this sense, the property rights of the integrated farmer are limited to the extent that the ultimate objective of the "contract is to make him disappear as an economic subject", i.e., to transform him into one more technical part of the large integrating company (Fenollar (1978) says it is "a form of submission of family farming to capital"), so that on the one hand, as a formal owner of land, he must take care of it and keep it in the best possible productive conditions, and on the other hand, as an "integrated artisan farmer", he cannot keep the surplus generated (p. 211).

In this sense, this first postulate also means the unfolding of a double movement: more "farmer dependence" (even in the case of farmers who are parts of associations) which reinforces the independence of the integrating LAC. This is observed in the fact that in most of the cases they are not obliged to guarantee the part corresponding to the depreciation of the equipment used during the production process (a cost that is therefore borne by the small and medium farmers) through the contract. In these favorable circumstances for the LAC, they have more room for maneuver in the management of their financial resources, since they can transfer their funds to other productions or other geographical areas, since the contracts previously signed are generally short term, and most of their duration is shorter than the "economic life" of the equipment used by the contracted small and medium producers.

Moreover, in a small number of cases in which the LAC is obliged to assume the "financial expense of investments", it does not alter "in any way the transfer of surplus through prices", insofar as the rent (withheld from the income of the small and medium contracted producers) guarantees a "normal" profitability level of the capital. Ultimately, the major change regarding the situation described above is that of less room for maneuver on the part of the contracting companies, since under these contractual conditions they cannot choose to shift their financial resources entirely to other activities before the end of the useful life of the equipment involved in agricultural production subject to the contract, since the integrating party has undertaken to support the depreciation costs of the equipment (Lebossé and Ouisse, 1979, p. 212).

On the other hand, the second postulate indicates that quasi-integration ensures the transfer of technical and economic risks from the integrating economic subject to the integrated legal subject, since it "makes it possible to control the integrated subject, to reduce its freedom of action by specializing it totally, imposing the exclusivity of supply, and often creating a debtor position vis-a-vis the integrator" (Lebossé and Ouisse, 1979, p. 214).

In the case of the former, i.e., the costs associated with the prevention of technical risks, e.g., droughts, floods, pests, diseases, etc., these are often borne by the farmer-owners of the land. They assume all the risks, except on exceptional occasions when they can prove that they were not responsible, as long as this circumstance is included in the contract. Thus, the most viable alternative that most small and medium contracted producers assume in the face of these disagreements is to pay for voluntary insurance to provide for this risk, which is always a possibility in agricultural production. As far as economic risks are concerned, Lebossé and Ouisse (1979) state that:

> it should be noted that they originate from the integrator's policy: the integrator pursues a certain freely chosen policy and the quasi-integration system allows him to reflect, in whole or in part, the consequences of the errors of his policy on the artisanal farmers he is part of (pp. 112-113).

Economic risks, in turn, may occur in the short and long term. Thus, in the short term, the following elements are present. First, "the prices assured to the integrator", although a positive element of the CF, is only partially attributable to the integrator since this is due to "a system of price equalization between producers and different periods of time". The intervention of the integrator LAC is weak, since "its quotations represent only a small part of the quotations of the integrated producers". Therefore, it is understood that its main performance is limited to carrying out accounting tasks and "anticipating certain quantities". From this perspective, even CF based on "protective" contracts or "risk-sharing" contracts, the small and medium integrated farmer "is obliged to finance half of the losses resulting from the integrator's errors (bad counter-cyclical policy, bad anticipations...)" (Lebossé and Ouisse, 1979, p. 213).

Secondly, "rectification of the quantities to be delivered" means that during the agricultural production period, when the contracting LAC is aware of a failure in its commercial strategy or senses a foreseeable change in market expectations that could cause difficulties in its business, it can (if included in the contract, and it generally is) impose a reduction or delay in deliveries by the integrated agricultural producers. Moreover, this type of contractual clause is combined with others, such as the exclusivity clause, whereby the contracted farmers can only sell their production to the contracting companies; all this undoubtedly translated into an arbitrary reduction in the income of the integrated farmers (even, it should be stressed, in those few cases where certain indemnities are provided for small and medium-sized farmers (Lebossé and Ouisse, 1979, p. 213)).

On the other hand, long-term economic risks are associated with the fall in the prices of agricultural products; this reduction is borne by the small and medium contracted producers, "since most of the contracts provide for remuneration based on the average value of sales over a given period". In this context, it can be inferred that these remunerations will tend to evolve "with the market", in such a way that they will not provide any guarantee on the effective prices in long-term periods (four or five years). And to the extent that it is necessary to amortize the equipment and installations, the contracted producer will be tied, "whatever the evolution of prices during this period". Thus, the contracting LAC can, at a given moment, modify its activity at its discretion because most of its means of production are adaptable (for example: transport, storage, conservation, marketing network, etc.) and, moreover, place on the shoulders of the contracted party the costs of this modification or "of its reduction of activity on the integrated farmer", who maintains "his relatively unsuitable equipment which, however, he has to amortize" (Lebossé and Ouisse, 1979, p. 214).

Thus, considering the above, it is logical to maintain that the social-agrarian structure that arises from this modality of integration generates "a strong sustenance", to the extent that the LACs are the ones that create and foster them (among other drivers), and, therefore, "their temporal perspectives are long-lasting" (Posada, 1999, p. 108).

In summary, from this theoretical current, LACs constitute the main determinant of CF growth, where this productive and commercial modality assumes an instrumental role, i.e., this is posed as a tool of capitalists to "refunctionalize" in their favor "the social-agrarian structure hegemonized by small-scale farms" (and, in fact, they achieve this to such an extent that during the corporate agrifood regime the dominant form of capital is the one linked to financialized corporate agribusiness (Bernstein, 2016, p. 632)), a characteristic land ownership structure in most European countries, different from the high concentration levels of fertile land in contexts such as Latin America in general, and Ecuador in particular. Therefore, analyzing the land ownership structure and how it evolves over time becomes necessary to understand the CF phenomenon within the framework of the business strategies developed by LACs in all their complexity, particularly in peripheral-dependent economies with high levels of agricultural land concentration.

2.2 The Anglo-Saxon current

From the Anglo-Saxon perspective, in contrast to the French and Latin American perspective, CF is just one modality in which the commercial relations of the LACs are observed. The main axis from which practically all the studies of this current related to the role played by LACs in promoting CF are based is the so-called "industrial organization theory", which studies how producers organize themselves in markets (Runsten and Nigel, 1996; Posada, 1999; García, Oreja, and González, 2002; Kirsten and Sartorius, 2002). In this sense, the study of the agrofood industry based on the theory of industrial organization has led to the formulation of an analytical framework focused on the following premise:

> The open market presents deficiencies in the transmission of information between the successive stages of the product marketing chain, with respect to quantity, quality, and delivery, as well as with respect to future consumer demand (Posada, 1999, p. 109).

From this school of thought, the entire agrifood chain is "the basic unit of analysis", starting from the primary stage and continuing through the intermediate stages to the final consumer. In relation to the origins and sources of this school, it is worth highlighting the article of Davis and Goldberg (1957, cited in Posada, 1999), in which both authors study the agro-industrial subsector, delving into a systemic analysis of the chain, emphasizing the vertical information flows that materialize between the different stages of the chain, in continuous adaptive processes in the face of structural transformations in the sector, especially those linked to technological developments, and their effects or results on the general functioning of markets.

Specifically, outstanding theoretical formulations have emerged around the theory of industrial organization. In this line, Bain (1968) synthesized the different contributions of numerous authors, establishing the bases of the paradigm known as structure-behavior-results (S-B-R) (Morales, 2000) as a basic and central theoretical tool for examining and evaluating the behavior of markets and for studying the relationships established between industrial structure and the results obtained by the corresponding subsector. Thus, it is understood that "the market structure influences but does not absolutely control the behavior of the firms, with both factors intervening in the results obtained" (Posada, 1999, p. 109).

According to the S-B-R paradigm, the variables to be analyzed to study the market structure are the following. First, structure (S), which is made up of a set of variables (the concentration degree of supply, the concentration degree of demand, product differentiation, the nature of the product, the technology available, and the input barriers) that remain relatively stable over time and influence the behavior of both suppliers and demanders. Second, behavior (B) which refers to the way in which suppliers and demanders behave between and within both agents. Among the variables studied are business strategies, the degree of investment in R+D+i, levels of marketing and advertising, collusion, etc. Finally, results (R) which are measured by comparing the company's results with those of the rest of the industry in terms of effectiveness and efficiency, using different ratios to assess the different degrees of profitability. In this case, the variables to be considered are price, quantity, product quality, distribution of resources, distribution of surplus between consumers and producers, efficiency degree in the production, introduction rate of new products, among others (Iglesias, 2000).

The S-B-R paradigm (associated with industrial organization theory) to the agrifood system and the CF-based business strategies have been widely applied. However, this theory has several significant limitations. First, it is a "static perspective", which is explained by the fact that the relationship that stands out is one of "cause-effect between the structure of the sector and its results", excluding behavior, and that "the role of the manager is one of passive adaptation to the environment" (Cuervo, 1996, cited in García, Oreja, and González, 2002; Machado, 2019). Thus, this paradigm must be adjusted and adapted to a context of active companies with a strategic vision that is constantly analyzing its potential consumers, substitute products and the bargaining power of suppliers and customers, elements that make up the structure of the sector and define its potential profitability, "while the real competition within the sector represents behavior" (Mili, 1980; Porter and Phillips-Howard, 1995, cited in García, Oreja, and González, 2002).

The second weakness of this paradigm is that it focuses on companies that compete with each other by offering products to the same demanders, i.e. it has been applied from the point of view of competition between companies that produce substitute products (analyzing, above all, the problems that arise from horizontal competition) paying little attention to the difficulties present in vertical competition, i.e. between companies that are part of the different stages of the system (Mili, 1996; Iglesias, 2000). Finally, the third limitation facing the S-B-R paradigm is the problem of defining "market". The market must include all companies and products that influence the price of the good. Thus, the market definition has a product and a geographic component. The product dimension involves determining those products that are demand substitutes. On the other hand, the geographic dimension implies determining the location of the companies that produce the same product (substitution on the supply). However, concentration measures are often taken from statistics that do not follow the economic and geographic market definition (for example, reference is usually made to the national market (Machado, 2019)).

In the face of these problems and limitations presented by the theory of industrial organization, it has been suggested that a correct analysis of the functioning of CF requires understanding that it should be studied from a "broader theoretical context", i.e., beyond the economic dynamics of the agricultural sector, understanding that the subcontracting of specific production processes covers a wide range of industries and sectors (primary, secondary or tertiary), and also encompassing aspects such as "decisions regarding manufacturing or purchasing, transaction costs, market failures and all the risk considerations normally taken into account by economic agents" (Runsten and Nigel, 1996, p. 2). In this sense, within the new institutional economics or neoinstitutionalism, the following theories have been collected: "transaction costs" (Coase (1937), Coase (1960), and Bartra (1994) and Williamson (1979), Williamson (1985), and Williamson (1988) being its main representatives; "agency theory") Jensen and Meckling (1976) are its most prominent exponents; and the theory of "property rights" (Alchian and Demsetz, 1972; Iglesias, 2000).

Thus, the combination of these theories has reformulated and reinterpreted Coase (1937) original perspective, moving from "a transactional approach to a contractual approach" (Williams, 1985), since the transaction is supported by a contract. These theories, usually defined as "contractualist theories" involve the introduction of a series of analytical contributions to the study of agribusiness markets in general and to the business strategies of LAC in CF, which are worth commenting on at this point.

In the first place, transaction costs, the main objective of this theory, study the best possible alternative for organizing exchanges in such a way that this choice is adapted to the characteristics of each transaction, i.e., it minimizes the risks, and, above all, the costs involved (Shelanski and Klein, 1995, cited in Iglesias, 2000). Thus, under certain conditions, a company develops the activities inherent to the production process; in other circumstances it outsources them to the market; and in others it may opt for intermediate positions such as entering contracts, and these in turn may be agreed with atomized agents or integrated into associations (Kirsten and Sartorius, 2002) (Figure 1). In short, companies and the economic system tend to organize themselves in such a way as to minimize transaction costs (Williamson, 1996).

Second, "asymmetric information" is important in CF and in the strategies adopted by LAC (Kirsten and Sartorius, 2002), since it describes the divergence in information between bidders. Likewise, from the neoinstitutionalism (linked to the branch of Development Economics), Bardhan (1989) argues that "the formation of institutions" is a logical reaction, in spaces of "generalized risks" and uncertainty for economic agents or "incomplete markets", and where information is generated and circulates in a markedly asymmetric way, as Morales (2000) and Bijman (2008) point out, by opportunistic conduct or behavior and limited rationality. Along these lines, this theoretical perspective has attempted to demonstrate that aspects such as the formation of agricultural associations, labor relations, land ownership and tenure, and existing agrarian institutions can be explained as the result of "rational economic behavior in a context of nonexistent or imperfect markets". Therefore, the main contribution of this current to the understanding of CF and contracting LAC is the following: "there are important costs not only in production, but also (due to the existence of imperfect markets) in transactions" (Runsten and Nigel, 1996, p. 3).

In general terms, it can be stated that contractual theories conceptualize vertical integration as an essential practical support that makes possible the circulation of information flows related to "the quality, timing and future price of products within the agri-food system". Thus, CF constitutes, from this perspective, "an intermediate coordinating position". In other words, a situation in which the central problem of vertical agroindustrial integration disappears "such as that of complementing very different production scales". The more efficient the mechanism, the greater the reduction of "the effectiveness of a given subsector of the open market" (Posada, 1999, p. 109).

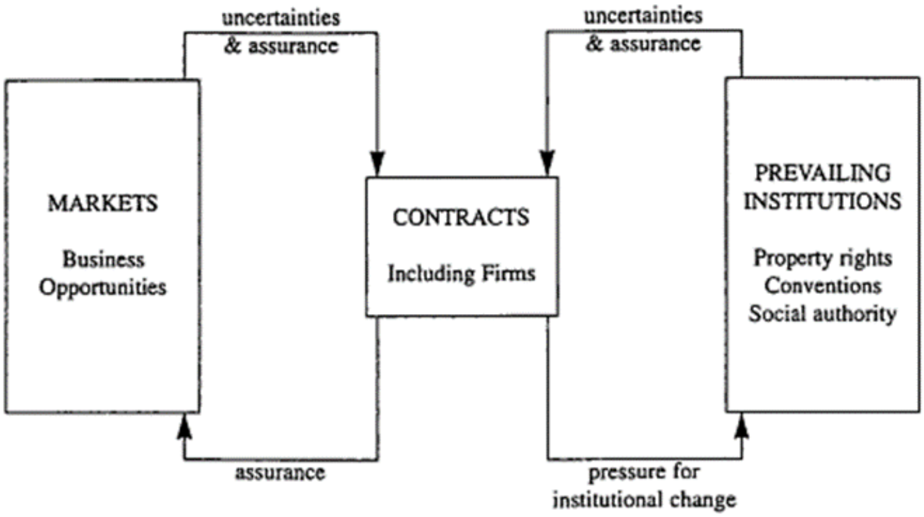

Figure 1. Contracts as intermediaries between markets and dominant institutions. **Source:** Hubbard (1997, p. 244).

2.3 The Latin American current

Finally, from Latin America it is postulated that the determining elements of the development of the productive and commercial modality of the "contract" in the agricultural sector are intimately linked to the development of agrarian capitalism (López, 1976); the fundamental axes of analysis are "the extension of capitalism on a universal scale" and, the verification of the existence of a hierarchy within the capitalist economic system on a world scale. From this perspective, strongly influenced by other contemporary theoretical postulations such as that of unequal exchange (Arghiri, 1972), the phenomenon would be related to the fact that the labor force in peripheral economies has lower remuneration levels than those found in central economies. From this point, this current was nourished by new contributions, such as, for example, the question of the distribution of profits from international trade, which would give greater theoretical substance to this perspective (Posada, 1999).

Thus, based on this analytical framework, various studies have been conducted on the formation and development of the agrifood system in peripheral economies in general, and particularly on the role of the LAC in promoting CF. These include the following works: Glover (1983), Glover (1984), and Glover (1987), Glover and Kusterer (1990), Feder (1984), Goldsmith (1985), Williams (1985), Giarracca (1983) and Giarracca (1985), Kesteren and Gutman (1981), Rama (1984), Rama and Vigorito (1979), Bartra (2006).

From this point of view, it is considered that LAC (in this case, transnationals) when they reach a certain level of development, policies of geographic and economic diversification represent a central axis of advantage over the competition. However, this expansionism occurs especially in peripheral economies, where they almost always manage to control and organize production and agrifood chains, since doing so within the central economies involves greater difficulties for them, such as higher wage and tax levels, relatively high levels of union organization, less bargaining power with the State, etc.

Thus, in this context, a profound debate began on the limitations and potentialities associated with the fact that Latin America was one of the territories most susceptible to receiving this type of investment. In this regards Feder (1984) states:

> Foreign capitalists in search of remunerative agricultural investments outside their own countries find in the Third World a set of elements which, alone or combined, enable them to earn substantially higher profits than similar investments in the industrial nations. Foreign investors occupy a dominant, if not monopolistic, position in the local economy.

> They benefit from the low value or low rents of land, cheap water, low construction costs, low wages of farm or factory workers, and low costs of services such as transportation and utilities. Additionally, there are two crucial elements: the abundance of resources not employed, or not fully employed, especially land, water and labor; and the possibility of going elsewhere as long as it is profitable (p. 371).

Taking up Malassis (1981) research, it is argued that large transnational corporations, within the framework of the corporate food regime, control "the agrarian phase directly or indirectly". Thus, the direct form would materialize through the ownership of land and the means of agricultural production. An example of this form of agriculture is plantations of various types. On the other hand, the indirect form consists of the participation of various agricultural holdings within agro-industrial complexes. The production of these agricultural units is controlled and focused on achieving the objectives set by the LAC. In this case, the links between the economic agents participating in this type of agriculture can be objectified in different ways, but CF is the most important and widespread.

Consequently, the most relevant characteristic of these contractual relations is the high level of business responsibilities to the medium or small farm "from the primary stage", and "has proved to be an effective means of assuring processors [LAC] an adequate supply that meets quality standards without the need to higher investment risks in agricultural production" (Goldberg, 1974, p. 52), from which it is understandable that LAC have increasingly promoted the CF modality in this type of scenario. Feder (1984) argues that:

> The problem of *developing country suppliers* across national borders is more complex. At first, international companies chose coordination through vertical integration, which was familiar but increasingly less accepted and more irritating. As developing countries formed a countervailing political and economic force, they precipitated the more widespread use of contractual methods of coordination, such as licensing, management contracts, franchising and production contracts (p. 364).

To summarize, it can be said that, for these authors, LAC promote the development of CF through a series of aspects in different spheres. In the first place, from the *market point of view* to the extent that contract production constitutes a highly secure source of supply, since the small and medium-sized producer does not have the control of his production except to deliver it to the contracting agroindustrial company, trying not to breach the clauses of his contract (a highly improbable event for various reasons, such as climate). Moreover, this dependence of the producers (López, 1976) (especially of small producers) increases considerably due to the oligopolistic and monopolistic role of the LAC in the territories in which they operate, in such a way that the latter have the necessary resources to "oppose and eliminate the production and commercialization of the products they do not manage, so that a producer has no outlet for his production if he is not contracted by the companies" (Feder, 1984, p. 365). However, the counterpart of this fact is the relative economic stability that the contracted producers generally obtain based on the considerable remunerative levels resulting from the CF.

Secondly, from the *contractual-informal* point of view, Feder (1984) states that, based on "observation in the field", it is argued that the contracted producers do not retain their autonomy and independence at all, a fact that is largely due to the "vague and partial" nature of the contracts between the companies and the producers. Vague to the extent that the most important conditions under which the small and medium contracted producers must transfer their agricultural production are not specified in detail, but depend on the decisions of the contracting LAC. This is largely explained by the fact that the producers are almost never organized and therefore have no bargaining power, and, in addition, the contracting companies promote conflicts among them to prevent them from organizing (with the connivance of the national and regional authorities on many occasions). Moreover, as Óman and Rama (1986) argue:

> the contract system allows firms to benefit from low wages without having to deal with the labor conflicts and social problems that would afflict them if they operated under the traditional way (. . .) Local farmers are often better able than plantation owners to restrict wage demands and unionization tendencies, mainly because smallholder farmers almost always work alongside their wage earners and labor relations are more personal.

> Moreover, the labor force is much more decentralized in the contract regime. On the other hand, while plantations employ mainly adult male workers, contract farmers often employ women and even children (p. 890).

And *partial* since the obligations of the contract are all referred to the contracted party but none for the contracting LACs, in this sense we speak of "leonine contracts". Specifically, Feder (1984) argues that:

> This implies that the acceptance or rejection of production is not determined by the contract but by the economic conditions (demand and supply) of the country or the world, so that the risks of *any fluctuations in prices and markets can be placed directly on the producers*. The conditions under which they are actually accepted or rejected are being decided by these companies with devious, disguised and often cruel methods, regardless of whether they are large or small producers, although the latter, are the first to suffer from the arbitrary and anti-peasant attitude of the international companies (...) In reality, what these [small and medium producers] do is almost to become mere farmers these companies (p. 367).

Thirdly, from the *technological* point of view, in general terms, the role of LAC as technology transferors is very limited, both in terms of content and scope but not in terms of duration. The contracting LAC maintain "*their role as innovators as a permanent function*", since renouncing their role as innovators would mean weakening their essential levers of control over the agro-industrial production-marketing systems (above all, this is a predominant factor of the LAC originating in the central economies), while the LAC of the periphery are mostly responsible for the distribution at the national level. Fundamentally, this advantage is "permanent" through at least three practices. First, limiting technological transfers (technical knowledge) to certain regions, excluding others. Second, transforming technology transfers into "a continuous source of profits" (genetically altered seeds are a good example of this, since each new planting requires the purchase of a new package of seeds, fertilizers, and specific pesticides). Third, discriminating with respect to the quality of the technology that these companies decide to transfer. Consequently, if it is interpreted that one of the main objectives of the LAC is to seek the least costly possible combination of inputs (seeds, fertilizers, pesticides, ripening agents, etc.), it is logical that technology transfers to small and medium contracted producers should be such as to raise costs unduly, "without investing in more technology than it is strictly necessary and transferring only enough for production and processing to continue without raising costs unduly" (Feder, 1984).

Thus, based on the conclusions obtained by Voll (1980), Freeman and Karen (1982), Goldsmith (1985) and Vigorito (1994), it can be inferred that there is "an integrating pole" or "*core*" in CF, formed by the large agribusiness companies, which establishes practically all the production conditions of the contracted farmers or "*satellites*". This asymmetrical position of power allows the contracting party to obtain a higher profitability than it would obtain in a vertical integration modality (one in which the LAC is the de facto owner of the land and the other means of production used in the production process). From this perspective, it is considered that the LAC conduct an "appropriation of the surpluses" generated by the peasants and "a transfer of long-term risks". Consequently, it is to be expected that large agroindustrial companies will tend to replace plantation agriculture with CF (Óman and Rama, 1986, cited in Posada, 1999).

Finally, from this school of economic thought, it is worth highlighting the contributions of economist Bartra (2006), who points out that, based on the characteristics of the current food regime in order to understand the role of the LAC in promoting CF, it is necessary to abandon any mechanical and simplistic vision that proposes a logical correspondence between the development of capitalism in agriculture and a "depeasantization" process of agriculture, i.e., it cannot be stated categorically that the penetration of capitalism in the rural area leads ineluctably and in a generalized way to the proletarianization of the labor force. "There are numerous examples of the development of agrarian capitalism which does not express itself in a linear process of proletarianization but as a dialectic of expropriation/reincarceration" (p. 357).

Thus, despite the relative increase in wage labor resulting from a modernization of agricultural production "when it develops in a pre-capitalist environment" this should not obfuscate the specificities of agrarian production and the demand for labor force it generates. In this sense, Bartra (2006) argues that in the capitalist system a significant part of agrarian labor and means of production can remain "subordinated to capital" through "mediations and with modalities considered *atypical*" such as, (for example: self-employed work, non-entrepreneurial mercantile production, subsistence economy, part-time wage labor, CF, among others), all of them fully functional to the system and fully embedded to the logic of capitalist accumulation (characterized as a "flexible accumulation" by Goodman and Watts (1994)); and where "capital hardly creates additional wealth; it simply takes advantage of locally produced wealth to concentrate and reuse it according to its own logic", a characteristic that is accentuated during the corporate agribusiness regime from the 1980s to the present (McMichael, 2013, p. 674).

For this reason, Bartra (2006, pp. 103-104) argues the following in relation to CF:

> small and medium peasant production, inserted in the cycle of capital and subjected to exploitation [...] constitutes at least one of the alternatives of the capitalist mode of production to the onerous extension of its own relations of production in the agrarian sector. The formally direct appropriation of the land by the peasants, subsumed in a real way to the cycle of capital, is presented as the alternative to the appropriation of the land of capitalist-landlord character. If the full extension of capitalist production to the agricultural sector entails a rent that cuts the general rate of profit of capital, the refunctionalized conservation (or reproduction) of peasant units, represents the possibility of a "rent in reverse"
>
> [...] a transfer of surplus-product metamorphosed into value that raises the general rate of profit. If the law of the maximum valorization of global capital is to impose itself there is no doubt as to what the choice will be.

In short, the Latin American current understands the growth of the productive and commercial modality of CF in the same expansionist and colonizing logic (in economic and geographic terms) of the capitalist system (despite being a modality that does not automatically lead, as seen in Bartra's contributions, to the integral and generalized conformation of a mass of salaried workers). In addition, CF is a mechanism of effective control of the LAC over small and medium contracted producers through multiple levers, specifically market, contractual-informal and technological.

3 Discussion and Conclusions

Throughout these sections we have analyzed the main studies and contributions from different schools that have been interpreted as relevant to the factors that have made a decisive contribution to the development of the productive and commercial modality of the contract in agriculture, especially from the perspective of the LAC. Thus, the contributions of three different currents have been presented: i) the French current; ii) the Anglo-Saxon current; and, iii) the Latin American current. Similarities and contrasts are presented below (Table 1):

In the first place, both the French and Latin American currents understand that the agrarian economy in general, and the business strategies deployed by the LAC, cannot be studied independently of the logics imposed by the capitalist system (among them, its constant expansion on a global scale). In this sense, the LAC through CF, within the framework of the corporate agrifood regime, "refunctionalize" the agrarian economy, in such a way that instead of transforming small and medium peasants into complete proletarians (i.e., without means of production and dependent for survival on a salary resulting from the sale of their labor power) they assume a role of "owner-proletarians" or, as Watts (1994) states CF "proletarianizes without dispossessing" farmers (cited in Dubb, 2018), i.e., their remuneration comes from two different sources: the first from the sale of their labor power, and the second from the ownership of the agrarian means of production (farm implements, land, water, etc.). The Anglo-Saxon-American trend, for its part, naturally assumes that the agrarian economy is capitalist per se, without going into the historical and structural aspects that explain this very specific characterization of agriculture.

Table 1. Economic currents analyzing contract farming.

	French current	Latin American current	Anglo-Saxon current
Theories	Agroindustrialization theory.	Agroindustrialization theory.	Industrial organization theory/Contractualist theories.
Logic of operation of the agricultural economy	The agrarian economy is embedded in the logic of the capitalist system.	The agrarian economy is subordinated to the expansion of capitalism on a global scale.	
Business expansion strategies	Vertical integration and indirect integration or quasi-integration.	Direct and indirect forms of control.	Agri-food chains.
Benefits for LAC of CF	Increased transfer of integrated surplus/ transfer of technical and economic risks.	Market, contractual-informal and technological springs.	Overcome the deficiencies in the transmission of chain information inherent to the open market.
Effects on agrarian social structure	Refunctionalization of agriculture.	CF is an "atypical" integration modality but fully functional to the process of capitalist accumulation.	

Secondly, it is worth mentioning that the three currents agree in affirming that the expansion strategies developed by LAC are, in general terms, threefold. First, vertical or direct integration, i.e., the company incorporates new stages of the agri-food chain into its ownership, such as plantations. Second, outsourcing, i.e., the company goes to the open market to acquire the necessary inputs for its production process. Third, indirect integration or quasi-integration, in which the business strategy consists of entering into agreements or pacts (especially in the form of contracts) with medium and small agricultural producers for the supply of agricultural products. Why does the last business strategy have several advantages over the previous ones and why has it expanded so much in recent years? The last point contains the answers offered by the different schools of thought.

Thirdly, regarding the elements available for LAC to increase CF levels in the territories, practically all three currents agree in pointing to the same elements (although each school address more effort to a particular advantage. Thus, these elements can be summarized as follows. First, more appropriation of the surplus extracted in the productive process (surplus as a synonym of surplus value in the case of the French and Latin American currents; surplus as a synonym of entrepreneurial profit for the Anglo-Saxon current). Second, transfer of risk to small and medium producers (according to the two Marxist-inspired currents), minimization of risk and uncertainty from the neo-institutionalist school. Third, from the Latin American school, two additional elements are raised, one contractual-informal (highlighting the loss of formal and real autonomy of the contracted farmers, and the laxity of the contracts for the contracting party, i.e., for the LAC); and the other technological (related to the scarce and limited transmission of technology from the *core* to the *satellites*).

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Characterization of pneumonia in alpacas (*Vicugna pacos*) in high Andean communities of Huancavelica, Peru

Caracterización de Neumonías en alpacas (*Vicugna pacos*) en comunidades altoandinas de Huancavelica, Perú

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Abstract

Pneumonic pathologies are clinically important in alpaca breeding; however, there is little information about pneumonic types and bacterial causes. The objective is to characterize pneumonias and bacteria causing pneumonias in neonates of dead alpacas in high Andean communities of Huancavelica, Peru. We sampled 365 dead alpaca pups under 45 days of age, from 10 communities, 216 of them were taken tracheal swabs for microbiological studies with peptone buffer because they were cases of pneumonia. Macroscopic identification of pneumonias was done by photodocumentation test. *Pasteurella multocida, Mannheimia haemolytica* were grown on Colombia agar enriched with alpaca blood; *Streptococcus pneumoniae* on Brain Heart Infusion agar supplemented with alpaca blood and identified by morphological and microscopic characterization, biochemical reactions and optokine. It was found 59.8% of mortality due to pneumonia and 40.2% by other causes, characterizing pneumonia by inflammation 55.1%, 44.9% by lesion and subtypes: Exudative 9.5%, Suppurative 3.8%, Proliferative 42.3%, Lobar 17.8%, Interstitial 26.6%, isolating strains *Streptococcus pneumoniae* 43.7%, *Mannheimia haemolytica* 14.9%, *Pasteurella multocida* 20.9%, predominant associates *Streptococcus pneumoniae-Pasteurella multocida* 10.7%. High frequency of types and subtypes of pneumonia associated to Gram (+) bacteria were observed as causal of pneumonia in alpaca pups, with predominance in three high Andean communities of Huancavelica, Peru.

Keywords: Alpaca, camelids, cleavage, fertilization, oocytes, reproduction.

Resumen

Las patologías neumónicas son de importancia clínica en crianza de alpacas; sin embargo, el conocimiento de tipos neumónicos y las causas bacterianas es muy limitado. El objetivo es caracterizar las neumonías y las bacterias causantes de neumonías en neonatos de alpacas muertas en comunidades altoandinas de Huancavelica, Perú. La muestra consistió en 365 crías muertas de alpacas menores de 45 días de edad, procedentes de 10 comunidades; se tomaron hisopados traqueales en 216 de ellas para estudios microbiológicos con buffer peptonada por resultar casos de neumonías. La identificación macroscópica de neumonías se hizo mediante el test foto documentador. Se cultivaron la *Pasteurella multocida* y *Mannheimia haemolytica* en agar Colombia enriquecidas con sangre de alpaca; *Streptococcus pneumoniae* en agar Infusión Cerebro Corazón suplementadas con sangre de alpaca e identificadas mediante caracterización morfológica, microscópica, reacciones bioquímicas y optoquina. Se encontraron 59,8% de mortalidad por neumonía y 40,2% por otras causas, caracterizándose neumonía por inflamación 55,1%; 44,9% por lesión y subtipos: Exudativa 9,5%, Supurativa 3,8%, Proliferativa 42,3%, Lobar 17,8%, Intersticial 26,6%, aislándose cepas de *Streptococcus pneumoniae* 43,7%, *Mannheimia haemolytica* 14,9%, *Pasteurella multocida* 20,9%, asociadas con *Streptococcus pneumoniae-Pasteurella multocida* 10,7%. Se evidenció una alta frecuencia de tipos, subtipos de neumonías asociados a bacterias Gram (+) como causales de neumonías en crías de alpaca con predomino en tres comunidades altoandinas de Huancavelica, Perú.

Palabras clave: Alpaca, camélidos, clivaje, fertilización, ovocitos, reproducción.

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1 Introduction

Alpaca breeding is an important source of economic income for alpaca producers in Andean communities (Carpio, 1991), and alpaca fiber is an essential resource for the breeder, due to its high demand in the textile industry, as it is considered a special fiber (Morales et al., 2017). Likewise, the garments produced are considered luxury items in the international market (Rosadio et al., 2012), and its meat is known for its high protein content with low cholesterol (Cirilo et al., 2012; Carhuapoma De la Cruz et al., 2020); therefore, this activity is paramount for the economy of families in the high Andean zone of Peru (Carhuapoma De la Cruz et al., 2020).

Despite the goodness presented in this species, there are multiple factors that predispose it to various infectious respiratory diseases, such as sudden changes in extreme microclimates (Rosadio et al., 2011), transport, long grazing routes, overcrowding, inadequate management in productive activities (sanitary, reproductive, shearing), stress and immune deficiencies (Svensson et al., 2003; Carbonero et al., 2011). Among these pathologies that affect alpaca pups more frequently in the winter season (calving season), is pneumonia in its different clinical presentations that are not reported and are not scientifically validated in the etiological, symptomatologic and diagnostic perspective as well as its treatment and control (Cirilo et al., 2012; Guzmán et al., 2013), resulting in high rates of neonatal mortality in alpacas, causing economic and genetic losses (Cirilo et al., 2012; Morales et al., 2017), and in many cases breeders tend to abandon the activity because it results in low economic profitability (Sicha et al., 2020; Carhuapoma De la Cruz et al., 2020); the responsible pathogen being Pasteurellosis of clinical virulence (Zanabria et al., 2000; Cozens et al., 2019), but in alpacas this pathogen is not well studied scientifically as other domestic species (Rosadio et al., 2011; Ramírez et al., 2012), making its treatment and control very complex.

There are reports indicating that *Pasteurella multocida, Mannheimia haemolytica* and *Streptococcus pneumoniae* are usually agents responsible for pneumonic processes in domestic species, taking part of the oral tract, respiratory tract, and gastrointestinal system (Boukahil and Czuprynski, 2018), managing to colonize lungs and alveoli and affecting mainly immunocompromised animals (Cirilo et al., 2012), developing clinical signs of different pneumonic types and subtypes in cattle, sheep, swine, and poultry with high mortality rates (Carbonero et al., 2011).

Two sub-types of pneumonia were reported superficially in South American camelids, especially in alpacas, and *Pasteurella multocida* was observed as a bacterial agent involved in pneumonic processes (Rosadio et al., 2011; Cirilo et al., 2012; Rímac et al., 2017), and possibly as part of the nasal microbiota, as occurs in other domestic animals (Rosadio et al., 2012; Rímac et al., 2017). However, there are few studies on the characterization of pneumonic cases, types, subtypes and the identification of bacterial agents, causing high mortality in alpacas. The aim of this study is to characterize pneumonias and bacteria causing pneumonias in dead neonatal alpacas in high Andean communities of Huancavelica, Peru, to obtain more knowledge of pneumonias and their etiological agents, so that control and prevention strategies can be developed to minimize alpaca mortalities, improving the socioeconomic conditions of the breeder in the high Andean areas, since alpaca breeding is essential for their subsistence.

2 Materials y Methods

2.1 Study Area

The field studies were between January and March 2020 in the high Andean communities of Pastales Huando, Cachimayo, Sacsamarca, Astobamba, Pucapampa, Asociación Lachocc, Santa Barbara, Carhuancho, Choclococha and Matipaccana in Huancavelica-Peru, located between 4200 and 5200 masl, with annual average temperature of $5-8°C$. However, the first step to start the research was to obtain the communal authorization and the informed consent of the breeder. Bacteriological studies were carried out in the Animal Health laboratory, in the microbiology area of the Universidad Nacional Huancavelica-Peru.

2.2 Characterization of pneumonias

365 dead Huacaya alpaca pups were collected, who apparently died due to pneumonia with ages less than 45 days old and less than 12 hours *post mortem*,

without distinction of sex, excluding animals with pharmacological treatments.

An independent *in situ* necropsy was performed on the 364 dead alpaca pups, following the necropsy technique in domestic animals (Aldrete, 2002) under strict biosecurity measures. The characterization of pneumonia cases, pneumonic types and sub-types was performed by inspection and macroscopic visualization of lesions and anatopathological inflammatory processes of lung structure, considering alterations at the level of lobes, internal parenchyma, change of morphology (color and/or consistency, appearance and distribution of exudate), hemorrhagic foci through the use of the Photo Documentary Test of Pneumonic Pathologies (Pijoan et al., 1999), recording cards as appropriate: inflammatory reaction pneumonia (exudative, suppurative bronchopneumonia, fibrinous pneumonia, proliferative pneumonia) and lesion diffusion pneumonia (bronchopneumonia, lobar pneumonia, interstitial pneumonia, embolic pneumonia, granulomatous pneumonia), as shown in Figure 1: a, b and c.

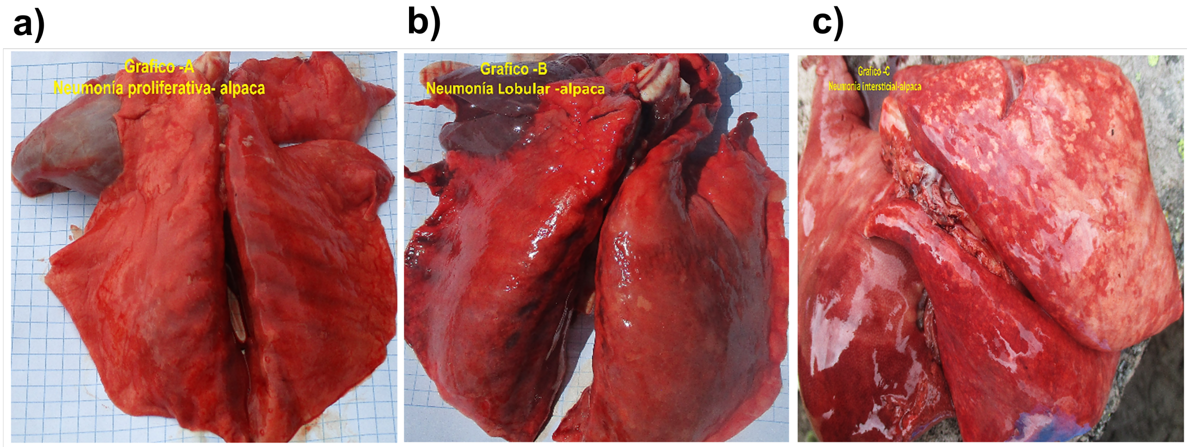

Figure 1. Characterization of pneumonia in alpaca pups. a) Proliferative pneumonia. b) Lobular pneumonia. c) Interstitial pneumonia.

2.3 Bacteriological studies

216 tracheal fluid samples were taken by tracheal swabbing (Schaefer et al., 2012) in sterile bottles with 5% peptonized buffer (10ml) from pneumonia-positive dead pups from 10 high Andean communities of Huancavelica-Peru (Figure 2: a, b and c, following biosafety measures. They were labeled and transported in a Vaccine Carrier Thermos at $8-10°C$ with biological ice (Gel Pack) to the Animal Health Laboratory: microbiology area-Universidad Nacional Huancavelica, for their microbiological study.

2.4 Isolation and bacterial identification

The 216 tracheal swab samples positive for pneumonia were inoculated independently in TSA broth (Tryptonga Soya Agar) and incubated at $37°C$ for 5 hours. Then, they were sown individually by exhaustion in Columbia Base Agar for *Pasteurella multocida, Mannheimia haemolytica* in Brain Heart Infusion Agar (BIH), both enriched with 5% sterile defibrinated alpaca blood (SAD), adding Gentamicin (0.75 μg/mL) as inhibitor of microorganisms and incubated at $37°C$ for 24 hours in aerobic processes (Avril et al., 1990; Moore et al., 1994). Brain Heart Infusion Agar (BIH) enriched with 5% sterile defibrinated alpaca blood (SAD) was used for the culture of *Streptococcus pneumoniae*, adding Gentamicin (0.75 μg/mL) and incubated at $37°C$ for 48 hours under anaerobic conditions in Gaspar Jar after adding Anaerocult® P (Moore et al., 1994).

Pasteurella multocida, Mannheimia haemolytica and *Streptococcus pneumoniae* strains were identified by macroscopic (shape, color, border, elevation and consistency), microscopic (Gram staining), hemolytic (β hemolytic, α hemolytic, γ hemolytic), biochemical tests (TSI, LIA, SIMON, SIM), Catalase (Fegan et al., 1995) and optochin susceptibility test (Rosadio et al., 2012).

2.5 Statistical analysis

The research was descriptive with a prospective cross-sectional approach (Campbell Donald and Stanley Julian, 1995). The frequency and relationship of mortality cases in alpaca pups, types, subtypes of pneumonia and pneumonic bacteria, between the number of animals diagnosed positive against the total number of alpaca pups evaluated were determined by the Chi-square test ($p < 0{,}05$), using the statistical package SPSS Vers. 21.0 and expressing the results in percentage frequencies.

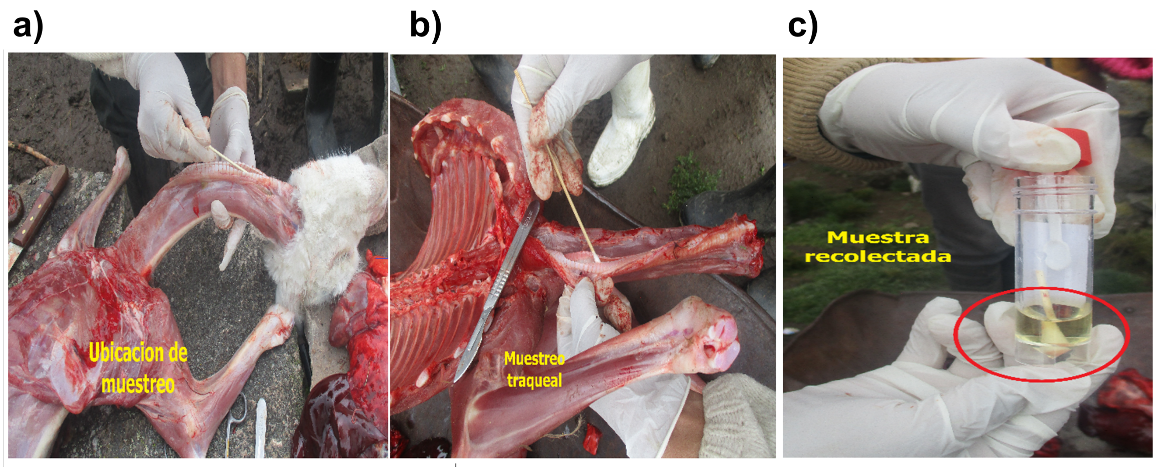

Figure 2. Sampling by tracheal swabbing in alpacas: a) Area of the collection. b) Tracheal sampling. c) Collected sample.

3 Results

A mortality rate of 59.8 % was found for pneumonia among the 10 communities. Out of this total, 3 communities were found to have higher mortality due to pneumonia; Choclocococha having 10.0 %, Pucapampa 8.3 % and Carhuancho 7.6 % (Table 1), showing a relationship between the causes of mortality and the community ($p = {,}000$) with a level of significance according to the Chi-square analysis ($p < 0{,}05$).

The most frequent type of pneumonia found was pneumonia due to inflammation (55.1 %) and the least frequent was pneumonia due to lesion (44.9 %), of which, the communities with higher cases of pneumonia due to inflammation and lesion were: Pastales de Huando (8.4 %; 2.8 %), Pucapampa (12.0 %; 2.3 %), Carhuancho (7.4 %; 5.6 %) and Choclococha (5.6 %; 10.6 %), while the rest of the communities resulted in lower proportion (Table 2), finding a relationship between types of pneumonia and community ($p = {,}000$) according to Chi-square analysis ($p < 0{,}05$).

Table 1. Mortality frequency in alpaca (*Vicugna pacos*) pups by pneumonia in the high Andean communities of Huancavelica ($n = 365$).

COMMUNITY	Mortality by pneumonia		Mortality by other causes	
	F	%	F	%
Pastales de Huando	24	7.0	7	2.0
Pucapampa	31	8.2	5	1.4
As. Lachocc	21	6.1	17	4.5
Santa Bárbara	26	7.1	9	2.4
Sacsamarca	15	4.1	27	7.3
Astobamba	12	3.3	20	5.4
Cachimayo	14	3.7	20	5.4
Carhuancho	28	7.6	10	2.5
Choclococha	35	10.0	8	2.2
Matipaccana	10	2.7	26	7.1
TOTAL	216	59.8	149	40.2

Legend: F= Frequency of mortality;%= percentage of mortality.

Types of inflammatory pneumonia and subtypes by lesion were identified, being exudative 9.5%, suppurative 3.8%, proliferative 42.3% and lobar 17.8%, Interstitial 26.6%; evidenced in 3 communities (Pastales Huando, Carhuancho, Choclococha) with high rates of cases of 5 subtypes of pneumonia (Table 3), finding relationship between subtypes of pneumonia and community with significance level ($p =$,000), according to Chi-square analysis ($p <$ 0,05).

Table 2. Frequency of types of pneumonia in alpaca (*Vicugna pacos*) pups in high Andean communities of Huancavelica-Peru ($n = 216$).

COMMUNITIES	Sub-types of pneumonia									
	Inflammatory pneumonia						Pneumonia by lesion			
	Exudative		Suppurative		Proliferative		Lobar		Interstitial	
	F	%	F	%	F	%	F	%	F	%
Pastales de Huando	4	1.8	2	0.9	12	5.6	2	0.9	4	1.8
Pucapampa	2	0.9	3	1.4	21	9.7	1	0.5	4	1.8
A. Lachocc	1	0.5	1	0.5	6	2.7	3	1.4	10	4.6
Santa Bárbara	1	0.5	-	0	9	4.2	8	3.6	8	3.6
Sacsamarca	3	1.4	1	0.5	8	3.6	3	1.4	-	-
Astobamba	-	-	1	0.5	7	3.2	4	1.8	-	-
Cachimayo	-	-	-	-	6	2.7	8	3.6	-	-
Carhuancho	4	1.8	-	-	12	5.6	3	1.4	9	4.2
Choclococha	4	1.8	-	-	8	3.6	5	2.3	18	8.3
Matipaccana	-	-	-	-	3	1.4	2	0.9	5	2.3
TOTAL	19	9.5	8	3.8	92	4.3	39	17.8	58	26.6

Legend: F= Frequency of cases;%= percentage of cases.

From a total of 216 cultured samples, strains of *Streptococcus pneumoniae* 43.1%, *Mannheimia Hemolitica* 14.7%, *Pasteurella Multocida* 20.5% and *Streptococcus pneumoniae - Pasteurella Multocida* (10.4%) with double associated predominance were isolated; observing high prevalence of independent and associated strains in the communities of Pucapampa, Pastales Huando and Choclococha, and low prevalence in the rest of the communities (Table 4; Figure 3: a, b and c), finding the relationship between bacterial agents and community ($p =$,000) according to Chi-square analysis ($p <$ 0,05).

Table 3. Frequency of pneumonia subtypes in alpaca (*Vicugna pacos*) pups in high Andean communities of Huancavelica-Peru ($n = 216$).

COMMUNITY	Types of pneumonia			
	Pneumonia by inflammation		Pneumonia by lesion	
	F	%	F	%
Pastales de Huando	18	8.4	6	2.8
Pucapampa	26	12.0	5	2.3
Lachocc	8	3.7	13	6.0
Santa Bárbara	10	4.6	16	7.4
Sacsamarca	12	5.6	3	1.4
Astobamba	8	3.7	4	1.9
Cachimayo	6	2.7	8	3.7
Carhuancho	16	7.4	12	5.6
Choclococha	12	5.6	23	10.6
Matipaccana	3	1.4	7	3.2
TOTAL	119	55.1	97	44.9

Legend: F= mortality rate in alpaca pups, %= mortality percentage of alpaca pups.

4 Discussion

The study reports 59.8% mortality of alpaca pups due to pneumonia in 10 high Andean communities of Huancavelica -Peru, resulting in 3 communities with high rates of pneumonic cases, which may be due to constant changes in extreme microclimates (prolonged snowfall, hailstorms, constant rainfall), long walks for grazing, inadequate management in livestock activities (Mamani et al., 2009), and aspiration of amniotic fluid at the time of delivery, or milk aspiration by false swallowing (Mamani et al., 2009), resulting in factors that are determinant for pneumonia in domestic animals (Guzmán et al., 2013).

Pneumonias are clinically important in the production of alpaca; however, there are few studies. Thus, Mamani et al. (2009) reported 31.12% of pneumonias in alpacas; Manchego et al. (1998) found mortalities due to pneumonia in alpaca pups with cyclical and irregular trends in rainy seasons in January (9.5%), February (17.5%), March (2.2%); Álvarez (2011) reported 48.1% mortality in pups due to CRB. The results of the study are usually slightly higher than the previous ones; therefore, apparently breeders do not practice adequate sanitary management strategies in alpaca pups.

Pneumonia types and subtypes are typical in cattle, swine and guinea pigs (Gonçalves et al., 2001; Boukahil and Czuprynski, 2018); reporting types of pneumonia: Inflammatory 55.1%, lesion 44.9%; subtypes of pneumonia: exudative 9.5%, suppurative 3.8%, proliferative 42.3%, lobar 17.8% and Interstitial 26.6%, observing high trends in 3 communities; these figures differ from those reported by Guzmán et al. (2013) who found 11 cases of multilobar pneumonia associated with fibrinous pleuritis and pulmonary edema in tuis alpacas. Cirilo et al. (2012) found 3 cases of suppurative fibrinnecrotizing bronchopneumonia, 10 cases of suppurative bronchopneumonia in neonatal alpacas; in other species Ramírez (2015) reported 8% of interstitial pneumonia, 6.3% of chronic suppurative bronchopneumonias, 12.2% of mucopurulent bronchitis in cattle. On the other hand, Guerrero (2011) found 48.6% of interstitial pneumonia in guinea pigs with predominance in adult males.

The high cases of types and subtypes of pneumonias found in neonatal alpacas would be linked to extreme changes of microclimates in high Andean communities (Rosadio et al., 2011; Rímac et al., 2017), complete or partial failure of transfer and low levels of immunoglobulins in colostrum (Rímac, 2016; Lucas et al., 2016), favoring massive proliferation of pneumonic bacteria (*M. Hemolytica, P. Multocida* and *S. pneumoniae*) that secrete Lipo poly saccharide (LPS), extracellular leukotoxin (Lkt) mediators of proinflammatory cytokines, hemorrhagic procoagulants and tumor necrosis alpha (TNF-α) in lungs of domestic animals (Kumar et al., 2009; Highlander, 2001), originating inflammatory tissue lesions in the lungs of alpaca pulps, making pharmacological treatment complex and in many cases unsuccessful (Gonçalves et al., 2001; Singh et al., 2011).

Table 4. Frequency of pneumonic bacterial agents present in alpaca (*Vicugna pacos*) pups in the communities of Huancavelica - Peru ($n = 216$).

COMMUNITY	Pneumonic bacterial agents											
	Streptococcus pneumoniae		***Mannheimia-Hemolytica***		***Pasteurella Multocida***		***Streptococcus Pneumoniae-Mannheimia Hemolytica***		***Streptococcus pneumoniae-Pasteurella Multocida***		***Mannheimia Hemolytica-Pasteurella Multocida***	
	F	%	F	%	F	%	F	%	F	%	F	%
Pastales Huando	14	6.3	0	-	7	3.2	0	-	3	1.3	0	-
Pucapampa	15	6.9	0	-	11	5.0	0	-	5	2.3	0	-
A. Lachocc	8	3.7	9	4.2	0	-	4	1.9	0	-	0	-
Santa Bárbara	12	5.5	10	4.6	0	-	4	1.9	0	-	0	-
Sacsamarca	4	1.9	3	1.3	3	1.3	2	0.9	2	0.9	1	0.5
Astobamba	3	1.3	5	2.3	0	-	4	1.9	0	-	0	-
Cachimayo	7	3.2	0	-	5	2.3	0	-	2	0.9	0	-
Carhuancho	11	5.0	5	2.3	4	1.9	3	1.3	2	0.9	3	1.3
Choclococha	16	7.4	0	-	12	5.5	0	-	7	2.8	0	-
Matipaccana	4	1.9	0	-	3	1.3	0	-	3	1.3	0	-
TOTAL	94	43.7	32	14.9	45	20.9	17	7.9	23	10.7	4	1.9

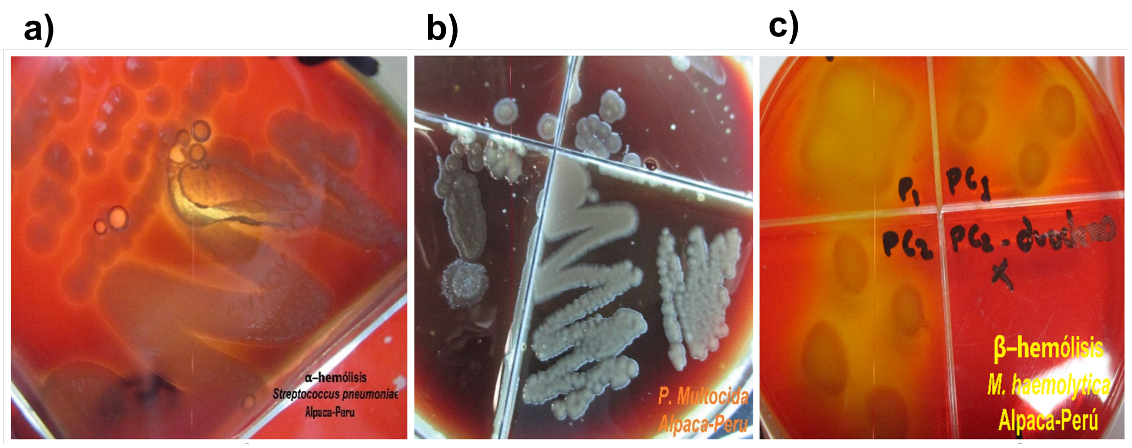

Figure 3. Isolated bacteria from alpaca pups with pneumonia (S-1, P-1 and M-1): a) Cepa S-1: α-haemolysis. b) Cepa P-1: *P. Multocida*. c) M-1: β-haemolysis.

The study reported *S. pneumoniae* 43.7 %, *M. Hemolitica* 14.9 %, *P. Multocida* 20.9 %, double associates between 10.7 % and 1.9 %; these results differ from those of Rímac et al. (2017), who isolated 24 strains of *P. Multocida* from 46 lungs of neonatal alpacas aged 1-2 months with pneumonia. Similarly, Cirilo et al. (2012) in 22 samples isolated 11 positive cases of *P. Multocida*, 7 of *M. Hemolitica* in alpaca neonates with acute pneumonias, results that differ from Pijoan et al. (1999), who isolated *P. Multocida* in 34 cases, *Salmonella* spp. in 12 cases and *Staphylococcus* spp. in 7 cases in calves with pneumonia. Tocqueville et al. (2017) found *P. Multocida* with a load 10^5, 10^7 and 10^8 in pneumonic swine; Gamal et al. (2016) found 5 isolates of *P. Multocida* KMT1 in sheep with 94 % homology to buffalo; regarding *S. pneumoniae* we differ to all reported, resulting as the first case reported in alpacas whose virulence would be originated by genes PM 2.5, luxA-E,ST615 (Herbert et al., 2018; Chen et al., 2020; Panagiotou et al., 2020).

P. multocida, *M. haemolytica* and *S. pneumoniae* are part of the microbiota of oral tract, respiratory tract, gastrointestinal system in domestic and wild animals (Boukahil and Czuprynski, 2018), being the first two bacteria frequent in alpacas (Cirilo et al., 2012; Rímac et al., 2017). However, *S. pneumoniae* is frequent in humans and rodents (Sandgren et al., 2005), justifying the presence of *P. multocida, M. haemolytica* in the study and its virulence would be influenced by serovars 10, 11, 12 ,15 of toxA, tbpA, pfhA genes of low genetic diversity (Rímac, 2016; Rímac et al., 2017), and by the excessive and improper use of growth-promoting antimicrobials to antibacterial resistance possibilities (Carhuapoma et al., 2018; Carhuapoma De la Cruz et al., 2020), promoting colonization processes and cross-reactivity to subclinical infections in the animal (Díaz et al., 2017), leading to respiratory failures and progressive death in alpaca pups.

The study shows that literature to characterize pneumonic conditions and their etiology is necessary to understand the clinical significance and to achieve an effective and timely therapeutic intervention for a sustainable production of alpacas and other domestic animals.

5 Conclusions

High rates of pneumonia of different types and subtypes associated with pneumonic bacteria such as *Mannheimia Hemolitica, Pasteurella Multocida* and *Streptococcus pneumoniae* were observed in dead alpaca neonates. Similarly, it is observed that three high Andean communities present high trends of pneumonias associated with Gram (+) bacteria.

Data confidentiality

The authors declare that they have followed a strict methodological approach to obtain the bases and adequate statistical model and program for data processing.

Right to privacy and informed consent

The authors declare that no patient or animal data appear in this article and that communal authorization and informed consent from the breeder were required.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Scientific paper/ Artículo científico
Agronomy

Response of the pea crop (*Pisum sativum* L.) to the application of organic fertilizers in the municipality of Pamplona, North of Santander

Respuesta del cultivo de arveja (*Pisum sativum* L.) a la aplicación de abonos orgánicos en el municipio Pamplona, Norte de Santander

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Abstract

In Pamplona pea production is based on the use of high doses of chemical fertilizers that cause environmental damage and human health. Therefore, in this study the effect of different organic fertilizers was compared with chemical fertilization through six treatments: T0: control; T1: vermicompost full dose (7831,00 kg/ha); T2: vermicompost half dose (3915.50 kg/ha) + chemical fertilizer (FQ 15N 15P_2O_5 15K_2O) half the dose (703.50 kg/ha); T3: chicken manure + goat manure + sugarcane residues full dose (ABOB: 10573.00 kg/ha): T4: ABOB half dose (1407.00 kg/ha) plus CF half dose (703.50 kg/ha); T5: CF full dose (1407.00 kg/ha); T6: CF half the dose (703.50 kg/ha). It was evaluated: plant height (AP), pods per plant (NVP); pod length (LV) and yield (kg/ha). A 5% analysis of variance and a Tukey test for separation of means were applied. The mean AP was higher in T2 (172.27 cm). NVP was higher in T3 and T5 with respect to the control, however, between treatments no statistical differences were observed. LV was statistically higher in the treatments compared to the control, although there was no variation between treatments. Although no statistical differences were observed in the performance between treatments, T1 and T4 presented a performance superior to the control of 42.85% and 39.99%, respectively. It is possible to substitute or supplement chemical fertilizer with organic amendments and reduce the negative effect of pollution that they generate on the environment and the health of the people.

Keywords: Vermicompost, yield, chemical fertilization.

Resumen

La producción de arveja en Pamplona se basa en el uso de altas dosis de fertilizantes químicos que generan daños ambientales y a la salud humana. Por lo tanto, en este estudio se comparó el efecto de diferentes abonos orgánicos con la fertilización química mediante seis tratamientos: T0: control; T1: vermicompost dosis completa (7831,00 kg/ha); T2: vermicompost mitad de la dosis (3915,50 kg/ha) + fertilizante químico (FQ 15N $15P_2O_5$ $15K_2O$) mitad de la dosis (703,50 kg/ha); T3: gallinaza + caprinaza + residuos de caña de azúcar dosis completa (ABOB: 10573,00 kg/ha): T4: ABOB mitad dosis (1407,00 kg/ha) más FQ mitad de la dosis (703,50 kg/ha); T5: FQ dosis completa (1407,00 kg/ha); T6: FQ mitad de la dosis (703,50 kg/ha). Se evaluó: altura de la planta (AP), vainas por planta (NVP); longitud de las vainas (LV) y rendimiento (kg/ha). Se aplicó un análisis de varianza al 5 % y una prueba de Tukey para la separación de medias. La AP promedio fue mayor en T2 (172,27 cm). El NVP fue más alto en T3 y T5 con respecto al control, sin embargo, no se observaron diferencias estadísticas entre tratamientos. La LV fue estadísticamente más alta en los tratamientos con respecto al control, aunque no hubo variación entre tratamientos. Sin embargo, no se observaron diferencias estadísticas en el rendimiento entre tratamientos, y T1 y T4 presentaron un rendimiento superior al control de 42,85 % y 39,99 %, respectivamente. Es posible sustituir o complementar el fertilizante químico con enmiendas orgánicas y reducir el efecto negativo de contaminación que generan sobre el ambiente y la salud de las personas.

Palabras clave: Vermicompost, rendimiento, fertilización química.

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1 Introduction

Growth of population leads to an increase in the demand of food, which must be met by increased agricultural production. In conventional agriculture it is achieved through the use of large quantities of chemical inputs, including fertilizers, which allows rapid plant growth. However, these products generate serious soil, water and air pollution problems (FAO, 2019; Latorre and Villamizar, 2019).

Pea (*Pisum sativum* L.) belongs to the Fabaceae family and it is an important source of protein (22-25%), carbohydrates, phosphorus, iron, magnesium, calcium, riboflavin, niacin, thiamine and ascorbic acid (Watt and Merrill, 1993; Dahl et al., 2012). Regarding agriculture, it plays an important role for its contribution to nitrogen fixation and soil fertility improvement (Davies et al., 1985; Gopinath and Mina, 2011).

In Colombia, it is the most important legume after beans. It is grown mainly in high altitudes with cold and medium climates (2200 and 3000 masl) and it is produced in Cundinamarca, Boyacá, Nariño and Tolima (Peñaranda and Molina, 2011; DANE, 2015; FENALCE, 2015). Small and medium producers crop this legume and it is considered a staple food in the family basket. Ninety-five percent of pea production at the national level is destined for direct human and animal consumption as a grain rich in protein and the remaining 5% for the production of dry peas as seed (Buitrago et al., 2006; FENALCE, 2015).

In the province of Pamplona, peas represent an alternative for the development of the local economy and the generation of employment and income. In addition, the area has optimal climatic conditions (between 10 and 17 °C) and the availability of labor for production (Peñaranda and Molina, 2011). In this crop, farmers traditionally fertilize with chemical formulas because they allow a rapid availability of elements for the plant, favoring some growth and yield variables. However, this implies high costs for obtaining inputs in addition to the environmental damage caused by chemical fertilization (González et al., 2015).

There is a growing awareness of the environmental damage caused by the use of non-renewable chemical resources in agriculture. Therefore, research has been directed towards alternatives such as the implementation of organic fertilizers, which is rapidly expanding worldwide (Willer et al., 2020; Flores et al., 2021; González-García et al., 2021). These techniques are less expensive and more environmentally friendly, so that the agricultural products obtained under these conditions are healthier and with better quality.

Additionally, the use of single or combined organic fertilizers with chemical fertilizers contributes to improve the physical, chemical and biological properties of soils (Heinze et al., 2010; Lalito et al., 2018; Mohammed et al., 2019; Flores et al., 2021). Specifically, they increase soil organic carbon content, provide nutrients necessary for the growth of microorganisms, temporarily reduce the toxicity of soluble and exchangeable aluminum due to the formation of chelates with organic substances in acid soils, all of which are observed in an increase in vegetative growth and plant yield (Suresh et al., 2004; Al-Bayati et al., 2019; Mohammed et al., 2019; Mátyás et al., 2020).

The aim of this research is to evaluate the effect of two organic fertilizers on the yield of pea (*Pisum sativum* L.) in the municipality of Pamplona, North of Santander, in comparison with chemical fertilization in order to offer an environmentally friendly, economically viable and socially acceptable production alternative.

2 Materials and Methods

2.1 Study area

The study was carried out in the experimental plots of Plant Health and Bioinputs Research Center (CISVEB) of Universidad de Pamplona, municipality of Pamplona, North of Santander. This area is located at 2331 meters above sea level; the climate in Pamplona is warm and temperate and according to the Köppen-Geiger climate classification it is maritime west coast (oceanic) (Cfb). The average annual temperature oscillates around 14.4 °C, with May being the hottest month of the year with an average of 15.0 °C and January the coldest month of the year with an average temperature of 13.3 °C. The average annual precipitation is 921 mm, there is precipitation throughout the year with the driest month being in January with 21 mm and the wettest month in April with an average of 141 mm (Climate-Data.Org, 2020).

Table 1 shows the results of the laboratory analysis of the soils in the area where the experiment was carried out.

Table 1. Physical and chemical characteristics of the soils of the Plant Health and Bioinputs Research Center (CISVEB) of Universidad de Pamplona, Pamplona municipality, North of Santander.

Soil variables	Value
Textural class	Sandy loam
Sand (%)	62.00
Clay (%)	22.00
Silt (%)	16.00
pH	6.00
Organic carbon (%)	2.46
Phosphorus (ppm)	15.1
Calcium (meq/100 g)	17.1
Magnesium (meq/100 g)	1.09
Sodium (meq/100 g)	0.12
Potassium (meq/100 g)	0.29
Boron (ppm)	0.24
Iron (ppm)	83.8
Manganese (ppm	2.76
Copper (ppm)	0.67
Zinc (ppm)	1.36

Source: Laboratorio químico de suelos (2018). Universidad Industrial de Santander.

2.2 Experimental design

A randomized block design with seven treatments and three replications was used. An experimental area of 504 m^2 was selected and divided into three blocks of 21 m × 7 m each (147 m^2 each block) and a separation of 1.5 m between blocks. Each block was divided into seven 7 m × 3 m (21 m^2/plot) for the distribution of treatments with a separation of 30 cm between plots. Three furrows were planted in each plot at a distance of one meter between them and approximately three centimeters between seeds, resulting in a population density of 333333.00 plants/ha.

Seven treatments were applied as described below: T0: Control; T1: Vermicompost full dose (VC100% = 7831.00 kg/ha or 16.44 kg/plot of 21 m^2); T2: Vermicompost half dose (VC50% = 3915.50 kg/ha or 8.22 kg/plot of 21 m^2) + Chemical Fertilizer (15N 15P_2O_5 15K_2O) half dose (FQ50% = 703.50 kg/ha or 1.48 kg/plot of 21 m^2); T3: Hen manure + goat manure + sugarcane residues full dose (GCR100% = 10573.00 kg/ha or 22.20 kg/plot of 21 m^2): T4: GCR half dose (GCR50% = 5286.50 kg/ha or 11.10 kg/plot of 21 m^2) + Chemical Fertilizer (15N 15P_2O_5 15K_2O) half dose (FQ50% = 703.50 kg/ha or 1.48 kg/plot of 21 m^2); T5: Chemical fertilizer full dose (FQ100% = 1407.00 kg/ha or 2.95 kg/plot of 21 m^2); T6: Chemical fertilizer (15N 15P_2O_5 15K_2O) half dose (FQ50% = 703.50 kg/ha or 1.48 kg/plot of 21 m^2).

The calculation of the doses of organic and chemical fertilizers in kg/ha was made based on the amount of nutrients available in the soil, according to the results obtained in the laboratory analysis and the nutritional requirements of the crop.

2.3 Application of organic manures, chemical fertilizer and crop management.

Two commercial organic fertilizers were used:

- Vermicompost: made from worm compost and commercially known as Ferticampo. This compost has a total nitrogen percentage of 2.70%, 0.82% total phosphorus, 3.06% total potassium, 2.63% calcium oxide, 0.68% magnesium, 13.70% total oxidizable carbon, a C/N ratio of 10.77 and a cation exchange capacity of 34.42 cmol(+)/kg of soil. The calculated dose of vermicompost was 7831.00 kg/ha according to the results of the soil analysis, while the equivalent dose was 16.44 kg/plot of 21 m^2 for the experimental area.

- GCR: organic fertilizer composed of hen manure, goat manure and sugarcane residues, commercially known as *Abonos orgánicos de Boyacá* (ABOB). This fertilizer has 2.00% of total nitrogen, 5.00% of total phosphorus (P_2O_5), 3.0% of water soluble potassium (K_2O), 10.00% of calcium (CaO), 24.00% of silicon (SiO_2), 35.00% of organic fertilizer, 9.00% oxidizable carbon, C/N ratio of 7.50 and cation exchange capacity of 25.00 cmol(+)/kg of soil. The calculated dose per hectare was 10573.00 kg, while it was 22.20 kg for the area of each plot.

- Chemical fertilizer: Triple 15 chemical fertilizer (15% N 15% P_2O_5 15% K_2O) was used at a calculated dose of 1407.00 kg/ha (2.95 kg/plot of 21 m^2).

The soil was prepared with a power tiller for a better soil conditioning at the time of planting, following the planting methods of the producers in the area. Both chemical and organic fertilization was carried out 15 days after planting. The trellising was established thirty days after planting to hang the pea plants to facilitate the crop and better manage weed and disease control. Weed control was carried out manually every eight days, removing weeds that could compete and reduce crop yield, since peas are not very competitive and needed strict weed control to avoid low yields at the end of the harvest.

Pest and disease control was carried out at the beginning of the crop due to the presence of slugs, birds and cuttings; it was done chemically and manually by applying different products such as Babosil at a dose of 20 kg/ha every 15 days. Lorsban (2.50% × 1 kg (Chlorpyrifos) of 3.00 to 5.00 cc/L) was applied to minimize damage to the plants during growth and development. In addition, scarecrows were placed in several points of the lots. For the control of diseases caused by some fungi such as anthracnose (*Ascochyta* spp), Mancoz33ed (dispersible granules: WG 75%) was applied preventively at a dose of 200 g/100 L of water during the initial growth stage of the plants and with a frequency of seven to 10 days. For the control of downy mildew (*Peronospora corda*), Ziram (zinc dimethyl-dithiocarbamate 760 g/Kg in dispersible granules: WG 76%) was applied at a dose of 240 to 300 g/100 L of water preventively before and after flowering, especially due to the climatic conditions of high rainfall that occurred during the development of the experiment.

Irrigation was carried out manually with an irrigation frequency of every two days depending on weather conditions. Approximately 250 to 380 mm of water were applied throughout the crop cycle, always guaranteeing good soil moisture availability. Harvesting was carried out 105 days after manual planting when the pea pods had reached maturity and was filled with fruit.

2.4 Study variables

Pea seeds (*Pisum sativum* L.) of *Rabo de gallo* variety were used for sowing, which is well known and preferred by local producers in Monte Adentro district due to its characteristics of good quality, large grain, large number of grains per pod and high resistance to pest attack. The variables evaluated were:

2.4.1 Plant height (AP)

Plant height was measured at 30, 60 and 90 (AP30, AP60 and AP90, respectively) days after planting. For this purpose, 20 plants were taken at random per experimental unit and the height was measured from the base of the plant to the last leaflet on the days indicated above, using a tape measure. The data were expressed in cm.

2.4.2 Length of green pods (cm) (LV)

The length of the pods was measured at the time of harvest. Twenty plants were taken from each experimental plot. From each plant, 5-10 pods were selected from the second third where the most developed pods were concentrated and the average length expressed in cm was obtained.

2.4.3 Number of green pods per plant (VP)

The total number of green pods per plant was counted from the 20 plants selected in each experimental plot.

2.4.4 Yield (kg/ha)

It was determined by weighing the total number of pods per experimental plot. The crop was harvested when the plants were at main stage number 7, code 79 according to the BBCH scale, in which the pods have already reached the typical size (green maturity), and were fully formed (Enz and Dachler, 1998).

2.5 Statistical analysis

For the statistical analysis of the data, a one-way analysis of variance (ANOVA) was applied. When the ANOVA was significant ($p<0.05$) a Tukey test was applied for separating means. Pearson's linear correlation analysis was used to analyze the relationship between the study variables. The SPSS version 21 statistical package was used to analyze the data at a significance level of 0.05.

3 Results and discussion

3.1 Plant height

According to the results presented in Table 2, 30 days after planting in the field, plant height (AP) in T0 was significantly higher ($p<0.05$) than the rest of the treatments, excepting T6, which did not show differences with T0.

AP was statistically lower 60 days after sowing ($p<0.05$) in T4 with respect to T2, T3 and T6, while no significant differences were observed between T0, T1, T2, T3, T5 and T6 ($p>0.05$). The AP at 60 days showed a better response of the crop to the treatments. The greatest height was observed in T6 (FQ50%) and T2 (VC50% + FQ50%), but there were no differences between them. The lowest height was observed in T4 with the mixture GCR + 50% + FQ50%, which shows that plants did not respond favorably to this combination.

The greatest plant height was found in T2 90 days after planting (172.27±12.70 cm) which was statistically higher than T0, T4 and T6 (Table 2). According to these results, maybe the PA in T0 was higher at the beginning of the trial because the organic fertilizers and the chemical fertilizer were not applied during sowing but 15 days after, therefore, they did not have enough time to solubilize (Álvarez-Sánchez et al., 2006; Flores et al., 2021).

Table 2. Height of pea plants at 30, 60 and 90 days after planting in response to organic and chemical fertilization in the municipality of Pamplona, North of Santander.

Treatments	Plant height (cm)		
	30 days	**60 days**	**90 days**
T0	25.72 ± 2.94^{ac}	97.80 ± 9.04^{ab}	144.36 ± 18.97^{a}
T1	23.81 ± 2.74^{b}	97.43 ± 12.75^{ab}	165.85 ± 14.92^{b}
T2	23.67 ± 2.99^{b}	102.13 ± 8.34^{b}	172.27 ± 12.70^{b}
T3	22.73 ± 2.98^{b}	101.88 ± 13.24^{b}	171.76 ± 20.43^{b}
T4	22.56 ± 3.33^{b}	93.96 ± 9.41^{a}	155.37 ± 16.10^{a}
T5	22.33 ± 3.03^{b}	99.56 ± 10.03^{ab}	165.55 ± 19.76^{b}
T6	24.81 ± 3.30^{c}	103.41 ± 12.18^{b}	162.13 ± 22.75^{a}

Mean values ± standard deviation accompanied by different lowercase letters indicate statistical differences ($p<0.05$) between treatments. T0: Control; T1: Vermicompost full dose (VC100% = 7831.00 kg/ha or 16.44 kg/plot of 21 m^2); T2: Vermicompost half dose (VC50% = 3915.50 kg/ha or 8.22 kg/plot of 21 m^2) + Chemical Fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50% = 703.50 kg/ha or 1.48 kg/plot of 21 m^2); T3: Hen manure + goat manure + sugarcane residues full dose (GCR100% = 10573.00 kg/ha or 22.20 kg/plot of 21 m^2): T4: GCR half dose (GCR50% = 5286.50 kg/ha or 11.10 kg/plot of 21 m^2) + Chemical Fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50% = 703.50 kg/ha or 1.48 kg/plot of 21 m^2); T5: Chemical fertilizer full dose (FQ100% = 1407.00 kg/ha or 2.95 kg/plot of 21 m^2); T6: Chemical fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50% = 703.5 kg/ha or 1.48 kg/plot of 21 m^2).

On the other hand, the height of pea plants was 19.33% higher 90 days after planting in the treatment that used the combination of half the recommended dose of vermicompost organic fertilizer equivalent to 3915.50 kg/ha plus half the dose of triple 15 chemical fertilizer (703.50 kg/ha) suggested according to the results of the soil analysis, with respect to the control treatment where no treatment was applied. In this sense, the combined use of the organic fertilizer based on vermicompost with the chemical fertilizer had a significant positive effect on plant height compared to the control treatment.

The literature has indicated that organic fertilizer can increase the efficiency of chemical fertilizer use when applied combined, being a strategy for the high costs of chemical fertilizers or when are not easy to find, since it allows maintaining and increasing yields in the long term (Van Zwieten, 2018; El-Salehein et al., 2019).

Although the use of vermicompost plus chemical fertilizer (T2) was statistically superior to the control, there was no difference between the treatments using full dose vermicompost (T1: 7831.00 kg/ha); full dose of commercial fertilizer composed of hen manure, goat manure and sugar cane residues (T3: 10573.00 kg/ha) and the full dose of triple 15 chemical fertilizer (T5: 1407 kg/ha). The fact that no differences in plant height were found among these treatments (T1, T2, T3 and T5) shows that the use of chemical fertilizers can be reduced or substituted by organic fertilizer, always taking into account that the availability of nutrients from organic fertilizers such as animal manure and compost is usually low in the short term, so the response of the crop is more visible in the long term.

On the other hand, the application of the mixture of half the dose of ABOB commercial fertilizer (T4: 5286.50 kg/ha) plus half the dose of triple 15 chemical fertilizer (T4: 703.50 kg/ha), and the use of only triple 15 chemical fertilizer at half the recommended dose (703.50 kg/ha^2) did not represent a significant increase in plant height compared to the control treatment. In this regard, scientific evidence points out that the supply of nutrients from organic fertilizer depends largely on the source and quality of organic fertilizers used, as well as on the mineralization rate of the organic compounds present (Mukai, 2018; Van Zwieten, 2018).

Similar results were reported by El-Salehein et al. (2019), where treatment with farmyard manure + NPK fertilizer at half the recommended dose resulted in significant increases in *Pisum sativum* L. growth, fruit set, leaf chemical content, green pod yield and its components, and seed quality. Lalito et al. (2018) also found that the combination of chemical fertilization, vermicompost and plant debris improved growth attributes, yield and soil properties relative to the control, such as: greater plant height, number of leaves per plant, number of branches per plant, number of pods per plant, number of seeds per pod, seed yield, higher values of N, P_2O_5 and K_2O available in the soil after crop harvest.

Bautista-Zamora et al. (2017) also found an analogous response in the effect evaluation of the application of organic fertilizer (compost and vermicompost) and commercial fertilizer on the growth and development of *Phaseolus vulgaris* var. Cerinza, where plant height 56 days after planting was significantly higher in the compost and vermicompost treatments compared to commercial fertilization.

When comparing the plant height observed in this study with other studies, it was found that it was much higher than the one by Checa et al. (2017), in an investigation in which they carried out an agronomic evaluation of different pea varieties at different sowing times. Santamaría et al. (2010) also obtained plant height values below those found in this study 60 days after sowing, although it is worth noting that the best response they found in plant height was when using organic manure compared to chemical fertilizer.

This result is probably due to the type of seed used. In this case, it is the variety known locally as *Rabo de gallo*, which is highly adaptable to the soil and climatic conditions of the area and has good yields. This is a non-certified seed, which is obtained by farmers in the area and marketed in different agricultural stores in municipalities such as Pamplona and Ragonvalia in the North of Santander (Amaya, 2017).

3.2 Number of pods per plant (NVP)

Figure 1 shows that T5 presented a higher number of pods per plant (NVP) compared to the rest of the treatments ($p<0.05$), except T3 where the full dose of commercial fertilizer composed of hen manure, goat manure and sugarcane residues was used (ABOB: 10573.00 kg/ha). T0 presented lower NVP ($p<0.05$) compared to T1, T2, T3, T5 and T6, but did not differ from T4. On the other hand, no differences were observed between T1, T2 and T4 as well as between T3 and T6. Finally, T4 resulted in lower NVP compared to T3, T5 and T6 ($p<0.05$).

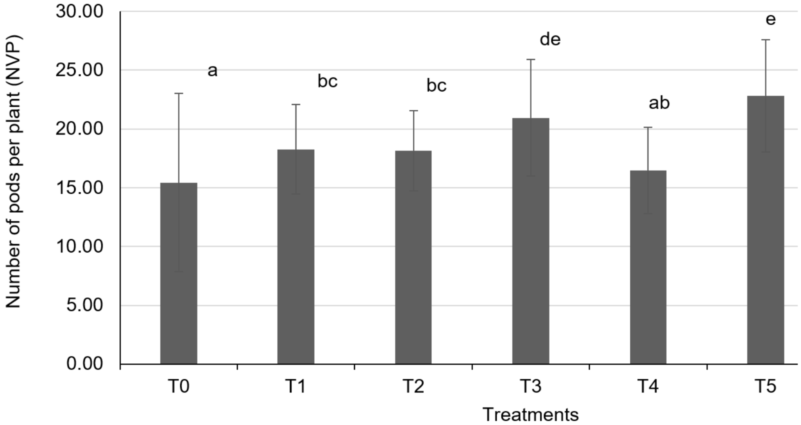

Figure 1. Number of pods per plant (NVP) in the pea crop in response to organic and chemical fertilization in the municipality of Pamplona, North of Santander. Bars with mean values ± standard deviation accompanied with different lowercase letters indicate statistical differences ($p<0.05$) among treatments. T0: Control; T1: Vermicompost full dose (VC100 % = 7831.00 kg/ha or 16.44 kg/plot of 21 m^2); T2: Vermicompost half dose (VC50 % = 3915.50 kg/ha or 8.22 kg/plot of 21 m^2) + Chemical Fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50 % = 703.50 kg/ha or 1.48 kg/plot of 21 m^2); T3: Hen manure + goat manure + sugarcane residues full dose (GCR100 % = 10573.00 kg/ha or 22.20 kg/plot of 21 m^2): T4: GCR half dose (GCR50 % = 5286.50 kg/ha or 11.10 kg/plot of 21 m^2) + Chemical Fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50 % = 703.50 kg/ha or 1.48 kg/plot of 21 m^2); T5: Chemical fertilizer full dose (FQ100 % = 1407.00 kg/ha or 2.95 kg/plot of 21 m^2); T6: Chemical fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50 % = 703.5 kg/ha or 1.48 kg/plot of 21 m^2).

The number of pods per plant is a very important yield component that determines crop productivity. The average NVP varied from 15.43 in the control treatment to 20.93 with the commercial fertilizer composed of hen manure, goat manure and sugarcane residues full dose (ABOB: 10573.00 kg/ha) and 22.83 with the full dose of the chemical fertilizer triple 15, finding no difference between the last two. In this regard, Jasim et al. (2016) found that both chemical and organic fertilization led to a significant increase in the number of pods per plant, pod length and seed yield of bean plants (*Visia faba* L.).

Likewise, Al-Bayati et al. (2019) found that the single or combined application of different doses of organic fertilizers and chemical fertilizers generated different responses in the pea crop (*Pisum sativum* L.). For example, the application of single organic fertilizer at 100 % of the required dose increased biological yield, number of seeds per pod and green seed yield. The combination of 3/4 chemical + 1/4 organic increased the number of branches per plant, dry matter percentage during vegetative growth, pod weight and pod length. The mixture of 1/4 chemical + 3/4 organic increased the number of pods per plant, pod yield per plant and total pod yield.

Additionally, there was a different response to the different sources of organic fertilizer, being the ABOB fertilizer the one that generated a better response. In this sense, Mukai (2018) showed that there can be a high variability in nutrient supply even within similar organic products, compared to chemical fertilizers where nutrients are immediately available to crops, so they are quickly absorbed by plants. Nevertheless, nutrient availability and crop yields can become similar for both organic and chemical fertilization when long-term effects are evaluated.

The average number of pods per plant obtained in the different treatments evaluated, as well as in the control was below those reported by Checa et al. (2017) who presented average values of number of pods per plant between 33 and 35 for the rainy seasons of the year under conditions of adequate moisture availability similar to those of this study. While Casanova et al. (2012) obtained average number of pods per plant (NVP) of 19.20, 20.25 and 20.10 at planting densities of 333333.00;

250000.00; and 200000.00 plants/ha, respectively. While in the treatment with the highest number of plants (666666.00 plants/ha) the NVP was statistically lower (16.05 pods per plant) in seven lines of bush pea (*Pisum sativum* L.).

The number of pods per plant is a variable sensitive to population density because the higher the density of plants, the greater the intraspecific competition and the lower the production of pods per plant. In this study, the population density was not evaluated, however, the population density was 333333.00 plants/ha equal for all treatments, and the values of number of pods per plant ranged from 15.43 in the control treatment to 22.83 in the chemical fertilization treatment at 100 % of the dose, indicating that the number of pods per plant is sensitive to the different doses and sources of both organic and chemical fertilization.

Galindo and Clavijo (2009) obtained average values between 9.70 and 9.90 pods/plant, relatively lower than those obtained in this study. These differences are probably due to factors such as natural soil fertility and the behavior of each of the varieties grown in each study area.

According to the results obtained in this study, it can be deduced that *Rabo de gallo* variety responds positively to both chemical fertilization and organic fertilization and that it has a good pod production both without fertilization and with organic or chemical fertilization, reason for which it is preferred by farmers in the area.

3.3 Length of green pods (LV)

Figure 2 shows that the LV of the pea crop was significantly lower in T0 than in the rest of the treatments, except in T1, which had no significant differences between them ($p>0.05$). No differences were observed among the rest of the treatments ($p>0.05$). This indicates that the pea crop responded favorably to the treatments applied with respect to the control, and the highest average value in the length of green pods was obtained (10.87 ± 0.947 cm) when applying organic fertilizer GCR100 % (T3).

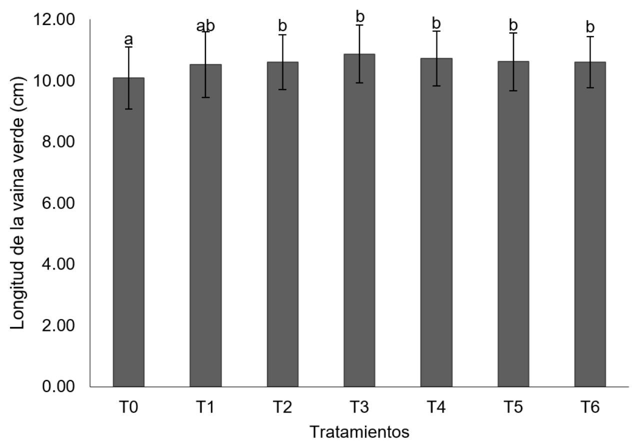

Figure 2. Length of green pods (LV) in the pea crop in response to organic and chemical fertilization in the municipality of Pamplona, North of Santander. Bars with mean values ± standard deviation accompanied with different lowercase letters indicate statistical differences ($p<0.05$) among treatments. T0: Control; T1: Vermicompost full dose (VC100 % = 7831.00 kg/ha or 16.44 kg/plot of 21 m^2); T2: Vermicompost half dose (VC50 % = 3915.50 kg/ha or 8.22 kg/plot of 21 m^2) + Chemical Fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50 % = 703.50 kg/ha or 1.48 kg/plot of 21 m^2); T3: Hen manure + goat manure + sugarcane residues full dose (GCR100 % = 10573.00 kg/ha or 22.20 kg/plot of 21 m^2): T4: GCR half dose (GCR50 % = 5286.50 kg/ha or 11.10 kg/plot of 21 m^2) + Chemical Fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50 % = 703.50 kg/ha or 1.48 kg/plot of 21 m^2); T5: Chemical fertilizer full dose (FQ100 % = 1407.00 kg/ha or 2.95 kg/plot of 21 m^2); T6: Chemical fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50 % = 703.5 kg/ha or 1.48 kg/plot of 21 m^2).

According to the results, pod length was statistically lower ($p<0.05$) in the control treatment where no fertilizer was used than in the treatments with organic fertilizer and chemical fertilizer, except in treatment where 100% vermicompost was used. However, no differences were observed between treatments. Therefore, as with the variable number of days to flowering, it is likely that this variety presents a genetic condition that makes it more stable for this parameter, despite the different doses and sources of organic fertilizers, as well as chemical fertilizer used.

The values obtained in this study are above the data reported by DANE (2015) for the Santa Isabel and Guatecana varieties with pod length of 4 to 6 cm long, 4 to 5 cm for Piquinegra, 4 to 8 cm for the Sindamanoy variety, while the Obonuco San Isidro and Alcalá varieties present green pod length of 7.00 to 9.60 cm and 7.00 to 9.20 cm, respectively. This is another reason why farmers prefer using it in the area, so it is necessary to delve deeper into the origin of the seeds, their proper identification, as well as the possibilities of carrying out a certification and marketing process.

3.4 Crop yield

The crop was harvested when the plants were at main stage number 7, code 79 according to the BBCH scale, in which the pods have already reached the typical size (green maturity), and were fully formed (Enz and Dachler, 1998).

According to the results presented in Table 3 regarding the effect of organic and chemical fertilization on pea crop yield, no statistically significant differences ($p>0.05$) were found between the different treatments. Yield ranged from 5079.36 kg/ha for T6 as the lowest value to 7936.51 kg/ha for T4 as the highest value.

The yield values (kg/ha) obtained in this study are relatively higher than the national average, especially in the treatments with organic fertilization. For example, according to data published by DANE (2015) in Colombia green pod yields of 4000 to 5600 kg/ha have been reported for the Santa Isabel variety; the yield for the Piquinegra variety is between 2000 to 4500 kg/ha; yields for the Guatecana variety range from 3000 to 5000 kg/ha; yield for the Sindamanoy variety is 4197 kg/ha; meanwhile the Andean Obonuco variety can reach yields of up to 6608 kg/ha; the highest yields are obtained with Alcalá and Sureña varieties with 12000 and 14990 kg/ha, respectively.

Table 3. Pea crop yield (kg/ha) in response to organic and chemical fertilization in the municipality of Pamplona, North of Santander.

Treatment	Performance (kg/ha)
T0	5555.56 ± 3993.57^a
T1	7777.78 ± 991.27^a
T2	7460.32 ± 1198.38^a
T3	6031.74 ± 1454.78^a
T4	7936.51 ± 3170.63^a
T5	6190.47 ± 4151.33^a
T6	5079.36 ± 1672.32^a

Bars with mean values ± standard deviation accompanied by different lowercase letters indicate statistical differences ($p<0.05$) between treatments. T0: Control; T1: Vermicompost full dose (VC100% = 7831.00 kg/ha or 16.44 kg/plot of 21 m^2); T2: Vermicompost half dose (VC50% = 3915.50 kg/ha or 8.22 kg/plot of 21 m^2) + Chemical Fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50% = 703.50 kg/ha or 1.48 kg/plot of 21 m^2); T3: Hen manure + goat manure + sugarcane residues full dose (GCR100% = 10573.00 kg/ha or 22.20 kg/plot of 21 m^2): T4: GCR half dose (GCR50% = 5286.50 kg/ha or 11.10 kg/plot of 21 m^2) + Chemical Fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50% = 703.50 kg/ha or 1.48 kg/plot of 21 m^2); T5: Chemical fertilizer full dose (FQ100% = 1407.00 kg/ha or 2.95 kg/plot of 21 m^2); T6: Chemical fertilizer (15N $15P_2O_5$ $15K_2O$) half dose (FQ50% = 703.5 kg/ha or 1.48 kg/plot of 21 m^2).

Authors such as Khan et al. (2013), found yields ranging from 3.74 to 10.43 $t{\cdot}ha^{-1}$ in rainy conditions; while Checa et al. (2017) reported yields between 6.04 $t{\cdot}ha^{-1}$ for the dry season; and values between 10.21 and 12.96 $t{\cdot}ha^{-1}$ during the rainy season. On the other hand, Mishra (2014) reported yields of 5.5 $t{\cdot}ha^{-1}$, while Celis and Prett (1995), obtained yields of 20 $t{\cdot}ha^{-1}$ for the pea crop, which is much higher than that reported in this research. Casanova et al. (2012) reported green pod yields between 4076.90, 4725.56, 5968.61 kg/ha for planting densities of 666666, 333333 and 200000 plants/ha.

Despite not finding statistical differences between treatments, it is important to highlight a trend in which the use of single vermicompost at 100% of the recommended dose (T1: 7831 kg/ha) represented an increase in yield with respect to the control treatment of 39.99%. The combined use of vermicompost at half the dose (3915.50 kg/ha) + triple chemical fertilizer 15 half the dose (703.50 kg/ha) resulted in an increase of 34.28%; while at T4 in which the combination of ABOB fertilizer at 50% (5286.50 kg/ha) plus half the dose of chemical fertilizer FQ50% (703.50 kg/ha) was applied, the yield was 42.85% higher than the control. The lowest yield increase corresponded to T3 (ABOB: 10573.00 kg/ha) with 8.57%, however in T5 with chemical fertilization at a dose of 1407.00 kg/ha of triple 15 the increase was only 11.42%. When half the chemical fertilizer dose was used, yield was 8.58% below the control.

A meta-analysis by Chivenge et al. (2011) reports yields of up to 60% with the use of organic fertilizers compared to the unamended control, while the combined use of organic fertilizers and nitrogen fertilizers can result in yield increases of up to 114%.

Relatively similar results were found by Rojas (2017) in agroclimatic conditions of Tiabaya-Arequipa, Peru, where the highest production of green pods of pea var. Quantum was obtained with organic fertilization in a mixture of 6 t·ha^{-1} of earthworm humus and 1 t·ha^{-1} of island guano and biol (liquid produced by a biodigester and used as foliar fertilizer at a concentration of 40%) with yields up to 12.80 t·ha^{-1} significantly higher than those obtained in this study. Santamaría et al. (2010) also found higher green pea pod yields when using Fertigran liquid organic fertilizer at three phenological stages of the crop, compared to conventional fertilization.

A review conducted by Van Zwieten (2018) explored the long-term role of organic fertilizers and presented strong evidence on the yield benefits of organic fertilizers compared to unfertilized controls. Organic fertilizers may not provide an adequate or balanced nutrient dose, so the amount of nutrients present in the fertilizer and the application of some additional fertilizer must be taken into account. Therefore, a basic understanding of the characteristics of the type of fertilizer used is needed, especially in the total N and P contents provided.

Vermicompost is an organic fertilizer obtained from the action of the Californian earthworm, also known as solid earthworm humus. The fertilizer used in this study is a certified product with adequate percentages of nitrogen, phosphorus, potassium, calcium and magnesium, as well as a high cation exchange capacity (34.42 cmol(+)/kg of soil). On the other hand, ABOB is a certified fertilizer produced commercially from hen manure, goat manure and sugar cane residues with a nitrogen and potassium content similar to vermicompost, but with much higher concentrations of phosphorus and silicon, and with a lower cation exchange capacity than vermicompost (25.00 cmol(+)/kg of soil).

Since these are fertilizers with different sources of organic material, the contribution and release of nutrients is different between these, which is expressed in differential increases in yield. In this study, the best performance was with single vermicompost and with the combination of ABOB with the chemical fertilizer.

3.4.1 Pearson correlation analysis between yield and the different development and growth variables evaluated in the pea crop in response to the treatments

Table 4 presents the Pearson correlation analysis performed on the data obtained in the different treatments. Yield was significantly and positively correlated with plant height at 90 days ($p<0.05$ and Pearson correlation 0.53), while the number of pods per plant (VP) presented a highly significant and positive correlation ($p<0.01$ and Pearson correlation 0.55) with plant height at 90 days (AP90D).

The analysis presented in Table 4 states that as plant height increases in response to the treatments, the number of pods per plant also increases and, consequently, yield is higher.

Table 4. Pearson correlation analysis between yield and the different growth and development variables evaluated in the pea crop in response to organic and chemical fertilization in the municipality of Pamplona, North of Santander.

Correlations								
		Performance	AP30D	AP60D	AP90D	DF	VP	LV
Performance	Pearson correlation	1	-0.06	0.27	**0.53***	-0.08	0.2	-0.12
	Sig. (bilateral)		0.81	0.23	**0.01**	0.72	0.4	0.62
	N	21	21	21	21	21	21	21
AP30D	Pearson correlation	-0.06	1	0.50*	0.04	-0.26	-0.07	-0.43
	Sig. (bilateral)	0.81		0.02	0.87	0.26	0.75	0.06
	N	21	21	21	21	21	21	21
AP60D	Pearson correlation	0.27	0.50*	1	0.72**	-0.06	0.51*	0.07
	Sig. (bilateral)	0.23	0.02		0	0.8	0.02	0.77
	N	21	21	21	21	21	21	21
AP90D	Pearson correlation	0.53*	0.04	0.72**	1	0.19	0.56**	0.32
	Sig. (bilateral)	0.01	0.87	0		0.42	0.01	0.16
	N	21	21	21	21	21	21	21
DF	Pearson correlation	-0.08	-0.26	-0.06	0.19	1	-0.23	0.43
	Sig. (bilateral)	0.73	0.26	0.8	0.42		0.31	0.05
	N	21	21	21	21	21	21	21
VP	Pearson correlation	0.2	-0.07	0.51*	**0.56****	-0.23	1	0.18
	Sig. (bilateral)	0.4	0.75	0.02	**0.01**	0.31		0.43
	N	21	21	21	21	21	21	21
LVS	Pearson correlation	-0.12	-0.43	0.07	0.32	0.43	0.18	1
	Sig. (bilateral)	0.62	0.06	0.77	0.16	0.05	0.43	
	N	21	21	21	21	21	21	21

*.The correlation is significant at the 0.05 level (bilateral).
**. The correlation is significant at the 0.01 level (bilateral).
AP30D: Plant height at 30 days; AP60D: Plant height at 60 days; AP90D: Plant height at 90 days; DF: Number of days to flowering; VP: Number of pods per plant; LV: Length of green pod.

The application of single or combined organic fertilizers with chemical fertilizer generated different effects on the growth and yield evaluated. Authors such as Pandey (2017), point out that the organic and inorganic combination of nutrient supply can be synergistic in pea crop because the organic source improves the physical and biological environment of the soil, increasing the availability of nutrients from the inorganic source. The increase in seed yield is due to the effect of growth and yield attributes.

Although the soils in this study have good fertility, it was possible to observe a positive response of the types of single or combined fertilizers with chemical fertilization. Organic fertilizers provide organic carbon to the soil, which is important for stimulating the growth of soil microbial biomass, especially in the long term. Increases in soil organic carbon of up to 49 % have been reported after adding organic fertilizers compared to an unfertilized control and 29 % over a fertilized control (Chen et al., 2018; Murillo-Montoya et al., 2020). Similarly, organic fertilizers can directly supply both macro and micronutrients, and as for the long-term supply of N, it is regulated by the mineralization rate of the added organic compounds. Additionally, the nutrient content depends on the source and quality of the added organic compounds (Van Zwieten, 2018).

4 Conclusions

Plant height was positively affected by the combined use of vermicompost at half the recommended dose (3915.50 kg/ha) plus half the dose of triple 15 chemical fertilizer (703.50 kg/ha) compared to the control treatment.

The number of pods per plant was higher with the fertilizer based on hen manure, goat manure and sugarcane residues at the full dose compared to chemical fertilization.

The length of green pods was above the national average and responded very well to the single or combined application of organic fertilizers and chemical fertilizers compared to the control. Although no statistical differences in yield were found among the different treatments, T1 and T4 presented a yield higher than the control of 42.85 % and 39.99 %, respectively.

Regarding vermicompost, the combination with chemical fertilizer favored plant height, probably due to faster nutrient availability.

The combination of organic fertilizers with chemical fertilizers generated positive effects on the pea crop. The *Rabo de gallo* variety responded very well to single or combined application of organic and chemical fertilizers, reason for which it is preferred by farmers in the area. Therefore, it is possible to substitute or complement chemical fertilizer with organic fertilizers and reduce the negative effect of contamination on the environment and people's health.

5 Recommendations

Combine the use of organic fertilizer with chemical fertilizer, due to the better response obtained in crop variables.

Evaluate different doses of organic fertilizer on crop growth variables.

Study the *Rabo de gallo* pea variety, investigate its origin, study different planting distances, different types of soils, include a greater number of crop variables and compare it with other varieties used at the national level.

Analyze the effect of organic fertilizer on the physical, chemical and biological properties of soils in the short, medium and long term.

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Agronomy

Baits for *Anastrepha fraterculus* with Hydrolyzed Protein from Agroindustrial By-products Pretreated with Gamma Radiation

Cebos para *Anastrepha fraterculus* con Proteína Hidrolizada de Subproductos Agroindustriales Pretratados con Radiación Gamma

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Abstract

The fruit fly (*Anastrepha fraterculus*) affects several Ecuadorian crops with export potential. Currently, a costly imported bait is used to monitor and control this pest. The aim of this research is to formulate baits for the fruit fly that could replace the commercial bait. Soy cake, palm kernel cake, bovine blood and whey were used as raw material. Each material was irradiated with a dose of 20 kGy with a Cobalt-60 source as a pretreatment. Then, the protein was extracted and hydrolyzed with a 0.025 AU mL^{-1} bromelain solution at pH 7,0 and 50°C, for 30 min. The baits were formulated with hydrolyzed protein, molasses, water, and borax, and they were placed in McPhail traps. The field evaluation was carried out in cherimoya (*Annona cherimola*) and guava (*Psidium guajava*) orchards. The hydrolysis degrees that were reached in the enzymatic process had values between 19.16 and 26.64%. According to an SDS-PAGE electrophoresis, the hydrolysates had peptides with molecular weights between 5 and 20 kDa. The bait made with palm kernel cake hydrolyzed protein and the commercial bait were statistically equal in the number of captured flies, whereas the bait made with whey protein had a higher FTD index (flies caught per trap, per day). The formulated baits could be an inexpensive alternative to the commercial bait for monitoring fruit flies in Ecuador.

Keywords: Attractant baits, protein hydrolysates, ionizing radiation, fruit fly.

Resumen

La mosca de la fruta (*Anastrepha fraterculus*) afecta a varios cultivos ecuatorianos con potencial de exportación. En la actualidad, para el monitoreo de esta plaga, se emplea un cebo importado que tiene un alto costo. La presente investigación tiene como objetivo formular cebos atrayentes de mosca de la fruta que puedan reemplazar al cebo comercial. Como materia prima se empleó torta de soya, torta de palmiste, sangre bovina y suero; cada material fue irradiado con una dosis de 20 kGy, en una fuente de Cobalto-60 como pretratamiento. Luego, la proteína se extrajo y se hidrolizó con una solución de bromelina de 0,025 UA mL^{-1}, a pH $7,0$ y 50°C durante 30 min. Los cebos fueron formulados con proteína hidrolizada, melaza, agua y bórax y se colocaron en trampas McPhail. La evaluación en campo se llevó a cabo en cultivos de chirimoya (*Annona cherimola*) y guayaba (*Psidium guajava*). En el proceso enzimático se alcanzaron grados de hidrólisis entre 19,16 y 26,64 %. Por electroforesis SDS-PAGE se determinó que los hidrolizados proteicos contenían péptidos con pesos moleculares entre 5 y 20 kDa. Se encontró que el cebo de proteína hidrolizada de palmiste y el cebo comercial fueron estadísticamente iguales en la cantidad de moscas atrapadas, mientras que el de suero presentó un mayor índice MTD (moscas capturadas por trampa, por día). Los cebos formulados podrían ser una alternativa más económica que el cebo importado para el monitoreo de la mosca de la fruta en el Ecuador.

Palabras clave: Cebos atrayentes, hidrolizados proteicos, mosca de la fruta, radiaciones ionizantes.

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1 Introduction

The increase in exports of non-traditional Ecuadorian products such as pitahaya, mango and passion fruit between 2012 and 2017 outpaced the growth of several traditional products such as bananas, cocoa and industrialized coffee (Verdugo and Andrade, 2018). However, markets for other fruits with high export potential have not been opened due to persistent inadequate fruit fly management (Vilatuña et al., 2010). There are international markets that do not admit the entry of fruit without adequate treatment from countries affected by this pest, such as the United States, Japan and the European Union (Vilatuña et al., 2016; IAEA, 2019). Phytosanitary requirements imposed by importing countries seek to prevent the entry of this pest into areas considered free (García-Rosero et al., 2015).

Fruit flies belong to the *Tephritidae* family of the *Diptera* order. In Ecuador predominates the genus Anastrepha, native to Central and South America, and *Ceratitis*, a genus introduced from the Mediterranean around 1976 (Vilatuña et al., 2010). They are considered one of the main pests of economic interest worldwide, due to the damage caused on fruits and crops in tropical and subtropical regions (White and Elson-Harris, 1992; Hafsi et al., 2016). Fruit flies can adapt to various environmental conditions and infect fruit at different ripening stages. The females are attracted by certain aromas that fruits emit when they begin to ripen, and when this occurs they deposit eggs inside the fruit. Upon hatching, the larvae feed on the pulp and form galleries that facilitate the entry of pathogenic agents such as fungi and bacteria, causing fruit rot and consequent rejection for consumption, export and agro-industry (INIAP, 2004).

In addition, the presence of fruit flies increases production costs because of research expenses and the application of monitoring and control measures (Salcedo-Baca et al., 2009). For example, the export of Ecuadorian mangoes to markets such as the United States has been achieved (Fundación Mango Ecuador, 2019), but by using pesticides during cultivation and post-harvest hydrothermal treatment of the fruits (AGROCALIDAD, 2016). Due to the growing importance of this and other crops in the country (MAG-CGINA, 2022), there is interest in controlling this pest through more eco-friendly options.

In 2014, the Agency for Phytosanitary and Zoosanitary Regulation and Control (AGROCALIDAD) started the "National Fruit Fly Management Project", with the purpose of declaring free or low prevalence areas for fruit production, through management strategies in the host crops of the fly (Vilatuña et al., 2016). Integrated management requires knowledge of the population density of the pest and its variations over time (Vilatuña et al., 2010). Baits formulated from organic compounds such as putrescine, ammonium acetate or liquid hydrolyzed protein have been effectively used as attractants in programs for the detection and monitoring of several fruit fly species (Heath et al., 1997; López-Guillén et al., 2010). Hydrolyzed protein is used, due to the content of nitrogenous compounds associated with the attraction of flies to fruit (Bateman et al., 1981; Mazor, 2009). The presence of essential amino acids for insects, such as methionine (Dadd, 1985), can also contribute to the attracting power of the baits (Díaz-Fleischer and Castrejón-Gómez, 2012).

The bait chosen by the National Fruit Fly Management Project is a commercial product that has hydrolyzed vegetable protein as its main component (Vilatuña et al., 2016); it is manufactured in Argentina and costs USD 14.60 per liter of concentrate (Edifarm, 2016). Therefore, there is an opportunity to formulate a bait that can be produced in the country at a lower cost, possessing similar or greater effectiveness and generating environmental and social benefits. From this perspective, agro-industrial by-products that contain protein and have a low commercial value can be used as raw material (Zahari and Alímon, 2005). In Ecuador, palm kernel and soybean cakes are available from vegetable oil extraction industries, as well as whey and bovine blood from cheese production and cattle slaughtering, respectively. These are produced in large quantities and are commonly discarded or destined for animal feed (Figueroa et al., 2012).

The inclusion of a pretreatment of the raw materials (agroindustrial by-products) can increase the yield of the extracted protein. Herrero et al. (2009) indicate that ionizing radiation could modify the structure of proteins and cause, depending on their intensity, even deamination, decarboxylation, reduction of disulfide bonds and other changes that would facilitate their separation in extraction processes. These proteins, after enzymatic hydrolysis with proteases such as bromelain (Guadix et al., 2000), could be used for creating attractant baits (Barrera, 2006), due to their high content of ammonia compounds and their high solubility (Benítez et al., 2008). In addition, irradiation at intermediate and high doses allows reducing the microbial load (Kuan et al., 2013).

The aim of the research was to formulate fruit fly (*Anastrepha fraterculus*) attractant baits, based on hydrolyzed protein obtained from the agro-industrial by-products mentioned above, and to evaluate their performance in monitoring the pest in the field. In addition, the pretreatment effect of raw materials with gamma irradiation on protein extraction yield was determined.

2 Materials and Methods

2.1 Assessment of baits in the field

2.1.1 Area of study

The field trial was conducted for 5 weeks, from August 18 to September 22, 2017, in Puéllaro, a rural cantonal head of Quito, Pichincha province. Four areas near the parish were identified, called Estadio, La Esperanza, La Merced and Sigsihuayco, in which there are custard apple (*Annona cherimola*) and guava (*Psidium guajava*) crops, which are host species of the fruit fly (Supplementary Material 1).

2.1.2 Traps

Two McPhail type traps were used for each type of bait, which were placed in custard apple or guava trees with a minimum separation of 30 m, on detection points determined based on the monitoring routes managed in the "National Fruit Fly Program". Each trap had 250 mL of attractant solution.

The attractant capacity of each bait was quantified every 7 days using the FTD index, which corresponds to the average number of flies captured per trap daily (Imbachi et al., 2012) (Supplementary Material 2). Then, traps were washed and 250 mL of fresh attractant solution (Asaquibay et al., 2010) were placed to keep them operative during the experimental period (Vilatuña et al., 2010).

2.1.3 Experimental design for evaluating baits in the field

The bait effectiveness evaluation described corresponded to a $5 \times 5 \times 4$ trifactorial randomized block design, in which the factors were bait type (5 levels), week (5 levels) and sector (4 levels), while the blocks corresponded to subdivisions made in each sector. The response variable was the FTD index. The data were analyzed in the Statgraphics Centurion XVIII program, through a variance analysis (ANOVA) with 95% confidence and a multiple range test with Fisher's method or least significant difference (LSD).

2.2 Raw materials and reagents

Raw materials used for preparing fruit fly attractant baits were: soybean cake, palm kernel cake, whey and bovine blood. The commercial bait currently used in Ecuador for fruit fly monitoring was also used (Supplementary Material 3).

The following reagents were used for protein extraction, enzymatic hydrolysis and bait formulation: NaOH (JTBaker; 98.5% purity), HCl (Riedel de Haen; 37%), bromelain (Sigma-Aldrich; B4882), flavourzyme (Granotec), casein (Merck, 102244, analytical grade), sodium dibasic phosphate (Sigma; S9763), trichloro acetic acid (Analar; 99% purity), bovine whey albumin (Sigma; A4503, electrophoresis grade), sodium carbonate (Merck; 99.5% purity), cupric sulfate (Sigma; 99% purity), sodium potassium tartrate (Merck, 99% purity), phenol reagent according to Folin-Ciocalteu (Sigma; F9252), ammonium persulfate (Promega; 99% purity), borax (technical grade) and 2-mercaptoethanol (Merck; CAS 60-24-2). The following reagents used for molecular determination had an electrophoresis grade: acrylamide (Bio-Rad), tetrabromophenol brilliant blue (Sigma), bisacrylamide (Bio-Rad), Coomassie brilliant blue (Merck), sodium dodecyl sulfate (Sigma) and Tris HCl (Sigma-Adrich). Biuret's reagent was prepared with cupric sulfate pentahydrate (Fluka, Biochemika; 99% purity), EDTA (J.T. Baker, analytical grade), potassium iodide (Panreac; 99% purity) and 6 N NaOH solution (J.T. Baker; 98.5% purity).

2.3 Obtaining of protein extracts

2.3.1 Soybean cake

Protein was extracted from ground soybean cake. For this, the cake was placed in water, in a 1:5 ratio of solid to liquid, and alkalinized with NaOH 6 N; then, an isoelectric precipitation of the solubilized protein was performed, as indicated in the method described by Vioque et al. (2001), which is detailed in Supplementary Material 4.

2.3.2 Palm kernel cake

The palm kernel flour was mixed with a 0.03 M NaOH solution, in a 1:30 ratio of solid to liquid. This mixture was stirred for 45 min at 35°C (Zarei et al., 2012) and filtered to obtain a supernatant free of solids and fat residues. Finally, the supernatant was dried for 16 h at 50°C and stored under refrigeration (Arifin et al., 2009).

2.3.3 Whey

Samples of 100 mL of whey were homogenized and pH was adjusted to 5.2 with a 6 N HCl solution. In order to separate the fat from the whey, the samples were centrifuged at 210*g* for 15 min; the aqueous phase was poured into beakers, and these were heated at 93°C for 30 min (Supplementary Material 5). The samples were cooled for 20 min and filtered for 3 h on filter paper (Vázquez et al., 2010).

2.3.4 Bovine blood

Samples of 100 mL of irradiated blood were dried at 110°C, for 6 h in a Memert DIN 40 050-IP 20 oven, until a concentrate with 5 to 10% humidity was obtained (Figueroa et al., 2012). The concentration of soluble protein in all samples was determined using the Biuret method described by Fernández and Galván (2010).

2.4 Pretreatment evaluation of raw materials with gamma radiation

2.4.1 Pretreatment with gamma radiation

The effect of gamma radiation on protein extraction yield was evaluated for each raw material. The doses evaluated for soybean and palm kernel cakes were 15, 20 and 25 kGy, while for whey and bovine blood were 10, 15 and 20 kGy. In each case, the protein percentage obtained was determined (Supplementary Material 6).

In the protein extraction assays, the experimental units for the soybean and palm kernel cakes were polyethylene bags with 1 kg of sample; for the whey and bovine blood they were 3 L bags. The samples were placed 30 cm from the Cobalt-60 source in the irradiation chamber of the Radiation Technology Laboratory of the National Polytechnic School. The samples were flipped halfway through the exposure time to guarantee dose uniformity (Maity et al., 2009). The experiment had three replicates.

2.4.2 Experimental design for pretreatment assessment

A completely randomized design was used for each raw material. In each of these designs, the design variable was the irradiation dose; its levels were 15, 20 and 25 kGy for raw plant materials, and 10, 15 and 20 kGy for raw animal materials. The response variable was the amount of protein obtained. There were three replicates. Statistical analysis was performed using Statgraphics Centurion XVIII program; an ANOVA was performed with 95% confidence and a multiple range test with Fisher's method.

2.5 Enzymatic hydrolysis of protein extracts

The substrate and enzyme concentrations for the hydrolysis processes were selected experimentally, as described below.

2.5.1 Selection of substrate concentration

Protein isolates were solubilized in 0.1 M pH 7.0 sodium phosphate buffer, at concentrations of 20, 40, 60, 90, 120, 120, 150 and 200 mg mL^{-1}; the solutions were shaken for 10 min at 900 rpm and subsequently centrifuged at 1,698 $\times g$ for 15 min. Then, 600 μL of the supernatant was mixed with 2,400 μL of phosphate buffer (Cheftel et al., 1989) and the amount of soluble protein was determined using the Biuret method (Fernández and Galván, 2010). The concentration that allowed obtaining the highest amount of soluble protein for each substrate was selected (Supplementary Material 7).

2.5.2 Selection of enzyme concentration and hydrolysis time

First, bromelain and flavourzyme enzymes were verified to retain their proteolytic activity according to the method described by Castillo et al. (2012) (Supplementary Material 8). Solutions of 0.020 AU mL^{-1} bromelain, 24.0 LAPU mL^{-1} flavourzyme and 10 mg mL^{-1} casein were prepared to guarantee that the enzyme concentration was lower than the substrate concentration and to generate saturation conditions (Nelson and Cox, 2013). Also, 100 μL of each enzyme solution were added to 1,100 μL of casein, and incubated for 20 min at 37°C. Then, 1,800 μL of 5% TCA were added to each mixture to stop the reaction and precipitate the soluble protein. Subsequently, the samples were centrifuged at 2,821 $\times g$ for 20 min; 1,000 μL aliquots were taken from the supernatant and their absorbance was measured at 280 nm in a Hitachi U-19000 UV-VIS spectrophotometer (Castillo et al., 2012). Blanks were prepared in the same way, but TCA was added immediately after mixing each enzyme solution with the substrate. The assay was performed in duplicate.

Hydrolysis assays were then performed with different concentrations of each enzyme. For each substrate, selected concentrations were used as indicated in section 2.4.1. The following concentrations were evaluated for bromelain: 0.002; 0.006; 0.015; 0.020 and 0.025 AU mL^{-1} and for flavourzyme: 2.0; 4.7; 6.0; 12.0; 24.0 and 40.0 LAPU mL^{-1}. The hydrolysis conditions for bromelain were pH 7.0; 40°C for 1 h and for flavourzime pH 7.0; 50°C for 5 h (Benítez et al., 2008). Then, the enzyme concentration that allowed obtaining the highest amount of soluble protein in the shortest time was chosen for each raw material (Supplementary Material 9).

In order to quantify the soluble protein, 200 μL aliquots of the reaction mixture were taken at different times. For reactions with bromelain, aliquots were extracted at 0, 1, 3, 5, 10, 20, 30 and 60 min; while for reactions with flavourzyme at 0, 15, 30, 60, 120, 180, 240 and 300 min. In each case, 2,000 μL of TCA (10%) were added to stop the enzymatic reaction. Then, the samples were centrifuged at 2,821 $\times g$ for 15 min, the supernatant was taken from each sample and the soluble protein content was measured by the Biuret method (Fernández and Galván, 2010).

Finally, product formation rate curves were made considering time for all concentrations of each enzyme, and the one that allowed obtaining the highest concentration of protein hydrolysates in the shortest reaction time was selected (Supplementary Material 10, Supplementary Material 11).

2.5.3 Degree of enzymatic hydrolysis

The degree of hydrolysis was determined with equation 1. The amount of soluble protein was determined during hydrolysis of each protein isolate for 1 h at the conditions previously indicated. Aliquots of 1 mL were taken at different times (0, 3, 5, 10, 20, 30 and 60 min), each aliquot was mixed with 1 mL of 10% TCA and centrifuged at 2,821 $\times g$ for 10 min (Molina-Ortiz and Wagner, 2002). Total protein content was determined by hydrolysis of 0.05 g of each isolate with 2 mL of 6 N HCl at 110°C for 48 h. The hydrolysates were then centrifuged at 2,821 $\times g$ for 10 min (Wilchek and Miron, 2003). Soluble and total protein contents were quantified in the supernatant by the Biuret method (Supplementary Material 12).

$$HD(\%) = \frac{\text{Soluble protein in TCA}(10\%)}{\text{Total protein}} \times 100 \quad (1)$$

2.5.4 Molecular size determination of the hydrolysates obtained by SDS-PAGE electrophoresis

The determination of molecular weights for each raw material was performed by SDS-PAGE electrophoresis (Supplementary Material 13), following the procedure described by Laemmli (1970) and modified by Castillo et al. (2012).

2.6 Formulation of fruit fly baits with hydrolyzed protein

To formulate the attractant baits, the concentration of soluble protein in the hydrolysates obtained was determined and the commercial bait was characterized (Supplementary Material 14). Then, the amount of each type of protein hydrolysate to be placed in the traps was established, so that all the resulting solutions have the same amount of protein (Supplementary Material 15, Supplementary Material 16). The reference formulation contains 10% of commercial bait, 3% borax and 87% water (OIEA, 2005).

3 Results

3.1 Evaluation of a pretreatment of raw materials with gamma irradiation

Figure 1 shows the amount of protein extracted from raw materials irradiated with doses from 0 (control) to 25 kGy. Irradiation had a significant effect ($p < 0,05$) on the extraction process in soybean cake, palm kernel cake and whey. The highest yield was obtained from the soybean cake irradiated with the 20 kGy dose, being 10 % higher than the one obtained with the control sample. Significant increases were observed in the palm kernel cake in the 20 and 25 kGy treatments; 12 % more protein was extracted at 20 kGy compared to the control sample, as shown in (a). At the same dose, the highest extraction yield was obtained for whey, with a 0.82 % increase with respect to the control sample. The blood samples did not show significant differences compared to the control, as shown in (b), because the extraction process included drying of the blood samples as a way of concentrating the protein. This method is not based on protein denaturation and precipitation (Figueroa et al., 2012), therefore, it was not benefited by the treatment with ionizing radiation, which, on the other hand, favors precipitation (Gaber, 2005).

Consequently, the irradiation dose selected for all the raw materials was 20 kGy, because it gave better yields from the soybean cake and whey. In the case of palm kernel cake, treatment with 20 kGy would generate results statistically equal to those obtained with the 25 kGy dose, but would represent less irradiation time and cost. As for bovine blood, no increase in the percentage of recovered protein would be achieved, but other benefits would also be obtained, such as microbial decontamination.

In general, radiation treatments increased the protein extraction yield. Consistently, Castillo et al. (2019) reported the increase in the percentage of extracted protein in chicken feathers exposed to 25 kGy. Likewise, Kuan et al. (2013) pointed out the modifications in the secondary structure of proteins when exposed to doses higher than 10 kGy, favoring their extraction.

3.2 Enzymatic hydrolysis of protein extracts

3.2.1 Selected substrate concentration

The substrate concentration of 15 % weight by volume (w/v) was selected for the soybean and palm kernel cake, because it produced the highest amount of soluble protein. In the case of whey and bovine blood, 12 and 15 % (w/v) were chosen, since the solubility of the protein decreased at higher concentrations, maybe due to the excess of solute (Cheftel et al., 1989).

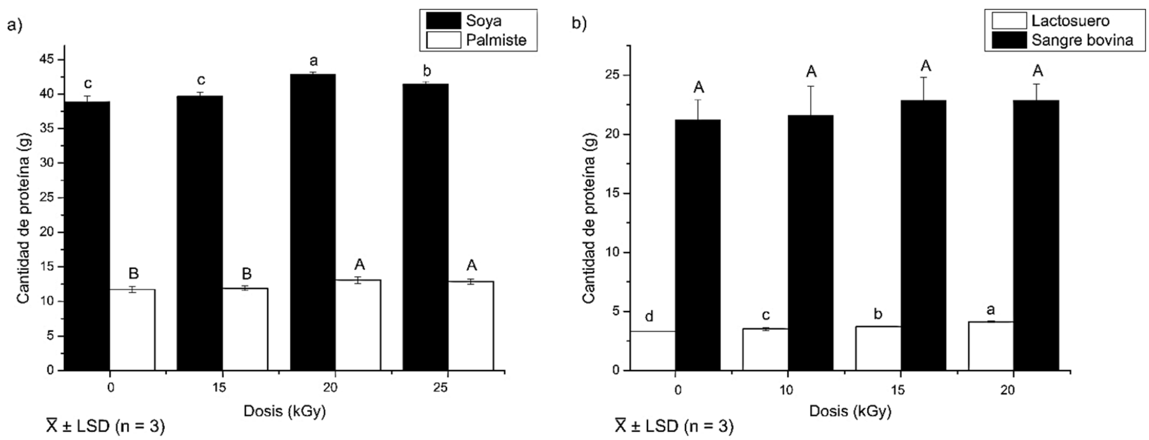

Figure 1. Effect of dose on the amount of protein extracted from irradiated raw materials a) soybean and palm kernel cakes, b) whey and bovine blood.

3.2.2 Selected enzyme concentration and hydrolysis time

Although both enzymes showed proteolytic activity to be used in the hydrolysis of protein isolates, the reactions catalyzed with flavourzyme required longer reaction time and higher enzyme concentration than the reactions with bromelain to achieve similar concentrations of soluble protein in the hydrolysates. Therefore, bromelain was selected for the subsequent trials. The combined use of bromelain and flavourzyme was discarded because no synergistic effects were observed.

Figure 2 shows the plots of soluble protein concentration considering time, which were obtained by hydrolyzing protein concentrates of the raw materials with bromelain at concentrations between 0.002 and 0.025 AU mL^{-1}. It is shown that the highest soluble protein concentration values are achieved for all substrates with a concentration of 0.025 AU mL^{-1} of bromelain. This is an advantage presented by the hydrolyzates compared to the original protein, because the number of polar groups increases when it is broken into peptides of lower molecular weight, due to the increased exposure of carboxylic groups and free amines, which improves its solubility (Benítez et al., 2008). The chosen reaction time was 30 min, since it allowed reaching protein concentrations of 11.94, 3.23, 7.24 and 8.53 mg mL^{-1} for soybean, palm kernel, whey and bovine blood, respectively, which were close to the maximum values observed at 60 min.

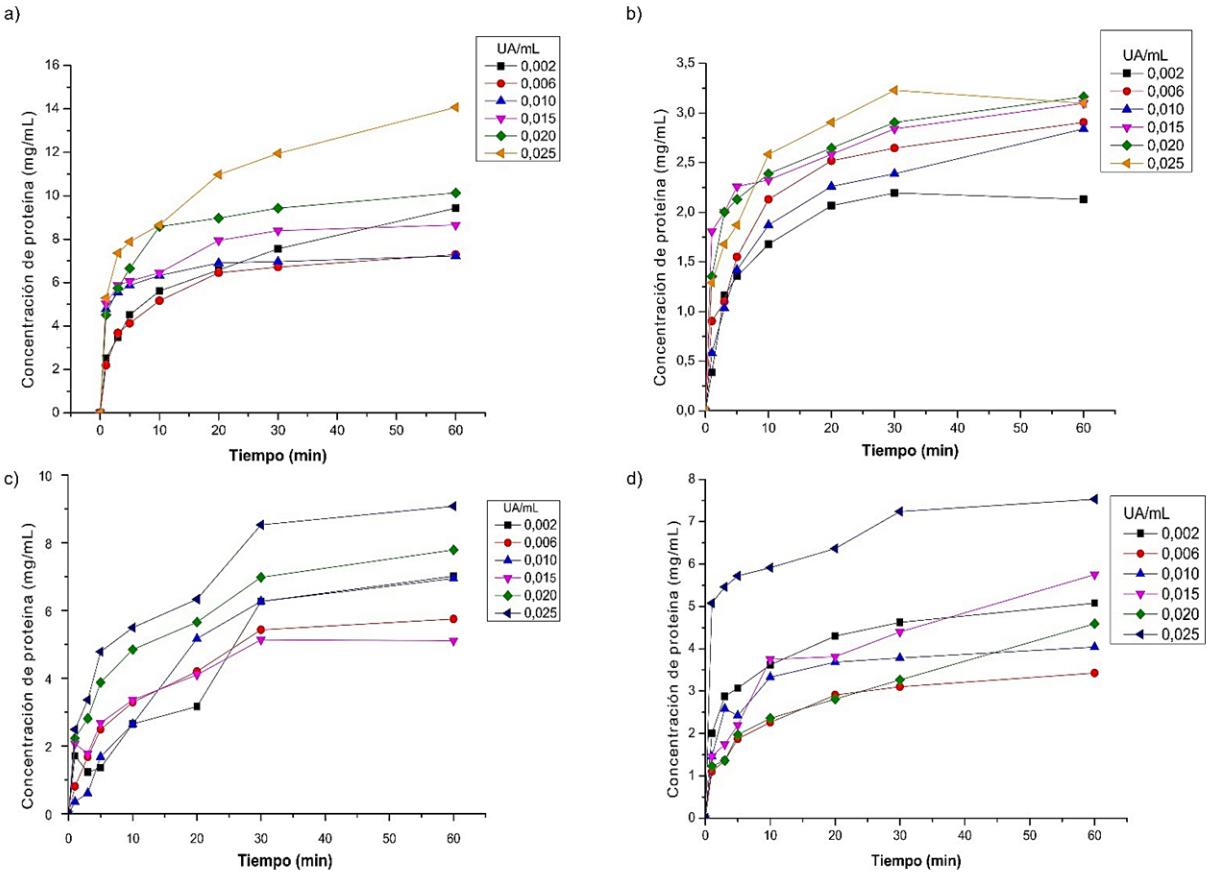

Figure 2. Soluble protein concentration according to hydrolysis time with bromelain at different concentrations, for a) soybean, b) palm kernel, c) whey, d) blood.

3.2.3 Hydrolysis degree

Figure 3 shows the hydrolysis degrees of the protein concentrates corresponding to each raw material, during 60 min of reaction, with 0.025 AU mL^{-1} of bromelain, under the pH and temperature conditions established.

It can be observed that after 30 min of reaction, hydrolysis percentages of 33.87 for soybean, 38.89 for palm kernel, 18.42 for whey and 20.24 for blood were obtained. These hydrolysates are extensive and can be used for the formulation of liquid protein substances, due to their high solubility and the fact that these types of hydrolysates are easily absorbed by living organisms (Vioque et al., 2001). the values of the hydrolysis degree did not present increases at 60 min that would justify a longer reaction time; for this reason, the hydrolysates corresponding to 30 min of reaction were used in the following stages.

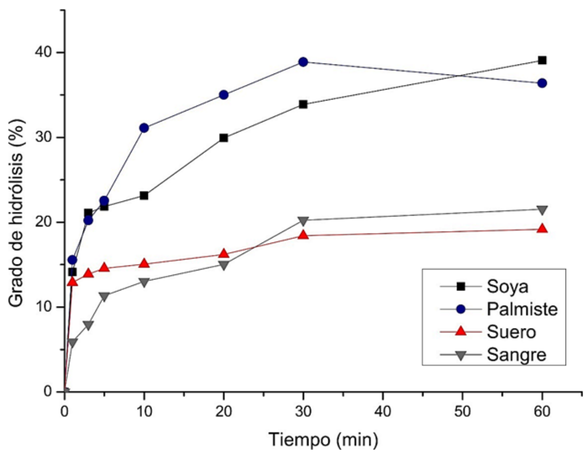

Figure 3. Hydrolysis degree for each raw material during the reaction with bromelain.

3.2.4 SDS-PAGE electrophoresis

Figure 4 shows the results of the molecular characterization by SDS-PAGE electrophoresis. Peptides with molecular weights between 10 and 70 kDa were identified in the commercial bait. The soybean hydrolysates exhibited bands corresponding to molecular weights of 10 to 20 kDa and in palm kernel, mostly lower than 15 kDa (Figure 4.a). Peptides from blood hydrolysate had molecular weights from 10 to 70 kDa and whey from 10 to 15 kDa (Figure 4.b). The commercial bait and the hydrolysates of the raw materials presented peptides with molecular sizes between 10 and 15 kDa, highly digestible fractions that would enable to formulate a bait that could replace the one currently imported.

3.3 Field evaluation of fruit fly baits

The attractant solutions placed in the traps for field evaluation were formulated to present a soluble protein concentration of 12.55 mg mL^{-1} equal to that of the commercial bait, i.e., different amounts of the protein extracts were placed to reach the same amount of protein in each bait.

The ANOVA indicated that the type of bait used did have a significant effect ($p < 0,05$) on the number of flies trapped. Figure 5 presents the means of the FTD index for the bait type. It is observed that the whey bait showed the highest FTD index (LSD, 95%). The effectiveness of the palm kernel and blood baits was statistically equal to that of the commercial bait. Therefore, these could be alternatives for fruit fly monitoring and control in Ecuador. The soybean bait showed a lower FTD index than the commercial bait.

The difference in effectiveness of the baits was possibly due to the molecular size and nature of the peptides obtained by the proteolytic action of bromelain. Bromelain is known to catalyze the hydrolysis of peptide bonds formed with non-polar residues (Benítez et al., 2008). The different result obtained with palm kernel and soybean bait could be due to the variation between the amino acid profiles of the corresponding protein extracts, since palm kernel protein has 7 out of the 8 hydrophobic amino acids (Alimon, 2004; Nelson and Cox, 2013), while soybean protein only has 5 (Calderón de la Barca et al., 2000). The efficiency of whey bait in capturing fruit flies was possibly due to the presence of nitrogen-rich peptides with molecular weights between 10 and 15 kDa. According to Canal et al. (2010), fruit flies easily perceive and assimilate short-chain peptides with low molecular weight. In addition, whey proteins have abundant presence of lysine, providing a higher amount of nitrogen than other protein sources (Jovanović et al., 2005). Nitrogen is a necessary factor for dipteran growth, which allows to reach sexual maturity (Montoya et al., 2010).

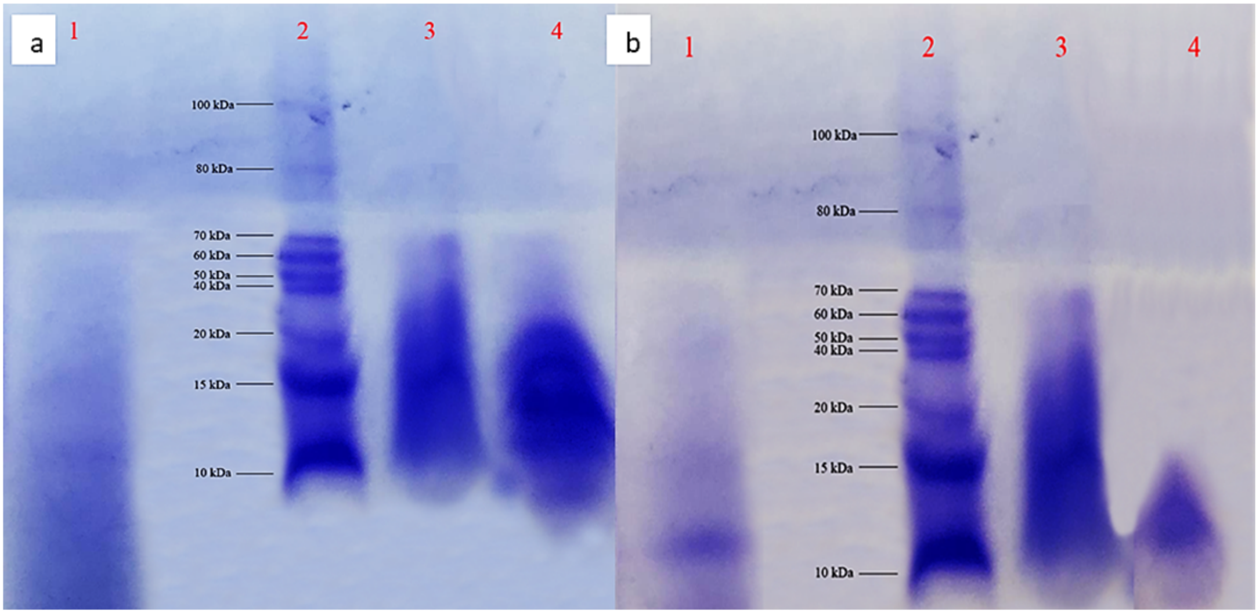

Figure 4. Electrophoresis gel a) 1. palm kernel hydrolysate, 2. standard, 3. commercial bait and 4. soy hydrolysate b) 1. blood hydrolysate, 2. standard, 3. commercial bait and 4. whey.

Figure 6 shows the FTD index considering time for each bait. In all cases, FTD values higher than 1 were obtained; therefore, Puéllaro can be considered a fruit fly infested area. Palm kernel and whey baits presented the highest values during the time evaluated. The FTD peaks could be due to the first rains of the season prior to the installation of the traps, which could favor the development of pest pupae and consequently increase the incidence of adults. Consistently, Tucuch-Cauich et al. (2008) pointed out a direct relationship between the presence of rainfall and the increase in the incidence of *Anastrepha* spp. It is believed that the affinity of fruit flies for the protein hydrolysates used in this research was influenced by factors such as climate, phenological state of the flies, crop type, trap location, trapping density and population level (Canal et al., 2010).

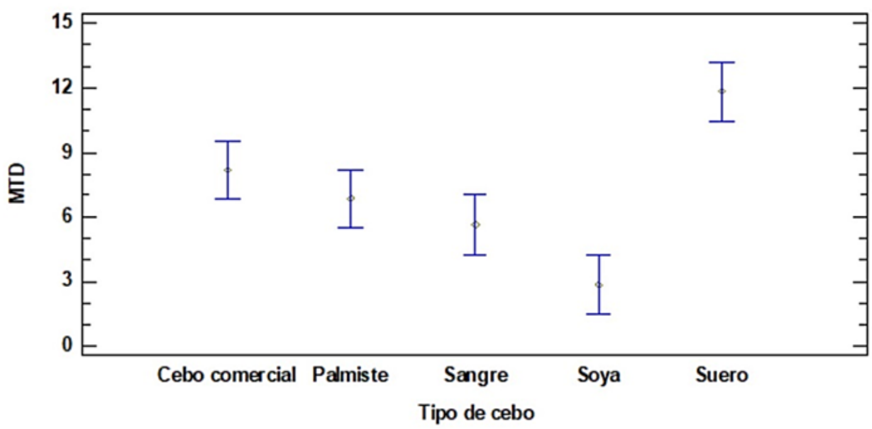

Figure 5. FTD index as a function of the type of bait during field evaluation (mean ± LSD, 95 %).

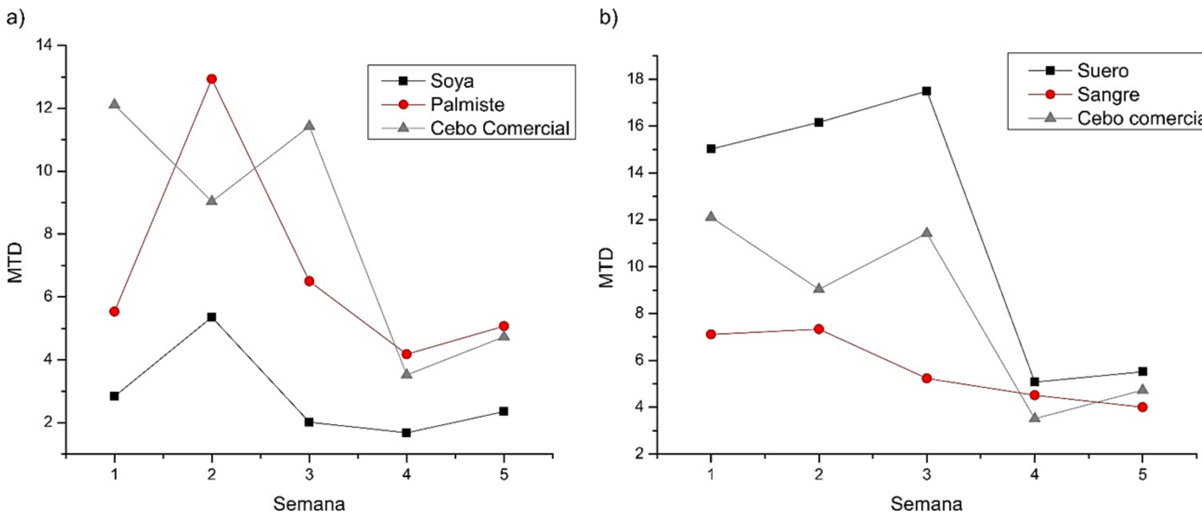

Figure 6. FTD variation of fruit fly from week 1 to week 5.

4 Conclusions

The application of a gamma radiation dose of 20 kGy as pretreatment allowed obtaining a higher yield in protein recovery for soybean with values of 67.24% for palm kernel 60.53% and 35.7% for whey. Radiation treatments did not show significant differences on the protein concentration yield for bovine blood.

The conditions selected for enzymatic hydrolysis were: pH 7.0; 50°C; substrate concentration (w/v) 15% (soybean cake, bovine blood and palm kernel cake) and 12% (whey); 0.025 AU mL^{-1} of bromelain and time of 30 min. The hydrolysis percentages were 33.87 for soybean, 38.89 for palm kernel, 24.78 for whey and 19.94 for blood, after 30 min of reaction. Protein hydrolysates with molecular weights similar to those found in commercial bait (10 to 50 kDa) were obtained.

Hydrolyzed whey protein in the first place, or palm kernel and bovine blood protein in the second could be an alternative to the protein used in the commercial bait, based on the results of the FTD index. It is necessary to investigate the performance of the proposed baits in larger experiments, in different crops and locations in Ecuador, where the presence of fruit flies has been detected. This is because one type of attractant may be effective for a given fruit fly species but not for another, even if they belong to the same genus (Aluja et al., 2001).

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Declaration of interest

No conflicts of interest are declared for any of the authors.

Authors contribution

MS was the director of the research project; he supervised the pretreatment of the raw materials with ionizing radiation, the experimental designs and statistical analyses, as well as the discussion of the results. PC supervised the processes of obtaining hydrolyzed protein. GJ supervised the field evaluation of the baits; CC and MC performed the laboratory tests and the traps.

Supplementary material

Supplementary material to this article can be found online at https://bit.ly/3DKQg5x.

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Agronomy

Physicochemical indicators of soil with conventional rice (*Oriza sativa* L.) management under irrigation

Indicadores fisicoquímicos del suelo con manejo convencional del arroz (*Oriza sativa* L.) bajo riego

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Abstract

The research evaluated the effect of conventional management of irrigated rice on the physicochemical indicators of the soil, in the Mercedes and Pérez farms, in Yurimaguas, Peru. It is a comparative non-experimental investigation, with statistical adjustment of a completely randomized design, where the treatments are made up of the secondary forest (BS), the conventional rice management of one (A1), five (A5) and nine years (A9), evaluating physicochemical indicators of the soil in strata from 0.0 to 0.2 and 0.2 to 0.4 m. The results showed differences in the fractions, with initial reduction of sand, silt and clay increase and in time slight recovery of the sand, silt fraction and clay reduction. The chemical indicators according to treatments and strata show differences, except K; management significantly affects the beginning of the production process (A1) reducing the levels of pH, MO, N, P, K^+, Ca^{2+} and Mg^{2+} and increasing Al^{3+}, AC and SAl, and there is a recovery over time (A9), except in MO and N which decrease to very low levels. In conclusion, conventional management shows significant effects between treatments and indicators evaluated in both strata, negatively affecting the beginning (A1) and recovering over time (A9); however, there are long-term negative effects on OM and N levels.

Keywords: Chemical fertilization, physical indicators, chemical indicators, organic matter, crop residues, Yurimaguas,

Peru.

Resumen

La investigación evaluó el efecto del manejo convencional del arroz bajo riego en indicadores fisicoquímicos del suelo, en los fundos Mercedes y Pérez, en Yurimaguas, Perú. Es una investigación no experimental comparativa, con ajuste estadístico de diseño completamente aleatorizado, donde los tratamientos lo constituyen el bosque secundario (BS), el manejo convencional del arroz de: uno (A1), cinco (A5) y nueve años (A9); evaluándose indicadores fisicoquímicos del suelo en estratos de 0,0 a 0,2 y 0,2 a 0,4 m. Los resultados mostraron diferencias en las fracciones, con reducción inicial de arena, limo e incremento de arcilla y en el tiempo ligera recuperación de la fracción arena, limo y reducción de arcilla. Los indicadores químicos según tratamientos y estratos presentan diferencias, excepto el potasio (K); el manejo afecta significativamente al inicio del proceso productivo (A1) reduciendo los niveles del potencial de hidrogeno (pH), materia orgánica (MO), nitrógeno (N), fosforo (P), potasio (K^{+}), calcio (Ca^{2+}) y magnesio (Mg^{2+}) e incrementando el aluminio (Al^{3+}), acidez cambiable (AC) y saturación de aluminio (SAl); de igual forma, se observa la recuperación en el tiempo (A9), excepto en MO y N que descienden a niveles muy bajos. En conclusión, el manejo convencional muestra efectos significativos entre tratamientos e indicadores evaluados en ambos estratos, afectando negativamente al inicio (A1) y recuperándose con el tiempo (A9); sin embargo, se observan efectos negativos a largo plazo en los niveles de MO y N.

Palabras clave: Fertilización química, indicadores físicos, indicadores químicos, materia orgánica, residuos de cosecha, Yurimaguas, Perú.

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1 Introduction

Peru has areas with great potential for irrigated rice production in different regions, which in the last 17 years (2001-2017) have had an upward trend in the national production, as the harvested area grew 2% and yields increased 0.4% on average per year. The main producing regions are San Martín with 27%, Lambayeque 13%, Piura 12%, Amazonas 10% and la Libertad with 7% (MINAGRI-DGESEP, 2018). The national average yield is 7,2$t\ ha^{-1}$, while Loreto (Yurimaguas) has an average of 2,9$t\ ha^{-1}$ ranking 13th nationally. Thus, production in this region is far from the national average and the average of regions such as Arequipa (13,9$t\ ha^{-1}$), Ancash (11,9$t\ ha^{-1}$), Tumbes (8,5$t\ ha^{-1}$), and Lambayeque with 8$t\ ha^{-1}$ (Contreras, 2016; MINAGRI-DGESEP, 2018; Quevedo et al., 2019). In addition, production in these areas is based on conventional crop management, with poor agricultural practices such as: pest control with agrochemicals, weed control with herbicides and intensive use of chemical fertilizers.

The application of conventional management is due to the low efficiency of the application of organic matter, which can affect the profitability of the crop (Alvarez et al., 2008), which has contributed in some cases to a decrease in average yield. Rice (*Oryza sativa* L.) is an essential food grain for most of citizens (Das et al., 2014; Çay, 2018; Lv et al., 2018), being a basic component in political, economic, social stability and our survival (Quevedo et al., 2019) and with important contributions in the economy. In Peru, it impacts in the generation of employment since cultivation is done manually in more than 95% of the cultivated area. The process requires on average 130 days$\cdot ha^{-1}$, generating in 2017 approximately a total of 222 thousand permanent jobs (Sanjinez, 2019). Therefore, economic stability and food security depends largely on the availability of this grain (Sanjinez, 2019; Effendi et al., 2021).

In general, agricultural practices such as monoculture, mechanization and the use of agrochemicals generally lead to changes in soil quality, degrading its structure and productive potential (Stehlíková et al., 2016; Florida and Núñez, 2020). Irrigated rice is a monoculture with special characteristics (Guzmán, 2006; Ruiz et al., 2005; Vignola et al., 2018); mechanization and fertilizer application and other activities in the development of this crop cause the degradation of soil physical properties: destruction of macropores, increased density (Çay, 2018), compaction, erosion, poor drainage, accumulation of P, K and others in the surface layer (Lv et al., 2015), hindering root growth and morphophysiological development of plants (Castillo, 2000; Pérez et al., 2002; Ruiz et al., 2005). Also, waterlogging can generate downward water circulation, causing the loss of clay and silt particles (Castillo, 2000; Alejandro, 2016) and accelerating the degradation of chemical characteristics, reducing the levels of OM, exchangeable bases and an acidification process, caused by the strong flushing (Castillo, 2000; Navarro et al., 2001; Alejandro, 2016; Ruiz et al., 2016).

In this context, it is necessary to evaluate the effects of rice cultivation on soil quality. Therefore, the aim of this research is to evaluate the effect of conventional rice (*Oryza sativa* L.) management under irrigation on the main physicochemical indicators of the soil, in the Mercedes and Pérez farms, in Yurimaguas, Alto Amazonas province-Loreto region, Peru.

2 Materials and Methods

2.1 Study area

The research was carried out in Mercedes and Pérez farms (Figure 1). Both farms are located in Suniplaya area, in the district of Yurimaguas, located in the southern part of the Alto Amazonas province in Loreto region.

2.2 Bio-climatic features

According to Holdridge (2000) classification of life zones or plant formations of the world, the area belongs to a Tropical rainforest (bh-T); according to Pulgar (2014) this area belongs to the Omagua Ecoregion or lowland rainforest. It has an equatorial, warm and humid climate with abundant rainfall, typical of the Amazon; the average temperature is $26,6°C$; the minimum relative humidity is 74.5% and the maximum is 81.5%, with an average annual rainfall of 2098 mm per year (World Climate Data, 2020). It is located on the left bank of the Huallaga River, about 100 km upstream from the confluence with the Marañon River, both belonging to the great Amazon River basin (Paredes, 2013). The soil type corresponds to Inceptisols, with a poorly developed B horizon and a medium floodable terrace.

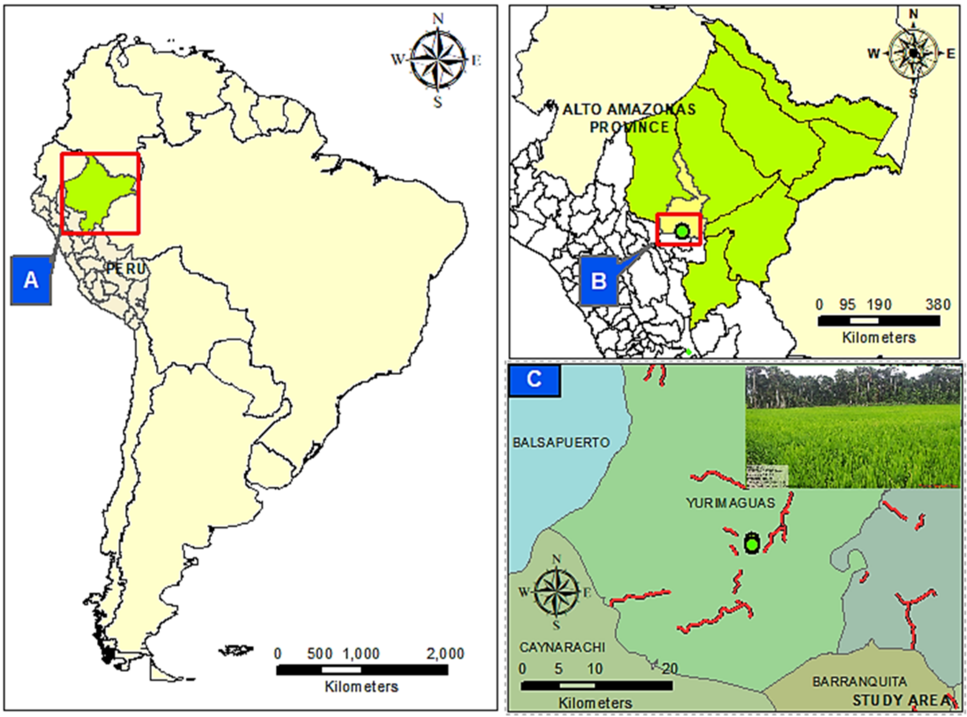

Figure 1. Geographical location of the area of study, Mercedes and Pérez farms (C), Yurimaguas in Alto Amazonas (B) Loreto-Perú (A).

2.3 Rice with conventional management

The areas with rice crops had a sequential process of intervention that is subdivided into:

(a) **First forest intervention** It was carried out on primary forest areas, using a caterpillar tractor which performed the clearing and cleaning of stumps and fallen trees in the area. Also, at this stage, the same machinery was used to level the land, build the edges, canals, access roads and drains; after this stage, the area was ready to begin the process of soil preparation and installation of the rice crop.

(b) **Soil preparation after the sow** In the dry season (June-October) the soil was harrowed with an agricultural tractor with a disc harrow, which allows the arable layer of the soil profile and the incorporation of the residues of the previous harvest. In the rainy season (between November and May), the soil is directly loosened with an agricultural tractor with a rotary plow, after flooding the land. Finally, the pits are leveled with an agricultural tractor equipped with a plow and the leveling is refined with motorized cultivators and the area is ready for rice planting.

(c) **Sowing** Once the soil is ready, in an independent space within the prepared area, the rice seedbed (variety HP 102 FL-THE VALUE) is planted for its subsequent transplanting of seedlings to the definitive field. The procedure involved soaking 80 kg of seed per hectare, the seed is sown broadcast and fertilized with urea at a dose of $8 kg\ ha^{-1}$; finally, when the seedlings from the nursery reach 25 to 30 days and about 20 cm in height, they are transplanted into the final field.

(d) **Crop management and fertilization plan at different productive stages.** Refer to Table 1.

(e) **Harvest** This stage was carried out approximately 135 days after the seedbed and crop installation, using a harvesting machine equipped with rubber tracks.

Table 1. Management plan of rice.

Description of the activity	Moment	Detail of the application
First fertilization	After the transplant	100 kg of di-ammonium phosphate and 100 kg of potassium chloride
Weed control	7 days after the transplante	Pre-emergent herbicide butachlor was applied
	10 days before the pre-sprout	Spraying was carried out with post-emergent herbicides Florpyrauxifen-benzyl (loyant), and Cyhalofop butyl (clincher) + its insecticide chlorpyrifos (typhoon).
Second fertilization	51 days after	100 kg of nitro s (ammonium nitrate) and 100 kg of potassium chloride were applied with a sheet of water.
Tilling treatment	60 days after	A biostimulant based on amino acid, fungicide carbendazin (protexin) and insecticide imidacloprid and benzoate (Agryben duo) are applied.
Third fertilization	70 days after	75 kg of ammonium nitrate (Nitro S) was applied with a water sheet.
Treatment for the formation and protection of ears	75 days after	Tebucunazole, Difeconazole, Propiconazole, imidacloprid and foliar insecticides of potassium, phosphorus, calcium, and boron were applied.
Yield	The last two harvests	A1 (7000$kg\ ha^{-1}$), A5 (8500$kg\ ha^{-1}$), A9 (8500$kg\ ha^{-1}$)

There are two harvests annually in these areas.

2.4 Secondary forest

The areas with conventional rice management were compared with secondary forest (SF), forest adjacent to these crops that present a large intervention of species with commercial value, whose current composition is based on species such as: moena (*Aniba amazónica* Meiz), pashaco blanco (*Macrolobium acaciaefolium* Benth), oje (*Ficus insípida* Willd.), Capirona (*Calycophyllum Spruceanum* (Bent.) Hook), palo lápiz (*Polyscias murrayi* F. Muel), ana caspi (*Apuleia proecox* C. Martius), bellaco caspi (*Himatanthus sucuuba* Woods), tornillo (*Cedrelinga cateniformis* D. Ducke), Cashimbo (*Cariniana periformis* Miers), setico (*Cecropia membranácea* Trécul), topa (*Ochroma pyramidale* Cav. Ex. Lamb), yarina (*Phytelephas macrocarpa* Ruiz et Pav), el huasai (*Euterpe oleracea* Mart.) and other species with low commercial value.

2.5 Soil sampling and physical-chemical analysis

Two harvests annually are obtained in the areas, in which sampling was carried out before the second harvest in 2020 (August-December), in rice plots with conventional management of one year (A1), five years (A5), nine years (A9) and secondary forest (SF) as reference. In them, a subarea of 2000 m^2 was selected and sampling was performed at 5 random points in each subplot, according to the methodology of Soil Taxonomy (2014), considering strata of 0.0 - 0.2 and 0.2 to 0.4 m depth; physical (Texture) and chemical indicators were evaluated: pH, organic matter $OM, N, P, K^+, Ca^{2+}, Mg^{2+}, Mg^{2+}, Al^{3+}$, cation exchange capacity (CEC), exchangeable acidity (EA) and aluminum saturation (SAl), following the protocols described by Bazán (2017).

2.6 Experimental design and statistical analysis

It is comparative non-experimental research (Hernández et al., 2014) statistically adjusted to the completely randomized design (CRD) with four treatments: secondary forest (SF), Rice with 1 year (A1), five years (A5) and nine years of management (A9) and a sample size $n = 5$ (40 samples in total), in strata from 0.0 to 0.2 m and 0.2 to 0.4 m and each experimental unit was formed by a subarea of 1000 m^2. Similar study methodologies have been applied by Navarro et al. (2018); Florida and Núñez (2020).

The data were subjected to ANOVA variance analysis and HSD-Tukey test with a significance level of 5% ($p < 0,05$) for the comparison of means, and the free software IBM-SPSS 25 was used to measure the management effects on soil physicochemical indicators in different strata.

3 Results and discussions

3.1 Physical indicators

The only physical indicator evaluated was soil texture. Table 2 shows that the different treatments evaluated present a clayey textural class (with% clay>42%) in both strata (0.0-0.2 and 0.2-0.4 m). In addition, changes are observed in the percentage of sand and silt fractions, showing variations with a tendency to decrease in A1, A5 and slight recovery in A9; on the contrary, the clay fraction increases in A1 and tends to decrease in A5 and A9 in both strata; this fraction is the least altered. In general, there was initially a reduction in the sand and silt fractions and a significant increase in clay, and an opposite effect was observed in both strata over time.

Table 2. Fraction statistics and texture class.

Treatments	Fractions			Texture class
	Sand	Clay	Silt	
Stratum 0.0-0.2 m				
SF	28.6 ± 3.29^{b}	48.8 ± 3.03^{a}	22.6 ± 2.61^{bc}	Clayey
A1	7.4 ± 0.89^{a}	80 ± 4.69^{b}	12.6 ± 4.56^{a}	Clayey
A5	9.4 ± 6.54^{a}	73.6 ± 9.94^{b}	17 ± 3.46^{ab}	Clayey
A9	14.6 ± 3.58^{a}	60 ± 6.78^{a}	25.4 ± 4.34^{c}	Clayey
EEM	16.8	44	14.6	
Sig.	0.00**	0.00**	0.00**	
CV (%)	61.93	21.07	31.85	
Stratum 0.2-0.4 m				
SF	21 ± 7.21^{b}	48.4 ± 6.07^{a}	30.6 ± 4.98^{b}	Clayey
A1	7.4 ± 1.67^{a}	79.6 ± 5.18^{b}	13 ± 4.24^{a}	Clayey
A5	7.8 ± 1.79^{a}	78.4 ± 5.55^{b}	13.8 ± 4.15^{a}	Clayey
A9	16.6 ± 9.21^{ab}	57.6 ± 8.41^{a}	25.8 ± 3.9^{b}	Clayey
EEM	35.7	41.3	18.8	
Sig.	0.005**	0.00**	0.00**	
CV (%)	61.36	22.67	42.07	

EEM: standard error of the mean, Sig.: Significance, **: highly significant, SF: secondary forest, A1,5 and 9 area with rice cultivation of 1 year, 5 and 9 years. Means followed by the same letter in the column do not differ from each other by Tukey's test ($p = 0,05$).

The results can be explained considering that the soil preparation system aims to prepare the soil prior to planting to create a suitable bed for plant growth and development (Vignola et al., 2018); therefore, the soil is lifted and turned from a depth of 10 to 20 cm, fractioning the aggregates, and affecting the soil-water relationship (Pérez et al., 2002; Ruiz et al., 2005). In addition, waterlogging generates downward water circulation, which causes the loss of fine particles, clay and silt (Castillo, 2000; Alejandro, 2016). These references explain why there is a reduction of the silt fraction in A1 and clay in A9 in both strata; however, they do not explain the reduction of sand and increase of clay in A1 and the recovery of the sand and silt fraction in A9; probably the initial conditioning of the plot that includes cuts and fills to flatten the terrain is responsible for the initial changes and it is only possible to observe in A5 and A9 as mentioned in the references.

3.2 Chemical indicators

Table 3 shows the means of fertility indicators; pH levels in both strata tend to decrease slightly in A1 and then increase in A5 and A9, the latter showing the highest mean. The mean *OM* and *N* in both strata tends to decrease in A1, A5 and A9 have the lowest mean; P decreases in A1 and then tends to stabilize and show recovery tendencies in A5 and A9, similar to SF. In addition, the mean levels of the superficial stratum are higher; on the contrary, in the case of K^+ the mean levels in A1, A5 and A9 are higher than SF in both strata and the highest means are in the 0.4 m stratum. In general, pH, P and K^+ decrease in A1 and then show recovery trends in A5 and A9, except for *OM* and *N*, which tend to decrease. In addition, significant differences were found in pH, *OM*, *N* and *P*, except for K^+, which shows no differences among the treatments and strata evaluated.

Table 3. Statistics of fertility chemical indicator.

Treatments	Indicators				
	pH	OM (%)	N (%)	P (ppm)	K (ppm)
			Stratum 0.0-0.2 m		
SF	4.74±0.14^a	3.82±0.68^b	0.19±0.03^b	7.04±0.48^a	72.37±0.99^a
A1	4.64±0.12^a	2.26±0.44^a	0.11±0.02^a	4.01±1.03^a	77.02±2.31^a
A5	4.97±0.16^b	2.48±0.55^b	0.12±0.03^a	6.79±2.26^{ab}	76.27±7.43^a
A9	4.76±0.08^{ab}	1.58±0.46^a	0.08±0.02^a	7.09±2.1^b	75.77±3.68^a
EEM	16	293	1	2706	18743
Sig.	0.007**	0.00**	0.000**	0.025*	0.367ns
CV (%)	3.56	38.19	38.46	32.1	5.8
			Stratum 0.2-0.4 m		
SF	4.76±0.13^{ab}	1.39±0.12^{ab}	0.07±0.01^{ab}	3.21±0.78^a	73.26±1.06^a
A1	4.63±0.1^a	1.52±0.32^{ab}	0.08±0.02^{ab}	2.6±0.8^a	80.98±5.38^a
A5	4.97±0.07^{bc}	1.65±0.52^b	0.08±0.03^b	5.43±1.47^b	82.46±7.31^a
A9	5.16±0.16^c	0.88±0.35^a	0.04±0.02^a	3.5±1.14^{ab}	81.37±5.56^a
EEM	0.01389	0.1269325	0	1178	28598
Sig.	0.00**	0.02*	0.021*	0.005**	0.056**
CV (%)	4.71	32.35	33.82	39.8	7.76

EEM: standard error of the mean, Sig.: Significance, **: highly significant, SF: secondary forest, A1,5 and 9 area with rice cultivation of 1 year, 5 and 9 years. Means followed by the same letter in the column do not differ from each other by Tukey's test ($p = 0,05$).

The behavior of the results in Table 3 can be explained considering that OM and N are indicators strongly altered by conventional management (Çay, 2018), as a consequence of being in conditions of high waterlogging (Castillo, 2000; Navarro et al., 2001; Alejandro, 2016), high transit of agricultural machinery that compacts the soil and alters the availability of oxygen (Alejandro, 2016), and the excessive use of herbicides for weed control (Ramírez et al., 2017). Therefore, the values of OM and N in A1, A5 and A9 are not ideal since Domínguez et al. (2020) consider normal values higher than 3 % OM for crop development. Although, soil preparation includes the incorporation of crop residues, this does not seem to help in increasing the levels of OM and N, as pointed out by Guzmán (2006); Alvarez et al. (2008); Li et al. (2011); in addition, very low levels are observed in A9 in both strata evaluated, which could affect the absorption levels of N, P and Mg, related to the production of green matter (Aguilar, 2010). Therefore, it is necessary to determine the fertilizer application rate to optimize N use efficiency and avoid adverse effects (Zhang et al., 2009).

Regarding pH, the intensive use of machinery and high volumes of water cause strong washing of exchangeable bases and an accentuated acidification process (Ruiz et al., 2016); although it is possible to improve or correct with the application of calcareous matter, in this case, flooding favors their rapid incorporation, raising pH levels (Morales, 2004). However, the results do not show this acidification process and according to Sanjinez (2019) are very close to the optimum levels for this crop (5.5 to 6.5 pH). Regarding K^+, no differences are observed and it tends to increase with time, which can be explained considering (Table 1) that 100 kg of potassium chloride is applied before transplanting, 100 kg more at 51 days after transplanting and foliar based on K, P, Ca^{2+} and B at 75 days, generating an accumulation that can alter the relationship that this element maintains with the cations Ca, Mg and with the nutrients N and P, and negatively influencing their absorption and limit production (Aguilar, 2010; Das et al., 2014), since the amount applied to the areas exceeds what is recommended by Alejandro (2016), doses between $80-150$ kg of K_2O ha^{-1} and by Paredes and Becerra (2015), who suggest using no more than 60 units of K^+ before transplanting. Therefore, it is necessary to consider the absorption curves of the crop to provide the necessary nutrients at each phenological stage of the crop (Tinoco and Acuña, 2009; Zhang et al., 2009). If the trend of K imbalance is not reversed, the potential for improving N and P fertilizer use efficiency and crop yield will be limited.

Table 4. Statistics of exchangeable chemical indicators.

Treatments	Indicators			
	Ca	**Mg**	**Al**	**CICe**
	Cmol(+)/kg			
	Horizon 0.0-0.2 m			
SF	4.41±0.78^a	0.65±0.09^a	7.42±1.16^{ab}	12.61±1.22^a
A1	4.04±0.82^a	0.61±0.11^a	13.1±1.57^c	18.36±2.58^b
A5	5.97±0.45^b	0.87±0.09^b	5.14±1.58^a	12.17±1.92^a
A9	4.95±0.59^{ab}	0.74±0.06^{ab}	7.94±1.09^b	13.93±1.56^a
EEM	459	8	1870	3561
Sig.	0.002**	0.001**	0.00**	0.00**
CV (%)	20.04	18.31	38.54	21.37
	Horizonte 0.2-0.4 m			
SF	4.59±0.73^{ab}	0.66±0.08^a	8.55±1.59^b	14.33±1.87^a
A1	4.05±0.69^a	0.61±0.09^a	12.26±2.13^c	18.15±2.2^b
A5	6.4±0.91^c	0.91±0.08^b	4.63±1.67^a	12.66±1.31^a
A9	5.85±0.58^{bc}	0.83±0.05^b	5.75±0.44^{ab}	12.91±0.76^a
EEM	539	6	2517	2652
Sig.	0.00**	0.00**	0.00**	0.00**
CV (%)	22.56	19.28	42.97	18.61

EEM: standard error of the mean, Sig.: Significance, **: highly significant, SF: secondary forest, A1,5 and 9 area with rice cultivation of 1 year, 5 and 9 years. Means followed by the same letter in the column do not differ from each other by Tukey's test ($p = 0,05$).

Table 4 shows that the means of Ca^{2+} and Mg^{2+} levels in both strata tend to decrease slightly in A1 and then increase in A5 and A9; on the contrary, Al^{3+}, AC and SAL levels increase in A1 and then decrease in A5 and A9. In general, all exchangeable indicators present highly significant differences according to treatment and stratum evaluated in comparison to the secondary forest soil.

The behavior of the exchangeable indicators (Table 4) can be explained by considering that rice soils lead to the establishment of a compact illuvial horizon, poorly permeable and enriched in iron and manganese, and an impoverished eluvial horizon, which is seen by an intense washout of bases (Castillo, 2000; Navarro et al., 2001); in addition, NH^{+4}, Fe^{2+} and Mn^{2+} ions released after flooding can displace considerable amounts of Mg^{2+} from exchange sites by strong scouring (Bacha, 2002; Ruiz et al., 2016). This explains the reduction of Ca^{2+}, Mg^{2+} and the increase of Al^{3+}, AC and SAl in A1; however, these references do not explain the recovery of Ca^{2+}, Mg^{2+} and the reduction of Al^{3+}, EA and SAl in A5 and A9, but may be due to the incorporation of crop residues and the contribution of fertilizers, in some cases in excess such as K^+ (Table 1) in the production process.

3.3 Multiple comparisons of physical indicators

All the fractions evaluated in the different treatments show significant differences (Table 3) and the HSD-Tukey multiple comparisons (Table 5) show that the sand fraction in treatments A1, A5 and A9 are different from SF, except A9 at 40 cm depth. In the case of the clay fraction, A1 and A5 present differences with SF in both strata and in the silt fraction A1 is different from SF in the superficial stratum, in the 40 cm stratum both A1 and A5 are different from SF.

Table 5. Tukey-HSD Test for physical indicators.

Dependent variable	Treatments		Mean differences (I-J)	Desv. Error	Sig.
Sand	SF20	A1	21.2*	2.59	0.000
		A5	19.2*	2.59	0.000
		A9	14*	2.59	0.000
	SF40	A1	13.6*	3.78	0.012
		A5	13.2*	3.78	0.014
		A9	4.4	3.78	0.657
Clay	SF20	A1	-31.2*	4.2	0.000
		A5	-24.8*	4.2	0.000
		A9	-11.2	4.2	0.072
	SF40	A1	-31.2*	4.06	0.000
		A5	-30*	4.06	0.000
		A9	-9.2	4.06	0.149
Silt	SF20	A1	10*	2.42	0.004
		A5	5.6	2.42	0.135
		A9	-2.8	2.42	0.660
	SF40	A1	17.6*	2.74	0.000
		A5	16.8*	2.74	0.000
		A9	4.8	2.74	0.332

*, Mean difference is significant at 0.05.

Multiple comparisons show differences between treatments and physical and chemical indicators evaluated in both strata. This result shows that the time of crop management, mainly mechanization and irrigation, has effects on the different soil fractions in both strata, compared to the secondary forest used as reference, being the affectation in the following order: sand>clay>silt.

3.4 Multiple comparisons of chemical indicators

The chemical indicators evaluated in the different treatments and strata also show significant differences (Table 4), except for K. The HSD-Tukey multiple comparisons (Table 6) show that pH in treatment A1 at 0.2 m and A9 at 0.4 m are different from SF; *OM* and *N* in treatments A1, A5 and A9 at 0.2 m; P in treatments A1 in surface stratum and A5 at 0.4 m and K^+ do not show differences compared to secondary forest SF.

Table 6. HSD-Tukey test for chemical fertility indicators.

Dependent variable	Treatments		Mean diffenrences (I-J)	Desv. Error	Sig.
pH	SF20	A1	0.09400	0.07977	0.648
		A5	-.23000*	0.07977	0.048
		A9	-0.02400	0.07977	0.990
	SF40	A1	0.13200	0.07454	0.322
		A5	-0.21000	0.07454	0.054
		A9	-.39200*	0.07454	0.000
OM	SF20	A1	1.56200*	0.34219	0.002
		A5	1.34000*	0.34219	0.006
		A9	2.24000*	0.34219	0.000
	SF40	A1	-0.13400	0.22533	0.932
		A5	-0.25800	0.22533	0.668
		A9	0.50600	0.22533	0.153
N	SF20	A1	0.08200*	0.01769	0.001
		A5	0.07000*	0.01769	0.006
		A9	0.11400*	0.01769	0.000
	SF40	A1	-0.00600	0.01179	0.956
		A5	-0.01400	0.01179	0.643
		A9	0.02600	0.01179	0.164
P	SF20	A1	3.03000*	1.04036	0.045
		A5	0.25000	1.04036	0.995
		A9	-0.05400	1.04036	1.000
	SF40	A1	0.61200	0.68649	0.809
		A5	-2.22200*	0.68649	0.024
		A9	-0.28600	0.68649	0.975
K^+	SF20	A1	-4.65200	2.73811	0.356
		A5	-3.89800	2.73811	0.504
		A9	-3.40000	2.73811	0.611
	SF40	A1	-7.72400	3.38217	0.144
		A5	-9.20600	3.38217	0.065
		A9	-8.11000	3.38217	0.118

*, Mean difference is significant at 0.05.

Table 7 shows the multiple comparisons according to the HSD-Tukey test, where Ca^{2+} in treatment A5 at 0.2 and 0.4 m shows differences with respect to the control treatment (SF); also, Mg^{2+} in A5 at 0.2 m and A5 and A9 at 0.4 m are different from SF; in the case of Al^{3+} in A1 at 0.2 m and A1 and A5 at 0.4 m shows differences with respect to SF and CICe in A1 at 0.2 and 0.4 m are different from SF. This multiple comparison test demonstrates that more than one treatment showed differences with respect to the control treatment (SF) in the different indicators and strata evaluated and negative effects are evidenced according to the treatments in the following order: A1>A5 >A9.

In general, the chemical indicators according to the treatments are severely affected at the beginning of the management (A1) and in most of them a recovery with time is observed, due to crop residues during soil preparation and the lack of precision in the management plan that so far (A9) seems a recovery process; however, negative effects are observed with time, as mentioned by Castillo (2000); Federación Nacional de Arroceros de Colombia (2001); Navarro et al. (2001); Ruiz et al. (2005); Alejandro (2016); Ruiz et al. (2016); Vignola et al. (2018); Domínguez et al. (2020).

Table 7. HSD-Tukey test for exchangeable chemical indicators.

Dependent variable	Treatments		Mean differences (I-J)	Desv. Error	Sig.
Ca^{2+}	SF20	A1	0.36600	0.42868	0.828
		A5	-1.56000*	0.42868	0.011
		A9	-0.54000	0.42868	0.600
	SF40	A1	0.54000	0.46432	0.658
		A5	-1.81000*	0.46432	0.006
		A9	-1.25600	0.46432	0.067
Mg^{2+}	SF20	A1	0.04000	0.05638	0.892
		A5	-.22000*	0.05638	0.006
		A9	-0.09400	0.05638	0.372
	SF40	A1	0.04600	0.04868	0.782
		A5	-.25600*	0.04868	0.000
		A9	-.17400*	0.04868	0.012
Al^{3+}	SF20	A1	-5.67400*	0.86488	0.000
		A5	2.28400	0.86488	0.076
		A9	-0.51600	0.86488	0.932
	SF40	A1	-3.70800*	1.00345	0.010
		A5	3.92000*	1.00345	0.006
		A9	2.80000	1.00345	0.057
CICe	SF20	A1	-5.74400*	1.19345	0.001
		A5	0.44200	1.19345	0.982
		A9	-1.31600	1.19345	0.693
	SF40	A1	-3.82200*	1.02998	0.009
		A5	1.66800	1.02998	0.396
		A9	1.41400	1.02998	0.533

*, Mean difference is significant at 0.05.

Finally, based on the yield background of the areas (Table 1) there is an average yield in the last two seasons of 7t ha^{-1} in A1 and 8,5t ha^{-1} in A5 and A9, the latter higher than the national average of 7,19t ha^{-1} (MINAGRI-DGESEP, 2018), to the 7,72t ha^{-1} reported by Gabriel (2014) combining biol and 20t ha^{-1} of bocashi, to the 6,88t ha^{-1} obtained by Jara (2003) with the Biflor variety in Tulumayo, Leoncio Prado and to the 5,5 and 5,3t ha^{-1}, respectively obtained by Barahona et al. (2019) in an Inceptisols soil in Coclé, Panama. However, they are slightly lower than the 9,5t ha^{-1} reported by Quevedo et al. (2019) and the 10,346t ha^{-1} reported by Contreras (2016), with the application of phosphorus and micronutrients in Tinajones Jequetepeque. According to Sanjinez (2019), the optimum temperature for germination varies between 10 and 35°C, and for stem, leaf and root growth it varies between 7 and 23°C; the area under study has an average of 26.6°C (World Climate Data, 2020). Secondly, the yields are due to the very disciplined fertilizer plan applied (Table 1) and thirdly to the crop residues incorporated in each soil preparation cycle.

4 Conclusions

Differences were found in the sand, silt and clay fractions in the different treatments evaluated (SF, A1, A5 and A9) and they correspond to a clayey textural class. Initially, a reduction was found in the mean values of the sand and silt fractions and an increase in clay, and over time the sand, silt and clay fractions tended to decrease.

The chemical indicators determine that the soils have an acid to slightly acid pH, with OM, N, P and Ca levels that vary from low to medium; K, Mg and CEC have low levels and Al has high levels, with significant differences between the treatments and strata evaluated, except for K, which shows no differences.

The analysis of variance and multiple comparisons show differences between treatments in the different physical and chemical indicators evaluated in both strata; the time of irrigated rice management has effects on the different soil fractions and on the chemical indicators in both strata, affecting severely at the beginning of management (A1) and a recovery is observed over time (A9), due to the incorporation of crop residues and a strict fertilization plan that has maintained yields above the national average; however, negative effects are observed in the long term.

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Geographic Sciences

Geomorphological restoration on tailing deposits: A study case applied to the Rio Blanco mining concession, Ecuador

Restauración geomorfológica sobre depósitos de relaves: Caso de estudio aplicado a la concesión minera Río Blanco, Ecuador

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Abstract

This study presents two separate assessments to contribute to the development of a sustainable mining industry with low environmental impact that minimizes possible social conflicts related with this activity. The first is how to apply the concept of geomorphological restoration on degraded spaces to establish geoenvironmental integration proposals, focused on the restoration of mining areas altered by the tailing deposit of the Rio Blanco project, Azuay province-Ecuador, based on the modeling with GeoFluv method and the Natural Regrade software. The second is an analysis of the general aspects of the mining activity in Ecuador, its cautions, and the environmental problems that could show tailing deposits. The main result of the current study has been a stable design that simulates natural conditions, which although it loses a storage capacity of 15% relative to the conventional design, it maximizes the volume of tailings to be accumulated. This significantly increases the stability and environmental integration that would have the tailing deposit.

Keywords: Geomorphological restoration, land modeling, environmental integration, tailings deposits, mining, environment.

Resumen

Con el fin de contribuir al desarrollo de una industria minera sostenible, de bajo impacto ambiental, que minimice posibles conflictos sociales asociados a esta actividad, este estudio da a conocer: 1) cómo aplicar el concepto de restauración geomorfológica sobre espacios degradados, para establecer propuestas de integración geoambiental, enfocadas a la restauración de zonas mineras alteradas por el depósito de relaves del proyecto minero Río Blanco, provincia del Azuay-Ecuador, basadas en el modelamiento con el método GeoFluv y el software Natural Regrade; y 2) un análisis de los aspectos generales de la actividad minera en el Ecuador, sus precauciones, y la problemática ambiental que pueden presentar los depósitos de relaves. Como resultado se obtuvo un diseño estable que imita condiciones naturales, en el que a pesar de perder una capacidad de almacenamiento del 15% en relación con el diseño convencional, logra maximizar el volumen de relaves a acumular, aumentando significativamente la estabilidad e integración ambiental que tendría el depósito.

Palabras clave: Restauración geomorfológica, modelación del terreno, integración ambiental, depósitos de relaves, minería, ambiente.

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1 Introduction

Ecuador is rapidly growing as a mining investment location in Latin America (Jamasmie, 2017). Ecuador recently ranked 92 out of the 109 most attractive nations to attract investors (Stedman et al., 2019). New campaigns and regulatory frameworks have changed the strategy for a nation that has traditionally based its economy on oil and agricultural exports (Verdugo and Andrade, 2018), specially with an average raw matter extractivity index of 36.93% between 2000 and 2011 (Hailu and Kipgen, 2017). Between 1999 and 2016, the share of mining GDP to total GDP was between 1.17% and 1.56%, with a gradual increase in recent years between 1.48% and 1.51% from 2013 to 2016 (Almeida, 2019). According to conservative studies, mining could become 4% of GDP (Vistazo, 2019).

Since 2016, Ecuador received approximately 420 concession applications, where 160 have already been approved obtaining an investment of more than 100 million dollars to explore areas rich in gold, copper, silver and molybdenum (Jamasmie, 2017). The country has a total area of 105,000 hectares concessioned for mining activities, distributed in seven provinces (BCE, 2021); for example, in 2013 Ecuador awarded the company Ecuagoldmining South America S.A., the Rio Blanco project in the province of Azuay (Figure 1), with reserves totaling 991,000 ounces of gold and 4.7 million ounces of silver, a resource equivalent to an amount not less than US$14 billion (Latinominería, 2012).

Likewise, it must be taken into account that mining has generated problems in the environment (Vásconez and Torres, 2018). Large extractive projects are also part of this controversy for not respecting human and nature rights (D'Angelo and Ruiz, 2018), deriving in social conflicts and legal suspensions (Ruiz, 2018), of projects such as Rio Blanco or Quimsacocha (Massa-Sánchez et al., 2018). In addition, the location of these large mining projects in highly fragile ecosystems, such as in hydric reloading areas, moorlands and wetlands, becomes relevant (Environmental Justice Atlas, 2017). As a result, on February 7, 2021, the people of the province of Azuay voted in a referendum for the prohibition of extractive metal mining activity in watershed areas with an important hydric reload (El Comercio, 2021). In this context, mining must necessarily consider its operational, legal and ethical responsibilities, aspiring to compatibility with respect for the environment and sustainability, for example, through the responsible exploitation of resources, generating benefits (environmental, social and economic) that equal or exceed the values that existed before exploitation (Oyarzún and Oyarzún, 2011).

On the other hand, the management of mining waste or tailings and their subsequent treatment are two factors of important environmental risk involved in mining (Oyarzún and Oyarzún, 2011), since their generation is much higher than the economic products they produce. For example, in the case of copper (Cu) mining, a ton of mineralized rock requires the extraction of 10 kg of the metal in the best case scenario. This means that 990 kg of material would be waste destined for a tailings deposit (Oyarzún et al., 2011), making this activity one of the most persistent sources of heavy metal pollution on the planet, due to: 1) its function is to store solid materials resulting from operations to separate and obtain metals (Espín et al., 2017), and 2) these wastes need to be stored in large areas (Serrato et al., 2010).

Regarding the environmental problems of tailings deposits, their potential to generate acid mine drainage (AMD) stands out, especially in places where deposits with sulfides are exploited (Sarmiento, 2007), and which are subsequently abandoned (Oyarzún et al., 2012). In addition, containment dams may break due to their intrinsic instability (plastic materials with high water content) (Mudd and Boger, 2013) and flood vast areas of land may happen (US EPA, 2015), and can cause huge human losses, such as the case of Brumadinho, Southeast Brazil, which occurred on January 25, 2019, where 257 people died and more than 182 dissapeared (Pereira et al., 2019).

These reservoirs generally have the classic design called "aguas arriba", which presents stability problems (WISE, 2019) such as: 1) the soil on which it sits is not adequate, so that there may be water infiltration and thereafter the base of the dam may give way, as happened in Aznalcóllar (Seville, Spain on April 25, 1998) (Rodríguez et al., 2009), 2) a poor response to seismic activity (relevant issue in Ecuador) (Oldecop and Rodríguez, 2007), and/or 3) the rise of the water level leading to two situations: one where the weight of water can induce rotational sliding type phenomena near the dam, with total or partial loss of the dam; and the other where water overtops the containment dam, and erodes the successive dam(s) leading to the breakage of the dam(s) (Owen et al., 2020).

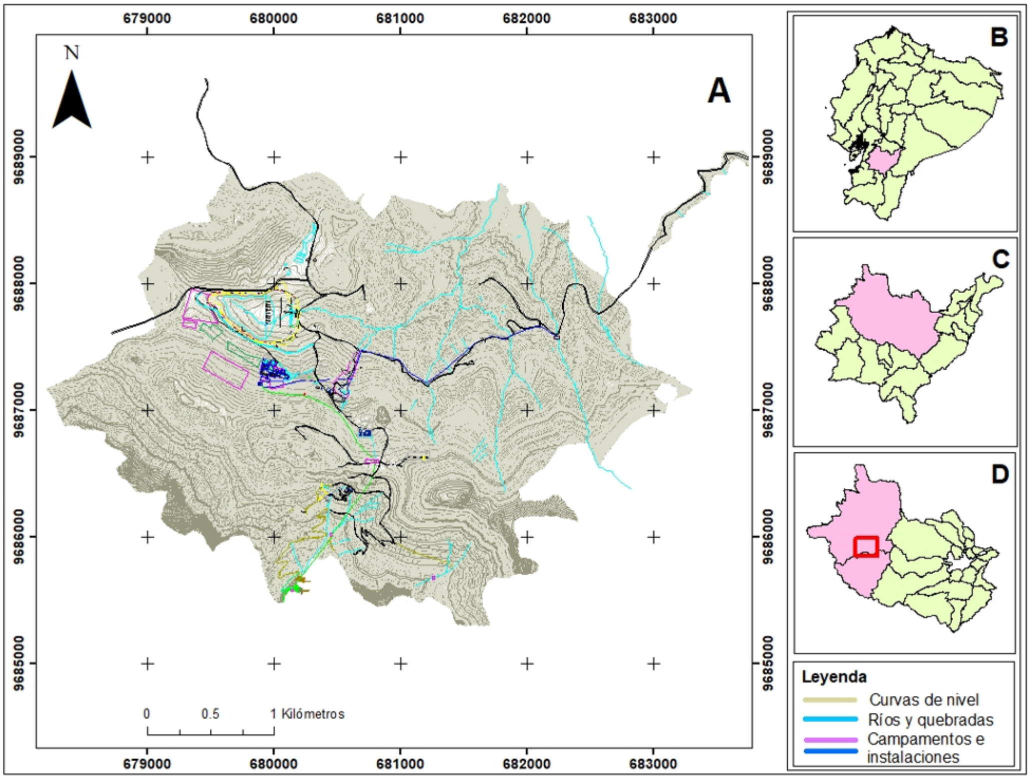

Figure 1. A (General map of Río Blanco mining project), B (location of the province of Azuay in the Republic of Ecuador), C (location of the Cuenca canton in the province of Azuay) and D (location of the mining project in the parishes Molleturo and Chaucha, Cuenca canton).

On the other hand, it is difficult to think of a human activity on the territory that does not involve moving earth or making changes on the first layers of soil, replacing ecosystems that once contributed to generate fertile soils with unfertile (Daily and Ehrlich, 1992). In this context, the application of geomorphological restoration, a recent discipline applied to "land moving" activities such as mining, linear infrastructures, civil works, urban planning, creation of green spaces, etc., is relevant. Currently, this discipline has become a tool for the mining sector (De la Villa and Martín Duque, 2018) because it is an activity that drastically transforms the relief, where restorations allow rebuilding new geoforms, landscapes and ecosystems (Universidad Complutense Madrid, 2021), as well as to create proposals for environmental integration, which tend to sustainability (Zapico et al., 2011), and help to reduce somehow the impacts of this activity (Bastidas-Orrego et al., 2018).

In this sense, GeoFluv method (patented in the USA) (GeoFluv, 2021) and the Natural Regrade software developed by Carlson Software Inc (2020), currently constitute one of the most advanced and complete tools at international level for the design of mature and stable geoforms that mimic natural conditions (Martín Duque et al., 2012) and that are generally applied in spaces affected by earthworks (Martín Duque and Bugosh, 2017). This study shows the application of the GeoFluv method on the tailings deposit of the Rio Blanco mining concession, Ecuador, with the aim of obtaining a conceptual, stable, environmentally integrated geomorphological design that mimics natural conditions.

2 Materials and methods

This section deals with the initial treatment and debugging operations of the starting information, followed by the performance of the restoration model on the tailings deposit using the GeoFluv method and the Natural Regrade software, and finally the definition of the design parameters used in the geomorphological terrain modeling.

2.1 Initial treatment of the information

the starting point of the study began with the treatment of the general information of the project in CAD format provided by the company1 (Terrambiente Consultores, 2012, 2016). Then, object selection and editing methods were used, performing some initial information debugging operations (quality control), such as: cleaning of repeated information, trimming of suitable working spaces, among others. At the end, a simplified map of the area was obtained (Figure 1A). The general topography of the project was detailed using contour lines with elevations, except for the tailings deposit, which did not offer elevations as it was represented as 2D polylines. Therefore, an initial step was to provide elevations to the lines defining this deposit, converting the 2D polylines to 3D. For information purposes, the crest of this tailings deposit is located at 3,763 masl.

2.2 Base design of the tailings deposit

Based on the information obtained in the Natural Regrade software, we focused on the design of the tailings deposit. Using layer management, we introduced: 1) boundary of the surface to be restored, simulating the edge of a river basin, 2) general layout of the fluvial channels and 3) design parameters (settings). Blue lines in Figure 2 represent the 2D polylines of the tailings deposit proposed by the company, and the white lines inside and surrounding it represent the design of the watershed boundary and the layout of the fluvial channels, respectively. Finally, as part of the preparatory process, an initial TIN (triangular irregular network) was performed. From this basic information, it was possible to start with the performance and definition of designs.

2.3 Restoration design parameters

Regarding parameters (Table 1), applying the Natural Regrade software (Carlson Software Inc, 2020), it is highlighted that most of these were obtained from a local or analog reference. Although the complete acquisition of these data was limited due to the intense field work required, the remaining parameters were obtained from similar projects (Martín Duque et al., 2012), obtaining a conceptual restoration design, which if adopted as a restoration solution, should be subsequently validated with local data.

3 Results and discussion

This section presents the results of the restoration design obtained on the tailings deposit, using the GeoFluv method and Natural Regrade software, highlighting the values of cut and fill, in addition to the analysis of slopes and orientations that quantitatively identifies the variation between designs, and finally addresses the discussion resulting from this study.

3.1 Results of the restoration design

The priority of the design was to seek stable morphologies that maximize the volume of tailings materials to be stored. Operations were carried out such as changing adjustments in the program and limiting the amount of affluents to try to generate fewer valleys (space that would not accumulate tailings with respect to the conventional design), distribute and balance the channels, reduce the value of the slope at the head of the channels, or maximize the convex section of the slopes (Figure 3). Finally, a restoration design was obtained, the results of which are detailed in Table 2. With the proposed design, 80,548.35 m^3 of tailings storage capacity is lost compared to the conventional (original) design, equivalent to 15% of the total mine waste (Table 2).

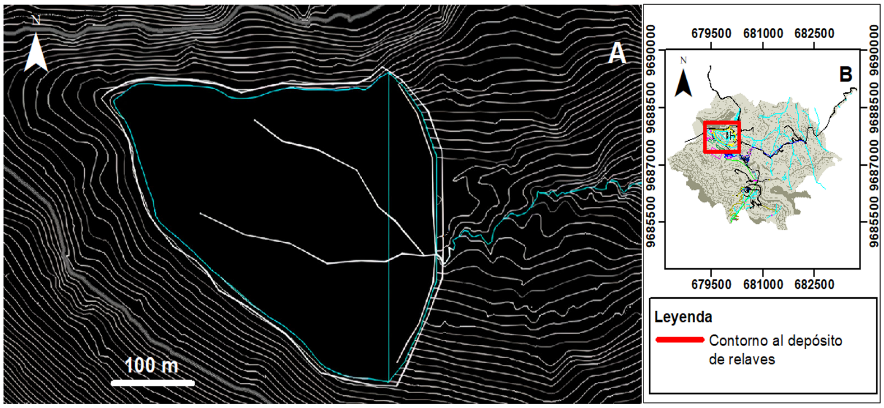

Figure 2. A (CAD map representation of the tailings deposit environment that is the subject of the restoration design and B (location of the tailings deposit within the overall mine project map).

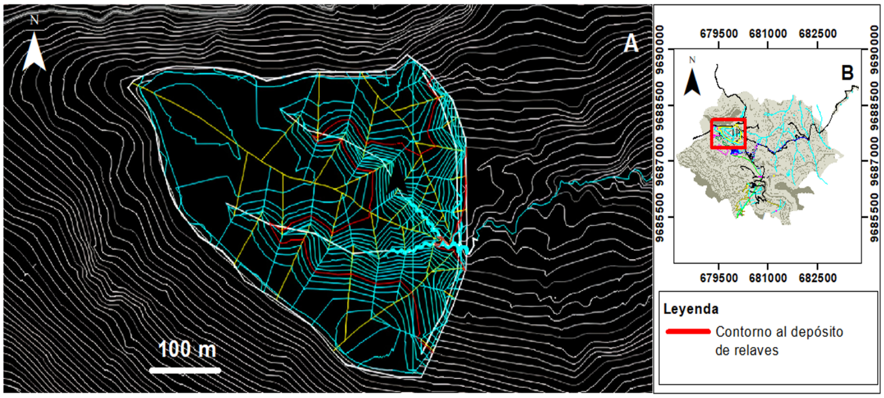

Figure 3. A (final adopted design. Blue shows contour lines and fluvial channels, red shows master contour lines), B (location of the tailings deposit in the general map of the mining project).

Figure 4 presents 3D views comparing images of the conventional design with their equivalents obtained in the proposed restoration design. One of the conditions observed in the results of the restoration design is that it limits the volume of tailings to be stored compared to the original (conventional) design, equivalent to a full reservoir, because the Natural Regrade program designs landscapes with valleys, which means that these spaces imply a reduction in the storage capacity. A design that accumulates 15 % less tailings material has been made in this study, and is much more integrated and stable. However, it will be up to the operating company to assess whether this reduction is feasible or not.

According to information from the general map of Rio Blanco mining project, made by Ecuagoldmining South America S.A., the storage capacity of the tailings deposit in the area would be 804 000 m^3 (Terrambiente Consultores, 2012, 2016). However, in our quantification carried out with accurate topography and using Carlson software, the capacity of the deposit is 535 273.5 m^3, taking the latter as a valid result. Given the environmental risk presented by tailings deposits in terms of: 1) static and dynamic stability; 2) effects associated with seismic response (Vanegas, 2011) and acid rock drainage (ARD), taking as a reference the quantification result obtained with the restoration design of this study implies working with a smaller amount of waste, prioritizing safety, and having a lower risk of breakage.

Table 1. Parameters used in the design using Natural Regrade software.

Parameters	Units	Tailing deposit
Maximum distance between connection channels	m	3
Maximum distance from the crest line to the head of the canal	m	45
Slope of the main valley to the mouth of the primary channel	%	Determined with AutoCAD
Main channel reach	m	36
2yr-1hr (value for a precipitation event that determines the dimensions of the main channel.)	cm	2.15
50yr-6hr (value for a precipitation event that determines the ddimensions of the flood-prone channel.)	cm	8.92
Drainage density variation	%	20
Angle of the subbridge to the channel perpendicular (upstream)	degrees	10
Maximum slopes on the North - East line	%	20
Maximum slopes in a straight line channel	%	33
Max Cut/Fill Variation	%	125
Max Cut/Fill variation	%	80
Overall magnification factor for cut stock		1
Overall reduction factor for fill material		1
Tolerable elevation at the head of the canal	m	1
Tolerable slope at the head of the channel	%	1
Channel settings		
Maximum waterflow velocity	m/s	1.37
Slope (upstream)	%	-12
Slope (downstream)	%	Determined with AUTOCAD
Width - depth ratio	slope>0.04	10
Sinuosity	slope >0.04	1.15
Watershed		
Runoff coefficient (reclaimed areas)		0.3
Runoff coefficient (areas without vegetation)		0.89

In the case of a real application, the results obtained with the restoration design could be contrasted with the information from the operating company, and if necessary the magnitude in relation to the storage capacity could be corrected. In this regards, the pros and cons between the methods used could be compared, a topic that would be investigated in a future research topic.

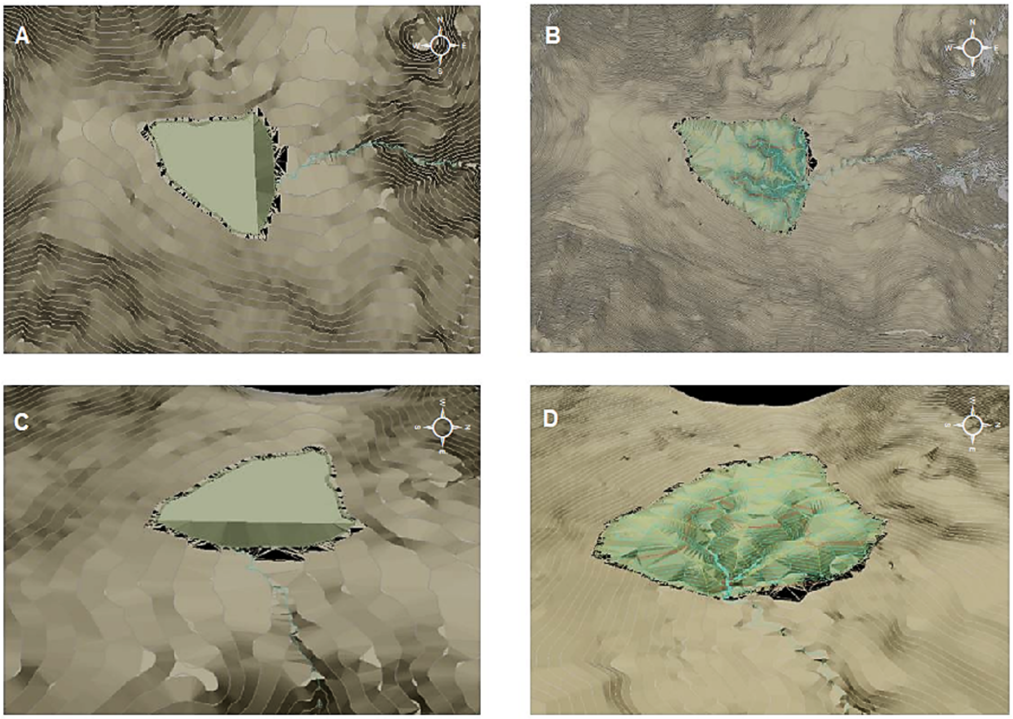

Figure 4. A and B (comparison designs viewed from facility), C and D (compare designs between frontal views).

Table 2. Data on volumes of the selected design version.

Description	Quantity (m^3)
Storage capacity of original deposit	535 237.50
Cut (capacity that does not store the geomorphologic design with respect to the conventional one, especially for the valleys)	142 739.00
Backfill (material that the geomorphological design can accumulate "in excess", with respect to the conventional one, by accumulating material in the hills of the interfluves).)	62 190.00
Difference of "cut-fill", i.e., volume of tailings that the geomorphological design stops accumulating with respect to the conventional design.)	80 548.35

3.2 Results of slope and orientation analysis

As complementary information to the restoration study, an analysis of slopes and orientations was performed for both the conventional and the proposed design, showing quantitatively (percentages of the different classes) the variation between the two designs. Regarding slope analysis: there is little variation in ranges or classes in the conventional (original) design (Table 3). On the contrary, the proposed design presents higher percentages, resulting in a larger area of habitats (Table 4). The most important aspect of this analysis is that there is a large difference in real land areas (sum of the areas of the triangle network) between the designs.

Regarding the orientation analysis: little variability is obtained in the conventional design, since the deposit is mostly flat, with its closing wall oriented towards the east (Table 5, Figure 4). The proposed restoration design has a greater diversity which is considered favorable, since it implies a greater biological diversity because some species will have a greater possibility of establishing in certain orientations, depending on different physical factors (light, humidity, etc.) (Table 6).

Table 3. Slope report, conventional design.

Slope report		
Average slope		4,40 %
Slope ranges or classes	Area (ha)	Percentage of total area in the slope range or class (%)
<10	7.76	83
10 a 20	0.12	1.4
20 a 30	1.46	15.7
30 a 40	0.00	0
>40	0.00	0
Total	9.36	100

Table 4. Slope report, proposed design.

Slope report		
Average slope		9.90 %
Slope ranges or classes	Area (ha)	Percentage of total rea in the slope ange or class (%)
<10	11.94	60.53
10 a 20	5.62	28.5
20 a 30	1.85	9.38
30 a 40	0.29	1.47
>40	0.02	0.12
Total	19.88	100

3.3 Discussion

Ecuador is progressively becoming a country that has embraced mining as a source of economic resources by taxes, royalties and employment, supporting the exploration and exploitation of various mining projects. However, mining activity generates a certain amount of distrust and fear in a context in which environmental and social concerns are becoming increasingly important, which has led to protests against extractivism, especially if these projects are located in environmentally sensitive areas. The high risk associated with mining can be minimized by applying proposals for environmental integration and restoration of areas altered by earthworks. It is possible to contribute to the development of mining that is compatible with sustainable development by applying new methods and software, such as the GeoFluv method and the Natural Regrade software, in such a way that it generates long-term environmental, social and economic benefits.

One of the main problems associated with mining activities at all scales is the disposal and subsequent treatment of tailings deposits. In the case of the Rio Blanco mining project, a natural high mountain valley (tailings deposit crown located at 3763 m.a.s.l.) will be filled with tailings. According to the literature consulted, this is not advisable from an environmental and geomorphological point of view, following conventional techniques and designs, such as the so-called "upstream" design, which presents a series of stability problems (breakage). In addition, according to information collected on the mineralogical studies of the project (Terrambiente Consultores, 2012, 2016) there is the presence of Pyrite in the area (FeS_2), a metallic sulfide that can cause the release of heavy metals and generate acid mine drainage under humidity and oxidation environmental conditions (AMD) (Gray, 1997).

In fact, the contaminating potential of this geochemical phenomenon continues once the activity has ceased. Covers refer to the techniques used for risks of breakage and environmental protection, which prevent tailings from coming into contact with the outside, but these covers would not be entirely effective, since mining disasters that relied on this methodology have occurred (Lottermoser, 2013). Therefore, in addition to the cover system, it is recommended to apply the GeoFluv method and the Natural Regrade software, which allow the design and construction of various geoforms, such as valleys through which a drainage network runs, as well as other sets that mimic natural conditions, designed to respond safely to extraordinary episodes of rain, making them more stable to water infiltration and the effect of water and fluvial erosion.

Conventional mining restoration methods focus on two aspects: 1) the conventional topographic berm-slope-ditch design and 2) the aesthetic aspect carried out through revegetation; these have had unsatisfactory results in different parts of the world, since this type of topography (which does not exist in nature) does not have the necessary capacity to evacuate high runoff values, which form streams and gullies with high erosion and sediment emission rates, also being scarce its landscape integration due to its rectilinear shapes (Nicolau et al., 2021). However, since 2005, the GeoFluv method and the Natural Regrade software have been applied on mining restorations, replacing conventional methods mainly in the United States, as well as others such as the reconstruction of La Revilla or el Alto Tajo (Spain), or large coal mines such as Drayton South (Australia), La Guacamaya and Puerto Libertador (Colombia) and Mina Invierno (Chile), with favorable results in terms of stability and reduction of erosion and sediments (Hancock et al., 2020). In this sense, geomorphological restoration can be used to solve intrinsic problems of tailings deposits.

Table 5. Orientation report, conventional design.

Orientation report		
Zone	Area (ha)	Surface percentage (%)
North	0.26	2.83
Northeast	0.51	5.46
East	2.49	26.65
Southeast	0.56	5.99
South	0.38	4.05
Southwest	2.04	21.87
West	1.79	19.17
Northeast	1.30	13.93
Total	9.36	100.00

Currently there is no real case (not even at the project stage), that may use these methods on high mountain tailings deposits. Hence, the potential importance of the results obtained in this study, specially by putting into practice the methodology and tools described to improve the environmental integration of the project in terms of risk reduction, reduction of environmental impact and ecological and landscape integration, and the improvement of the image of the company from the operational and social point of view.

To standstill the mining activity and the closure of the tailings deposit, a cover system must be applied, whose objective is to keep tailings as stable as possible (in chemical terms), avoiding the formation of acid mine drainage (Matos et al., 2016), an essential cover system when complementing a geomorphological restoration. The proposal of a cover system was not addressed in this study, which can be further investigated.

Table 6. Orientation report, proposed design.

Orientation report		
Zone	Area (ha)	Surface percentage (%)
North	2.91	14.79
Northeast	5.08	25.79
East	3.03	15.39
Southeast	2.93	14.89
South	3.76	19.09
Southwest	1.18	6
West	0.42	2.15
Northeast	0.36	1.87
Total	19.73	100.00

4 Conclusions

The geomorphological restoration proposal offers an optimal result, capable of providing maximum stability and environmental integration with only a 15% reduction of the total storage capacity. Since there are no designs or projects applied with the GeoFluv method and the Natural Regrade software for tailings deposits, there were unknown aspects, such as the contribution (in fact, an essential condition) of placing a series of perimeter channels in front of the deposit (dam). The quantification of the increase in real land area that occurs between designs (flat top and inclined plane at the dam, with respect to a complex topography with valleys, hills, slopes, etc.), is almost double.

Considering that the mining project is located within sensitive high mountain areas and close to other water recharge areas, the best possible extractive management should be sought, since inappropriate management, such as the lack of adequate environmental control, could lead to terrible environmental consequences for the natural and social environment. Therefore, the methodology applied with GeoFluv and Natural Regrade in the Rio Blanco mining project contributes to minimize the associated environmental risk.

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