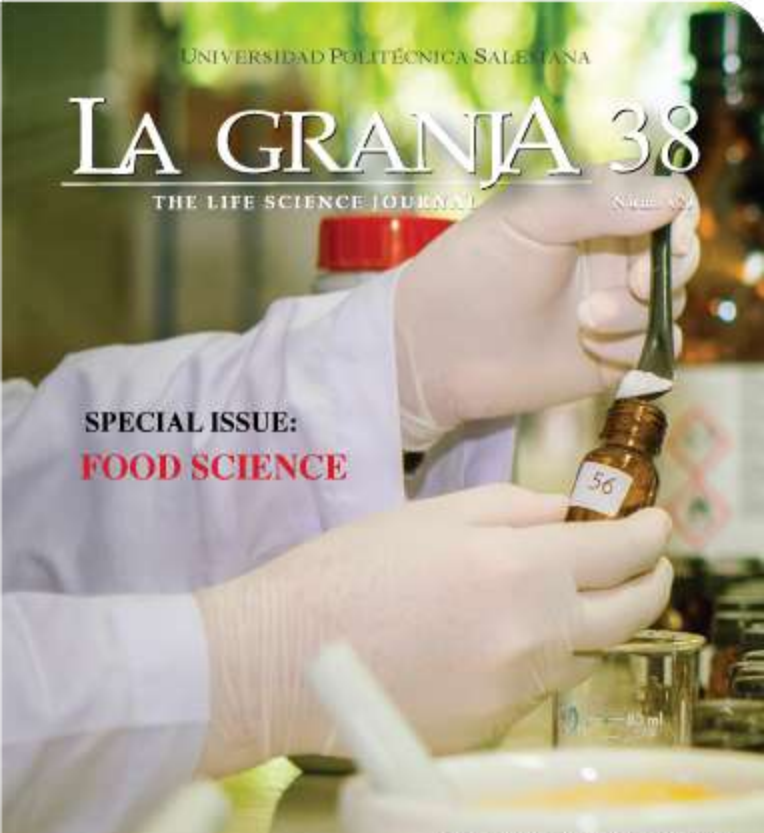
UNIVERSIDAD POLITÉCNICA SALESIANA

LA GRANJA 38

THE LIFE SCIENCE JOURNAL

Volume 38

SPECIAL ISSUE:
FOOD SCIENCE



ISSN Impreso: 1390-3799 / ISSN electrónico: 1390-8596

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Chemical analysis of amazonian essential oils in ecuadorian Shuar community

Solvatochromic behavior of mortiño's natural dye

Endophytic bacteria isolated from *Senecio glaucus* L. in Egypt

Review: reproductive parameters in the production of tilapia

LA GRANJA REVISTA DE CIENCIAS DE LA VIDA

La Granja Revista de Ciencias de la Vida • Volumen 38 • Número 2 • septiembre 2023 - febrero 2024. Revista semestral de Ciencias de la Vida de la Universidad Politécnica Salesiana del Ecuador. Publicación internacional bilingüe dedicada a estudios relacionados con ciencias ambientales, de la tierra, agropecuarias, biológicas, y sus líneas transversales de conocimiento. Las ideas y opiniones expresadas en las colaboraciones son de exclusiva responsabilidad de los autores y autoras.

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La granja:
Revista de
Ciencias de la Vida

Editorial

ABYA YALA | UNIVERSIDAD POLITÉCNICA SALESIANA

Dear reader:

We are pleased to present our Special Issue in Food Science in volume 38 of La Granja. Food is the basis of human life and health, and today it faces challenges and threats ranging from the search for healthier and more varied foods, as well as shortages of quality raw materials due to intensive agriculture, land degradation and climate change. Thus, the importance of a discipline such as food science, which is a multidisciplinary field of knowledge that studies food from the raw material to the final product, covering chemistry, biochemistry, nutrition, microbiology and engineering for its production, transformation, conservation and consumption, as well as the use and valorization of its by-products and wastes. The papers published in this volume relate to this topic.

Firstly, the volume presents the paper by Sebastián Yánez-Segovia, Leticia Silvestre, Ignacio Chamorro-Warnken, researchers from the University of Talca- Chile, Universidad Central del Ecuador, Universidad Austral de Chile and the University of Reading- UK, who present a quick method to determine the content of foliar chlorophyll as an effective tool to estimate the quality of potato tuber. Then, Luis Intriago, Viviana Talledo, Rudyard Arteaga, Anderson Pazmiño and Gerardo Cuenca-Nevárez, from the Technical University of Manabí, study the use of propolis in the conservation of red tilapia filets to propose it as a promising organic preservative for the food industry.

Likewise, Mayra Montalvan, Omar Malagón, Nixon Cumbicus, Fani Tanitana and Gianluca Gilardoni from Universidad Técnico Particular de Loja, analyze the chemical composition of the essential oils from four Amazonian species of the Shuar Antuash center in Morona, province of Morona Santiago - Ecuador. Finally, the article by Tatiana Mora, Martha Suárez, Carlos Brito and Dennys Almachi, from the Central University of Ecuador, presents the solvatochromic behavior of the natural dye of mortiño (*Vaccinium floribundum Kunth*), whose effect shows potential applications.

As for the productive and veterinary point of view, Leonardo Reyes-Trigueros, María del Carmen Monroy-Dosta, Erika Torres-Ochoa, Alejandro De Jesús Cortés-Sánchez and Luis Daniel Espinosa-Chaurand from the Autonomous University of Baja California Sur, the Metropolitan Autonomous University and Xochimilco, along with the CONACYT of Mexico analyze the reproductive parameters of Tilapia. They present the main factors for an efficient reproduction of this new species that has become widely known and accepted.

Regarding meat and milk, from the veterinary sciences, the risk factors of burcellosis in the livestock industry are analyzed. Omar Santiago Andrade Guzmán, Andrea Elizabeth Vintimilla Rojas, Mateo Damián López Espinoza, Guillermo Emilio Guevara Riera and Sergio Emiro Rivera Pirela, in a collaboration between the University of Cuenca in Ecuador and the University of Zulia in Venezuela, carry out an exhaustive study of the main risk factors.

From Egypt, Mohammed Sabry Sultan, Ashraf Elsayed, and Yasser Ahmed El-Amier from the Botany Department of Mansoura University for the first time isolated endophytic bacteria from the internal cells of *Senecio glaucus*'s roots, stems, leaves and capitular tissue.

Then, from the environmental sciences, Renato Sánchez, Carlos Cerón and Karla Landeta from Universidad Politécnica Salesiana UPS of Ecuador, evaluate the fluvial load of pesticides in the Pisque River, a highly productive sector in Floriculture of the Community of Cayambe.

Likewise, from the UPS, Ernesto Delgado, Maribel León, Carlos Cantos Guamán and Martha Guzmán Juárez analyze the effects of mining activity on biodiverse areas of the country, which is valuable research at crucial moments for the country when in August 2023, the citizens will vote on the permanence in certain areas of this controversial activity.

Finally, Oscar Portilla, César Leiva, Marco Luna and Izar González, in a joint work between the ESPE University of Armed Forces and the companies Geoint and ETSI, carry out a thorough evaluation of digital terrain and geopotential models in Ecuador. In this way, we hope that the following selection of scientific articles will be useful and serve in more socially relevant research in the region and abroad.

Sincerely,

PhD. Ignacio de los Ríos
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La Granja:*Revista de Ciencias de la Vida* 38(2) 2023: 7-8

La granjA:
Revista de
Ciencias de la Vida
pISSN:1390-3799; eISSN:1390-8596
http://doi.org/10.17163/lgr.n38.2023.01

Special Issue/ Número Especial

Food Science

ABYA YALA UNIVERSIDAD POLITÉCNICA SALESIANA

Solvatochromic behavior of the natural colorant of blueberry (*Vaccinium floribundum Kunth*)

Comportamiento solvatocrómico del colorante natural de mortiño (*Vaccinium floribundum Kunth*)

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Received on October 23rd, 2021. Accepted, after review, on June 06th, 2023. Published on September 1st, 2023.

Abstract

The solvatochromic effect is the modification of the absorption spectrum of a solute by varying the solvent. This research evaluated the solvatochromic characteristics of Malvidin-3-glucoside hydrochloride. The method to obtain maximum wavelengths was by spectral scanning. In primary standard CAS No. 7228-78.6, the variation was determined in binary mixtures of ethanol-water: 40,55, and 70%v/v. The solvatochromic effect when modifying the pH of the solvent (water) was evaluated in natural dye and stabilized. The results suggest that the intermolecular hydrogen connections between Malvidin-3-glucoside and the ethanol-water binary mixtures are responsible for the solvatochromic changes: 565.2586 $\pm$ 3.2784nm, 472.5498 $\pm$ 2.5128nm and 457.3589 $\pm$ 6.2586nm, produced by the analyzed combinations. When anthocyanins are stabilized in a chelating matrix, the solvatochromic changes produced by varying the pH of the water solvent are not significant compared to the unstabilized natural dye.

Keywords: malvidin-3-glucoside, secondary metabolites, dye, natural products, solvatochromism, anthocyanins, pectin.

Resumen

El efecto solvatocrómico es la modificación del espectro de absorción de un soluto al variar el solvente. El presente trabajo evaluó las características solvatocrómicas de Malvidina-3-glucósido clorhidrato. El método para obtener longitudes de onda máxima fue por barridos espectrales. En estándar primario CAS *N°* 7228-78.6, la variación fue determinada en mezclas binarias de etanol-agua: 40,55 y 70%v/v. El efecto solvatocrómico al modificar el pH del solvente (agua) fue evaluado en colorante natural y estabilizado. Los resultados indican que los enlaces hidrógeno intermoleculares entre Malvidina-3-glucósido y las mezclas binarias de etanol-agua son responsables de los cambios solvatocrómicos: 565,2586 $\pm$ 3.2784nm, 472,5498 $\pm$ 2.5128nm y 457,3589 $\pm$ 6.2586nm, producidos por las combinaciones analizadas. Al estabilizar antocianinas en una matriz quelante los cambios solvatocrómicos producidos al variar

el pH del solvente agua, son no significativos en comparación con el colorante natural sin estabilizar.

Palabras clave: Malvidina-3-glucósido, metabolitos secundarios, colorante, productos naturales, solvatocromismo, antocianinas, pectina.

Suggested citation: Mora, T., Suárez, M., Brito, C. and Almachi, D. (2023). Solvatochromic behavior of the natural colorant of blueberry (*Vaccinium floribundum Kunth*). La Granja: Revista de Ciencias de la Vida. Vol. 38(2):9-16. http://doi.org/10.17163/lgr.n38.2023.01.

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1 Introduction

Vaccinium floribundum Kunth is a fruit native of Ecuador (Torres and Pulgar, 2017). During ripening, this berry undergoes color changes (Xu et al., 2010): initially it is green when the fruit is immature, pink when it reaches physiological maturity and finally black when it reaches full maturity (Arteaga et al., 2014). Cyanidine, malvidine and delfinidine are anthocyanins typically in this type of fruit (Jin et al., 2020). Narváez and Suárez (2016) reported that they obtained 3.92 mg of cyanidine-3-o-glucoside for each gram of dry extract of this fruit (Rahman et al., 2021).

Plant-origin anthocyanins are natural dyes (Yépez and Suaárez, 2019) with protective properties for plants against UV light, oxidants and free radicals (Enaru et al., 2021). Its usefulness in the cosmetic, food and pharmaceutical industries has increased by its bioactive properties (Buchweitz et al., 2013) among which are: antidiabetic, antitumor, anti-inflammatory and anticancer effects (Garzón, 2008). In addition, they provide a variety of colors ranging from red to blue in various fruits (Nguyen et al., 2018). One of the reasons for this variety of colors is the structure of the B-ring present in the Malvidine 3-O-Glucoside structure (Sánchez, 2013). This ring has variations in its radicals (Table 1), in which greater methoxylation can be differentiated with displacements towards blue colorations, on the other hand, fewer methoxylations are related with red tones (Rahman et al., 2021).

Table 1. Anthocyanins present in nature. Modified by Rahman et al. (2021).

anthocyanins	**Radical**		$\lambda_{max}[nm]$	**Color**
	R1	R2	Visible spectrum	
Pelargonidin	H	H	494	orange
Cyanidin	OH	H	506	orange-red
Dephinidin	OH	OH	508	blue-red
Peonidin	OCH_3	H	506	orange-red
Peninidin	OCH_3	OH	508	blue-red
Malvidin	OCH_3	OCH_3	510	blue-red

To increase their solubility anthocyanins have glycosidic replacements in radicals 3 and/or 5 (Putra et al., 2023). For example, malvidine in nature presents as Malvidine 3-glucoside (Ayala et al., 2018) see Figure 1.

Aromatic acylations can substitute glycosidic groups, producing purple shades (Kader et al., 1998). In addition, the colored pigments are found in four different chemical forms depending on the pH of the medium (Castañeda-Ovando et al., 2009). Thus, the flavilio cation at $pH = 1-3$ is formed, which is soluble in water and is also responsible for the colors red and purple (Vasco et al., 2009). When the pH increases between 8 and 10, the quinoidal blue species is abundant (Belmonte et al., 2016) while the pseudobase of carbinol and a colorless chalcona appear at pH between 12 and 14. However, all four compounds are soluble in polar solvents and can coexist in a wide pH range (Enaru et al., 2021).

Solvatocromism is commonly used in many fields to study global and local polarity in macrosystems (Reichardt, 1994). Its study includes phenomena involving intermolecular and dynamic forces coupled to the solvent (Marini et al., 2010).

Anthocyanins under experimental conditions have solvatochromic properties by contact with solvents that could produce hydroxylations, methoxylations, changes in pH or aromatic substitutions (Iosub et al., 2014). Based on spectral data Iosub et al. (2014) correlations were established between the solvatochromic properties and the polarity parameters of the solvent. The study determined that anthocyanin extracts are useful in the study of the solvatochromic effect in solvents of different polarity.

The importance of studying solvatochromic behavior, specifically in natural dyes, with structure similar to anthocyanins lies in obtaining the quantification method (Klymchenko, 2017). Emphasizing spectral data, "Beer's law explains the quantitative aspects of absorption measurements by the linear dependence of solute concentration" [p. 1386]Linying2022. The Lorentz-Lorenz and Clausius-Mosotti equations are known for determining dipolar moments, and the purpose "is to predict a strong coupling between the solute and solvent oscillators" [p. 2]Mayerhofer2020. When intermolecular forces are involved with the solvent, it is necessary to establish variation ranges, specifically with regard to polarity (Lee et al., 2013).

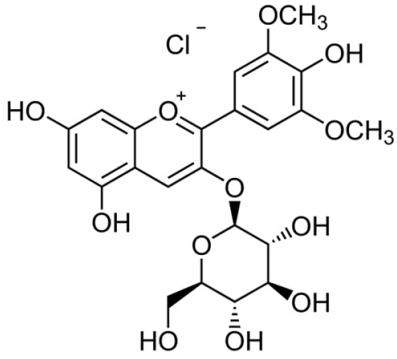

Figure 1. The structure of Malvidine 3-O-Glycoside. Modified by Pubchem (2022).

Codex Alimentarius, (2021), during the manufacturing process it is essential that the natural dye is not affected by wavelength variations when in contact with the ingredients of the formulation (Loving et al., 2010). The solvatochromic effect of anthocyanins in a pharmaceutical, cosmetic or food product is reflected as a degradation (Cai et al., 2020).

Based on the latter, this study evaluates the solvatochromic behavior of Malvidine -3-glycoside hydrochloride primary standard CAS *N°*. 7228-78.6, produced by the solute-solvent coupling, by varying the molar fraction of the solvent. This analysis determined the maximum wavelength for each molar fraction of solvent.

The solvatochromic behavior was also evaluated by the diversification of the maximum wavelength of Malvidine -3- glycoside in natural and stabilized dye, by modifying the pH of the solvent (water) to 4 and 6, with HCl and NaOH respectively. The analysis evaluated whether the solvatochromic behaviors in these pigments are statistically significant, by varying the pH of the solvent.

2 Materials and Methods

2.1 Solvatochromic study: primary standard

The solvatochromic behavior was evaluated in Malvidine-3-glycoside hydrochloride primary standard CAS *N°*. 7228-78.6, with variation in the proportion of the solvent (ethanol-water: 40,55,70%v/v. During the experimental determination of spectral sweeps with primary standard the pH was maintained at the value of 2, with the addition of a solution of HCl 1M to preserve the flavilion ion structure, which is consistent with the literature (Iosub et al., 2014).

The maximum wavelength change of Malvidine 3-O-Glycoside was analyzed in duplicate during three days in a VARIAN 50Bio spectrophotometer. The variation of the data was analyzed by standard deviation at each solvent concentration.

2.2 Solvatochromic study: natural dye

2.2.1 Extraction

The extraction method was a modification of the research (Almachi, 2018). Obtaining the natural dye of *Vaccinium floribundum Kunth* began with degreasing the previously dried and ground mortiño fruit, using Söxleth with n-hexane for 8 hours, followed by maceration in filter paper caps with ethanol 96% for 24 hours. Each cap was individually percolated at a rate of 20 drops per minute until the test for phenolic compounds with 5% ferric chloride was negative. Each ethanolic extract was concentrated in the RapidVap equipment: Evaporation heat 205 Kcal/Kg, Speed 45% and Vacuum 175 Mbar. The dry extracts were stored in a desiccator protected from light.

2.2.2 Stabilization

The natural dye from dry extract of *Vaccinium floribundum Kunth* was stabilized in a commercial pec-

tin matrix (Ceampectin RS 4710), by the absorption method with the following conditions: ethanol concentration 60%v/v, extract concentration 5%w/v and contact time 25 hours.

2.2.3 Solvatocromism

The solvatochromic behavior was evaluated by the maximum wavelength variation of Malvidine -3-glycoside in natural and stabilized dye, by modifying the pH of the solvent (water) to 4 and 6 with HCl and NaOH, respectively. The study was based on ANCOVA multiple covariance analysis of two factors, each with 2 levels on 1 response variable, i.e., K= 2 and n= 1. Four experimental runs were developed with a complete replica of the design to determine the reproducibility of the model, giving a total of 8 experimental runs.

3 Results and Discussion

3.1 Evaluation of the solvatochromic behavior of Malvidine -3- glycoside hydrochloride

The absorption maxima of Malvidine with their respective standard deviation are observed in Table 2, showing a solvatochromic effect at different solvent concentrations.

Table 2. Results obtained by solvatochromism of Malvidine.

Ethanol oncentration	40%	55%	70%
Wavelengthnm>nm	565.2586 ± 3.2784	472.5498 ± 2.5128	457.3589 ± 6.2586

The effect of the solvent produced by these ternary systems (water:ethanol:anthocyanin) is determined by the polarity parameters of the ethanol: water ratio. Solvatochromism was evaluated by the ability of the solvent to produce dipole changes in the malvidine molecule caused by the change of the solvent molar fraction. Mayerhöfer and Popp (2020) describe that the polar changes produced by the solvent present spectral modifications of the absorption bands. Polar variations produced by the solvent are evidenced by the maximum wavelength alterations in Table 2. Therefore, when developing a quantification method with molecules with similar structure to Malvidine-3-glycoside hydrochloride, it is advisable to maintain the molar fraction of the solvent in order to avoid solvatochromic behaviors of the analyte.

3.2 Evaluation of solvatochromic behavior of natural dye of *Vaccinium floribundum Kunth*

3.2.1 Extraction

The standardization process of dry extract of mortiño started from a dry and ground sample of the fruit with the following specifications: particle size 595μm, moisture 3.05±0.05% and total fat content of 2.5±0.2%. The extraction method of natural dye and its stabilization in pectin described in materials and methods allowed to obtain a yield as dry extract of 53.1±4.4% and stabilized dye of 91.8±4.6%.

Table 3. Two-factor ANCOVA.

	Coloring Type(X_1)	
pH (X_2)	Wavelength [nm] Stabilized dye	Wavelength [nm] Natural dye
4	511.5092±1.4934	517.0453±4.3861
6	511.9522±1.2678	512.5500±1.3859

Table 3 presents maximum wavelengths of malvidine in natural dye and stabilized in pectin when there is a pH variation in water. The ANCOVA analysis determined that the pH of the natural dye is significant (p Value $<$ 0,05), with a 95% confidence interval. The pigment when exposed to a pH variation of 4 and 6 exhibits changes in the maximum wavelength. These displacements are produced by Malvidine -3- glycoside present in the dry extract.

In an environment between 2 and 4 it leaves the anthocyanin form to be present as the quinoidal species, while at pH 6 it is found as chalcone (Enaru et al., 2021). When comparing the maximum wavelengths of the natural dye with stabilizer, the two-factor ANCOVA analysis determined that there is a significant difference (p-Value $<$ 0,05) with a 95% confidence interval, which can be observed in Figure 2. The binding of Malvidine -3- glycoside with pectin by hydrogen bridging with oxygen of the anthocyanin methoxyl group shows a change in maximum wavelengths (Koh et al., 2020).

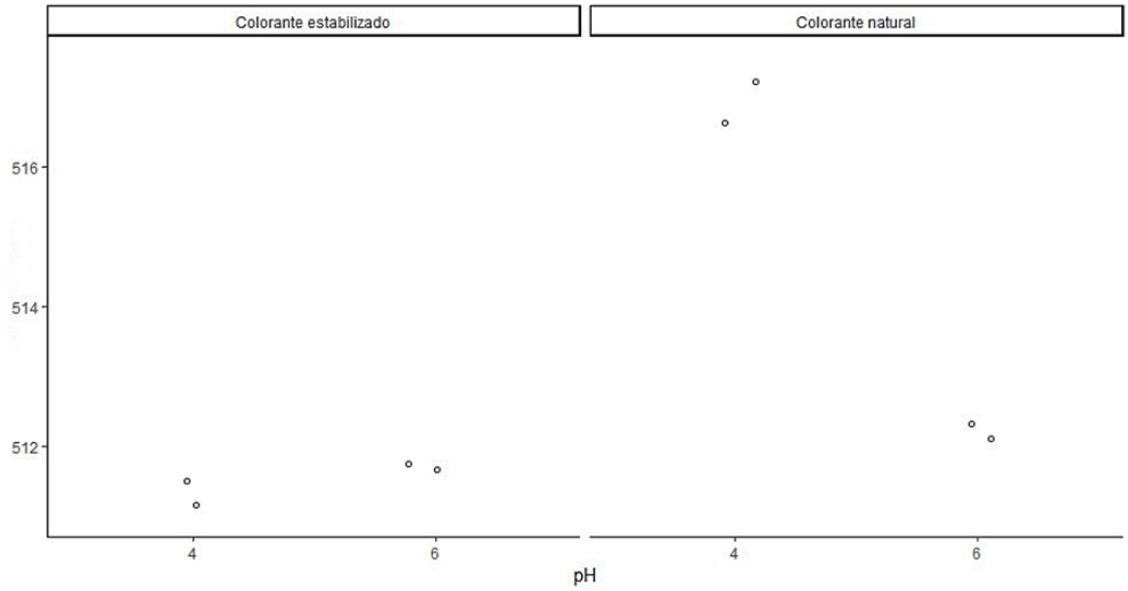

Figure 2. Maximum Wavelength Variation in Natural Dye and Stabilized Dye.

Natural dyes when used for pharmaceutical, cosmetic or food purposes should be stable against changes in pH during manufacturing processes. The study determined that there is no significant difference (p-Value $> 0{,}05$), when varying the pH of the solvent when the natural dye is stabilized. The stabilizing matrix pectin prevents the Malvidine -3-glycoside from interacting with the OH^-, H^+ ions of the solvent, preventing solvatochromic shifts.

Analyzes were performed at specific pH values of 4 and 6. However, a food study found that industrialized foods have slightly more acidic pH values than natural foods. This is probably related to the preservation methods used and the addition of vitamins. Cereals showed pH values ranging from 7.95 -y 5.4 [p. 91]Casaubon2018. Therefore, it is necessary to determine solvatochromic changes in a higher pH range.

The dry extract, when exposed to a variation of pH of 4 and 6, presents changes in the maximum wavelength. These displacements are produced by Malvidine -3- glycoside present in the pigment. The ANCOVA analysis determined that the pH variation in the natural dye produces significant variations (p-Value $< 0{,}05$), with a 95% confidence interval.

4 Conclusions

The present research evaluated the change in polarity of malvidine -3- glycoside hydrochloride when using ethanol as solvent in three different proportions. The study determined that there are solvatochromic displacements by the variation of the maximum wavelengths. The results indicate that the intermolecular hydrogen bonds between the solute and the solvent are responsible for the solvatochromic changes in the analyzed binary mixtures. The study in primary standard was not based on determining significance in solvatochromic changes. The analysis was the reproducibility evaluation of each wavelength in different days.

The stabilization of the pigment in a natural matrix determined that there is no significant difference (p-Value $> 0{,}05$), when varying the pH of the solvent. Solvatochromic displacements produced by the presence of hydrogen and hydroxyl ions at pH 4 and 6 were eliminated by the polysaccharide-polyphenol binding.

Acknowledgements

The authors acknowledge Carlos Brito for his great contribution in the statistics of this research. Also, Dr. Martha Suárez Heredia, by her contribution that made this research possible and the *Spanish Agency for Development Cooperation* (AECID). Finally, the authors thank the Ministry of the Environment.

References

Almachi, D. Suarez, M. (2018). Estudio de la influencia de colorantes naturales sobre el color y las propiedades reológicas de una formulación alimenticia. Master's thesis, Universidad Central del Ecuador.

Arteaga, M., Andrade, M., and Moreno, C. (2014). Relación del desarrollo del color con el contenido de antocianinas y clorofila en diferentes grados de madurez de mortiño (*Vaccinium floribundum*). *Enfoque UTE*, 5(2):14–28. Online:https://n9.cl/4tu14.

Ayala, K., Pineda, J., Duarte, A., Soto, C., and Soto, Pineda, C. (2018). Toxicidad de los colorantes sintéticos: de lo global al ecuador. *Revista Biorrefinería*, 1(1):40–48. Online:https://n9.cl/kpepv.

Belmonte, J., Arroyo, I., Vázquez, M., Cruz, D., and Peña, E. (2016). Colorantes artificiales en alimentos. *Revista Naturaleza Y Tecnología Universidad de Guanajuato*, 10(8):24–38. Online:https://n9.cl/8z4rr.

Buchweitz, M., Speth, M., Kammerer, D., and Carle, R. (2013). Impact of pectin type on the storage stability of black currant (ribes nigrum l.) anthocyanins in pectic model solutions. *Food chemistry*, 139(1-4):1168–1178. Online:https://n9.cl/z7256.

Cai, L., Sun, X., He, W., Hu, R., Liu, B., and Shen, J. (2020). A solvatochromic aie tetrahydro [5] helicene derivative as fluorescent probes for water in organic solvents and highly sensitive sensors for glyceryl monostearate. *Talanta*, 206:0039–9140. Online:https://n9.cl/z7256.

Castañeda-Ovando, A. Pacheco-Hernández, M., Páez-Hernández, M., Rodríguez, J., and Galán-Vidal, C. (2009). Chemical studies of anthocyanins: A review. *Food chemistry*, 113(4):859–871. Online:https://n9.cl/8nvxi.

Enaru, B., Dreţcanu, G., Pop, T., Stănilă, A., and Diaconeasa, Z. (2021). Anthocyanins: Factors affecting their stability and degradation. *Antioxidants*, 10(12):1967. Online:https://n9.cl/wtuvw.

Garzón, G. (2008). Anthocyanins as natural colorants and bioactive compounds: A review. *Acta Biológica Colombiana*, 13(3):27–36. Online:https://n9.cl/e31th.

Iosub, S., Meghea, A., and Geana, I. (2014). Solvatochromic parameters of some anthocyanin derivatives concentrated from selective natural extracts. *UPB Scientific Bulletin, Series B: Chemistry and Materials Science*, 76(1):25–34. Online:https://n9.cl/q9lcm.

Jin, W., Xiang, L., Peng, D., Liu, G., He, J., Cheng, S., Li, B., and Huang, Q. (2020). Study on the coupling progress of thermo-induced anthocyanins degradation and polysaccharides gelation. *Food Hydrocolloids*, 105:105822. Online:https://n9.cl/t4p9w.

Kader, F., Haluk, J., Nicolas, J., and Metche, M. (1998). Degradation of cyanidin 3-glucoside by blueberry polyphenol oxidase: kinetic studies and mechanisms. *Journal of agricultural and food chemistry*, 46(8):3060–3065. Online:https://pubag.nal.usda.gov/catalog/1371074.

Klymchenko, A. (2017). Solvatochromic and fluorogenic dyes as environment-sensitive probes: design and biological applications. *Accounts of chemical research*, 50(2):366–375. Online:https://n9.cl/dqp7t.

Koh, J., Xu, Z., and Wicker, L. (2020). Binding kinetics of blueberry pectin-anthocyanins and stabilization by non-covalent interactions. *Food Hydrocolloids*, 99:105354. Online:https://n9.cl/gybb5.

Lee, J., Chang, H., An, H., Ahn, S., Shim, J., and Kim, J. (2013). A protective layer approach to solvatochromic sensors. *Nature Communications*, 4(1):2461. Online:https://n9.cl/oa2lc.

Loving, G., Sainlos, M., and Imperiali, B. (2010). Monitoring protein interactions and dynamics with solvatochromic fluorophores. *Trends in biotechnology*, 28(2):73–83. Online:https://n9.cl/rii2t.

Marini, A., Muñoz-Losa, A., Biancardi, A., and Mennucci, B. (2010). What is solvatochromism?

The Journal of Physical Chemistry B, 114(51):17128–17135. Online:https://n9.cl/lvkye.

Mayerhöfer, T. and Popp, J. (2020). Beyond beer's law: Revisiting the lorentz-lorenz equation. *ChemPhysChem*, 21(12):1218–1223. Online:https://n9.cl/h4cw5.

Narváez, G. and Suárez, M. (2016). Estabilización de las antocianinas presentes en el fruto de mortiño (*Vaccinium floribundum Kunth*) mediante el método de copigmentación intermolecular para la obtención de pigmentos antociánicos con potencial aplicación en la industria farmacéutica y cosmé. Master's thesis, Universidad Central del Ecuador.

Nguyen, T., Phan-Thi, H., Pham-Hoang, B., Ho, P., and Tran, T.and Waché, Y. (2018). Encapsulation of hibiscus sabdariffa l. anthocyanins as natural colours in yeast. *Food research international*, 107:275–280. Online:https://n9.cl/g8yca.

Pubchem (2022). Malvidin 3-glucoside. Pubchem0. Online:https://n9.cl/3yw9c.

Putra, N., Rizkiyah, D., Idham, Z., Zaini, M., Yunus, M., and Aziz, A. (2023). Optimization and solubilization of interest compounds from roselle in subcritical ethanol extraction (see). *Alexandria Engineering Journal*, 65:59–74. Online:https://n9.cl/g1igc.

Rahman, S., Mathew, S., Nair, P., Ramadan, W., and Vazhappilly, C. (2021). Health benefits of cyanidin-3-glucoside as a potent modulator of nrf2-mediated oxidative stress. *Inflammopharmacology*, 29:907–923. Online:https://n9.cl/6bhp6.

Reichardt, C. (1994). Solvatochromic dyes as solvent polarity indicators. *Chemical reviews*, 94(8):2319–2358. Online:https://n9.cl/vcpl6.

Sánchez, J. (2013). La química del color en los alimentos. *Química Viva*, 12(3):234–246. Online:https://n9.cl/jbcd2.

Torres, F. and Pulgar, N. (2017). Evaluación de la estabilidad del pigmento natural obtenido a partir de mortiño (*Vaccinium myttillus L*) como colorante para la industria de alimentos. *SATHIRI*, 12(1):171–186. Online:https://n9.cl/9fim7.

Vasco, C., Riihinen, K., Ruales, J., and Kamal-Eldin, A. (2009). Chemical composition and phenolic compound profile of mortiño (*Vaccinium floribundum Kunth*). *Journal of Agricultural and Food Chemistry*, 57(18):8274–8281. Online:https://n9.cl/es/s/nrbxl.

Xu, M., Bower, K., Wang, S., Frank, J., Chen, G., Ding, M., Wang, S., Shi, X., Ke, Z., and Luo, J. (2010). Cyanidin-3-glucoside inhibits ethanol-induced invasion of breast cancer cells overexpressing erbb2. *Molecular cancer*, 9(1):1–14. Online:https://n9.cl/byd7l.

Yépez, M. and Suaárez (2019). Adición de antocianinas extraídas del fruto de *Vaccinium floribundum Kunth* y antocianinas estabilizadas en nanopartículas de zeína en yogur natural, como alternativa a los colorantes de síntesis. Master's thesis, Universidad Centra del Ecuador.

La granjA:
Revista de
Ciencias de la Vida
pISSN:1390-3799; eISSN:1390-8596
http://doi.org/10.17163/lgr.n38.2023.02

Special Issue/ Número Especial

Food Science

ABYA YALA UNIVERSIDAD POLITÉCNICA SALESIANA

Analysis of the inhibition of proteolytic microorganisms in red tilapia (*Oreochromis spp*) fillets preserved with propolis (*Apis mellifera Linnaeus*)

Análisis de la inhibición de microorganismos proteolíticos en filetes de tilapia roja (*Oreochromis spp*) conservados con propóleo (*Apis mellifera Linnaeus*)

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Received on January 30th, 2023. Accepted, after review, on July 3rd, 2023. Published on September 1st, 2023.

Abstract

The food industry is focused on replacing chemical preservatives with organic alternatives for food preservation and safety. The present study seeks to analyze the use of propolis in the conservation of red tilapia fillets. Propolis was applied in two concentrations (15% and 30%) and two impregnation times (1.5 and 3 hours) to red tilapia fillets stored at 4-5°C for 30 days. Several parameters, including pH, water-holding capacity (WRC), and basic volatile nitrogen concentration (N-BVT), were evaluated at 10-day intervals. In addition, a microbiological analysis of mesophilic microorganisms and *E. coli* present was carried out. From day 20, significant differences were observed in the color of the fillet according to the chromatic coordinates L^*, a^* and b^*. The sensory analysis showed that the sensory properties were maintained when the acceptance values were higher than 6.5. The most effective treatment was propolis in a concentration of 15% with a soaking time of 1.5 hours and preserved for 20 days. This approach showed that propolis effectively extends the shelf life of fillets by preventing proteolytic damage. In addition, it inhibits the proliferation of microorganisms by maintaining the load of mesophiles and *E. coli*, as well as the physicochemical parameters (pH, CRA and N-BVT) according to the NTE-INEN 183-2013 standard. In conclusion, propolis is a promising organic preservative for the food industry.

Keywords: Bacteria, fillet, inhibition, normative, proteolytic.

Resumen

La industria alimentaria se centra en reemplazar los conservantes químicos con alternativas orgánicas para la conservación y seguridad de los alimentos. El presente estudio, busca analizar el uso de propóleo en la conservación de filetes de tilapia roja. Se aplicó propóleo en dos concentraciones (15% y 30%) y dos tiempos de impregnación (1,5 y 3 horas) a filetes de tilapia roja almacenados a 4-5 °C durante 30 días. Se evaluaron varios parámetros, incluidos el pH, la capacidad de retención de agua (CRA) y la concentración básica de nitrógeno volátil (N-BVT), a intervalos de 10 días. Además, se realizó un análisis microbiológico de microorganismos mesófilos y *E. coli* presentes. A partir del día 20 se observaron diferencias significativas en el color del filete según las coordenadas cromáticas L^*, a^* y b^*. El análisis sensorial mostró que las propiedades sensoriales se mantuvieron cuando los valores de aceptación fueron superiores a 6,5. El tratamiento más efectivo fue el propóleo en concentración del 15% con un tiempo de impregnación de 1,5 horas y conservado durante 20 días. Este enfoque mostró que el propóleo extiende efectivamente la vida útil de los filetes al prevenir el daño proteolítico. Además, inhibe la proliferación de microorganismos al mantener la carga de mesófilos y *E. coli*, así como los parámetros fisicoquímicos (pH, CRA y N-BVT) según la norma NTE-INEN 183-2013. En conclusión, el propóleo es un conservante orgánico prometedor para la industria alimentaria.

Palabras clave: Bacteria, filete, inhibición, normativa, proteolítica.

Suggested citation: Intriago, L., Talledo, V., Arteaga, R., Pazmiño, A. and Cuenca-Nevárez, G. (2023). Analysis of the inhibition of proteolytic microorganisms in red tilapia (*Oreochromis spp*) fillets preserved with propolis (*Apis mellifera Linnaeus*). La Granja: Revista de Ciencias de la Vida. Vol. 38(2):17-31. http://doi.org/10.17163/lgr.n38.2023.02.

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1 Introduction

The shelf life of fish is very important in the industrial and commercial field involving fish farming, hence, the use of biopreservation agents for whole fish or in vacuum-packed filet is an alternative to reduce the use of chemical preservatives that affect the health of the consumer, and avoid the proliferation of microorganisms that cause their deterioration, maintaining the quality and safety traits established by the control bodies (Ahmad et al., 2017; Rodríguez-Pérez et al., 2020).

Tilapia stands out for being a very desired fish because of its great taste, texture, color and great versatility when making various preparations; thus, its cultivation has spread potentially in recent years, and this new business opportunity has given rise to new ideas that contribute to improvements in exports and different methods of consumption and marketing of the product (Jácome et al., 2019).

Likewise, there is an increase interest on the part of consumers, industrialists, and researchers to resort to natural sources of food additives that can be used to preserve food without affecting human health (Vargas-Sánchez et al., 2013). In this way, a natural product such as propolis has been successfully introduced into the food industry, which can offer these properties (Rodríguez-Pérez et al., 2020; Farag et al., 2021).

Propolis is a resinous substance produced by bees whose characteristics vary depending on the weather season, geographical area, type of bees and existing vegetation; it contains between 50 to 60% resins and balms, 30 to 40% wax, 5 to 10% pollen, and 8 to 10% essential oils; it is composed of around 180 substances, mainly flavonoids and phenolic acids or esters in 50% (Rodríguez-Pérez et al., 2020; Sarıkahya et al., 2021; Farag et al., 2021; Salleh et al., 2021). Propolis is used by bees to maintain the optimal conditions of the hive and its honey, avoiding the growth of microorganisms that alter it, so that its properties can be studied as a natural food additive. Considering the relationship of its flavonoid content and its biological effect, propolis is a bioactive product that stands out for its antimicrobial and antioxidant activity, strong flavor, and typical aroma, capable of establishing multiple synergistic combinations with other components (Viloria et al., 2012; Rodríguez-Pérez et al., 2020; Peixoto et al., 2021; Salleh et al., 2021).

Propolis has useful characteristics for the food industry such as its antioxidant, antimicrobial, antifungal and antiparasitic (antiprotozoal) activity (Peixoto et al., 2021; Vică et al., 2021; Afata et al., 2022), reason for which it can be used in meat products (beef, chicken, pork, fish or shellfish), vegetable oils, unpasteurized dairy products, fruits and fruit juices. The antibacterial activity of propolis has been studied in different microorganisms, including Gram-positive and Gram-negative bacteria. The Gram-positive bacteria evaluated included *Staphylococcus aureus, Streptococcus sp., Micrococcus sp., Bacillus sp. Listeria monocytogenes*; as for the Gram negative bacteria evaluated are *Salmonella typhi, Salmonella typhimuriu, Pseudomona aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli, Helicobacter pylori, Shigella* spp. For propolis, the minimum inhibitory concentrations (MIC) for these microorganisms vary depending on the way they are applied and the chemical composition of the geographical area of origin.

According to the information collected by Przybyłek and Karpiński (2019), the MIC of the ethanolic extract of propolis for Gram positive bacteria (*S. aureus* as representative) is within 8-1500 μg mL^{-1}, while the MIC for Gram negative bacteria (*E. coli* as representative) is within 116-5000 μg mL^{-1}. Thus, higher efficacy on Gram positive bacteria and to a lesser extent on Gram positive bacteria has been established as Gram-negative due to the action of bioactive compounds present in propolis and that directly affect the cell wall of these bacteria, making them susceptible to cell breakdown and subsequent lysis (Nedji and Loucif-Ayad, 2014; Santos et al., 2017; Zhang et al., 2017; Torres et al., 2018; Afata et al., 2022).

This study aims to investigate the effect of propolis (*Apis mellifera Linnaeus*) on the inhibition of proteolytic microorganisms in vacuum-packed red tilapia (*Oreochromis* spp.) filets by measuring their physicochemical and microbiological parameters, as well as their sensory attributes during the storage period. This study responds the need to address the challenge faced by the fishing industry to preserve the properties of packaged products, without increasing the use of synthetic chemical preservati-

ves.

2 Materials and Methods

This research was carried out in the food analysis laboratories of the Faculty of Zootechnical Sciences of the Technical University of Manabí, located at kilometer 2 $^1/_2$ of Ánima via Chone-Boyacá, in Chone, Manabí province, area with a potential evapotranspiration of 107.04 mm, average annual temperature of 25.2 °C and average annual precipitation of 54.63 mm (Cabrera-Estupiñán et al., 2017).

The propolis used in the study was obtained from a poultry farm located in the city of Flavio Alfaro, Manabí province. The red tilapia (*Oreochromis* sp.) was acquired in the local market of Chone, taking specimens of 525 g of average weight. Then the tilapias were eviscerated, and cut into filets of an average weight of 370 g. Each filet maintained its skin to maintain the stability of the muscle. In each filet parallel cuts were made of 5 mm with the aim of getting the propolis through these cuts and impregnate the fillets.

To evaluate the preservative action of propolis in vacuum sealed red tilapia filets ,an experimental design was established applying a cubic factorial model attenuated with the factors A) storage time (10, 20 and 30 days), B) propolis concentration in relation to the weight of the filet (0, 15 and 30%), and C) impregnation time (1.5 and 3 h). Each filet of the experimental design was packed in 2/60 gage transparent polyester (PET) bags, U seal, Zipack, Avidditi®brand, ISO 9001:2008 certified (Manuli Fitasa, Brazil) using a vacuum atmosphere generated by a chamber with high-capacity vacuum generator pump (20 m3 h^{-1}), VM400TE/B model 440 x 420 x 75 mm and double seal bar 400 x 100 mm, from Fin brand Teck S.A. Subsequently, the packaged filets were stored at refrigeration temperatures of 4.5 $\pm$ 0,5 °C for 30 days. The analyzes were performed in triplicate both at the physicochemical and sensory levels.

2.1 Physicochemical parameters

2.1.1 pH analysis

For determining this parameter, a potentiometer Orion A:211 was used (Thermo Scientific™, United States) which has a 6mm electrode, which is inserted directly into tilapia filets.

2.1.2 Percentage of water holding capacity

A sample of 2 g of raw filet was placed on circular filter paper. Then, it was placed between two glass plates with a weight of 5 kg, for five minutes. Water retention capacity (WRC) was determined by the difference in both initial and final weights (Rebouças et al., 2020).

$$\%WRC = \frac{P_i - P_f}{P_i} \times 100 \quad (1)$$

Where P_i is the initial weight of the filet, and P_f is the final weight of the filet.

2.1.3 Stability to proteolytic degradation

The amount of total volatile nitrogen bases (N-BVT) was used for Valencia-Junca et al. (2019) method with modifications. A sample of 10 g of tilapia filet was ground with 50 mL of distilled water into an Oster®food processor; the mixture formed was placed in a 500 mL Erlenmeyer with 200 mL of distilled water. Then, it was distilled incorporating 2 g of MgO and a drop of silicone was added to inhibit the formation of foams. The distilled product was placed in a 250 mL flask with a 3% boric acid solution and 0.04 mL of methyl red and methylene blue as an indicator of the presence of ammonium. The titration of the distillate was performed with 0.1N HCl until obtaining a turn from green to pink. The following equation was used to calculate N-BVT in mg $100g^{-1}$ of fish filet.

$$\%mg\ N-BVT = \frac{(V \cdot C \cdot 14 \cdot 100)}{10} \quad (2)$$

Where, V = Volume of added hydrochloric acid;
C = Normal concentration of hydrochloric acid;
14 = Atomic weight of N;
10 = Weight of sample

2.1.4 Instrument color determination

Color determination was performed using a colorimeter (Kónica, Minolta Chroma Meter CR400, Japan), with illuminant D65 and observer of 2° (calibrated equipment with a standard plate with reference values Y = 89.5 x = 0.3176 y = 0.3340). The measurements were expressed in terms of luminosity L^* and chromaticity parameters a^* and b^*.

2.2 Microbiological parameters

For the microbiological analysis, the standard solution was prepared using 10 g of tilapia filet homogenized with 90 mL of peptone water to conduct serial dilutions of each of the bacterial groups to be quantified.

2.2.1 Count of aerobic mesophilic micro-organisms

For determining aerobic mesophilic microorganisms, planting in stretch marks was performed using the Agar Plate Count (APC), then incubation at 35°C was carried out for 48 hours. After the incubation period, the colony forming units (log CFU g^{-1}) were counted.

2.2.2 *Escherichia coli* count

This analysis was carried out using the Most Probable Number (MPN g^{-1}) methodology, for which test tubes were taken inside containing Durham bells and were subsequently incubated at 35°C for 48 hours. Test tubes showing turbidity and gas presence were taken as presumptively positive. Then, the presence of coliforms was confirmed using the Kovacs reagent. The positive tubes were inoculated in Bright Green Lactose Bile broth and incubated at 35°C for 48 hours. In addition, the test tubes that showed turbidity and gas production that were classified as positive were planted in Methylene Blue Eosine Agar (MBE) and incubated for 24 hours to confirm the presence of *Escherichia coli*.

2.3 Sensory analysis

The sensory panel was composed of twenty semi-trained judges, who evaluated the organoleptic attributes such as color, aroma, flavor and texture, for which a 9-point hedonic scale valued was used (Jonaidi-Jafari et al., 2018), where 1 is "extremely disliked" and 9 "extremely liked". The value of 4 was considered as a minimum acceptability benchmark. For the sensory evaluation, the filets were cut into small pieces, were covered with breadcrumb, and then were fried. The portions of filet were placed in containers labeled with the code of the treatments analyzed. A glass of water at room temperature was provided to each judge to allow tasting the different treatments.

2.4 Statistical Analysis

For data analysis, the statistical software InfoStat version 2020 was used. A multifactorial ANOVA was performed to analyze the physicochemical and microbiological variables, in order to evaluate the effect of storage time, propolis concentration, impregnation time and their interactions.

For the sensory analysis, a simple ANOVA was performed to evaluate the existence of significant differences in each of the attributes evaluated. The post hoc analysis of Tukey ($p < 0{,}05$) was applied to check the existing differences between treatments.

3 Results and Discussion

3.1 Physicochemical parameters of red tilapia filets (*Oreochromis* sp.)

3.1.1 pH analysis

The pH values in the red tilapia filets impregnated with propolis determined that this parameter ranged from 6.12 to 7.53, with a mean of 6.85 (Figure 1). The pH tended to decrease as the storage days progressed, existing a similar behavior considering both impregnation times of propolis design. Until the end of the storage period, the control treatment obtained pH values of 5.91 and 5.96 for the two impregnation times; the pH for treatments with 15% propolis with impregnation time of 1.5 hours was 6.46 and 3 hours 6.27; in contrast, treatments with 30% propolis and impregnation times of 1.5 hours and 3 hours had pH of 6.24 and 6.18, respectively.

The analysis of variance determined a significant difference in the pH value in factor A (storage time) and factor B (propolis concentration), even in the interactions AC (storage time - impregnation time) and BC (propolis concentration - impregnation time) (Table 1). The pH values reported are related to those determined by Montoya Camacho et al. (2021) in studies conducted with black tilapia to evaluate the biochemical changes undergone by the muscle of this fish when stored in a temperature between 0 and 5°C.

The pH values recorded are within the ranges established by the Ecuadorian technical regulation NTE-INEN 183-2013 (INEN, 2014), which establis-

hes that the pH during its commercialization is 6.5 in the internal part of the muscle and 6.8 in the external part of the organism.

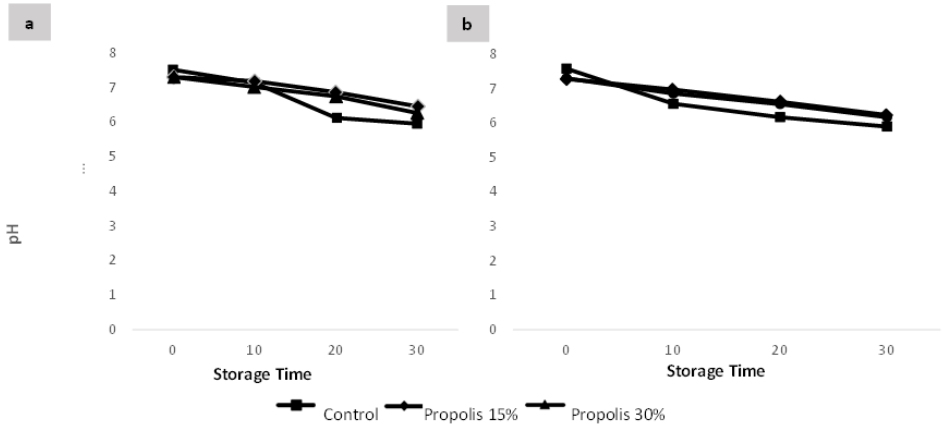

Figure 1. pH behavior in red tilapia filets (*Oreochromis* sp.) impregnated with propolis, a) impregnation of propolis at 1.5 hours, b) impregnation of propolis at 3 hours.

Table 1. Analysis of variance of the treatments employed in this study for pH.

Source	SC	gl	CM	p - value
A-Storage time	6.05	1	6.05	<0.0001
B-Propolis concentration	0.4413	1	0.4413	<0.0001
C-Time impregnation	0.0702	1	0.0702	0.0558
AB	0.0137	1	0.0137	0.3928
AC	0.2491	1	0.2491	0.0005
BC	0.4052	1	0.4052	<0.0001
ABC	0.0026	1	0.0026	0.7085
Residual	1.05	57	0.0184	
Total Cor	11.39	71		

Equation 3 determines the multiple regression of the pH parameter and allows predicting the response of each factor by identifying and comparing the coefficients thereof.

$$\begin{aligned} pH = {} & 6,52 + (-1,49 \cdot A) + (-0,1535 \cdot B) + \\ & (-0,0667 \cdot C) + (-0,0226 \cdot AB) + \\ & (-0,0789 \cdot AC) + (-0,0919 \cdot BC) + \\ & (-0,0099 \cdot ABC) \end{aligned} \quad (3)$$

The pH values vary between 6.81 and 6.91 in the treatments using propolis at 15% and 30% at an impregnation time of 1.5 hours, while the values were 6.68 and 6.70 respectively at impregnation times of 3 hours. Hence, conserving tilapia filets with propolis is an organic and feasible alternative.

3.1.2 Water Retention Capacity (WRC)

WRC results of propolis-preserved filets range from 46.43% to 86.99% (Figure 2). The results indicate a decrease in the WRC as the storage time progressed. The control treatment obtained WRC values of 51.85% at 1.5 h of impregnation and 46.45% at 3 h of impregnation. Treatments with 15% propolis with impregnation times of 1.5 and 3 hours had WRC values of 62.92% and 58.19%, respectively, while treatments with 30% propolis and impregnation times of 1.5 hours and 3 hours had WRC values of 60.92% and 55.95%, respectively.

The statistical analysis of variance for the WRC (Table 2) determines that the main effects of the

model were A (storage time), B (propolis concentration), and interactions AB (storage time- propolis concentration) and AC (storage time - impregnation time) with p values of <0.0001 in all detailed cases, there being significant differences in the values of WRC.

According to Campus et al. (2010) WRC is a parameter that measures the ability of muscle to retain free water by capillarity and stress forces that have been subjected to the sample, in this case the red tilapia filets; thus, WRC ranges between 70-80 % are considered optimal values to estimate the freshness of fish.

For this study, the WRC values were 71.88, 77.34 and 75.96 % in red tilapia filets with an impregnation time of 1.5 hours and up to 10 days of storage time and with 3 concentrations of propolis; meanwhile, the WRC values were 73.12, 78.37 and 75.84 % for the impregnation time of 3 hours, up to the 10th day of storage and 3 concentrations of propolis. After day 10, the WRC reduced to values between 53.53 and 58.56 % in both concentrations in impregnation times of 3 and 1.5 hours respectively.

$$\begin{aligned} WRC = {} & 72,15 + (-15,02 \cdot A) + 1,32 \cdot B + \\ & (-0,3919 \cdot C) + 1,93 \cdot AB + \\ & (-2,33 \cdot AC) + (-0,1328 \cdot BC) + \\ & (-0,0237 \cdot ABC) \end{aligned} \tag{4}$$

Equation 4 determines the multiple regression of the WRC parameter. In terms of real factors, this equation can be used to make predictions about the response for given levels of each factor, thus identifying the relative impact of the factors by comparing their coefficients.

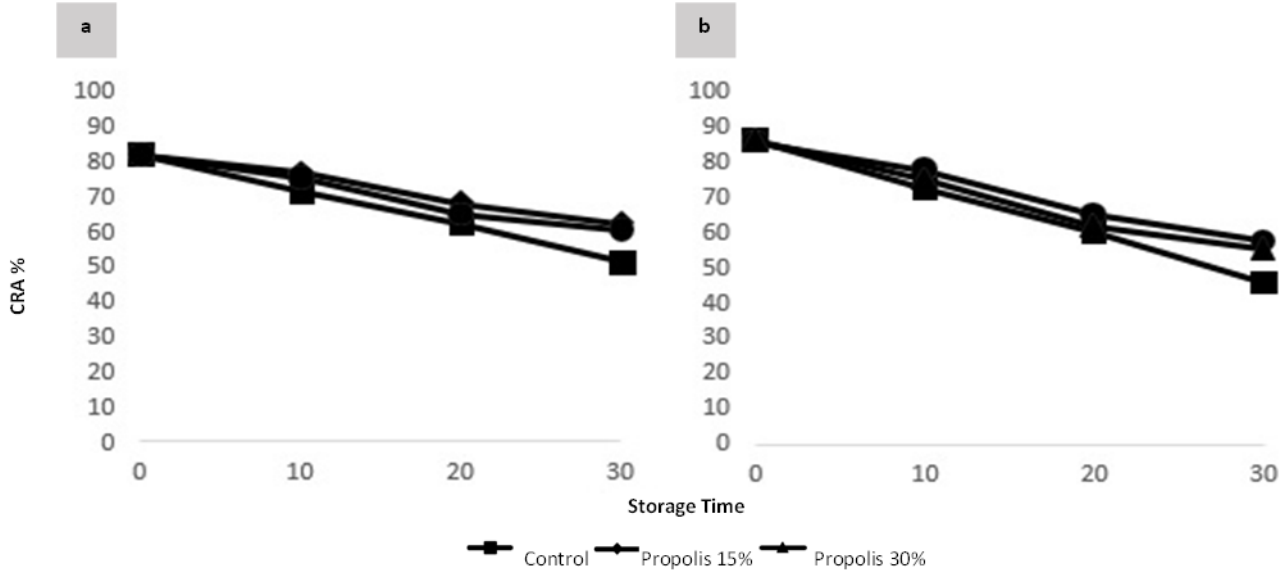

Figure 2. WRC values in red tilapia filets (*Oreochromis sp.*) preserved with propolis, a) impregnation of propolis at 1.5 hours, b) impregnation of propolis at 3 hours.

The values of WRC are favorable until the day of storage in the treatments performed with propolis at 15 % and 30 % for the impregnation time of 1.5 hours and 3 hours, because the filets lose juiciness after these days, affecting unfavorably the sensory parameter taste. According to Melody et al. (2004), the space for water to be retained in myofibrils is reduced as rigor progresses, and the fluid can be forced into extramiofibrillary spaces where it is more easily lost as a drip as a result of the lateral contraction of myofibrils that occurs during rigor, which can be transmitted to the entire cell if proteins that

Table 2. Analysis of variance of the treatments considered in the study for the WRC.

Source	SC	gl	CM	p-value
A-Storage time	600.75	1	600.75	<0.0001
B-Propolis concentration	31.13	1	31.13	<0.0001
C-Time impregnation	2.41	1	2.41	0.0488
AB	99.34	1	99.34	<0.0001
AC	215.75	1	215.75	<0.0001
BC	0.8262	1	0.8262	0.2433
ABC	0.0149	1	0.0149	0.8747
Residual	33.28	56	594	
Total Cor	9171.03	70		

bind myofibrils to each other and myofibrils to the cell membrane are not degraded.

3.1.3 Analysis of total volatile nitrogen bases (N-BVT)

According to Cicero et al. (2014), fish has high protein index and hence it is a highly recommended product mainly in balanced and healthy diets, but these organisms suffer internal alterations, where the degradation of nitrogen compounds occurs mainly by the action of bacteria, which mainly form trimethylamine (TMA) and ammonium, increasing the pH at the end of the rigor mortis phase. The measurement of the amount of N-BVT in a fish filet sample is used to determine the state of the deterioration process, this being an indicator of its freshness (Howgate, 2010).

As for the N-BVT values for red tilapia filets, significant differences were observed between treatments from day 20 of storage (Figure 3). The behavior of N-BVT in red tilapia filets preserved under vacuum determined a value of 22.58 mg N-BVT $100g^{-1}$ for the control treatment, while treatments with propolis showed a decreasing behavior in this parameter according to the impregnation time and concentration of propolis; thus, the treatment at 15% resulted in 19.69 mg N-BVT $100g^{-1}$ (1.5 hour impregnation) and 14.68mg N-BVT $100g^{-1}$ (3 hours impregnation).

From day 20, the control treatments showed increments in the N-BVT value, exceeding the permissible limit value of 30 mg N-BVT $100g^{-1}$ to be considered fresh fish filet acceptable for consumption, established in the regulations issued by the Comunidad Europea (2008) and stated in the Ecuadorian standard NTE-INEN 183-2013. The treatments with 15% and 30% propolis did not exceed the established normal value, presenting values between 10.77 and 36.22 mg $100g^{-1}$. The values recorded within the permissible limit determined over the storage time of red tilapia filets could be influenced by the antibacterial activity of propolis, which prevents proteolytic degradation in the filet, as mentioned by Basiri et al. (2015) on a seafood conservation study.

The statistical analysis of variance for the effect (Table 3) determined that the variation sources that were significant were A (storage time), B (propolis concentration), C (impregnation time), interactions AB, AC and ABC, with significant differences in the values of the N-BVT.

Equation 5 determines the multiple regression of the N-BVT parameter and can be used to predict about the response for given levels of each factor, thus identifying the relative impact of the factors by comparing the coefficients thereof.

$$\begin{aligned} N-BVT = {} & 17,09+6,12\cdot A+(-2,16\cdot B)+ \\ & 3,57\cdot C+(-1,99\cdot AB)+ \\ & 0,7075\cdot AC+0,0865\cdot BC+ \\ & 0,5514\cdot ABC \end{aligned} \tag{5}$$

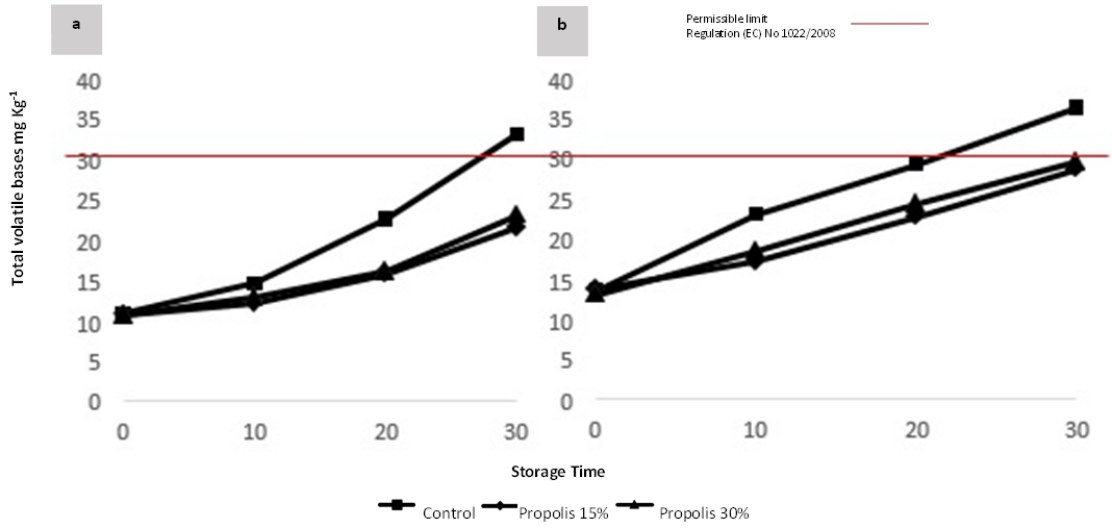

Figure 3. N-BVT values in red tilapia filets (*Oreochromis sp.*) preserved with propolis and with its permissible regulation a) Impregnation of propolis at 1.5 hours b) Impregnation of propolis at 3 hours.

Table 3. Analysis of variance of the treatments considered in the study for N-BVT.

Source	SC	gl	CM	p-value
A-Storage time	102.08	1	102.08	<0.0001
B-Propolis concentration	87.71	1	87.71	<0.0001
C-Time impregnation	201.50	1	201.50	<0.0001
AB	105.11	1	105.11	<0.0001
AC	20.02	1	20.02	<0.0001
BC	0.3588	1	0.3588	0.2934
ABC	8.11	1	8.11	<0.0001
Residual	18.18	57	0.3190	
Total Cor	3847.46	71		

3.1.4 Instrument color determination

Regarding the values of the chromatic coordinates L*, a* and b* for red tilapia filets, significant differences were observed between treatments from day 20 of storage. The chromatic coordinate L* (luminosity) decreases as the days of storage increase. In any case, the impregnation time of 1.5 hours reduced these values in contrast to the impregnation time of 3 hours. This behavior is similar to that reported by Magalhães et al. (2019), who determined the quality of snacks from mechanically separated red tilapia filets. Likewise, Zapata and De la Pava (2018) determined that the red tilapia filets used in the preparation of sausages lose their brightness as the storage time.

The analysis of variance for the chromatic parameters in this study (Table 4), shows that for L the significant terms of model are A, AB, BC and ABC. For the parameter a* (red), the significant terms of the model are A, B, AB and AC. The a* values increased steadily as the storage time and the concentration of propolis employed in the filets increased. In parameter b* (yellow), the significant terms were A, B, C, AB, AC and BC.

Parameters a and b (red and yellow) increase significantly and progressively during the 30 days of refrigerated storage and independently of the factors analyzed in this research.

$$\begin{aligned} L^* = {} & 19,56 + (-1,84 \cdot A) + 0,0213 \cdot B + \\ & (-0,0120 \cdot C) + 0,9873 \cdot AB + \\ & (-0,1229 \cdot AC) + (-0,1829 \cdot BC) + \\ & (-0,2893 \cdot ABC) \end{aligned} \tag{6}$$

$$a^* = 7,42 + 1,70 \cdot A + (-1,45 \cdot B) + (-0,0802 \cdot C) + (-1,21 \cdot AB) + (-0,1909 \cdot AC) + (-0,0756 \cdot BC) + 0,0001 \cdot ABC \quad (7)$$

$$b^* = (-0,5200) + 2,12 \cdot A + (-0,2152 \cdot B) + (-0,6360 \cdot C) + (-0,3244 \cdot AB) + (-0,1975 \cdot AC) + (-0,0594 \cdot BC) + 0,0334 \cdot ABC \quad (8)$$

Equations 6, 7 and 8 are presented in terms of codified factors and can be used to make predictions about the response for each factor, and allow to identify the relative impact of the factors by comparing their coefficients.

Table 4. Analysis of p-values of treatments considered in the study for the CieLab color scale.

Source	p-value		
	L*	a*	b*
A-Storage time	<0.0001	<0.0001	<0.0001
B-Propolis concentration	0.8830	<0.0001	<0.0001
C-Time impregnation	0.9389	0.2731	<0.0001
AB	<0.0001	<0.0001	<0.0001
AC	0.2164	<0.0001	<0.0001
BC	0.0462	0.0739	0.0020
ABC	0.0196	0.9982	0.1798
Residual	6.20	4.72	0.9182
Total Cor	852.82	379.10	117.25

3.2 Microbiological analysis

Figure 4 shows the microbial growth results for mesophilic bacteria in red tilapia filets for each factor during storage days. The count of mesophiles in filets without propolis showed continuous growth during storage, while the microbial load of mesophiles tended to decrease in treatments with propolis at 15 and 30%. The control treatments showed an increase until day 30 of mesophilic bacteria of 11.74 and 11.92 log CFU g^{-1} for the impregnation times of 1.5 and 3 hours, respectively.

The mesophilic count at the end of the storage days for the concentration of 15 and 30% of propolis at 3 hours of impregnation had values of 6.03 and 6.27 log CFU g^{-1}. The tilapia filets impregnated with propolis during a 1.5 h impregnation time obtained values of 4.39 and 4.13 log CFU g^{-1} for the concentrations of propolis at 15 and 30%, respectively. The results at 1.5 h of impregnation are within the ranges determined by the NTE-INEN 183-2013 and the International Commission on Microbiological Specifications of Food (ICMSF) (Roberts et al., 2005).

The inhibition effect of mesophilic microorganisms in the concentrations of propolis at 15 and 30% in an impregnation time of 1.5 h during the 30 days of storage was superior to other investigations that use propolis as a preservative; thus the count of mesophilic in these conditions were lower than those reported by Suarez et al. (2014) and Duman and Özpolat (2015), who obtained a count of mesophilic higher than 5.4 log CFU g^{-1} during 20 days storage, using propolis solutions on fish filets in a concentration of 1.2 mg mL^{-1} and 0,5%, respectively, under similar storage conditions as in the present study.

The results determined a development of *E. coli* at 10 days of storage time of the red tilapia filets, both in the impregnation times of propolis of 1.5 and 3 hours and with all concentrations of propolis evaluated; however, some values obtained in this study are below the highest limit allowed by the NTE INEN 183:2013, which establishes a maximum of 2.69 log UFC g^{-1} of *E. coli* concentration.

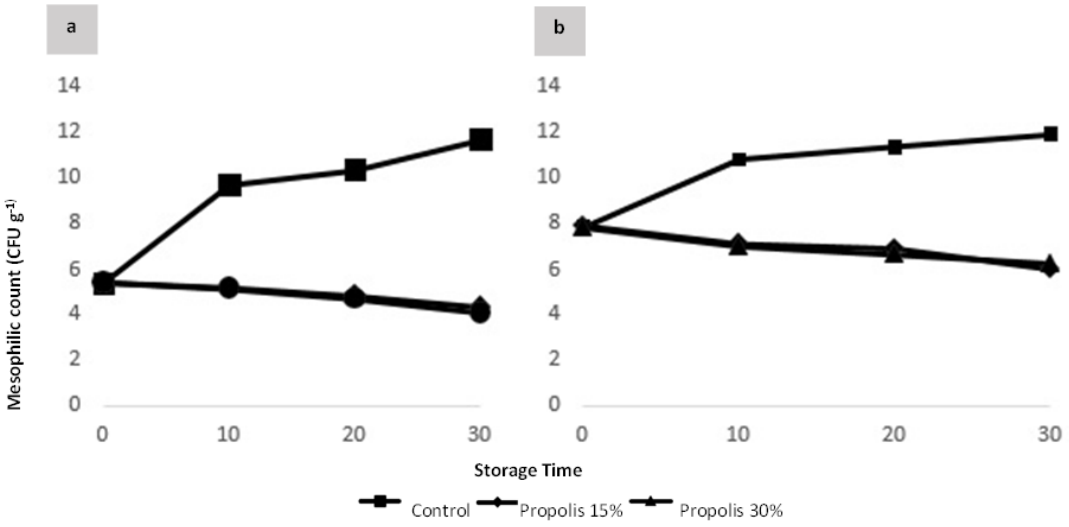

Figure 4. Concentration of mesophiles in red tilapia filets (*Oreochromis sp.*) preserved with propolis and with its permissible regulation a) Impregnation of propolis at 1.5 hours b) Impregnation of propolis at 3 hours.

The values obtained during the 30 days of storage for the impregnation time of 1.5 h are between 1.73 and 6.61 log CFU g^{-1} for the 0% of propolis concentration (control), from 1.71 to 3.57 log CFU g^{-1} with the 15% of propolis concentration and from 1.74 to 3.27 log CFU g^{-1}, for 30% of propolis concentration; likewise, the values during the 30 days of storage for the impregnation time of 3 hours were 2.13 to 6.87 log CFU g^{-1} for 0% propolis concentration and 2.08 to 5.11 and 2.14 to 5.15 log CFU g^{-1} for 15 and 30% propolis concentration (Figure 5). These values are lower than those mentioned by Talledo-Solórzano et al. (2020) who obtained presence of these bacteria between 3.32 and 5.17 log CFU g^{-1} in tilapia filets treated with lactic acid bacteria.

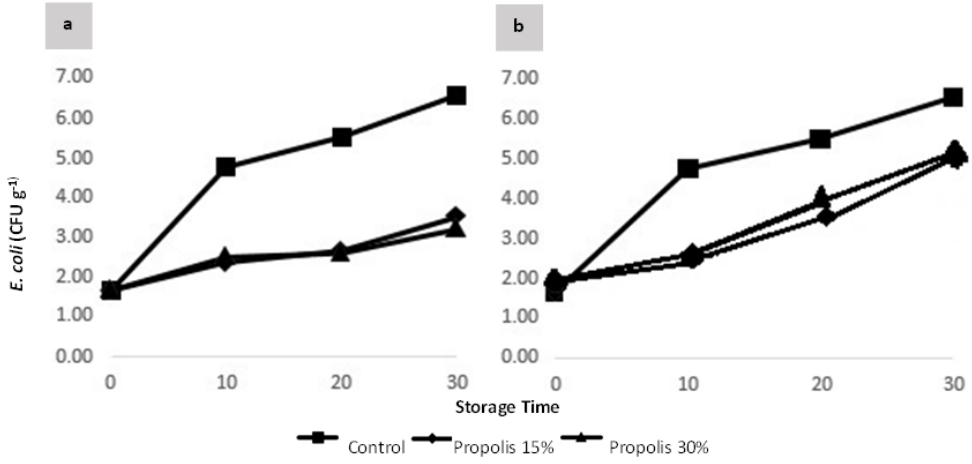

Figure 5. Count of *E. coli* in red tilapia filets (*Oreochromis sp.*) preserved with propolis and with its permissible regulations. a) Impregnation of propolis at 1.5 hours; b) Impregnation of propolis at 3 hours.

The results of the microbiological analysis indicate that the concentrations of propolis at 15 and 30% with an impregnation time of 1.5 h are adequate to maintain the mesophilic count within the limits allowed in the different regulations of quality in force during the storage of 30 days, and also these conditions are adequate to maintain the levels of *E. coli* in acceptable quality levels for up to 20 days in the storage conditions evaluated.

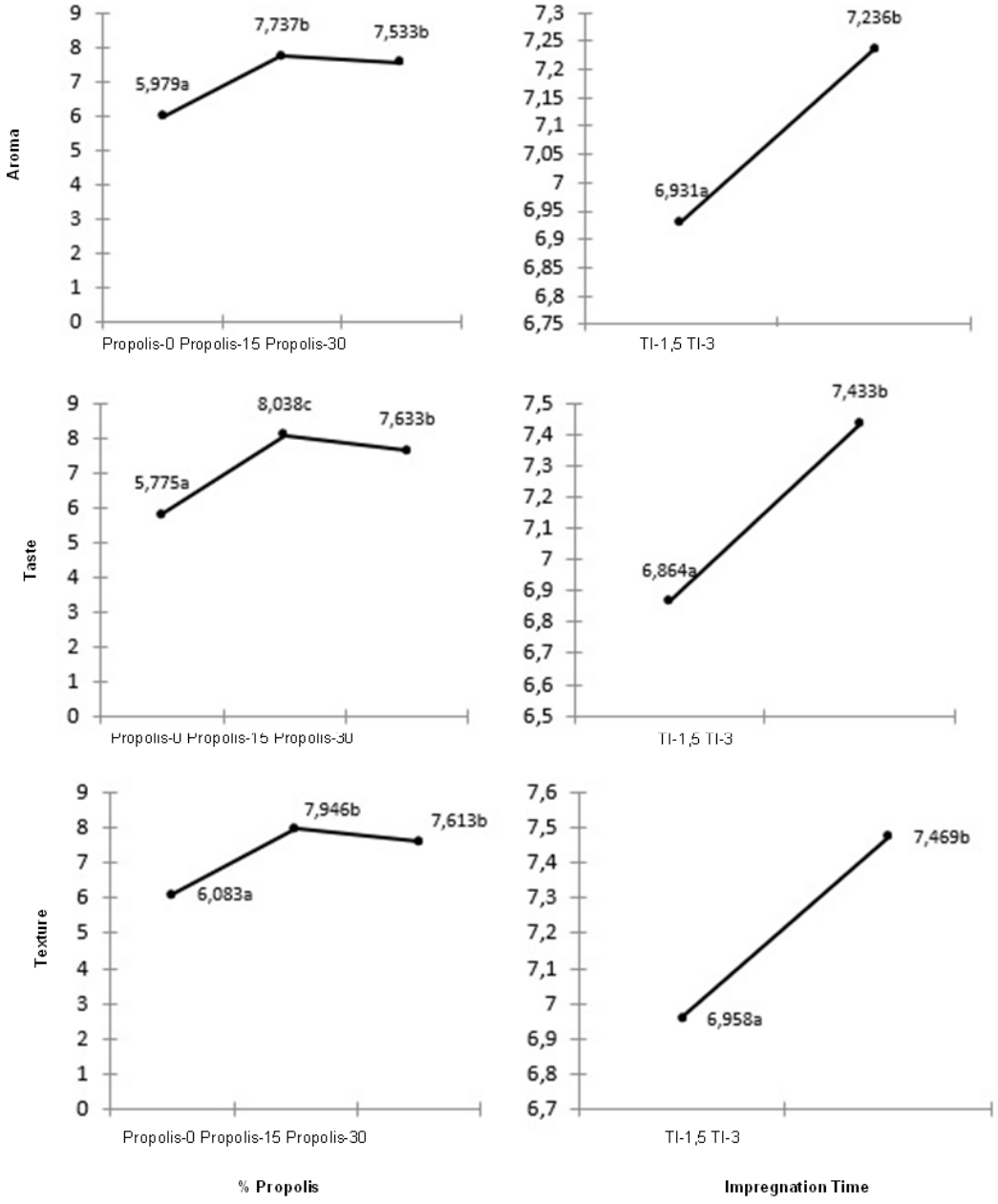

Figure 6. Analysis of the organoleptic parameters of red tilapia filets (*Oreochromis sp.*) impregnated with two doses of propolis (15 and 30%), and two impregnation times (1.5 and 3 h) to 10 days of storage for the sensory characteristics of aroma, flavor and texture

On the other hand, both concentrations of propolis with 3 h of impregnation do not allow to maintain the concentration of allowed mesophiles during the days of storage, and they maintain the concentration of *E. coli* in acceptable quality levels until 10 days of storage; for this reason, the impregnation time of 3 h is inadequate to avoid the proteolytic deterioration of the filets by the proliferation of microorganisms evidenced.

Thus, the most appropriate treatment to maintain the microbiological quality within the limits allowed by the standard NTE-INEN 183-2013 is 15% propolis with an impregnation time of 1.5h, for a storage time of 20 days at 4-5°C vacuum-packed.

3.3 Sensory analysis

Figure 6 shows the sensory analysis results of propolis-impregnated red tilapia filets stored at 4-5°C. The exposed data show the results of the sensory analysis applied to tilapia filets with ten days of storage, time in which all treatments comply with the physicochemical and microbiological quality values established in the current quality regulation.

The treatments of the present study with both concentrations of propolis (15 and 30%), two impregnation times (1.5 and 3 h) and with a storage time of 10 days obtained an acceptability level higher than 6.5 in the attributes of aroma, flavor and texture.

Regarding the propolis concentration, this analysis determined that in the attributes of aroma, flavor and texture tilapia filets with 15% propolis with acceptance levels between 7.7 and 8 have greater acceptance. Regarding the impregnation time, the sensory panel preferred the treatments with impregnation time of propolis at 3 hours, with acceptance values between 7.2 and 7.4. Considering the storage time of ten days, the treatments had acceptance values between 6.5 and 6.7 and there was no difference with respect to the control treatment, demonstrating the conservation of the organoleptic characteristics within this storage time at 4-5 °C (4-11°F).

Overall, the acceptance was greater than 80% for all the sensory characteristics highlighted in the present study. Likewise, studies carried out by Talledo-Solórzano et al. (2020) demonstrated a great acceptance by the sensory panel for red tilapia filets preserved with lactic acid bacteria, where the average acceptance value per attribute was higher than 4.

4 Conclusions

By evaluating the effect of propolis at concentrations of 15 and 30% as a preservative agent in the preservation of red tilapia filets (*Oreochromis sp.*) vacuum-packed and stored at 4-5°C, it is possible to affirm that propolis can maintain the physicochemical and microbiological properties of these filets. Under these conditions, the treatment of 15% propolis with an impregnation time of 1.5 h for a storage time of 20 days maintains acceptable levels of quality within the limits allowed by the standard NTE-INEN 183-2013 and European Community Regulation (EC) N° 1022/2008.

Propolis is also useful as a preservative in the preservation of red tilapia filets (*Oreochromis sp.*), since it is directly involved in the inhibition of proteolytic deterioration of fish fibers, maintaining low concentration levels of mesophilic microorganisms, *E. coli* and the physicochemical parameters immersed in the deterioration process and analyzed in the present study (pH, N-BVT, CRA and color). Finally, sensory analysis of red tilapia (*Oreochromis sp.*) filets showed that the use of propolis as a preservative allows maintaining the organoleptic quality during the storage time.

Acknowledgments

The authors of this research thank the Faculty of Zootechnical Sciences of the Technical University of Manabí for supporting them with technical and professional resources to conduct this research.

References

Afata, T., Nemo, R., Ishete, N., Tucho, G., and Dekebo, A. (2022). Phytochemical investigation, physicochemical characterization, and antimicrobial activities of ethiopian propolis. *Arabian Journal of Chemistry*, 15(7):103931. Online:https://n9.cl/lx0ma.

Ahmad, I., Babitha, A., Verma, A., and Maqsood, M. (2017). Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. *Aquaculture international*, 25:1215–1226. Online:https://n9.cl/geusw.

Basiri, S., Shekarforoush, S., Aminlari, M., and Akbari, S. (2015). The effect of pomegranate peel extract (ppe) on the polyphenol oxidase (ppo) and quality of pacific white shrimp (litopenaeus vannamei) during refrigerated storage. *LWT-Food Science and Technology*, 60(2):1025–1033. Online:https://n9.cl/w48fgs.

Cabrera-Estupiñán, Pérez Leira, R., and Moreira Santos, J. (2017). Régimen de precipitaciones y evaporación para riego en el multipropósito chone, ecuador. *Revista de Investigaciones en Energía, Medio Ambiente y Tecnología: RIEMAT ISSN: 2588-0721*, 2(2):6–15. Online:https://n9.cl/lcka2.

Campus, M., Addis, M., Cappuccinelli, R., Porcu, M., Pretti, L., Tedde, V., Secchi, N., Stara, G., and Roggio, T. (2010). Stress relaxation behaviour and structural changes of muscle tissues from gilthead sea bream (sparus aurata l.) following high pressure treatment. *Journal of Food Engineering*, 96(2):192–198. Online:https://n9.cl/ploa8i.

Cicero, L., Furlan, E., Tomita, R., Prisco, R., Savoy, V., and Neiva, C. (2014). Estudo das metodologias de destilação na quantificação do nitrogênio das bases voláteis totais em pescada, tilápia e camarão. *Brazilian Journal of Food Technology*, 17:192–197. Online:https://n9.cl/k9mke1.

Comunidad Europea (2008). Reglamento (ce) n 1022/2008. Diario Oficial de la Unión Europea. Online:https://tinyurl.com/2b9nrsgo.

Duman, M. and Özpolat, E. (2015). Effects of water extract of propolis on fresh shibuta (barbus grypus) fillets during chilled storage. *Food Chemistry*, 189:80–85. Online:https://n9.cl/0cy3h.

Farag, M., Abdelnour, S., Patra, A., Dhama, K., Dawood, M., Elnesr, S., and Alagawany, M. (2021). Propolis: Properties and composition, health benefits and applications in fish nutrition. *Fish & Shellfish Immunology*, 115:179–188. Online:https://n9.cl/gfx74.

Howgate, P. (2010). A critical review of total volatile bases and trimethylamine as indices of freshness of fish. part 2. formation of the bases, and application in quality assurance. *Electronic Journal of Environmental, Agricultural & Food Chemistry*, 9(1):58–88. Online:https://n9.cl/tv7is.

INEN (2014). Nte inen 183 pescado fresco, refrigerado y congelado requisitos. Online:https://tinyurl.com/265zhffu .

Jácome, J., Quezada Abad, C., Sánchez, O., Pérez, J., and Nirchio, M. (2019). Tilapia en ecuador: paradoja entre la producción acuícola y la protección de la biodiversidad ecuatoriana. *Revista peruana de biología*, 26(4):543–550. Online:https://n9.cl/dknts.

Jonaidi-Jafari, N., Kargozari, M., Ranjbar, R., Rostami, H., and Hamedi, H. (2018). The effect of chitosan coating incorporated with ethanolic extract of propolis on the quality of refrigerated chicken fillet. *Journal of Food Processing and Preservation*, 42(1):e13336. Online:https://n9.cl/jt2gq.

Magalhães, A., Máralém, E., Júnior, M., and Monteiro, M. (2019). Evaluation of the technological quality of snacks extruded from broken grains of rice and mechanically separated tilapia meat flour. *Boletim do Instituto de Pesca*, 45(2):1–9. Online:https://n9.cl/jyl7x.

Melody, J., Lonergan, S., Rowe, L., Huiatt, T., Mayes, M., and Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. *Journal of animal science*, 82(4):1195–1205. Online:https://n9.cl/p3k87.

Nedji, N. and Loucif-Ayad, W. (2014). Antimicrobial activity of algerian propolis in foodborne pathogens and its quantitative chemical composition. *Asian Pacific Journal of Tropical Disease*, 4(6):433–437. Online:https://n9.cl/x6o2o.

Peixoto, M., Freitas, A., Cunha, A., Oliveira, R., and Almeida-Aguiar, C. (2021). Antioxidant and antimicrobial activity of blends of propolis samples collected in different years. *Lwt*, 145:111311. Online:https://n9.cl/g93nd.

Przybyłek, I. and Karpiński, T. (2019). Antibacterial properties of propolis. *Molecules*, 24(11):2047. Online:https://n9.cl/y2ps7.

Rebouças, L., do Vale Figueiredo, J., Alves, V., da Silva, M., de Oliveira, P., Souza, J., Firmino, S., da Silva P., da Silva, J., and de Oliveira, P. (2020).

Diferentes processos de salga na qualidade de filés de tilápia (oreochromis niloticus). *Research, Society and Development*, 9(10):e1029108251. Online:https://n9.cl/ipoyi.

Roberts, T., Cordier, J., Gram, L., Tompkin, R., Pitt, J., Gorris, L., and K., S. (2005). *Micro-organisms in foods 6: microbial ecology of food commodities*, chapter Fish and fish products, page 174–249. Springer.

Rodríguez-Pérez, B., Canales, M., Penieres, J., and Cruz, T. (2020). Composición química, propiedades antioxidantes y actividad antimicrobiana de propóleos mexicanos. *Acta universitaria*, 30:e2435. Online:https://n9.cl/b02542.

Salleh, S., Hanapiah, N., Johari, W., Ahmad, H., and Osman, N. (2021). Analysis of bioactive compounds and chemical composition of malaysian stingless bee propolis water extracts. *Saudi journal of biological sciences*, 28(12):6705–6710. Online:https://n9.cl/b9vwh.

Santos, T., Queiroz, R., Sawaya, A., Lopez, B., Soares, M., Bezerra, D., A., R., De Paula, V., and Waldschmidt, A. (2017). Melipona mondury produces a geopropolis with antioxidant, antibacterial and antiproliferative activities. *Anais da Academia Brasileira de Ciências*, 89:2247–2259. Online:https://n9.cl/yuesa.

Sarıkahya, N., Gören, A., Okkalı, G., Çöven, F., Orman, B., Kırcı, D., Yücel, B., Kışla, D., Demirci, B., Altun, M., Önem, A., and Nalbantsoy, A. (2021). Chemical composition and biological activities of propolis samples from different geographical regions of turkey. *Phytochemistry Letters*, 44:129–136. Online:https://n9.cl/bg9l9.

Suarez, H., Jiménez, Á., and Díaz, C. (2014). Determination of microbiological and sensoryparameters of fish fillets with propolis preserved under refrigeration. *Revista MVZ Córdoba*, 19(3):4214–4225. Online:https://n9.cl/ev171.

Talledo-Solórzano, V., Chavarría, L.and Zambrano, S., and Cuenca, G. (2020). Efecto del uso de bacterias ácido lácticas en la inhibición del deterioro microbiológico de filetes de tilapia roja (oreochromis sp.). *Journal of Science and Research*, 5(CININGEC):363–383. Online:https://n9.cl/90w8c.

Torres, A., Sandjo, L., Friedemann, M., Tomazzoli, M., Maraschin, M., Mello, C., and Santos, A. (2018). Chemical characterization, antioxidant and antimicrobial activity of propolis obtained from melipona quadrifasciata quadrifasciata and tetragonisca angustula stingless bees. *Brazilian Journal of Medical and Biological Research*, 51(6):Online:https://n9.cl/8m4v9.

Valencia-Junca, M., Valencia, C., Flórez López, E., Delgado-Ospina, J., Zapata, P., Solano, M., and Grande Tovar, C. (2019). Chitosan beads incorporated with essential oil of thymus capitatus: Stability studies on red tilapia fillets. *Biomolecules*, 9(9):458. Online:https://n9.cl/wme2i.

Vargas-Sánchez, R., Urrutia, G., and Escalante, A. (2013). El propóleos: conservador potencial para la industria alimentaria. *Interciencia*, 38(10):705–711. Online:https://n9.cl/1yp4e.

Vică, M., Glevitzky, M., Tit, D., Behl, T., Heghedűş-Mîndru, R., Zaha, D., Ursu, F., Popa, M., Glevitzky, I., and Bungău, S. (2021). The antimicrobial activity of honey and propolis extracts from the central region of romania. *Food Bioscience*, 41:101014. Online:https://n9.cl/1y0in.

Viloria, J., Gil, J., Durango, D., and García, C. (2012). Caracterización fisicoquímica del propóleo de la región del bajo cauca antioqueño (antioquia, colombia). *Biotecnología en el sector agropecuario y agroindustrial*, 10(1):77–86. Online:https://n9.cl/lgnqd.

Zapata, J. and De la Pava, G. (2018). Physicochemical analysis of frankfurter type sausages made with red tilapia fillet waste (oreochromis sp) and quinoa flour (chenopodium quinoa w.). *Brazilian Journal of Food Technology*, 21:e2016103. Online:https://n9.cl/3bht9.

Zhang, C., Shen, X., Chen, J., Jiang, X., and Hu, F. (2017). Identification of free radical scavengers from brazilian green propolis using off-line hplc-dpph assay and lc-ms. *Journal of food science*, 82(7):1602–1607. Online:https://n9.cl/lb1t6.

La Granja:
Revista de
Ciencias de la Vida

pISSN:1390-3799; eISSN:1390-8596

http://doi.org/10.17163/lgr.n38.2023.03

Special Issue/ Número Especial

Food Science

Chemical analysis of amazonian essential oils of an Ecuadorian Shuar Community

Análisis químico de aceites esenciales amazónicos de una Comunidad Shuar Ecuatoriana

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Received on January 30th, 2023. Accepted, after review, on August 05th, 2023. Published on September 1st, 2023.

Abstract

This research was carried out with the aim of determining the chemical composition of the essential oils of four Amazonian species from the Antuash community, Morona canton, province of Morona Santiago. These species belong to the main aromatic families of Ecuador. The essential oils (EO) of *Critoniopsis pycnantha* (Benth.) H. Rob., *Myrcia aliena* McVaugh, *Piper macrotrichum* C. DC. and *Siparuna schimpffii* Diels were obtained from the dry leaves by analytical steam distillation; a percentage yield of 0.24%, 0.80%, 0.44%, and 0.32% was achieved respectively. EO were qualitatively analyzed by gas chromatography coupled to mass spectrometry (GC-MS) and quantitatively analyzed by gas chromatography coupled to flame ionization detector (GC-FID) with a DB-5ms apolar column. The compounds were identified based on mass spectra and the Van Den Dool and Kratz retention indices. They were quantified by calculating the relative response factors based on the combustion enthalpies. *M. aliena* and *P. macrotrichum* resulted rich in monoterpenes, and *C. pycnantha* and *S. schimpffii* in sesquiterpenes. The major compounds for the essential oils of *C. pycnantha* were γ-muurolene, bicyclogermacrene, (E)-caryophyllene α-ylangene and α-humulene; *M. aliena*: α-pinene and β-pinene; *P. macrotrichum* δ-3-carene, eugenol and chavibetol acetate; and *S. schimpffii*: spathulenol, 2-undecanone, bicyclogermacrene and (E)-Isocroweacin.

Keywords: *Critoniopsis pycnantha*, *Piper macrotrichum*, *Myrcia aliena*, *Siparuna schimpffii.*

Resumen

La presente investigación se realizó con el propósito de determinar la composición química de los aceites esenciales de cuatro especies amazónicas del centro shuar Antuash en el cantón Morona, provincia de Morona Santiago; las cuales pertenecen a las principales familias aromáticas del Ecuador. Los aceites esenciales de *Critoniopsis pycnantha* (Benth.) H. Rob., *Myrcia aliena* McVaugh, *Piper macrotrichum* C. DC. y *Siparuna schimpffii* Diels, fueron obtenidos de las hojas secas mediante destilación analítica por arrastre de vapor, determinándose un rendimiento por peso con respecto a las hojas secas de 0,24 %, 0,80 %, 0,44 % y 0,32 %, respectivamente. Estos fueron analizados cualitativamente mediante cromatografía de gases acoplada a espectrometría de masas (GC-MS) y cuantitativamente mediante cromatografía de gases acoplada a detector de ionización de llama (GC-FID), con columna apolar DB-5ms. Se realizó la identificación de los compuestos en base a los espectros de masas y los índices de retención de Van Den Dool Kratz, y se cuantificó calculando los factores de respuesta relativos con base a las entalpías de combustión. Así se determinó riqueza en monoterpenos para el aceite de *M. aliena* y *P. macrotrichum* y riqueza en sesquiterpenos para el aceite de *C. pycnantha* y *S. schimpffii*. Los compuestos mayoritarios en el aceite esencial de *C. pycnantha* fueron γ-muuroleno, biciclogermacreno, (*E*)-cariofileno α-ylangeno y α-humuleno; para *M. aliena* α-pineno y β-pineno; para *P. macrotrichum* δ-3-careno, eugenol y acetato de chavibetol; y, para *S. schimpffii* espatulenol, 2-undecanona, biciclogermacreno y (*E*)-Isocroweacina.

Palabras clave: *Critoniopsis pycnantha*, *Piper macrotrichum*, *Myrcia aliena*, *Siparuna schimpffii.*

Suggested citation: Montalván, M., Malagón, O., Cumbicus, N., Tanitana, F. and Gilardoni, G. (2023). Chemical analysis of amazonian essential oils of an Ecuadorian Shuar Community. La Granja: Revista de Ciencias de la Vida. Vol. 38(2):32-44. http://doi.org/10.17163/lgr.n38.2023.03.

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1 Introduction

There are about 295 families of plants with medicinal use in Ecuador of which 60 to 80 families produce essential oils, such as *Asteraceae, Lamiaceae, Lauraceae, Myrtaceae, Rosaceae, Rutaceae, Apiaceae* and *Pinaceae* (Aguirre-Mendoza et al., 2017). The study of the aromatic fraction of plant species is one of the most researched approaches in relation to the great diversity of plant species distributed in the four regions of the country. Essential oils are complex mixtures of secondary metabolites mainly monoterpenes, diterpenes and sesquiterpenes produced by different parts of plants. They are widely used in the food, cosmetic and pharmaceutical industry for presenting antiseptic, antibacterial, antiviral and antifungal properties (Camus and Trujillo, 2011; Ochoa-Pumaylle et al., 2012; León-Méndez et al., 2015; Noriega-Rivera, 2009). For this reason, the aim of this research was to perform the qualitative and quantitative chemical identification of the volatile fractions of four Amazonian species *Critoniopsis pycnantha, Myrcia aliena, Piper macrotrichum* and *Siparuna schimpffii* at the center of Antuash, Morona Santiago province, from which there is very little information. The chemical analysis was performed by gas chromatography coupled to mass spectrometry and flame ionization detector.

Critoniopsis pycnantha is a native shrub or tree that grows at a height between 1500 and 3000 m.a.s.l. (Missouri Botanical Garden, 2022) and presents beekeeping utility (De la Torre et al., 2008). *Myrcia aliena* is a tree native to the Andean and Amazon region that grows at an altitude between 500 and 2500 m.a.s.l. (Missouri Botanical Garden, 2022), known locally as "awapit", its stem is timber and its fruit feeds animals (De la Torre et al., 2008). *Piper macrotrichum* is a subshrub or native shrub found in the provinces of Morona Santiago, Napo, Pastaza at an altitude between 0 and 1000 m.a.s.l. (Missouri Botanical Garden, 2022). *Siparuna schimpffii* is a native shrub or tree located at an altitude between 0 and 1500 m.a.s.l. in the provinces of Tungurahua, Napo, Pastaza, Morona Santiago and Zamora Chinchipe (Missouri Botanical Garden, 2022), known as "chiri wayusa, mal aire panka, ardillón". This species has some medicinal uses, using its leaves in infusion to combat fatigue and bark to relieve the general pain of the body produced by fever. It is also used to clean the "bad energies" and its stem is used as a pole for construction (De la Torre et al., 2008). In addition, the species of the genus Siparuna are used in traditional medicine for relieving pain, inflammation, fever and infections (Ferreira-Silva et al., 2021).

2 Materials and Methods

2.1 General data

The analysis of essential oils was performed in a Thermo Scientific (Wal-than, MA, USA) Trace 1300 gas chromatograph coupled to a simple quadrupole mass spectrometer (GC-MS) ISQ 7000 and a traditional flame ionization detector (GC-FID). A DB-5MS apolar column with a stationary phase 5%-phenyl-methylpolysiloxane of 30 m of length, 0.25 mm in internal diameter and 0.25 μm in thickness was used.

The solvents and standards used were analytical grade with a purity greater than 99% and acquired in Sigma Aldrich (San Luis, Missouri, USA). In the case of isopropyl caproate, it was synthesized in the laboratories of the Technical Private University of Loja with a purity of 98.8%, determined by GC-FID.

2.2 Collection of species

The four Amazonian species *Critoniopsis pycnantha, Myrcia aliena, Piper macrotrichum* and *Siparuna schimpfii* were collected at the shuar center Antuash-Morona Santiago, with the following coordinates: 02°39'41.7348" S 77°42'44.9496" W, 02°39'44.7372" S 77°42'55.8072" W", 02°39'49.8744" S 77°43'21.2484" W and 02°39'44.5248" S 77°43'28.8588" W, respectively. The four species were found in a flowering state and were collected during the rainy period. The study was conducted according to the MAAE-ARSFC-2021-1233 research permit, under the free, prior and informed consent of access to traditional knowledge, associated with biodiversity (Biological and genetic resources), and its scope, signed freely and voluntarily between the UTPL, ProAmazonia and the community of Antuash under the legal protection of the Nagoya Protocol and Ecuadorian law. It was subscribed to the Shuar Antuash Center on April 22, 2022. The species were entered into the herbarium of the Universidad Tecnico Particular de Loja (HUTPL), with the following codes of Váucher

14684 (*C. pycnantha*), 14685 (*M. aliena*), 14691 (*P. macrotrichum*) and 14692 (*S. schimpfii*).

2.3 Obtaining and yielding essential oils

The essential oils (EO) of the aforementioned species were obtained from the dry leaves by analytical distillation by steam drag, a process that lasted three hours. The leaves were dried at a temperature of 35°C for 48 hours. The essential oil was collected on two milliliters of cyclohexane, which in turn contained nonane as an internal standard and which was placed before starting distillation. It was then recovered and stored in amber vials under refrigeration at -4°C. Yields were calculated with the quantity in volume of distilled EO in cyclohexane and nonane solution with respect to the weight of the dry leaves of *Critoniopsis pycnantha* (61.8 g), *Myrcia aliena* (81.5 g), *Piper macrotrichum* (40.6 g) and *Siparuna schimpffii* (95.5 g).

2.4 Preparation of essential oil samples

The four essential oils obtained were diluted for injection into the gas chromatograph. For the species *M. aliena* and *C. pycnantha* a 1 in 1000 dilution was performed with cyclohexane and for *P. macrotrichum*, and for the species *M. aliena* and *C. pycnantha* a 1 in 1000 dilution was performed with cyclohexane and for *P. macrotrichum* and *S. schimpffii* a 1 in 500 dilution with cyclohexane.

2.5 Qualitative analysis

2.5.1 Chromatographic method

The method used for injections of EOs in GC-MS consisted of injector temperature: 200° C; injection mode: split, with split 10 ratio, injection volume: 2 μl for *C. pycnantha, P. macrotrichum* and *S. schimpffii*, and 1 μL for *M. aliena*; DB-5ms column with a flow of He of 1 mL/min; thermal program: oven temperature 50° C for 10 minutes, with a temperature ramp of 3° C/min up to 2555° C for 5 minutes. Total running time: 81.66 minutes. A mass spectrometer transfer line temperature of 200° C and an ion source temperature of 230° C, a mass range of 40-400 m/z and two minutes waiting for the detector ignition were used.

2.5.2 Identification of compounds

With the essential oil samples and with the same chromatographic method described for mass spectrometry, a mixture of hydrocarbons of the C9 to C22 series was injected to obtain the equations that allowed to determine the Van Den Dool and Kratz indexes of each compound (Van Den Dool and Dec Kratz, 1963). The identification was based on the mass spectrum and a difference of no more than twenty units between the calculated indexes and the retention indexes described in Adams (2017).

2.6 Quantitative analysis

2.6.1 Chromatographic method

Injections in GC-FID were performed with the following conditions: injector temperature of 230° C, split injection mode, with split radius 10 for *P. macrotrichum* and 40 for *C. pycnantha, S. schimpffii* and *M. aliena*, being the injection volume 1 μL; column DB-5ms with flow of 1 mL/min; thermal program: oven temperature 50° C for 10 minutes and temperature ramp of 2° C/min to 170° C and 10° C min to 230° C for 10 minutes. The total running time was 83 minutes. The detector temperature was 230° C.

2.6.2 Quantification

The quantification of the compounds was performed with the FID detector, according to the method proposed by Tissot et al. (2012). For this, the relative response factor (RF) of each compound was calculated with respect to isopropyl caproate, which was used as the standard of quantification. The FRRs were based on combustion enthalpies and determined using the formula described by Tissot et al. (2012). Nano was used as internal standard and isopropyl caproate as calibration standard. Four replicates per sample of EO and six standards were injected under the same chromatographic conditions. These standards were prepared by weighing constant amounts of nonane (7.13 mg) and increasing amounts of isopropyl caproate (0.6, 1.4, 4.2, 8.3, 16.4 and 33.6 mg) gaging with cyclohexane. The curve presented a R^2 of 0.9998 and the obtained equation allowed us to obtain the milligrams of each compound. A detection limit of 0.1% was considered, calculating the percentages of each component in

relation to the total mass of EO. The mean and standard deviation for each compound were calculated.

3 Results and discussion

3.1 *Critoniopsis pycnantha*

In the essential oil of *C. pycnantha* 63 compounds were identified, being the predominant compounds γ-muurolene in 34.45%, bicycles-germacean in 12.04%, (E)-cariophyllenein 11.05%, α-ylangen in 5.37% and α-humulene in 4.68%. These compounds are shown in the chromatogram in Figure 1 and are detailed in Table 1.

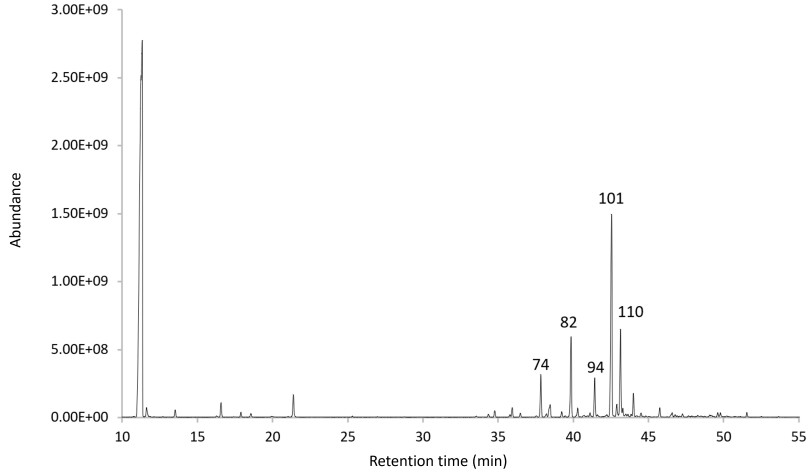

Figure 1. *C. pycnantha* essential oil chromatogram in column DB-5ms.

3.2 *Piper macrotrichum*

In the essential oil of *P. macrotrichum*, 66 compounds corresponding to 98.71% were determined. It was found that δ-3-carene (58.21%), eugenol (9.75%) and chavibetol acetate (7.81%) were major compounds, showing a greater amount of monoterpene hydrocarbons in 73.92% of the total. The identified compounds are described in Table 1 and can be observed on the chromatogram in Figure 2.

3.3 *Myrcia aliena*

In the essential oil of *M. aliena* 43 compounds were identified, representing 98.92%, being the major compounds two monoterpenes α-pinene (72.19%) and β-pinene (15.82%). The compounds can be seen on the chromatogram in Figure 3 and are detailed in Table 1.

3.4 *Siparuna Schimpffii*

In the essential oil of *S. schimpffii* 125 compounds were found, which represent 93.65% of the total. The major compounds identified were spatulenol (12.10%), 2-undecanone (10.87%), (E)-isocroweacin (6.41%) and bicyclogermacrene (5.84%), which can be seen on the chromatogram in Figure 4 and are described in Table 1.

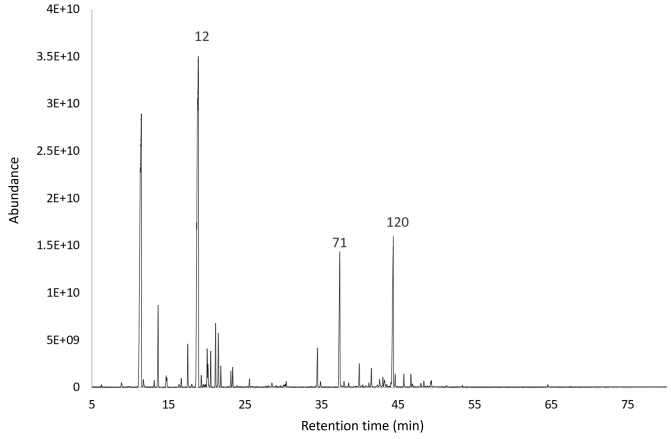

Figure 2. Essential oil chromatogram of *P. macrotrichum* in column DB-5ms.

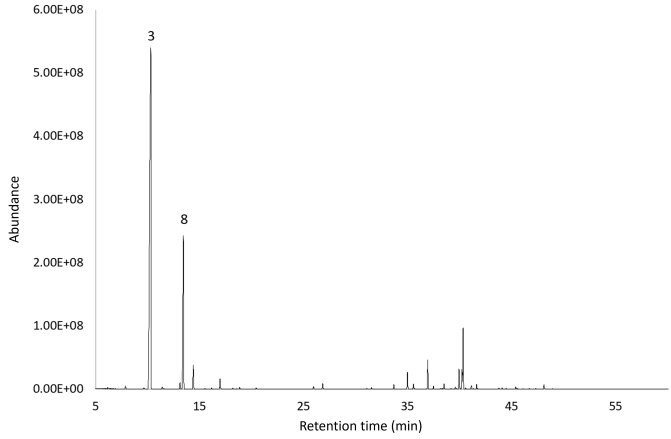

Figure 3. Essential oil chromatogram of *M. aliena* in column DB-5ms.

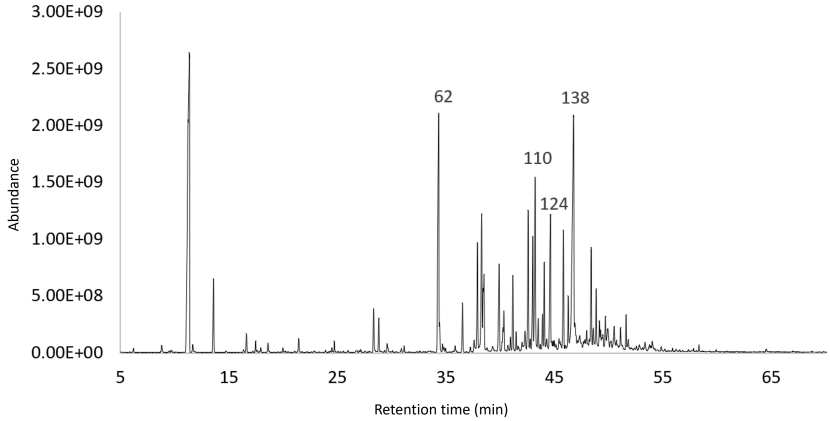

Figure 4. Essential oil chromatogram of *S. schimpffii* in column DB-5ms.

Table 1. Chemical composition of the essential oil of *Critoniopsis pycnantha, Myrcia aliena, Piper macrotrichum* and *Siparuna schimpfii* in column DB-5ms.
IRC: Calculated retention rate. **IRL**: Literature retention index. **ND**: Not determined. **PM**: Molecular weight. σ: standard deviation.

N°	Compounds	IRC	IRL	*C.pycnantha*		*M.aliena*		*P. macrotrichum*		*S.schimpffii*	
				%	σ	%	σ	%	σ	%	σ
1	(2*E*.4*E*)- hexadienol	905	912	TRAZA	0.03	-	-	TRAZA	-	TRAZA	-
2	α-Thujene	928	924	-	-	TRAZA	-	0.25	0.01	2.00	0.11
3	α-pinene	933	932	1.08	0.03	72.19	0.21	3.21	0.1	-	-
4	α-fenchene	948	945	-	-	-	-	0.44	0.02	TRAZA	-
5	Canphene	950	946	-	-	0.13	0.01	0.39	0.01	-	-
6	benzaldehyde	972	952	-	-	-	-	-	-	0.06	0.01
7	Sabineno	973	969	0.14	0.01	0.48	0.02	0.10	0.01	-	-
8	β-pinene	977	974	2.15	0.03	15.82	0.07	0.34	0.01	0.51	0.03
9	myrcene	990	988	-	-	1.60	0.03	1.60	0.05	0.32	0.01
10	2-Pentyl furan	990	984	-	-	-	-	-	-	0.10	0.01
11	δ-2-carene	1009	1001	-	-	TRAZA	-	-	-	-	-
12	δ-3-carene	1009	1008	0.04	0.02	TRAZA	-	58.21	1.72	0.22	0.01
13	α-terpinene	1017	1014	-	-	-	-	0.34	0.02	-	-
14	ρ-cymene	1023	1020	0.04	0.02	-	-	0.07	0.01	-	-
15	ND (PM 136)	1024	1025	-	-	-	-	0.14	0.01	-	-
16	o-cimene	1027	1022	-	-	0.05	0.01	TRAZA	-	TRAZA	-
17	Limonene	1029	1024	0.52	0.02	TRAZA	-	1.40	0.04	0.11	0.01
18	β-felandrene	1031	1025	0.47	0.02	0.75	0.01	0.59	0.06	TRAZA	-
19	1.8-cineol	1033	1026	-	-	-	-	TRAZA	-	TRAZA	-
20	(*Z*)-β-ocimene	1037	1032	0.12	0.03	-	-	1.15	0.04	TRAZA	-
21	*cis*-arbusculone	1045	1046	-	-	-	-	-	-	TRAZA	-
22	(*E*)-β-ocimene	1048	1044	TRAZA	-	TRAZA	-	2.74	0.27	0.05	-
23	ND (PM 120)	1052	1055	4.36	0.21	-	-	-	-	0.12	-
24	ND (PM 152)	1053	-	-	-	-	-	-	-	0.54	0.06
25	ND (PM 150)	1054	-	-	-	-	-	1.11	0.03	-	-
26	γ-terpinene	1057	1054	-	-	0.10	0.01	0.68	0.02	-	-
27	-tolualdehyde	1057	1062	-	-	0.26	0.08	-	-	-	-
28	(2*E*)-octen-1-al	1066	1049	-	-	-	-	-	-	TRAZA	-
29	linalool *trans*-oxide (furanoid)	1073	1084	-	-	-	-	-	-	TRAZA	-
30	Isoterpinolene	1082	1085	-	-	-	-	0.53	0.01	-	-
31	Terpinolene	1086	1086	-	-	0.07	0.01	0.65	0.02	TRAZA	-
32	ND (PM 154)	1094	-	-	-	-	-	0.06	0.01	-	-
33	2-nonanone	1095	1087	-	-	-	-	TRAZA	-	TRAZA	-
34	pinene α-oxide	1098	1099	-	-	-	-	TRAZA	-	-	-
35	n-nonanal	1110	1100	-	-	-	-	TRAZA	-	0.02	0.01
36	ND (PM 152)	1122	-	-	-	-	-	TRAZA	-	-	-
37	1-terpineol	1125	-	-	-	-	-	0.27	0.01	-	-
38	neo-*allo*-ocimene	1131	1140	-	-	-	-	TRAZA	-	-	-
39	α-canfolenal	1133	1122	-	-	-	-	-	-	TRAZA	-
40	*trans*-pinocarveol	1147	1135	-	-	-	-	-	-	TRAZA	-
41	Nopinone	1149	1135	-	-	-	-	-	-	0.05	0.01
42	*trans*-verbenol	1153	1140	-	-	-	-	-	-	0.12	0.01
43	Canfor	1155	1141	-	-	-	-	-	-	TRAZA	-
44	*cis*-pinocarveol	1166	1166	-	-	-	-	TRAZA	-	-	-
45	1-dodecene	1168	1187	-	-	-	-	-	-	TRAZA	-
46	Pinocarvone	1171	1160	-	-	-	-	-	-	TRAZA	-
47	α-felandren-8-ol	1172	1172	-	-	-	-	0.05	0.01	-	-
48	n-nonanol	1178	1165	-	-	-	-	-	-	0.95	0.28
49	Borneol	1179	-	-	-	TRAZA	-	-	-	-	-
50	octanoic acid	1184	1167	-	-	-	-	-	-	0.95	0.04
51	4-terpineol	1186	1174	-	-	0.17	0.01	-	-	-	-
52	ρ-cimen-8-ol	1197	1197	-	-	-	-	TRAZA	-	-	-
53	α-terpineol	1203	1186	-	-	0.33	0.01	-	-	0.22	0.09
54	ND (PM152)	1203	-	-	-	-	-	0.06	0.01	-	-
55	Safranal	1209	1197	-	-	-	-	-	-	TRAZA	-
56	ND (PM 204)	1211	-	-	-	-	-	0.08	0.01	-	-

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Table 1 – Continued from previous page

N°	Compounds	IRC	IRL	*C.pycnantha*		*M.aliena*		*P. macrotrichum*		*S.schimpffii*	
				%	σ	%	σ	%	σ	%	σ
57	n-decanal	1212	1201	-	-	-	-	TRAZA	-	TRAZA	-
58	octanol acetate	1215	1211	-	-	-	-	TRAZA	-	-	-
59	Verbenone	1219	1204	-	-	-	-	-	-	TRAZA	-
60	β-cyclocitral	1223	1217	-	-	-	-	0.28	0.02	0.13	0.01
61	ND (PM 164)	1234	-	-	-	-	-	-	-	0.12	0.01
62	2-undecanone	1298	1293	1.01	0.03	0.06	0.02	-	-	10.87	0.4
63	Safrol	1299	1285	-	-	-	-	TRAZA	-	0.17	0.17
64	methyl (*Z*)-cinnamate	1301	1299	-	-	-	-	1.61	0.06	-	-
65	1-tridecene	1307	1290	-	-	-	-	-	-	0.13	0.01
66	Hexenyl (*3E*)-tiglate	1310	1315	-	-	-	-	0.22	0.01	0.13	0.02
67	n-nonyl acetate	1313	1311	-	-	-	-	-	-	TRAZA	-
68	isopulegol *neo iso*-acetate	1328	1312	0.37	0.05	-	-	-	-	-	-
69	δ-element	1332	1335	0.37	0.05	-	-	-	-	TRAZA	-
70	α-cubebene	1346	1348	0.49	0.04	0.16	0.01	-	-	0.97	0.03
71	Eugenol	1363	1356	-	-	-	-	9.75	0.36	0.11	0.02
72	Cyclosativene	1368	1369	0.14	0.02	TRAZA	-	-	-	-	-
73	Isoledene	1371	1374	-	-	-	-	TRAZA	-	-	-
74	α-ylangen	1373	1373	5.37	0.09	0.58	0.01	-	-	TRAZA	-
75	α-copaene	1377	1374	-	-	-	-	0.19	0.01	3.54	0.31
76	β-borbonene	1383	1387	0.34	0.03	-	-	-	-	2.87	0.08
77	β-cubebene	1387	1387	1.33	0.03	0.18	0.04	-	-	0.46	0.46
78	β-element	1389	1389			-	-	0.10	0.01	1.01	0.43
79	α-gurjunene	1404	1409	0.71	0.02	-	-	-	-	-	-
80	ND (PM 204)	1408	-	-	-	-	-	-	-	0.09	0.01
81	*cis*-α-bergamotene	1410	1411	0.14	0.01	-	-	-	-	-	-
82	(*E*)-cariophyllene	1420	1417	11.05	0.19	1.28	0.02	-	-	-	-
83	(*Z*)-cariophyllene	1422	1408	-	-	-	-	0.85	0.03	2.59	0.06
84	(*E*)-α-ionone	1428	1428	-	-	-	-	0.06	0.01	0.93	0.12
85	ND (PM 204)	1429		0.70	0.02	-	-	-	-	-	-
86	γ-element	1431	1434	-	-	-	-	-	-	TRAZA	-
87	β-gurjunene	1432	1431	-	-	-	-	-	-	0.16	0.07
88	α-trans-bergamotene	1433	1432	-	-	0.13	0.01	-	-	-	-
89	Aromadendrene	1437	1439	0.15	0.02	-	-	-	-	0.10	0.02
90	ND (PM 204)	1438		0.09	0.03	-	-	-	-	-	-
91	ND (PM 204)	1447	1458	-	-	-	-	-	-	0.29	0.02
92	(Z)-β-farnesene	1449	1440	0.32	0.02	-	-	-	-	-	-
93	Sesquisabinene	1453	1457	-	-	TRAZA	-	-	-	1.23	0.03
94	α-humulene	1457	1452	4.68	0.07	-	-	-	-	0.38	0.01
95	Linalool isovalerate	1457	1466	-	-	0.20	0.11	-	-	-	-
96	ND (220)	1459	-	-	-	-	-	0.48	0.21	-	-
97	ND (PM 222)	1460	1458	0.11	0.03	-	-	-	-	-	-
98	*allo*-aromadendrene	1463	1458	TRAZA	-	-	-	-	-	0.08	0.03
99	*cis*-cadin 1(6).4-diene	1464	1461			-	-	-	-	TRAZA	-
100	(*E*)-9-epi-cariophyllene	1474	1464	0.34	0.03	-	-	-	-	0.14	0.02
101	γ-muurolene	1480	1478	34.45	0.6	-	-	0.22	0.24	0.36	0.06
102	*trans*-cadina1(6).4-diene	1483	1475	-	-	0.13	0.01	-	-	-	-
103	germacrene D	1485	1480	-	-	-	-	-	-	3.52	0.12
104	*trans*-muurola 4(14).5-diene	1485	1493	-	-	-	-	0.20	0.09	-	-
105	δ-selinene	1487	1492	TRAZA	-	-	-	-	-	-	-
106	ND (PM 204)	1490	-	-	-	-	-	-	-	0.09	0.03
107	β-selinene	1491	1489	1.62	0.2	0.68	0.02	-	-	-	-
108	2-tridecanone	1495	1500	-	-	TRAZA	-	-	-	-	-
109	α-selinene	1499	1498	-	-	2.89	0.09	-	-	-	-
110	Biciclogermacrene	1499	1500	12.04	0.28	-	-	0.31	0.04	5.84	0.45
111	α-murolene	1502	1500	-	-	-	-	0.33	0.02	-	-
112	(*E.E*)-α-farnesene	1502	1505	0.83	0.05	-	-	-	-	2.05	0.03
113	β-bisabolene	1506	1505	0.83	0.05	-	-	-	-	-	-
114	Myristicine	1507	1517	-	-	TRAZA	-	TRAZA	-	TRAZA	-
115	germazrene A	1509	1508	0.55	0.04	-	-	-	-	-	-
116	ND (PM 204)	1512	-	-	-	-	-	-	-	0.15	0.01
117	γ-cadinene	1517	1513	-	-	-	-	-	-	0.13	0.01

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Table 1 – Continued from previous page

N°	Compounds	IRC	IRL	*C.pycnantha*		*M.aliena*		*P. macrotrichum*		*S.schimpffii*	
				%	σ	%	σ	%	σ	%	σ
118	δ-amorfene	1518	1511	2.77	0.04	-	-	-	-	-	-
119	δ-cadinene	1521	1522	-	-	0.12	0.01	TRAZA	-	0.82	0.02
120	chavibetol acetate	1526	-	-	-	-	-	7.81	3.19	-	-
121	*cis*-calamenene	1527	1528	-	-	-	-	-	-	1.54	0.13
122	(*Z*)-carpacine	1531	1540	0.19	0.02	-	-	-	-	-	-
123	*trans*-cadin-1.4-diene	1534	1533	0.13	0.01	-	-	-	-	-	-
124	(*E*)-isocroweacin	1534	1553	-	-	0.15	0.01	0.63	0.03	6.41	1.19
125	ND (PM 222)	1538	-	-	-	-	-	-	-	0.07	0.04
126	α-cadinene	1540	1537	0.06	0.04	-	-	-	-	0.29	0.01
127	(*Z*)-nerolidol	1544	1531	-	-	-	-	-	-	0.37	0.01
128	seline 3.7(11)-diene	1546	1545	-	-	-	-	-	-	0.13	0.01
129	α-calacorene	1548	1544	-	-	-	-	-	-	TRAZA	-
130	Hedicariol	1555	1546	-	-	-	-	-	-	TRAZA	-
131	Elemycin	1558	1555	-	-	-	-	-	-	0.17	0.03
132	ND (PM 220)	1560	-	-	-	-	-	-	-	0.08	0.01
133	(*E*)-nerolidol	1563	1561	-	-	-	-	0.30	0.18	-	-
134	germazrene B	1564	1559	1.35	0.03	-	-	-	-	3.07	0.06
135	isoeugenol (*Z*)-acetate	1565	1566	-	-	-	-	0.12	0.22	-	-
136	ND (PM 218)	1573	1573	-	-	-	-	-	-	1.86	0.79
137	ND (PM 220)	1576	1576	-	-	-	-	-	-	5.57	0.48
138	Spatulenol	1585	1577	0.75	0.06	-	-	-	-	12.10	1.33
139	cariophyllene oxide	1589	1582	0.20	0.03	TRAZA	-	0.10	0.03	-	-
140	ND (PM 220)	1592	-	-	-	-	-	-	-	0.58	0.28
141	Guaiol	1600	1600	0.39	0.03	-	-	-	-	-	-
142	ND (PM 238)	1603	-	0.06	0.01	-	-	0.09	0.01	-	-
143	ND (PM 222)	1603	-	-	-	-	-	-	-	0.12	0.01
144	ND (PM 220)	1608	-	-	-	-	-	-	-	0.06	0.04
145	Ledol	1610	1602	0.14	0.03	-	-	-	-	-	-
146	β-oplopenone	1612	-	-	-	-	-	-	-	TRAZA	-
147	ND (PM 220)	1616	-	-	-	-	-	-	-	0.34	0.2
148	Humulene epoxide II	1616	1607	0.07	0.01	-	-	-	-	-	-
149	Isoeugenol (*E*)-acetate	1619	1614	-	-	-	-	TRAZA	-	-	-
150	ND (PM 220)	1621	-	-	-	-	-	-	-	0.46	0.02
151	dill apiol	1630	1620	0.23	0.03	0.11	0.01	0.20	0.01	3.21	0.05
152	1-*epi*-cubenol	1634	1627	0.15	0.03	TRAZA	-	-	-	-	-
153	ND (PM 222)	1637	-	-	-	-	-	-	-	0.12	0.04
154	epoxide *allo*-aromadendrene	1644	1639	0.12	0.02	-	-	-	-	0.80	0.16
155	ND (PM 220)	1644	-	-	-	-	-	-	-	1.58	0.02
156	ND (PM 220)	1647	-	-	-	0.05	0.01	-	-	-	-
157	β-eudesmol	1649	1649	-	-	-	-	TRAZA	-	-	-
158	Cubenol	1650	1645	-	-	TRAZA	-	-	-	-	-
159	*epi*-α-cadinol	1653	1638	-	-	-	-	-	-	TRAZA	-
160	α-muurolol (torreyol)	1648	1644	0.38	0.12	-	-	-	-	-	-
161	ND (PM 204)	1653	-	0.38	0.12	-	-	-	-	-	-
162	*epi*-α-muurolol	1655	1640	0.38	0.12	-	-	-	-	0.24	0.15
163	(*Z*)-14-*hydroxy*-cariophyllene	1656	1666	0.38	0.12	-	-	-	-	-	-
164	ND (PM 220)	1658	-	-	-	-	-	-	-	0.48	0.15
165	α-cadinol	1659	1652	0.44	0.12	TRAZA	-	0.15	0.01	0.81	0.16
166	neo-intermedeol	1668	1658	0.41	0.11	TRAZA	-	-	-	-	-
167	ND (PM 238)	1673	-	-	-	-	-	0.05	0.01	-	-
168	ND (PM 220)	1673	-	-	-	-	-	-	-	0.26	0.07
169	ND (PM 220)	1678	-	-	-	-	-	-	-	0.09	0.03
170	ND (PM 220)	1681	-	-	-	-	-	-	-	0.07	0.04
171	ND (PM 220)	1689	-	-	-	-	-	-	-	0.75	0.23
172	ND (PM 222)	1698	-	-	-	-	-	-	-	0.08	0.01
173	2-pentadecanone	1704	1697	-	-	0.20	0.01	-	-	-	-
174	ND (PM 220)	1705	-	-	-	-	-	-	-	0.47	0.06
175	ND (PM 220)	1710	-	-	-	-	-	-	-	0.22	0.08
176	tridecenol (2*E*)-acetate	1714	1703	0.45	0.01	-	-	-	-		
177	ND (PM 218)	1719	-	-	-	-	-	-	-	2.13	0.08
178	ND (PM 220)	1725	-	-	-	-	-	-	-	0.09	0.03

Continued on next page

Table 1 – Continued from previous page

N°	Compounds	IRC	IRL	*C.pycnantha*		*M.aliena*		*P. macrotrichum*		*S.schimpffii*	
				%	σ	%	σ	%	σ	%	σ
179	ND (PM 220)	1735	-	-	-	-	-	-	-	0.10	0.01
180	ND (PM 220)	1741	-	-	-	-	-	-	-	0.11	0.02
181	Renal isocyclothermacrene	1747	1733	-	-	-	-	-	-	0.17	0.05
182	ND (PM 220)	1753	-	-	-	-	-	-	-	0.23	0.06
183	ND (PM 220)	1760	-	-	-	-	-	-	-	0.06	0.06
184	ND (PM 220)	1762	-	-	-	-	-	-	-	0.09	0.06
185	ND (PM 220)	1768	-	-	-	-	-	-	-	0.36	0.07
186	15-al γ-curcumene	1777	1766	-	-	-	-	-	-	0.13	0.02
187	14-*hydroxy*-α-murolene	1787	1779	-	-	-	-	-	-	TRAZA	-
188	14-*hydroxy*-δ-cadinene	1810	1803	-	-	-	-	-	-	0.05	0.01
189	ND (PM 222)	1820	-	-	-	-	-	-	-	0.07	0.01
190	ND (PM 262)	1887	-	0.12	0.02	-	-	-	-	-	-
191	ND (PM 268)	1891	-	-	-	-	-	-	-	0.05	0.01
192	(*5Z.9E*)-farnesyl acetone	1912	1889	-	-	-	-	-	-	0.12	0.01
193	methyl hexadecanoate	1927	1921	-	-	-	-	TRAZA	-	-	-
194	Phytol	1942	1942	-	-	-	-	-	-	TRAZA	-
195	ND (PM 268)	1963	-	-	-	-	-	-	-	0.14	0.01
196	(*6E.10Z*)-pseudophytol	2024	2018	-	-	-	-	-	-	TRAZA	-
197	ND (PM 316)	2031	-	-	-	0.06	0.1	-	-	-	-
198	ND (PM 300)	2097	-	0.19	0.04	-	-	-	-	-	-
199	ND (PM 222)	2101	-	-	-	-	-	0.07	0.04	-	-
200	ND (PM 328)	2106	-	0.54	0.02	-	-	-	-	-	-
201	ND (PM 296)	2108	-	-	-	-	-	-	-	0.14	0.01
202	oleic acid	2125	2141	-	-	-	-	-	-	TRAZA	-
203	ND (PM 300)	2188	-	0.06	0.01	-	-	-	-	-	-
	Monoterpene hydrocarbons			4.58		91.19		73.92		3.24	
	Oxygenated monoterpenes			1.01		0.56		12.19		12.29	
	Sesquiterpene hydrocarbons			80.12		6.14		2.72		32.02	
	Oxygenated sesquiterpenes			3.68		0.45		0.76		29.32	
	Others			5.56		0.58		9.12		16.77	
	TOTAL (%)			94.94		98.92		98.71		93.65	

In this study it was shown that the essential oil of *C. pycnantha* is composed mainly of sesquiterpenes, which represent 80.12% of its total composition. Tran and Cramer (2014) describe terpenes in general as compounds with great economic importance when used as aromas, flavors, spices and drugs, and help plant species by attracting pollinators (Xu et al., 2017).

Sesquiterpenes are compounds with antitumor, antimicrobial activity and effects on the central nervous system according to Da Silveira e Sá et al. (2015), who also mention that the major compounds are those that usually establish the biological activity of an essential oil. Among the major compounds was (E)-cariophyllene (11%). Shan et al. (2016) mentioned it as one of the most important sesquiterpenes for possessing anticancer properties, antioxidants, antimicrobials, anti-inflammatory and local anesthetic action. (E)-cariophyllene is also used in the food and cosmetics industry as a flavor (Montanari et al., 2011).

Regarding species of the genus *Myrcia*, Barbosa de Moraes et al. (2022) and De Cerqueira et al. (2007) mention that the essential oils of the leaves of *Myrcia paivae* O.Berg and *Myrcia myrtifolia* DC are oils rich in monoterpenes with 77.0% and 94.1% of the total of its composition, which was observed in the essential oil of the species *Maliena*, where 91.19% of these compounds were determined. In addition, Barbosa de Moraes et al. (2022) and De Cerqueira et al. (2007) describe α-pinene as one of the major compounds with 6.39% in the EO of *Myrcia paivae* O.Berg and 61.5 to 90.9% in essential oils of leaves, flowers and fruits of *Myrcia myrtifolia* DC. In our study α-pinene was found in 72.2% and β-pinene in 15.8%. Both compounds are also described as majority in the EO of the species *Myrcia mollis*, in which α-pinene and β-pinene were found in 29.2% and 31.3% (Montalván et al., 2019).

Monoterpenes protect plant species against insects, herbivores, and mammals and have allelopathic functions by blocking seed germination (Thoss et al., 2007). The monoterpenes α-pinene and β-

pinene are structural isomers commonly found in essential oils. They have antiviral, antifungal, antimicrobial, anticancer, antispasmodic, antimalarial, anti-inflammatory and antioxidant activity (Zielińska-Błajet and Feder-Kubis, 2020). In addition, α-pinene and β-pinene act as bacteriostatic and fungistatic agents (Talebi-Kouyakhi et al., 2008).

Navickiene et al. (2006) mention that the essential oils of leaves, stems and fruits of the species *Piper aduncum, Piper arboreum* and *Piper tuberculatum* share certain common compounds, which are α-pinene, limonene, mircene, (E)-ocimeno, (Z)proved-ocimeno and *piper tuberculatum*. In the essential oil of *Piper macrotrichum* these compounds were also found except for linalool. The main compounds δ-3-carene (58.21%), eugenol (9.75%) and chavibetol acetate (7.81%) were determined, of which eugenol was found to be the majority of the EO of *Piper divaricatum* leaves with 37.5%, and δ-3-carene with 9.6% and 35.3% in the EO of *Piper aff*. Hispidum and *Piper sanctifelicis*, respectively (Jaramillo-Colorado et al., 2019). The δ-3-carene is also one of the main compounds of the EO of Piper nigrum with 14.4%. Arunachalam et al. (2023) describe anticonvulsant activity for eugenol and linalool, while Woo et al. (2019) mentions δ-3-carene as a compound used in perfumery and cosmetics, in addition to having antifungal, anti-inflammatory and sedative activity.

Noriega-Rivera et al. (2014) report as main compounds of essential oil of *Siparuna schimpffii* to germacrene D (35.34%), bicyclogermacrene (8.73%), γ-muurulene (7.04%), germacrene B (6,34%) and cadina-1(2), 4-diene trans (5.16%), of which only bicyclogermacrene (5.8%) were determined as one of the majority in the analyzed essential oil of *S. schimpffii*, together with spatulenol (12.10%), 2-undecanone(10.87%) and (E)-isocroweacin (6.41%). However, it can be observed that the main composition in both essential oils corresponds to sesquiterpene hydrocarbons. As described by Durán et al. (2007); Ruiz et al. (2015); Silva et al. (2021), essential oil composition differences may be due to several biotic and abiotic factors such as the presence or absence of pests, collection place, soil type, amount of moisture and light, as well as the climatic conditions in which the species develop. Spatulenol has moderate antimycobacterial action (Do Nascimento et al., 2018). Silva et al. (2007) mention antimicrobial activity for bicyclogermacrene and fungitoxic activity for germacrene B; Xu et al. (2017) detail some functions of bicyclogermacrene, including its antioxidant, fungistatic, cytotoxic, allelopathic and acetylcholinesterase inhibitor activity. Noriega-Rivera et al. (2014) also state that *S. schimpffii* is used by Shuar communities in Ecuador as an analgesic.

4 Conclusions

It was possible to determine the chemical composition of essential oils of the Amazonian species *C. pycnantha, M. aliena, P. macrotrichum* and *S. schimpffi*. A higher concentration of monoterpenes was observed in the EOs of *M. aliena* and *P. macrotrichum* and a higher content of sesquiterpenes in the EO of *C. pycnantha* and *S. schimpffii*. In addition, the main compounds of each essential oil were identified and some of its functions were described bibliographically, redirecting future studies and applications that Antuash residents can give to these species.

Acknowledgments

We are grateful to the Ministries of Agriculture (MAG), Environment, Water and Ecological Transition (MAATE), and the PROAmazonía program, which together with the Universidad Técnico Particular de Loja made possible the access to samples of the Amazonian species selected for this study, with the aim of providing valuable information for future research and applications that can be provided to essential oils of these species.

References

Adams, R. (2017). *Identification of essential oil components by gas chromatography/mass spectrometry*. Texensis Publishing.

Aguirre-Mendoza, Z., Aguirre-Mendoza, N., and Muñoz, J. (2017). Biodiversidad de la provincia de loja, ecuador. *Arnaldoa*, 24(2):523–542. Online:https://n9.cl/6fhn5.

Barbosa de Moraes, Â. Pereira-Franco, C., Oliveira-Ferreira, O., Pompeu-Valera, E., Do Nascimento, L., Moraes-Cascaes, M., Pereira da Silva, D., Percário, S., Santana de Oliveira, M., and Aguiar-Andrade, E. (2022). Myrcia paivae o. berg (myrtaceae) essential oil, first study of the chemical composition and antioxidant potential. *Molecules*, 27(17):5460. Online:https://n9.cl/b8sece.

Camus, J. and Trujillo, M. (2011). Contribucion a la quimica de los aceites esenciales provenientes del oregano. *Revista Boliviana de Química*, 28(1):1–5. Online:https://n9.cl/ay9ke.

Da Silveira e Sá, R., Andrade, L., and De Sousa, D. (2015). Sesquiterpenes from essential oils and anti-inflammatory activity. *Natural product communications*, 10(10):1767–1774. Online:https://n9.cl/at83u.

De Cerqueira, M., Souza-Neta, L., Passos, M. adn Lima, E., Roque, N., Martins, D., Guedes, M., and Cruz, F. (2007). Seasonal variation and antimicrobial activity of myrcia myrtifolia essential oils. *Journal of the Brazilian Chemical Society*, 18:998–1003. Online:https://n9.cl/rnkxv.

De la Torre, L., Navarrete, H., Muriel, P., Macía, M., and Balslev, H. (2008). *Enciclopedia de las Plantas Útiles del Ecuador*. Pontificia Universidad Católica del Ecuador.

Do Nascimento, K., Moreira, F., Alencar, J., Kassuya, C., Croda, J., Cardoso, C., Vieira, M., Góis, A., Ann, M., de Carvalho, J., and Formagio, A. (2018). Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities of the essential oil of psidium guineense sw. and spathulenol. *Journal of ethnopharmacology*, 210:351–358. Online:https://n9.cl/rju7y.

Durán, D., Monsalve, L., Martínez, J., and Stashenko, E. (2007). Estudio comparativo de la composición química de aceites esenciales de lippia alba provenientes de diferentes regiones de colombia, y efecto del tiempo de destilación sobre la composición del aceite. *Scientia et technica*, 13(33):435–438. Online:https://n9.cl/eawn4.

Ferreira-Silva, I., Oliveira, R., and Oliveira, F. (2021). Siparuna aublet genus (siparunaceae): from folk medicine to chemical composition and biological activity. *Trends in Phytochemical Research*, 5(4):168–189. Online:https://n9.cl/1ycn2.

Jaramillo-Colorado, B., Pino-Benitez, N., and González-Coloma, A. (2019). Volatile composition and biocidal (antifeedant and phytotoxic) activity of the essential oils of four piperaceae species from choco-colombia. *Industrial Crops and Products*, 138:111463. Online:https://n9.cl/k4jfs.

León-Méndez, G., Osorio-Fortich, M., and Martínez-Useche, S. (2015). Comparación de dos métodos de extracción del aceite esencial de c itrus sinensis l. *Revista Cubana de Farmacia*, 49(4):742–750. Online:https://n9.cl/gw4c1.

Missouri Botanical Garden (2022). Tropicos. Missouri Botanical Garden0. Online:https://n9.cl/8ghbb.

Montalván, M., Peñafiel, M., Ramírez, J., Cumbicus, N., Bec, N., Larroque, C., Bicchi, C., and Gilardoni, G. (2019). Chemical composition, enantiomeric distribution, and sensory evaluation of the essential oils distilled from the ecuadorian species myrcianthes myrsinoides (kunth) grifo and myrcia mollis (kunth) dc.(myrtaceae). *Plants*, 8(11):511. Online:https://n9.cl/gi23y.

Montanari, R., Barbosa, L., Demuner, A., Silva, C., Carvalho, L., and Andrade, N. (2011). Chemical composition and antibacterial activity of essential oils from verbenaceae species: Alternative sources of (e)-caryophyllene and germacrene-d. *Química Nova*, 34(9):1550–1555. Online:https://n9.cl/hmpe3.

Navickiene, H., Morandim, A., Alécio, A., Regasini, L., Bergamo, D., Telascrea, M., Cavalheiro, A., Lopes, M., Bolzani, V., Furlan, M., Marques, M., Young, M., and Kato, M. (2006). Composition and antifungal activity of essential oils from piper aduncum, piper arboreum and piper tuberculatum. *Química Nova*, 29:467–470. Online:https://n9.cl/97key.

Noriega-Rivera, P. (2009). Extracción, química, actividad biológica, control de calidad y potencial económico de los aceites esenciales. *LA GRANJA. Revista de Ciencias de la Vida*, 10(2):3–15. Online:https://n9.cl/h87yx.

Noriega-Rivera, P., Guerrini, A., and Ankuash-Tsamaraint, E. (2014). Composición química del aceite esencial de hojas de siparuna schimpffii diels (limoncillo). *Revista Cubana de Plantas Medicinales*, 19(2):128–137. Online:https://n9.cl/ehm63.

Ochoa-Pumaylle, K., Paredes-Quiroz, L., Bejarano-Luján, D., and Silva-Paz, R. (2012). Extraction, characterization and evaluation of antibacterial activity of essential oil of senecio graveolens wedd (wiskataya). *Scientia agropecuaria*, 3(4):291–302. Online:https://n9.cl/vl1bg.

Ruiz, C., Díaz, C., and Rojas, R. (2015). Composición química de aceites esenciales de 10 plantas aromáticas peruanas. *Revista de la Sociedad Química del Perú*, 81(2):81–94. Online:https://n9.cl/n5wj4.

Shan, J., Chen, L., and Lu, K. (2016). Protective effects of trans-caryophyllene on maintaining osteoblast function. *IUBMB life*, 69(1):22–29. Online:https://n9.cl/469kt.

Silva, L. d., Oniki, G., Agripino, D., Moreno, P., Young, M., Mayworm, M., and Ladeira, A. (2007). Biciclogermacreno, resveratrol e atividade antifúngica em extratos de folhas de cissus verticillata (l.) nicolson & jarvis (vitaceae). *Revista Brasileira de Farmacognosia*, 17:361–367. Online:https://n9.cl/v46dx.

Silva, R., Costa, J. d., Figueiredo, R. d., Setzer, W., Silva, J., Maia, J., and Figueiredo, P. (2021). Monoterpenes and sesquiterpenes of essential oils from psidium species and their biological properties. *Molecules*, 26(4):965. Online:https://n9.cl/q0tk3.

Talebi-Kouyakhi, E., Naghavi, M., and Alayhs, M. (2008). Study of the essential oil variation of ferula gummosa samples from iran. *Chemistry of Natural Compounds*, 44:124–126. Online:https://n9.cl/zbfko.

Thoss, V., OâReilly-Wapstra, J., and Iason, G. (2007). Assessment and implications of intraspecific and phenological variability in monoterpenes of scots pine (pinus sylvestris) foliage. *Journal of Chemical Ecology*, 33:477–491. Online:https://n9.cl/wspe8.

Tissot, E., Rochat, S., Debonneville, C., and Chaintreau, A. (2012). Rapid gc-fid quantification technique without authentic samples using predicted response factors. *Flavour and fragrance journal*, 27(4):290–296. Online:https://n9.cl/i0xyru.

Tran, D. and Cramer, N. (2014). Biomimetic synthesis of (+)-ledene,(+)-viridiflorol,(-)-palustrol,(+)-spathulenol, and psiguadial a, c, and d via the platform terpene (+)-bicyclogermacrene. *Chemistry- A European Journal*, 20(34):10654–10660. Online:https://n9.cl/zru25n.

Van Den Dool, H. and Dec Kratz, P. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of chromatography*, 11:463â471. Online:https://n9.cl/ohxwg.

Woo, J., Yang, H., Yoon, M., Gadhe, C., Pae, A., Cho, S., and Lee, C. (2019). 3-carene, a phytoncide from pine tree has a sleep-enhancing effect by targeting the gabaa-benzodiazepine receptors. *Experimental neurobiology*, 28(5):593–601. Online:https://n9.cl/ijwxu.

Xu, Y., Wu, B., Cao, X., Zhang, B., and Chen, K. (2017). Citrus cmtps1 is associated with formation of sesquiterpene bicyclogermacrene. *Scientia Horticulturae*, 226:133–140. Online:https://n9.cl/art13.

Zielińska-Błajet, M. and Feder-Kubis, J. (2020). Monoterpenes and their derivativesârecent development in biological and medical applications. *International Journal of Molecular Sciences*, 21(19):7078. Online:https://n9.cl/pr456k.

La Granja: Revista de Ciencias de la Vida
pISSN:1390-3799; eISSN:1390-8596
http://doi.org/10.17163/lgr.n38.2023.04

Special Issue/ Número Especial

Food Science

Chlorophyll content in leaves of highland potatoes for estimating tubers quality

Contenido de clorofila en hojas de papas de altura para estimar la calidad de los tubérculos

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Article received on May 05th, 2022. Accepted, after review, on July 24th, 2023. Published on September 1st, 2023.

Abstract

In this study, we assessed the relationship between tubers quality of three varieties (INIAP Libertad, INIAP Josefina and Diacol Capiro) of Ecuador highland early of potato and two formulations of edaphic fertilizer sources. Leaf chlorophyll content (LCC) was evaluated with Chlorophyll Meter SPAD-502Plus. Samples were taken at different heights in three phenological stages: vegetative grow, flowering–formation of tubers and ripening-thickening. Several responses were found in the three potato varieties. Correlation between SPAD value and weight of tubers (W), dry matter (DM), specific gravity (SG) and good chips (GC) were significantly correlated with potato leaves chlorophyll content. For Libertad variety and formulation (KNO_3 + $NH_4H_2PO_4$ + KCl), at vegetative grow in upper strata of plant, the optimal mathematic function for SPAD value and W, DM, SG and GC were: $y = 0.262x - 9.460$ ($R^2 = 0.9938$), $y = 42.948\ e^{-0,01x}$ ($R^2 = 0.5240$), $y = 10^{0,13}x^{-0,05}$ ($R^2 = 0.3277$) and $y = 10^{-0,36}x^{1,41}$ ($R^2 = 0.8681$); at plant flowering-formation of tuber stage, the optimal function models were: $y = 10^{-1,57}\ x^{1,06}$ ($R^2 = 0.8553$), $y = 28.789\ e^{-0,0024}$ ($R^2 = 0.9103$), $y = 10^{0,07}\ x^{-0,02}$ ($R^2 = 0.7543$) and $y = 0.468x + 64.361$ ($R^2 = 0.9935$); at plant ripening-thickening, the optimal function models were: $y = 0.664\ e^{0,02x}$ ($R^2 = 0.7924$), $y = 29.370\ e^{-0,003x}$ ($R^2 = 0.9572$), $y = 10^{0,07}\ x^{-0,02}$ ($R^2 = 0.8247$) and $y = 0.576x + 62.675$ ($R^2 = 0.9690$), respectively. Our results showed that the use of SPAD-520PLUS proved to be a rapid method for the determination of LCC, being an effective tool for estimating potato tuber quality.

Keywords: Correlation analysis, Ecuador highlands, photosynthesis, *Solanum tuberosum*, SPAD-502 plus®.

Resumen

En este estudio se evaluó la relación de la calidad de tubérculos de tres variedades de papa (INIAP Libertad, INIAP Josefina y Diacol Capiro) del Altiplano ecuatoriano y dos formulaciones de fertilizante de fuentes edáficas. El contenido de clorofila foliar (CCF) se evaluó con el medidor de clorofila SPAD-502Plus. Las muestras se tomaron a diferentes alturas en tres etapas fenológicas: crecimiento vegetativo, floración-formación de tubérculos y maduración-espesamiento. Se encontraron varias respuestas en las tres variedades de papa. El valor de *SPAD* y peso de tubérculos (*W*), materia seca (*MS*), densidad específica (*GS*) y buenas papas (*GC*) se correlacionaron significativamente con el contenido de clorofila en las hojas de papa. Para la variedad y formulación Libertad (KNO_3 + $NH_4H_2PO_4$ + KCl), en el crecimiento vegetativo en estratos superiores de la planta, la función matemática óptima para SPAD y W, DM, SG y GC fueron: $y = 0,262x - 9,460$ ($R^2 = 0,9938$), $y = 42,948e^{-0,01x}$ ($R^2 = 0,5240$), $y = 10^{0,13}x^{-0,05}$ ($R^2 = 0,3277$) e $y = 10^{-0,36}x^{1,41}$ ($R^2 = 0,8681$); en la etapa de floración-formación de la planta de tubérculo los modelos de función óptima fueron: $y = 10^{-1,57}x^{1,06}$ ($R^2 = 0,8553$), $y = 28,789e^{-0,0024x}$ ($R^2 = 0,9103$), $y = 10^{0,07}x^{-0,02}$ ($R^2 = 0,7543$) e $y = 0,468x + 64,361$ ($R^2 = 0,9935$); en la maduración-espesamiento de las plantas, los modelos de función óptima fueron: $y = 0,664e^{0,02x}$ ($R^2 = 0,7924$), $y = 29,370e^{-0,003x}$ ($R^2 = 0,9572$),$y = 10^{0,07}x^{-0,02}$ ($R^2 = 0,8247$) $y = 0,576x + 62,675$ ($R^2 = 0,9690$), respectivamente. Los resultados mostraron que el uso de SPAD-520PLUS demostró ser un método rápido para determinar CCF como una herramienta efectiva para estimar la calidad del tubérculo de papa.

Palabras clave: Análisis de correlación, tierras altas del Ecuador, fotosíntesis, *Solanum tuberosum*, SPAD-502 plus®.

Suggested citation: Yánez-Segovia, S., Silvestre, L. and Chamorro- Warnken, I. (2023). Chlorophyll content in leaves of highland potatoes for estimating tubers quality. La Granja: Revista de Ciencias de la Vida. Vol. 38(2):45-57. http://doi.org/10.17163/lgr.n38.2023.04.

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1 Introduction

Chlorophyll is an essential pigment for photosynthesis. Consequently, chlorophyll content is the main index reflecting the photosynthetic capacity and health status of the plant (Chou et al., 2020). The common way to measure leaf chlorophyll content (LCC) usually need to extract leaf tissue with organic solvents such as acetone, ethanol or *N, N*-dimethyl formamide (Lan et al., 2011). Although this method is relatively accurate, extraction is laborious, destructive, time-consuming, and expensive. During this process, significant pigment losses may occur through the extraction and dilution and lead to highly variable results (Kaspary et al., 2019). LCC measurements, such as those performed with the SPAD-502 plus® (Konica Minolta, Tokyo, Japan) are a non-destructive, simple and portable diagnostic tool that measures the greenness or the relative chlorophyll content of leaves (Padilla et al., 2019).

By measuring the leaf transmittance in two wavelenght bands (400-500 nm and 600-700 nm), this device quantifies the relative amount of chlorophyll with a reading in arbitrary unit (SPAD-502 plus® Chlorophyll Index) that is proportional to the leaf chlorophyll concentration (Sim et al., 2015), resulting in substantial savings in time and resources. High correlations between SPAD-502 plus® value and leaf chlorophyll content have been shown for several species of rice (Yuan et al., 2016), soybean (Kühling et al., 2018), wheat (Yue et al., 2019), muskmelon (Azia and Stewart, 2001), maize (Casa et al., 2015), coffee (Netto et al., 2005) and tomato (Padilla et al., 2018). While the correlation in potato relationship was comparatively weak (Uddling et al., 2007). However some research also presented mathematical relationships between SPAD-502 plus® readings and leaf chlorophyll readings with plant growth stage (Yuan et al., 2016; Roslan et al., 2019), growing conditions (Giletto and Echeverría, 2013; Kühling et al., 2018) and genotype (Noulas et al., 2018).

Potato (*Solanum tuberosum* L.) is an important food crop for human nutrition together with wheat and rice (De Jong, 2016). Potato have better yield under cool conditions, but elevated soils reduce yields (Zommick et al., 2014). Since the 1950s, chipping potatoes have been selected for high dry matter content and for their ability to produce light-colored chips (Lulai and Orr, 1979). Tuber dry matter content (DM), which consists primarily of starch, also decreases when potato is grown at highest than upper optimal (Raymundo et al., 2018). High tuber dry matter content is beneficial because it reduces oil absorption during frying and increases of good chips (Camps and Camps, 2019). Nissen (1955) analyzed the data collected for approximately 18 years and concluded that the DM content of the potatoes is a linear function of their weight in water and not depending on the specific gravity (SG) of the tuber. Reducing sugars, glucose and fructose accumulate after harvest as a result of sucrose hydrolysis by vacuolar invertase acid, this sugars react with amino groups in a non-enzymatic Maillard reaction to produce dark-colored pigments during chip frying (Wiberley-Bradford and Bethke, 2018). Low quality tubers produce bad chips that can be rejected at processing plants, representing a financial risk for producers and may lead to supply problems (Busse et al., 2019).

Mathematical correlations between SPAD value and yield and tubers quality can be important to optimize advanced interpretations of data from the chlorophyll meter. This study was carried out to determine if there was a correlation of LCC (SPAD value) of three of early potato varieties with different sources fertilizer. The information was used to build a mathematical function to describe relationship between LCC of different plant stages and tubers quality in order to optimize a model to provide more precise, reliable and easier method for estimation of tubers quality in the industrial process.

2 Material and Methods

2.1 Plant Material and Growth Conditions

Improved commercial varieties of potatoes (V) including INIAP Libertad (v1) (crosses 380479.15 × Bk Precoz-84), INIAP Josefina (v2) (cross between the Bolona variety with a hybrid between *S. phureja* and *S. pausissectum*) and Diacol Capiro (v3) (crosses with Tuquerreña (CCC 61) × 1967 (C) (9) (CCC751), from the germplasm collection of the International Potato Center – Quito, were used for the study. Two formulations of edaphic fertilizers (F) were used. The composition of the formulations was as follows: (f1: 23-24-45) and (f2: 20-31-40) with the sources: KNO_3 + $NH_4H_2PO_4$(MAP) + KCl(MOP)

and NH_4NO_3 + MAP + K_2SO_4 (SOP) respectively. The first fertilization was 25 days after planting (DAP) at a dose of 90 kg ha^{-1} of N, 198 kg ha^{-1} of P and 180 kg ha^{-1} of K. The second fertilization was 60 DAP at a dose of 130 kg ha^{-1} of N, 20 kg ha^{-1} of P and 250 kg ha^{-1} of K.

In March of 2018, the experimental plots were located in Pujilí, Cotopaxi, central highlands of Ecuador at 3,060 m.a.s.l. (01° 03′ 0.7″ South / 78° 41′ 29.8″ West). The soil was silty sandy – Inceptisol, with 2% slope and surface irrigation was weekly by furrows. During the experiment, temperature was 16±1.2°C, 6 h $light^{-1}$ day^{-1} average annual. Mean relative humidity was maintained above 60%. The pH of the soil solution and the electrical conductivity were monitored periodically and maintained at approximately 6.5 and 2.0-3.0 dS m^{-1}, respectively. Six treatments were evaluated, resulting from the interaction of the study factors (varieties and formulations). Three repetitions were performed. In each block, the six treatments (F × V) were distributed randomly. No pesticides were applied during the experiment. Experiment was implemented in 900 m^2. Each net plot was 41.25 m^2. In each net plot, six rows of 1.1 m were made between them and 30 cm between plants. The rows were planted east-west. Plants randomly selected of the four central furrows of each net plot were evaluated to avoid the edge effect of the treatments. The plants were in complete competition, located inside each plot and completely surrounded by other plants.

2.2 SPAD-502 plusSPAD Value Measurement

After selection, the plants were evaluated for chlorophyll content with a SPAD-502 plus® KONICA MINOLTA, in consecutive and non-destructive readings between 10 and 12 AM. Before measurement, the SPAD-502 plus® was calibrated using the reading checker supplied by the manufacturer. Readings were performed in completely irradiated leaflets at 30, 60 and 90 DAP in the lower, middle and upper third part of the plant. Each leaf SPAD value obtained was the average of thirty readings in mature terminal leaflets (Matsuda and Fujiwara, 2014).

2.3 Tubers Yield and Quality Parameters

At harvest, 120 DAP, a classification by size and/or categories of the tuber were made according to their diameter (Huaraca et al., 2009). This variable was expressed in kg total tubers plant−1, with their respective categories (first, second and third). A Mettler Toledo (SB 8001) balance of 0.1 g precision was used.

Quality parameters analyzes were performed in the frying laboratories of the National Roots and Tubers Program (NRTP) and in the Department of Nutrition and Food Quality of National Institute of Agricultural Research (INIAP) located at the Santa Catalina Experimental Station, Quito, Ecuador. All quality variables were evaluated at 20 and 40 days after harvest (DAH). The percentage of Dry Matter (DM) and Specific Gravity (SG) in tubers was determined in the laboratory. The calibration and lecture followed the protocol established by the user manual of the PW-2050 weighing system equipment (Weltech International Limited, Cambridgeshire, U.K.). Five tubers per treatment were used for reducing sugars (RS). The Clerget Method (micro colorimetric method with hydrochloric acid inversion) and protocols manual for the genetic improvement of potatoes (Cuesta et al., 2015) was followed. The results were expressed in mg 100 g^{-1}. A sample of five tubers was selected and washed, peeled and cut potato. The best 100 flakes were selected and fried (ECOSERV 25 lb. min^{-1} industrial potato peeler, 10 Lb ROBOT COUPLE CL 50 cutters, and 11-liter CROYDON electric fryer) at a temperature of 175°C ± 5°C for 2.5 minutes. After frying, the chips were classified into three categories: good, regular and bad (Cuesta et al., 2015).

2.4 Statistical Analysis

Correlation analysis and regression analysis was done with Excel 2019 (v19.0) (Microsoft, USA) and InfoStat version 2016 software and Analysis of Variance (ANOVA) test at level of significance $\alpha = 0.05$. When differences in interaction were found, depending on the number of treatments, the Tukey's test was performed. Correlation tests of Pearson and regressions analysis were performed between: weight of tubers, dry matter, specific gravity, reducing sugars, good chips, and the chlorophyll content of the leaves. In order to determine the trend and establishing the best model: linear, logarithmic, power and

index, in the significant cases.

3 Results

3.1 SPAD value

The relationships between crop phenological age and LCC (SPAD-502 plus®) are shown in Table 1. With increase of phenological age, the SPAD values showed a trend of quadratic increase. SPAD value was constant and incremental in leaves of the three strata evaluated until sixty DAP, independent of variety and edaphic formulation. There was a highly significant effect ($p < 0{,}01$) in the edaphic fertilizer formulations with LCC at 30 DAP in the lower strata of the plant and significant effect ($p < 0{,}05$) at 90 DAP in the middle strata; that is, during the stages of vegetative development and thickening of the tuber. Highly significant differences were observed between potato varieties and LCC at 30 and 60 DAP in all strata; and at 90 DAP, only in the middle strata of plants. Significant differences in the LCC were found in the interaction (FxV) in leaves at 60 DAP in the middle stratum of the plants (Table 1). Tukey test $\alpha = 0{,}05$ presented three ranges of statistical significance. The f1v1 treatment maintained the highest values; while the f1v2 treatment had the lowest chlorophyll content means.

Table 1. SPAD value and descriptive statistics of the factors of study in the crop phenology.

Factors	LEAF CHLOROPHYLL CONTENT (SPAD)															
	30 DAP				60 DAP						90 DAP					
	lower		upper		lower		midlle		upper		lower		midlle		upper	
Formulation (F)	$p < 0{,}001$		$p = 0{,}027$		$p = 0{,}453$		$p = 0{,}168$		$p = 0{,}497$		$p = 0{,}187$		$p = 0{,}009$		$p = 0{,}349$	
f1	42.31 ± 2.83	b	44.5 ± 2.29	b	49.83 ± 3.18	a	54.69 ± 3.67	a	52.72 ± 5.16	a	42.82 ± 2.24	a	43.75 ± 2.80	b	46.00 ± 4.97	a
f2	44.52 ± 2.52	a	45.93 ± 3.04	a	50.77 ± 2.62	a	53.48 ± 1.83	a	52.06 ± 3.19	a	44.49 ± 2.62	a	46.39 ± 2.77	a	47.56 ± 2.90	a
Variety (V)	$p < 0{,}001$		$p < 0{,}001$		$p = 0{,}041$		$p < 0{,}01$		$p0{,}001$		$p = 0{,}073$		$p < 0{,}01$		$p = 0{,}031$	
v1	41.02 ± 1.45	c	44.12 ± 1.07	b	48.55 ± 2.22	b	55.13 ± 2.79	a	55.48 ± 4.12	a	45.83 ± 1.85	a	48.11 ± 2.22	a	50.01 ± 3.25	a
v2	42.62 ± 1.81	b	42.86 ± 0.87	b	49.59 ± 2.18	ab	51.36 ± 2.27	b	53.85 ± 2.00	b	42.73 ± 2.80	a	43.27 ± 2.18	b	43.89 ± 3.77	b
v3	46.61 ± 1.43	a	48.67 ± 1.62	a	52.77 ± 2.18	a	55.77 ± 1.43	a	47.85 ± 1.51	a	42.41 ± 1.23	a	43.82 ± 2.18	b	46.44 ± 2.82	ab
Interaction (FxV)	$p = 0{,}387$		$p = 0{,}396$		$p = 0{,}224$		$p = 0{,}0104$		$p = 0{,}063$		$p = 0{,}971$		$p = 0{,}8038$		$p = 0{,}195$	
f1v1	40.08 ± 0.74	d	43.75 ± 0.72	c	49.39 ± 1.96	a	57.67 ± 0.56	a	57.57 ± 4.79	a	44.97 ± 1.63	a	46.51 ± 2.10	ab	49.75 ± 3.85	a
f1v2	41.08 ± 1.05	d	42.35 ± 0.15	c	47.69 ± 3.09	a	50.06 ± 2.06	c	47.50 ± 1.82	a	41.73 ± 2.23	a	42.33 ± 2.48	b	40.99 ± 2.16	a
f1v3	45.77 ± 1.32	ab	47.41 ± 1.26	ab	52.42 ± 2.35	a	56.33 ± 1.67	ab	53.08 ± 1.66	a	43.06 ± 0.71	a	42.39 ± 1.22	b	47.25 ± 3.74	a
f2v1	41.95 ± 1.39	cd	44.49 ± 1.22	bc	47.71 ± 2.18	a	52.58 ± 1.50	bc	53.38 ± 1.48	a	46.69 ± 1.64	a	49.71 ± 0.64	a	50.26 ± 2.48	a
f2v2	44.15 ± 0.88	bc	43.37 ± 0.98	c	51.49 ± 0.52	a	52.68 ± 1.65	bc	48.19 ± 2.11	a	43.73 ± 2.95	a	44.21 ± 1.25	b	46.79 ± 2.64	a
f2v3	47.46 ± 0.96	a	49.94 ± 0.70	a	53.11 ± 0.58	a	55.2 ± 0.80	ab	54.62 ± 0.77	a	43.06 ± 1.30	a	45.25 ± 1.99	ab	45.64 ± 0.72	a
MEAN	43.42 ± 2.83		45.22 ± 2.78		50.3 ± 2.95		54.08 ± 2.96		52.39 ± 4.30		43.66 ± 2.58		45.07 ± 3.08		46.78 ± 4.15	
CV	2.07		2.59		5.06		3.19		3.76		5.72		3.92		7.20	

Fisher´s Test for factors and Tukey for interaction at $\alpha = 0.05$
Means with different letters are statistically different (p-value)
Coefficient of variation (*CV*)
Standard error (±)

3.2 Yield and after-harvest tubers quality

Regarding tubers yield, the weight for category presented the following mean values. f1: 1.54 and f2: 1.84 kg plant^{-1}. The formulations f1:1.05 of first, 0.62 of second and 0.17 kg plant^{-1} of third category; while f2 fertilizers obtained yields per category of 1.01 of first, 0.38 of second and 0.15 kg plant^{-1} of third. The difference in second category tubers is reflected in statistical significant values (Fig. 1A). INIAP Libertad presented the best response with 2.18, followed by INIAP Josefina with 1.51, and DIACOL Capiro with 1.39 kg plant^{-1} (Fig. 1B). The statistical differences found between varieties occur mainly in the yield expressed in the weight of tubers of the first and third category, according to orthogonal comparisons. The greatest relevance is provided by the first category tubers. No significance was observed for interactions FxV.

Overall, there is a trend in DM and SG increase with storage for all treatments. However, there was no statistical significant difference. The content of RS and chips good for industrial processing of tubers decreases from 20 to 40 DAH ($p < 0{,}01$). No significance was found in fertilizer formulations for the percentage of DM at 20 and 40 DAH. The effects of potato varieties in DM are significant at 20 DAH ($p < 0{,}01$) and 40 DAH ($p < 0{,}05$) (Fig. 2A). INIAP Libertad presented the highest percentages of DM with a mean of 24.45%. Capiro and INIAP Josefina are statistically similar, with a DM content of 22.29 and 21.82%, respectively (Fig. 2A). The Tukey test for interaction ($\alpha = 0{,}05$), presented three

ranges of significance. The f1v1 treatment had the highest DM content, with a mean of 25.17%. The f1v2 treatment presented the lowest DM contents, with a mean of 21.50%.

There was no significant difference of the formulations in SG. Significant effects were observed between potato varieties and SG in the values at 20 and 40 DAH. INIAP Libertad presented the highest specific gravity value (1.101) followed by Capiro (1.091) and INIAP Josefina (1.088), in the same range of significance (Fig. 2B). Significant differences were found for the interaction at 20 DAH, as showed for the variable DM. According to the Tukey test $\alpha = 0{,}05$ for treatments, two ranges of significance were observed. The f1v1 treatment showed the highest SG, with a mean of 1.104; while the f1v2 treatment, the lowest values, with 1.086. No significance was observed for formulations, varieties and interaction in RS content.

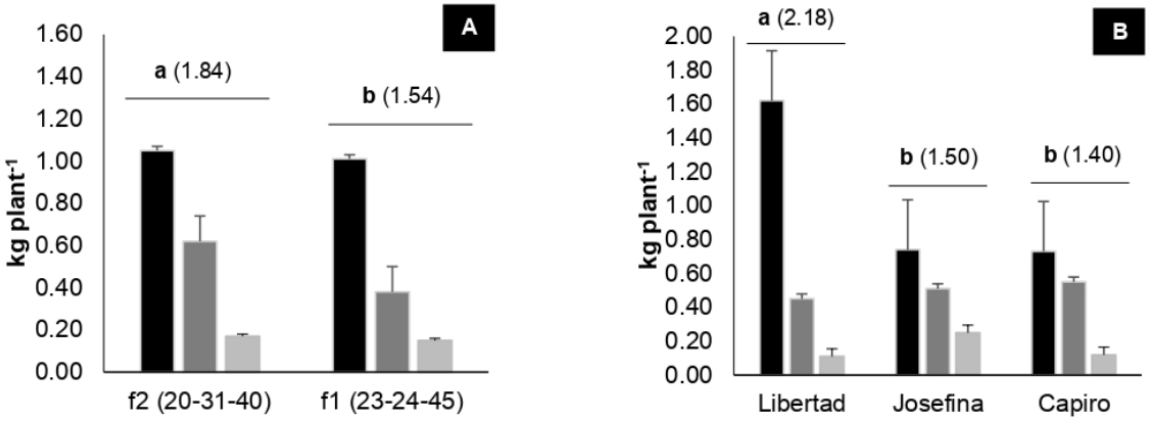

Figure 1. Effect of the fertilizer formulations and varieties on tuber yields.
A) Test: LSD Fisher $\alpha = 0.05$ DMS = 0.19. B) Test: LSD Fisher$\alpha = 0.05$ DMS = 0.24.
Means with different letters are statistically different ($p < 0.05$). Values in parentheses are totals. Black bars: first, gray: second and clearer: third category.

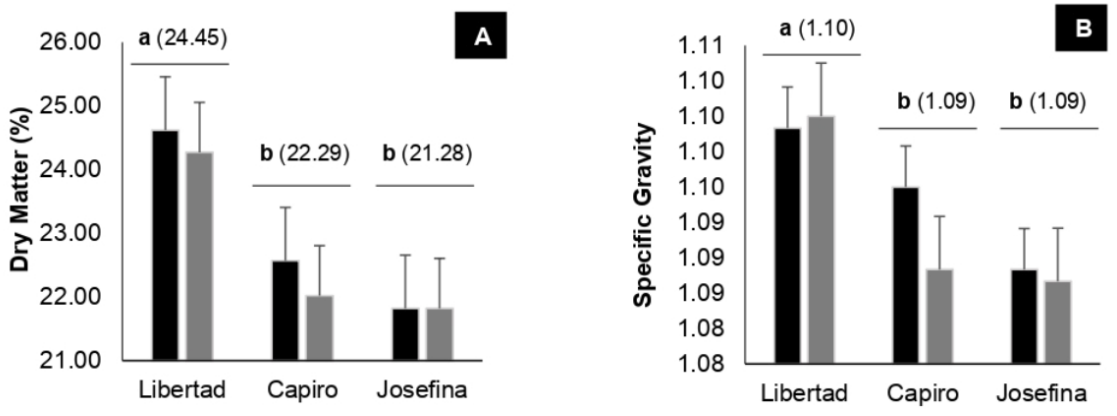

Figure 2. Dry matter and Specific gravity for effect of potatoes varieties.
A) Test: LSD Fisher $\alpha = 0.05$ DMS = 3.93. B) Test: LSD Fisher $\alpha = 0.05$ DMS = 0.06.
Means with different letters are statistically different ($p < 0.05$). Values in parentheses are totals. Black bars: 20 days after harvest; gray: 40 days after harvest.

For the variable lost by frying, there is no significance for fertilizers. There were highly significant differences for varieties in the percentage of good and regular chips, and significant differences for the percentage of bad chips. The Capiro and INIAP Libertad varieties obtained the lowest percentages of frying losses with 4.4 and 4.6% respectively, leaving a total of approximated 95.5% of usable chips. INIAP Josefina presented the greatest losses due to frying, with 19.4% of bad chips. There were significant effects for the interaction in the percentages of good and regular chips. Tukey test $\alpha = 0{,}05$, showed that the f1v1 treatment maintained the highest values and the f1v2 treatment presented the lowest percentages.

3.3 Correlation and regression analysis of SPAD value with yield and tubers quality

Dependent effects were observed among other variables at upper strata of plant (Fig. 3-4). Correlation analyses of yield (kg plant^{-1}) were highest ($p < 0{,}01$) at 90 DAP, independent of plant height.

For INIAP Libertad variety and formulation (KNO_3 + $NH_4H_2PO_4$ + KCl), at different stages of crop, in upper strata of plant, different mathematic modeling functions, correlations between LCC (SPAD value), as x, weight of tubers (kg plant^{-1}); DM; SG; GC (data 20 DAH), as y, were significantly different and correlations changed with different adjustment for model (Tab. 2). For W (kg plant^{-1}), the highest correlation occurred in different model function. At 30 DAP linear model function, with y = 0.262x – 9.460 ($R^2 = 0.9938$); at 60 DAP power model function, with $y = 10^{-1,57}\ x^{1,06}$ ($R^2 = 0.8553$) and 90 DAP index model function, with $y = 0.664\ e^{0,02}x$ ($R^2 = 0.7924$).

For DM, the highest correlation occurred in the third stages in index model function. At 30 DAP with $y = 42.948\ e^{-0,01x}$ ($R^2 = 0.5240$); $y = 28.789\ e^{-0,0024}$ ($R^2 = 0.9103$) at 60 DAP and $y = 29.370\ e^{-0,003x}$ ($R^2 = 0.9572$) at 90 DAP. For SG, the highest correlation occurred in the third stages in power model function. At 30 DAP with $y = 10^{0,13}\ x^{-0,05}$ ($R^2 = 0.3277$); $y = 10^{0,07}$ x-0.02 ($R^2 = 0.7543$) at 60 DAP and $y = 10^{0,07}\ x^{-0,02}$ ($R^2 = 0.8247$) at 90 DAP. For GC, the highest correlation occurred in different model function. At 30 DAP power model function, with $y = 10^{-0,36}\ x^{1,41}$ ($R^2 = 0.8681$). For 60 and 90 DAP the highest correlation occurred in linear model function, with $y = 0.468x + 64.361$ ($R^2 = 0.9935$) and $y = 0.576x + 62.675$ ($R^2 = 0.9690$) respectively.

4 Discussion

It is important to study the correlation between LCC and canopy spectra, which could reflect the characteristics of crop groups and the comprehensive information of canopy spectra (Guo et al., 2018). In respect to potatoes plant architecture, upper parts of the canopy constitute about 50% of the aerial biomass and together with different growth stages, would determine the best chlorophyll content at the top of the canopy (Clevers et al., 2017). On the other hand, chlorophyll is sensitive to high temperatures (chlorophyll "a" more than chlorophyll "b"). High temperatures disintegrate the cell structure, leaving the pigment exposed to various enzymatic and non-enzymatic reactions. Reports indicate that the optimum temperature for chlorophyllase activity (the enzyme that catalyzes chlorophyll degradation) ranges from 60 to 82.2°C (Todorov et al., 2003). Qiqige et al. (2017) and (Kamrani et al., 2019) have determined a positive relationship of LCC with DM tubers and plant yield under different levels of influence. In addition, we demonstrate that in some early varieties, there is a correlation with the SG and good chips.

Regression analysis (showed in Table 2) presented different optimal mathematic function model of correlations between SPAD value with yield and tubers quality based on coefficient value of R^2. Previous research usually reported a single mathematic regression. The linear model is mostly used to perform regression analyze relationships between SPAD values and yield (Netto et al., 2005; León et al., 2007; Hawkins et al., 2009). Uddling et al. (2007), determined that relationships in potato were non-linear with an increasing slope with higher SPAD unites. The relationships of potato was comparatively weak ($R^2 = 0.5$).

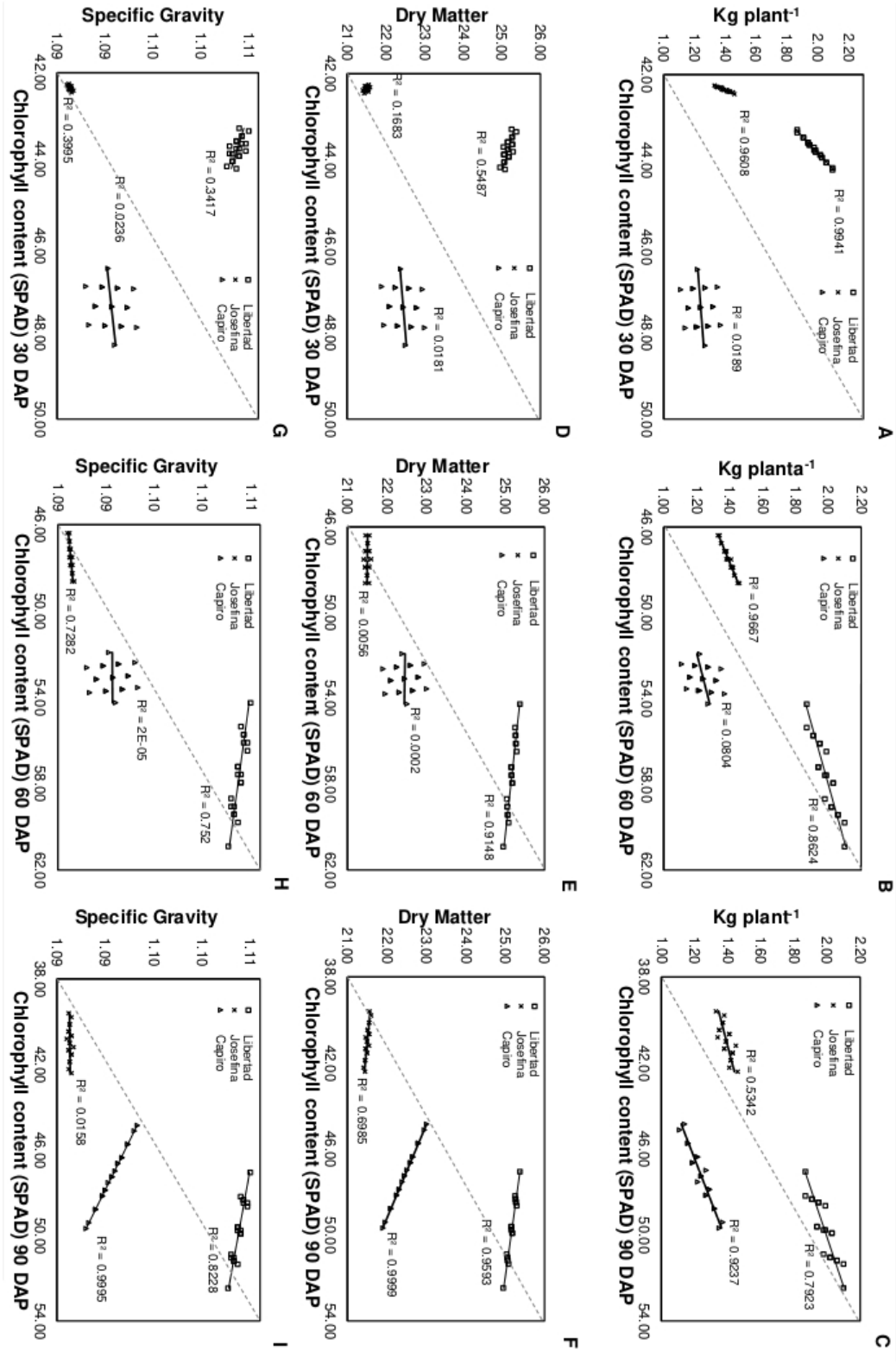

Figure 3. Correlation coefficient of the SPAD value with kg plant^{-1} (A, B, C), Dry matters (D, E, F) and Specific gravity (G, H, I).

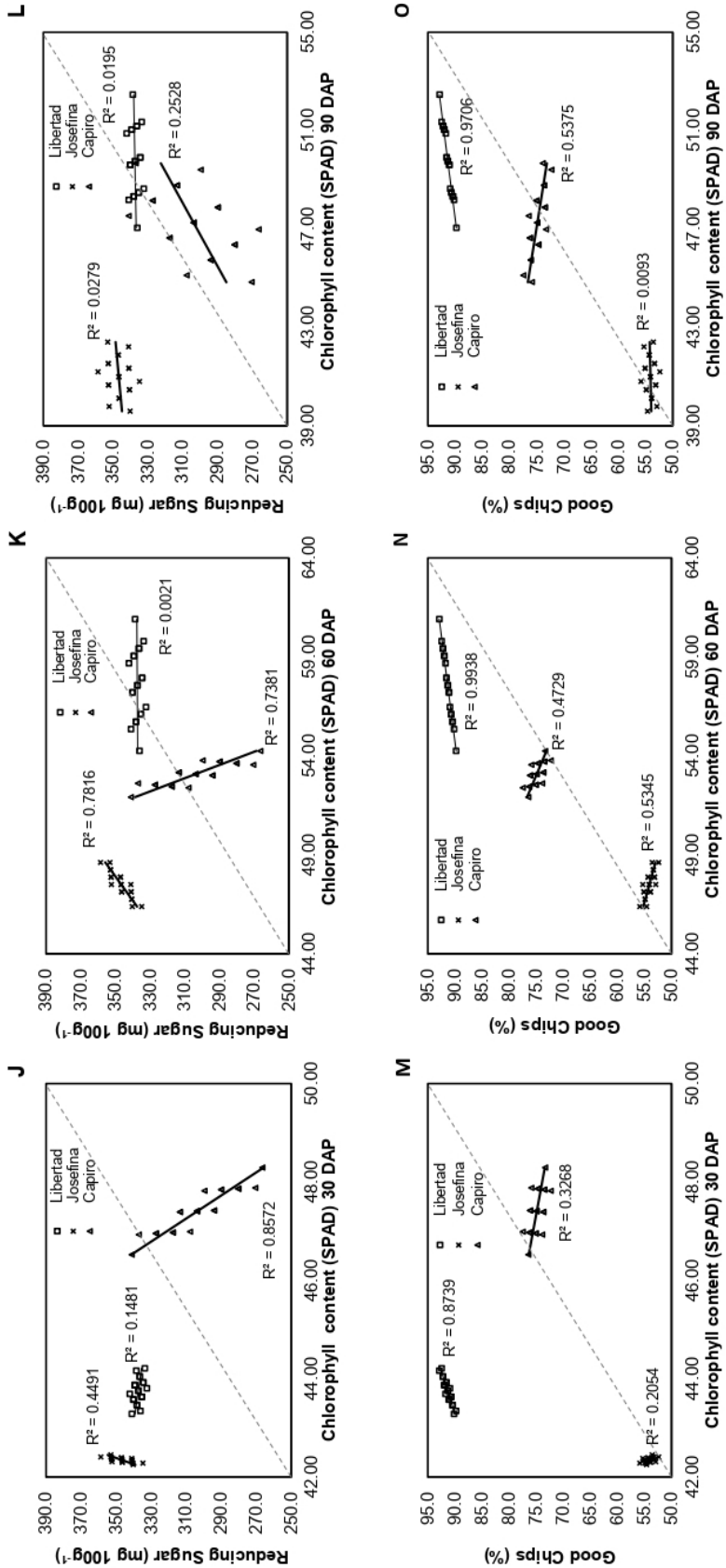

Figure 4. Correlation coefficient of the SPAD value with Reducing sugar (J, K, L) and Good chips (M, N, O).

Table 2. Regression analysis of correlations and SPAD value with several mathematic models of yield 242 and tubers quality in different vegetative stages.

Days after planting	Component	Linear model, $y = ax + b$	Logarithmic model, $y = aln(x) + b$	Power model, $y = ax^b$	Index model, $y = ae^{bx}$
vegetative growth (30 DAP)	Weight of tubers (kg plant^{-1})	$y = 0{,}262x - 9{,}460$ $R^2 = 0{,}9938$ $p < 0{,}0001$	$y = 11{,}430ln(x) - 41{,}203$ $R^2 = 0{,}9937$ $p < 0{,}0001$	$y = 10^{-9{,}22}x^{5{,}80}$ $R^2 = 0{,}9936$ $p < 0{,}0001$	$y = 0{,}0062e^{0{,}13x}$ $R^2 = 0{,}9934$ $p < 0{,}0001$
	Good chips (%)	$y = 2{,}934x - 37{,}041$ $R^2 = 0{,}8669$ $p < 0{,}0001$	$y = 128{,}200ln(x) - 339{,}070$ $R^2 = 0{,}8669$ $p < 0{,}0001$	$y = 10^{-0{,}36}x^{1{,}41}$ $R^2 = 0{,}8681$ $p < 0{,}0001$	$y = 22{,}421e^{0{,}03x}$ $R^2 = 0{,}8675$ $p < 0{,}0001$
	Dry matter of tubers (%)	$y = -0{,}309x + 38{,}670$ $R^2 = 0{,}5236$ $p = 0{,}0002$	$y = -13{,}480ln(x) + 76{,}104$ $R^2 = 0{,}5235$ $p = 0{,}0002$	$y = 10^{2{,}28}x^{-0{,}54}$ $R^2 = 0{,}5181$ $p = 0{,}0002$	$y = 42{,}948e^{-0{,}01x}$ $R^2 = 0{,}5240$ $p = 0{,}0002$
	Specific gravity of tubers	$y = -1{,}4E^{-03}x + 1{,}164$ $R^2 = 0{,}3052$ $p = 0{,}0068$	$y = -0{,}061ln(x) + 1{,}333$ $R^2 = 0{,}3050$ $p = 0{,}0068$	$y = 10^{0{,}13}x^{-0{,}05}$ $R^2 = 0{,}3277$ $p = 0{,}0049$	$y = 1{,}162e^{-0{,}0012x}$ $R^2 = 0{,}2898$ $p = 0{,}0084$
flowering–formation of tubers (60 DAP)	Weight of tubers (kg plant^{-1})	$y = 0{,}036x - 0{,}115$ $R^2 = 0{,}8548$ $p < 0{,}0001$	$y = 2{,}098ln(x) - 6{,}517$ $R^2 = 0{,}8543$ $p < 0{,}0001$	$y = 10^{-1{,}57}x^{1{,}06}$ $R^2 = 0{,}8553$ $p < 0{,}0001$	$y = 0{,}691e^{0{,}02x}$ $R^2 = 0{,}8548$ $p < 0{,}0001$
	Good chips (%)	$y = 0{,}468x + 64{,}361$ $R^2 = 0{,}9935$ $p < 0{,}0001$	$y = 26{,}944ln(x) - 17{,}868$ $R^2 = 0{,}9931$ $p < 0{,}0001$	$y = 10^{1{,}44}x^{0{,}30}$ $R^2 = 0{,}9928$ $p < 0{,}0001$	$y = 68{,}033e^{0{,}01x}$ $R^2 = 0{,}9933$ $p < 0{,}0001$
	Dry matter of tubers (%)	$y = -0{,}060x + 28{,}605$ $R^2 = 0{,}9101$ $p < 0{,}0001$	$y = -3{,}431ln(x) + 39{,}076$ $R^2 = 0{,}9099$ $p < 0{,}0001$	$y = 10^{1{,}64}x^{-0{,}14}$ $R^2 = 0{,}9074$ $p = 0{,}0002$	$y = 28{,}789e^{-0{,}0024}$ $R^2 = 0{,}9103$ $p < 0{,}0001$
	Specific gravity of tubers	$y = -3{,}1E^{-04}x + 1{,}122$ $R^2 = 0{,}7382$ $p < 0{,}0001$	$y = -0{,}018ln(x) + 1{,}176$ $R^2 = 0{,}7384$ $p < 0{,}0001$	$y = 10^{0{,}07}x^{-0{,}02}$ $R^2 = 0{,}7543$ $p < 0{,}0001$	$y = 1{,}116e^{-0{,}00027x}$ $R^2 = 0{,}7210$ $p < 0{,}0001$
ripening-thickening (90 DAP)	Weight of tubers (kg plant^{-1})	$y = 0{,}043x - 0{,}179$ $R^2 = 0{,}7808$ $p < 0{,}0001$	$y = 2{,}161ln(x) - 6{,}456$ $R^2 = 0{,}7804$ $p < 0{,}0001$	$y = 10^{-1{,}57}x^{1{,}09}$ $R^2 = 0{,}7818$ $p < 0{,}0001$	$y = 0{,}664e^{0{,}02x}$ $R^2 = 0{,}7924$ $p < 0{,}0001$
	Good chips (%)	$y = 0{,}576x + 62{,}675$ $R^2 = 0{,}9690$ $p < 0{,}0001$	$y = 28{,}610ln(x) - 20{,}444$ $R^2 = 0{,}9686$ $p < 0{,}0001$	$y = 10^{1{,}43}x^{0{,}31}$ $R^2 = 0{,}9680$ $p < 0{,}0001$	$y = 66{,}686e^{0{,}01x}$ $R^2 = 0{,}9687$ $p < 0{,}0001$
	Dry matter of tubers (%)	$y = -0{,}076x + 28{,}951$ $R^2 = 0{,}9570$ $p < 0{,}0001$	$y = -3{,}775ln(x) + 39{,}919$ $R^2 = 0{,}9567$ $p < 0{,}0001$	$y = 10^{1{,}66}x^{-0{,}15}$ $R^2 = 0{,}9548$ $p < 0{,}0001$	$y = 29{,}370e^{-0{,}003x}$ $R^2 = 0{,}9572$ $p < 0{,}0001$
	Specific gravity of tubers	$y = -4{,}0E^{-04}x + 1{,}124$ $R^2 = 0{,}8129$ $p < 0{,}0001$	$y = -0{,}020ln(x) + 1{,}182$ $R^2 = 0{,}8128$ $p < 0{,}0001$	$y = 10^{0{,}07}x^{-0{,}02}$ $R^2 = 0{,}8247$ $p < 0{,}0001$	$y = 1{,}127e^{-0{,}00036x}$ $R^2 = 0{,}7967$ $p < 0{,}0001$

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The effect of non-uniformly distributed chlorophyll is likely to be more important in explaining the nonlinearity in the empirical relationships, since the effect of scattering was predicted to be comparatively weak. We determined that the regression coefficient between SPAD value and yield (kg plant^{-1}) were significant in the three strata of plant at evaluation 90 DAP (Table 2). Our results confirmed that plant age condition would affect the accuracy of correlation analysis (Retta et al., 2016; Ucar et al., 2018).

Our results suggest that highland potato has a different behavior than other crops regarding the mathematical fit of the studied relationships. It was necessary to adjust research means according to specific plant and growth stage. Meanwhile, the significant difference of coefficient values suggested the importance and need of calculation method in estimation.

5 Conclusions

There was a significant relationship between SPAD-520 plus® value in potato leaves with yield and tubers quality. However, the optimized mathematical model for estimation tubers quality with SPAD value of leaves at different growth stages and high of plant were different.

Highest correlation efficiency of four mathematic modeling functions at different growth stage was showed. In general, for INIAP Libertad variety and formulation (KNO_3 + $NH_4H_2PO_4$ + KCl), the results demonstrated that optimal model for yield was linear function at 30 DAP; index model for DM at 90 DAP; power model for SG at 90 DAP and linear model for GC at 60 DAP.

Further work is planned to evaluate late varieties and planting density for adequate correlation interpretation in the estimate.

Acknowledgments

This research was supported by ECUAQUIMICA [No. 2-229], National Institute of Agricultural Research (INIAP) [Program roots and tubers] and EGEEMIP-YAinnova. The authors thank professor Guillermo Schmeda-Hirschmann (Universidad de Talca, Talca 3460000, Chile) for critical reading of the manuscript.

References

Azia, F. and Stewart, K. (2001). Relationships between extractable chlorophyll and spad values in muskmelon leaves. *Journal of Plant Nutrition*, 24(6):961–966. Online:https://n9.cl/jf8i1.

Busse, J., Wiberley-Bradford, A., and Bethke, P. (2019). Transient heat stress during tuber development alters post-harvest carbohydrate composition and decreases processing quality of chipping potatoes. *Journal of the Science of Food and Agriculture*, 99(5):2579–2588. Online:https://n9.cl/ah7rz.

Camps, C. and Camps, Z. (2019). Optimized prediction of reducing sugars and dry matter of potato frying by ft-nir spectroscopy on peeled tubers. *Molecules*, 24(5):967. Online:https://n9.cl/2wokl.

Casa, R., Castaldi, F., Pascucci, S., and Pignatti, S. (2015). Chlorophyll estimation in field crops: an assessment of handheld leaf meters and spectral reflectance measurements. *The Journal of Agricultural Science*, 153(5):876–890. Online:https://n9.cl/7n60q.

Chou, S., Chen, B., Chen, J., Wang, M., Wang, S., Croft, H., and Shi, Q. (2020). Estimation of leaf photosynthetic capacity from the photochemical reflectance index and leaf pigments. *Ecological Indicators*, 110:105867. Online:https://n9.cl/x6mo9.

Clevers, J., Kooistra, L., and Van den Brande, M. (2017). Using sentinel-2 data for retrieving lai and leaf and canopy chlorophyll content of a potato crop. *Remote Sensing*, 9(5):405. Online:https://n9.cl/2zdl7.

Cuesta, X., Rivadeneira, J., and Monteros, C. (2015). *Mejoramiento Genético de papa: Conceptos, procedimientos, metodologías y protocolos*. Instituto Nacional de Investigaciones Agropecuarias.

De Jong, H. (2016). Impact of the potato on society. *American journal of potato research*, 93:415–429. Online:https://n9.cl/tok4h.

Giletto, C. and Echeverría, H. (2013). Chlorophyll meter for the evaluation of potato n status. *American journal of potato research*, 90:313–323. Online:https://n9.cl/9bnoc.

Guo, T., Tan, C., Li, Q., Cui, G., and Li, H. (2018). Estimating leaf chlorophyll content in tobacco based on various canopy hyperspectral parameters. *Journal of Ambient Intelligence and Humanized Computing*, 10:3239–3247. Online:https://n9.cl/h19dcv.

Hawkins, T., Gardiner, E., and Comer, G. (2009). Modeling the relationship between extractable chlorophyll and spad-502 readings for endangered plant species research. *Journal for Nature Conservation*, 17(2):123–127. Online:https://n9.cl/88936.

Huaraca, H., Montesdeoca, F., and Pumisacho, M. (2009). *Guía para facilitar el aprendizaje sobre el manejo del tubérculo-semilla de papa*. INIAP, Estación Experimental Santa Catalina.

Kamrani, M., Rahimi, A., and Hosseinniya, H. (2019). Effects of different growing media on yield and growth parameters of potato minitubers (solanum tuberosum l.). *Communications in soil science and plant analysis*, 50(15):1838–1853. Online:https://n9.cl/2rt8l.

Kaspary, T., Cutti, L., Bellé, C., Casarotto, G., Groth, M., Da Silva, G., and De Aguiar, A. (2019). Non-destructive analysis of photosynthetic pigments in'avena strigosa'and'avena sativa'. *Australian Journal of Crop Science*, 13(3):354–359. Online:https://n9.cl/fb6wr.

Kühling, I., Hüsing, B., Bome, N., and Trautz, D. (2018). Soybeans in high latitudes: effects of bradyrhizobium inoculation in northwest germany and southern west siberia. *Organic Agriculture*, 8:159–171. Online:https://n9.cl/mlzf4.

Lan, S., Wu, L., Zhang, D., Hu, C., and Liu, Y. (2011). Ethanol outperforms multiple solvents in the extraction of chlorophyll-a from biological soil crusts. *Soil Biology and Biochemistry*, 43(4):857–861. Online:https://n9.cl/etx1o.

León, A., Viña, S., Frezza, D., Chaves, A., and Chiesa, A. (2007). Estimation of chlorophyll contents by correlations between spad-502 meter and chroma meter in butterhead lettuce. *Communications in soil science and plant analysis*, 38(19-20):2877–2885. Online:https://n9.cl/vnl5k.

Lulai, E. and Orr, P. (1979). Influence of potato specific gravity on yield and oil content of chips. *American Potato Journal*, 56:379–390. Online:https://n9.cl/dfarc.

Matsuda, R., O. N. and Fujiwara, K. (2014). Leaf photosynthesis, plant growth, and carbohydrate accumulation of tomato under different photoperiods and diurnal temperature differences. *Scientia Horticulturae*, 170:150–158. Online:https://doi.org/10.1016/j.scienta.2014.03.014.

Netto, A., Campostrini, E., de Oliveira, J., and Bressan-Smith, R. (2005). Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and spad-502 readings in coffee leaves. *Scientia Horticulturae*, 104(2):199–209. Online:https://n9.cl/hgafm.

Nissen, M. (1955). The weight of potatoes in water. *American Potato Journal*, 32:332–339. Online:https://n9.cl/9rh3a.

Noulas, C., Herrera, J., Tziouvalekas, M., and Qin, R. (2018). Agronomic assessment of nitrogen use efficiency in spring wheat and interrelations with leaf greenness under field conditions. *Communications in soil science and plant analysis*, 49(7):763–781. Online:https://n9.cl/km8ys.

Padilla, F., de Souza, R., Peña-Fleitas, M., Grasso, R., Gallardo, M., and Thompson, R. (2019). Influence of time of day on measurement with chlorophyll meters and canopy reflectance sensors of different crop n status. *Precision Agriculture*, 20:1087–1106. Online:https://n9.cl/s1j29.

Padilla, F., Thompson, R., Peña-Fleitas, M., and Gallardo, M. (2018). Reference values for phenological phases of chlorophyll meter readings and reflectance indices for optimal n nutrition of fertigated tomato. In *V International Symposium on Ecologically Sound Fertilization Strategies for Field Vegetable Production 1192*, pages 65–72. Online:https://n9.cl/xg11j.

Qiqige, S., Jia, L., Qin, Y., Chen, Y., and Fan, M. (2017). Effects of different nitrogen forms on potato growth and development. *Journal of Plant Nutrition*, 40(11):1651–1659. Online:https://n9.cl/d09iw.

Raymundo, R., Asseng, S., Robertson, R., Petsakos, A., Hoogenboom, G., Quiroz, R., Hareau, G., and Wolf, J. (2018). Climate change impact on global potato production. *European Journal of Agronomy*, 100:87–98. Online:https://n9.cl/anli9.

Retta, M., Yin, X., van der Putten, P., Cantre, D., Berghuijs, H., Ho, Q., Verboven, P., Struik, P., and Nicolaï, B. (2016). Impact of anatomical traits of maize (zea mays l.) leaf as affected by nitrogen supply and leaf age on bundle sheath conductance. *Plant science*, 252:205–214. Online:https://n9.cl/s88vm.

Roslan, N., Aznan, A., Ruslan, R., Jaafar, M., and Azizan, F. (2019). Growth monitoring of harumanis mango leaves (mangifera indica) at vegetative stage using spad meter and leaf area meter. In *IOP Conference Series: Materials Science and Engineering*, volume 557, page 012010. Online:https://n9.cl/1oku5.

Sim, C., Zaharah, A., Tan, M., and Goh, K. (2015). Rapid determination of leaf chlorophyll concentration, photosynthetic activity and nk concentration of elaies guineensis via correlated spad-502 chlorophyll index. *Asian Journal of Agricultural Research*, 9(3):132–138. Online:https://n9.cl/25fhu.

Todorov, D., Karanov, E., Smith, A., and Hall, M. (2003). Chlorophyllase activity and chlorophyll content in wild and mutant plants of arabidopsis thaliana. *Biologia plantarum*, 46:125–127. Online:https://n9.cl/hz5df.

Ucar, E., Ozyigit, Y., Eruygur, N., Güven, D., Yur, S., Turgut, K., Özek, T., and Kütük, N. (2018). The effect of the plant age and growth period on the nutritional substance, chlorophyll and steviol glycoside rates in stevia (stevia rebaudiana bertoni) leaves. *Communications in Soil Science and Plant Analysis*, 49(3):291–302. Online:https://n9.cl/z42or.

Uddling, J., Gelang-Alfredsson, J., Piikki, K., and Pleijel, H. (2007). Evaluating the relationship between leaf chlorophyll concentration and spad-502 chlorophyll meter readings. *Photosynthesis research*, 91:37–46. Online:https://n9.cl/iagn0.

Wiberley-Bradford, A. and Bethke, P. (2018). Suppression of the vacuolar invertase gene delays senescent sweetening in chipping potatoes. *Journal of the Science of Food and Agriculture*, 98(1):354–360. Online:https://n9.cl/29jcz.

Yuan, Z., Ata-Ul-Karim, S., Cao, Q., Lu, Z., Cao, W., Zhu, Y., and Liu, X. (2016). Indicators for diagnosing nitrogen status of rice based on chlorophyll meter readings. *Field crops research*, 185:12–20. Online:https://n9.cl/zxqxjo.

Yue, X., Hu, Y., Zhang, H., and Schmidhalter, U. (2019). Evaluation of both spad reading and spad index on estimating the plant nitrogen status of winter wheat. *International Journal of Plant Production*, 14:67–75. Online:https://n9.cl/pfdjh.

Zommick, D., Knowles, L., Pavek, M., and Knowles, N. (2014). In-season heat stress compromises postharvest quality and low-temperature sweetening resistance in potato (solanum tuberosum l.). *Planta*, 239:1243–1263. Online:https://n9.cl/k14er.

La granja:
Revista de
Ciencias de la Vida

pISSN:1390-3799; eISSN:1390-8596

http://doi.org/10.17163/lgr.n38.2023.05

Scientific paper/ Artículo científico

Earth sciences

Evaluation of digital land and geopotential models in Ecuador

Evaluación de los modelos digitales de terreno y geopotenciales en el Ecuador

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Article received on October 23rd, 2022. Accepted, after review, on June 06th, 2023. Published on September 1st, 2023.

Abstract

Engineering uses digital elevation models to perform calculations and modeling phenomena, since it allows determining the scale at which they can be used and the quality of the by-products obtained. Two groups of models were evaluated, the digital terrain models (DTMs): Shuttle Radar Topography Mission (SRTM), ASTER Global Digital Elevation Map (ASTER GDEM), ALOS PALSAR and the DTM generated by the Instituto Geográfico Militar del Ecuador (IGM), and the geopotential models (GMs): EGM96, EGM08 and the GM created by the IGM. For the evaluation, the geometric leveling points and ellipsoidal height raised in one of the IGM projects were used to determine atypical values, calculate the mean square error (RMSE) and define the precision and scale at which the different ones can be used. The heights between the DTMs were compared to know their difference. It was determined that the SRTM 30, ALOS PALSAR and IGM DMTs can be used for jobs that require an accuracy of less than 10 meters. The GM EGM08 together with high precision ellipsoidal heights could generate elevation models that can reach an accuracy of 1.25 meters, while the GMs EGM96 and IGM can generate models that achieve an accuracy of 2.5 meters. The ellipsoidal heights of the SRTM 30, ALOS PALSAR and IGM DTMs obtained with the EGM 96 and EGM 08 GMs can only be used in jobs that require an accuracy of less than 10 meters.

Keywords: SRTM, ASTER GDEM, ALOS PALSAR, EGM 96, EGM 08, orthometric height, ellipsoidal height.

Resumen

Los trabajos de ingeniería utilizan los modelos digitales de elevación para realizar cálculos y modelar fenómenos, conocer su precisión permite determinar la escala de uso y la calidad de los subproductos que se obtienen. Existen modelos libres que son muy utilizados en la práctica, como es el caso de los modelos digitales del terreno (MDTs): Shuttle Radar Topography Mission (SRTM), ASTER Global Digital Elevation Map (ASTER GDEM), ALOS PALSAR, el MDT generado por el Instituto Geográfico Militar del Ecuador (IGM) y los modelos geopotenciales (MGs): EGM96, EGM08 y el MG creado por el IGM. Se evaluaron los modelos utilizando los puntos de nivelación geométrica y altura elipsoidal levantados por el IGM. Se determinaron los valores atípicos, se compararon las alturas entre los MDTs para conocer su diferencia, se calculó el error cuadrático medio (RMSE) y se definió la precisión y escala a la que se pueden emplear los diferentes modelos. Se concluyó que los MDTs SRTM 30, ALOS PALSAR e IGM pueden utilizarse para trabajos que requieran una precisión inferior a los 10 metros. El MG EGM08 junto con alturas elipsoidales de alta precisión podrían generar modelos de elevación que alcancen una precisión de 1.25 metros, mientras que los MGs EGM96 e IGM pueden generar modelos que alcancen una precisión de 2.5 metros. Las alturas elipsoidales de los MDTs SRTM 30, ALOS PALSAR e IGM obtenidos con los MGs EGM 96 y EGM 08 se pueden utilizar si se requiere una precisión inferior a los 10 metros.

Palabras clave: SRTM, ASTER GDEM, ALOS PALSAR, EGM 96, EGM 08

Suggested citation: Portilla, O., Leiva, C., Luna, M. and González, I. (2023). Evaluation of digital land and geopotential models in Ecuador. La Granja: Revista de Ciencias de la Vida. Vol. 38(2):58-79. http://doi.org/10.17163/lgr.n38.2023.05.

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1 Introduction

The characteristics of the terrain limit the activities that humans can perform; for this reason, engineering analyzes the characteristics of the terrain and determine the accuracy the models require to conduct the studies. For example, civil engineers analyze the land before building, geomorphologists understand the shape and processes that gave rise to it, surveyors measure and describe the land surface. There are different digital models that can be used depending on the vertical reference system required for the study. The digital terrain models, known as MDT, have their heights referring to the natural characteristics of the territory under study. While digital surface models, known as MDS, refer to their heights above the ground (Li et al., 2004).

The importance of having high-quality digital elevation models lies in the large number of applications that exist. Agriculture (Sinde-González et al., 2021), civil works (Abbondati et al., 2020), archeology (Peña Villasenín et al., 2017; Gil-Docampo et al., 2023), environmental management (McClean et al., 2020) or territorial planning (Zafar and Zaidi, 2019), among others, are among the most current and require more precision. However, on a planetary scale, centimeter-level precisions are not required and therefore global models are used. In this case, the applications focus on studies of geodynamics (Luna et al., 2017) and geodesy (Orejuela et al., 2021).

The definition of the SIRGAS Vertical Reference System is identical to the definition of the International Height Reference System (IHRS). Both point out the importance of using physical heights for conducting engineering works (Sánchez, 2015). Orthometric height is the most used physical height and is obtained by dividing the geopotential dimension for a mean gravity value (Drewes et al., 2002). Geoidal undulation depends on the ellipsoid used, but its variability is approximately within *pm* 100 (m) (Seeber, 1993). As known, GNSS positioning provides high-precision ellipsoidal heights efficiently, but to obtain high-precision orthometric heights it is necessary to generate high-precision MGs (Martínez and Bethencourt, 2012).

1.1 MDT Shuttle Radar Topography Mission (SRTM)

It was created by an initiative of the National Aeronautics and Space Administration (NASA), the German Aerospace Center, DLR, and the Italian Space Agency, ASI. It is an MDT with two resolution levels, one of 1 (30 meters) and another of 3 seconds of arc (90 meters), which covers 80% of the earth's surface from the 60° north to the 57° south. The horizontal accuracy of the MDT is greater than $\pm$ 20 (m), while the vertical accuracy meets $\pm$ 16 (m) for 90% of the data across the mission (Rabus et al., 2003). The type of height of the MDT SRTM is orthometric, since the MG EGM 96 was used to transform the ellipsoidal heights (Lemoine et al., 1998).

1.2 MDT generated by the Military Geographic Institute (IGM)

It was generated from the curve level obtained by restitution of the mapping generation project 1:5 000. These curves were generalized and interpolated to obtain a TDM with a resolution of 30 (m). The type of heights of the IGM MDT is orthometric generated with the MG EGM96 and its use is recommended for generating cartography 1:50 000.

1.3 MDT ASTER GDEM

Obtained by NASA and METI efforts in mid-October 2011. This model covered the Earth's surface from the 83° north to the 83° south. Its spatial resolution reached 1 second of arc (30 meters) and the vertical precision is around 20 meters with a confidence level of 95%. The orthometric heights of the MDT ASTER GDEM were obtained by using the MG EGM 96 (Tachikawa et al., 2011).

1.4 MDT ALOS PALSAR RTC

Distributed by Alaska Satellite Facility (ASF), it converted the orthometric heights of SRTM or NED MDTs into ellipsoid heights using the ASF MapReady geoid_adjust tool. This tool applies a geoid correction so that the resulting MDE is related to the ellipsoid (Alaska Satellite Facility, 2021).

Table 1 details the technical characteristics of the TDMs used in the research.

Table 1. Technical characteristics of the MDTs.

MDT	Vertical Accuracy	Spatial resolution	Height type
SRTM	± 16.0 m	30 m	Orthometric
IGM	± 12.5 m	30 m	Orthometric
ASTER GDEM	± 20,0 m	30 m	Orthometric
ALOS	-	30 m	Ellipsoid

1.5 MG EGM 96

It has a spatial resolution of approximately 56 kilometers, incorporating surface gravity data, ERS-1 and GEOSAT Geodetic Mission gravity anomalies, position and altimetry satellite data from various systems. The model is defined up to 360 degrees, allowing to calculate 131000 harmonic coefficients (Lemoine et al., 1998).

1.6 MG EGM 08

It has a spatial resolution of approximately 9 kilometers. It was developed by the combination of least squares of the ITG-GRACE03S gravitational model and its error covariance matrix. For its generation, gravitational information was extracted from a 5-minute-of-arc equiangular grid. This set of gravity anomalies was obtained by merging data from ground and airborne sensors with values derived from altimetry. The least squares adjustment was performed in terms of ellipsoidal harmonics; this conversion retained the order but not the degree, originating coefficients of grade 2190 and order 2159 (Pavlis et al., 2012).

1.7 MG generated by IGM

It used GPS techniques and geometric leveling to structure and train an artificial neural network of the type Radial Basis Functions (RBF) that allows calculating the geoidal undulation at any point by interpolation Tierra Acurio, 2014).The MG of the IGM obtained errors less than 40 cm and a mean quadratic error of 15 cm (Tierra and Acurio, 2014).

Engineering requires that the models and cartographic products meet a certain precision, not knowing the accuracy can cause economic and logistic problems. The TDMs and GMs used in this research, except for the TDM and the GM generated by the IGM, have been generated worldwide and have scientific literature that supports their accuracy worldwide, but has this accuracy been met in Continental Ecuador? In this way, the aim is to determine the accuracy of the models and the maximum scale at which they can be implemented for elaborating cartographic products in Continental Ecuador.

2 Materials and methods

The data used can be observed in Figure 1: one of the four MDTs and the points of geometric leveling and ellipsoidal height raised in one of the IGM projects. Although it is true that the geometric level heights would not be useful to evaluate the orthometric heights of the physical MDTs, it was determined by previous evaluations of the MDTs presented in the introduction that the accuracy of the MDTs reaches 15 meters, and as in the Continental Ecuador it has been determined that the difference between the level height and the orthometric height reaches the meter (Cañizares, 2015).

In addition, in the present investigation the difference between level height and orthometric height was rejected, since the accuracy of the MDTs would absorb the difference. There were points with ellipsoidal height that served to evaluate the transformation of orthometric heights of the MDTs in ellipsoidal heights and a pseudo geoidal ripple was calculated to evaluate the MGs at the points where the data had the level height and ellipsoidal height.

The equation of physical geodesy (Equation 1) considers geoidal undulation (N) as the vertical separation between the ellipsoidal height (h) and orthometric height (H). This consideration is used because of the ease of transforming the ellipsoidal heights into orthometric and vice versa, thus avoiding using gravimetric models and gravity measures to obtain physical heights, which make the costs of the projects more expensive.

$$N = h - H \tag{1}$$

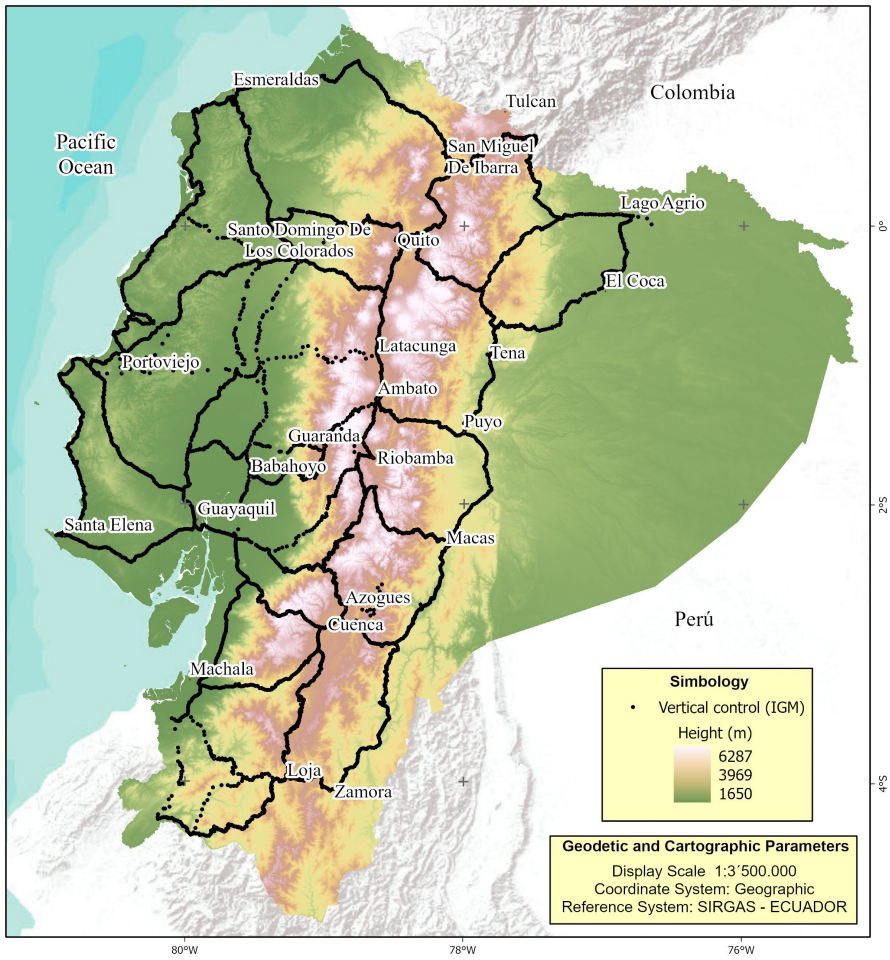

Figure 1. Elements used for evaluating the MDTs and MGs.

A spatial table was generated to evaluate the different MDTs and MGs. For this purpose, the leveled and ellipsoidal elevation performed by the IGM was georeferenced. At each point of the survey, the height value present in each pixel of the different MDTs was extracted, without resorting to any interpolation method for the extraction, because each point of the survey was located within a single pixel.

The geographical coordinates of each point of the survey were calculated and the spatial table was transformed into a .dat file that served as input to calculate the geoidal undulations with the MG EGM 96, EGM 08 and IGM. Using ETL software, the geo-ideal ripples present in each of the .dat files were added to the spatial table of points. The geoidal pseudo-undulation was calculated with the Geographic Information System (GIS), for each point of the uplift where the level height and ellipsoidal height existed at the same time. Hence, Equation 1 was used, where the level height of the ellipsoidal height was subtracted.

Subsequently, the original orthometric height of the MDTs was evaluated. The orthometric heights of all models except for the MDT ALOS PALSAR were obtained by using the MG EGM 96. The original heights of the MDT ALOS PALSAR are ellipsoidal heights, reason for which equation 1 was used to transform the ellipsoidal heights into orthometric heights, using the MG EGM 96.

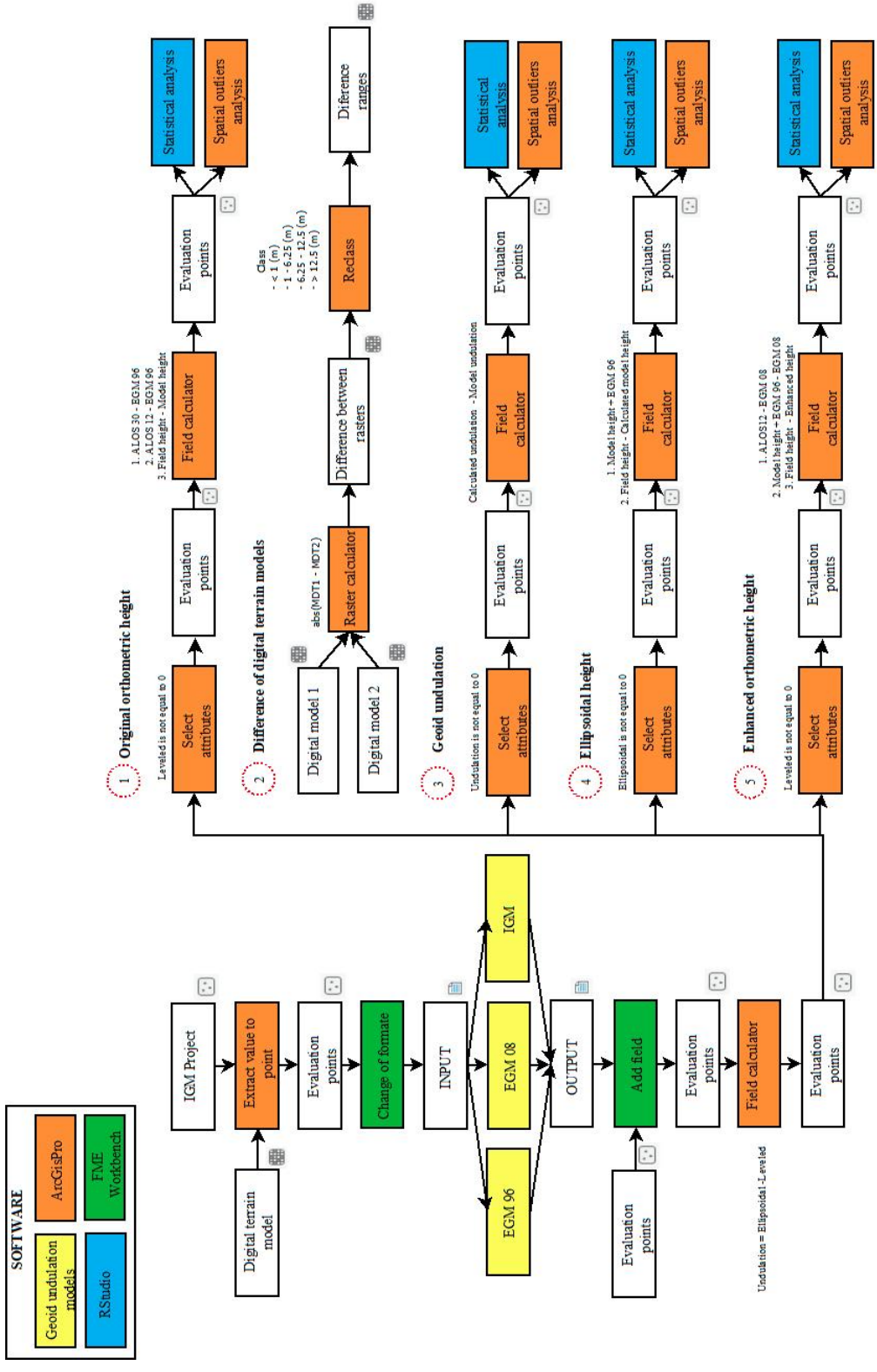

Figure 2. Mapping model for assessing TDMs and GMs.

Also, 3931 points were used, which had level heights of the IGM project to evaluate the vertical accuracy of the MDTs. It required calculating the difference of the captured value in the field with respect to the value of the MDT. Subsequently, it was proceeded to analyze the distribution of the differences with box diagrams, we plotted the dispersion of the differences with respect to the height at which the differences were calculated and calculated the RMSE of each BAT. The accuracy reported with the RMSE reflects all uncertainties, including errors in data acquisition, compilation and final calculation of heights (Federal Geographic Data Committee, 1998).

The differences were analyzed spatially using the local Moran´s I value in order to understand how the difference of a point is related to the differences that surround it, thus determining spatial clusters and atypical values (Anselin, 1995). The local Moran´s I uses a z-score, a pseudo-P-value to represent the statistical significance of the calculated index values. A negative value for I indicates that an entity has neighboring entities with different values; this entity is an outlier. In both instances, the P-value for the entity must be small enough for the outlier to be considered statistically significant. An outlier can be of two types, a high value surrounded primarily by low values (high - low) and a low value surrounded primarily by high values (low-high). Statistical significance is set at a 95% confidence level (ESRI, 2020).

Once MDTs were statistically and spatially analyzed, the second step was to determine the difference between each MDT. As mentioned, the MDT ALOS PALSAR has ellipsoidal heights, while the other MDTs have orthometric heights, reason for which no difference raster was generated with the MDT ALOS PALSAR. The differences raster served to classify the differences, visualize their spatial behavior, analyze the coverage percentage of each difference range and determine to what extent the DMTs can be considered similar to be able to use them together and overcome their weaknesses.

The MGs were evaluated, where 1253 points of the IGM project were used since they had the value of the geoidal pseudo-undulation. The difference between the geoidal pseudo-ripple captured in the field was determined with respect to the ripple calculated with the MGs EGM 96, EGM 08 and IGM model. Once with the calculated differences, the distribution of the differences was analyzed, the dispersion of the differences was plotted with respect to the height at which they were calculated. The RMSE of each model was calculated and the outliers were spatially analyzed using the local Moran´s I.

1253 points of the IGM project were used to evaluate the ellipsoidal height of the MDTs. For this reason, the orthometric heights of the MDTs SRTM, ASTER GDEM and IGM were transformed into ellipsoidal heights using equation 1 and the MG EGM 96. It was not necessary to transform the heights of the MDT ALOS PALSAR, because the original heights of the MDT are ellipsoidal heights. We proceeded to determine the difference between the ellipsoidal height captured in the field with respect to the ellipsoidal height of the MDTs, analyze their distribution, plot their dispersion with respect to the height at which the differences were calculated, calculate the RMSE and spatially determine the outliers using local Moran´s I.

The last step was to determine if using MG EGM 08 with the ellipsoidal heights calculated in the previous step could achieve MDTs of more accurate orthometric heights. In the 3931 points that had the level height of the IGM project, the original orthometric heights of the MDTs were transformed into ellipsoidal heights, using equation 1 and the MG EGM 96. Then the ellipsoidal heights were transformed into orthometric heights using again equation 1 and the MG EGM 08. As in the previous steps, the difference between the obtained value in field with respect to the value of the model was determined, its distribution was analyzed, the dispersion of the differences with respect to the height to which they were calculated was plotted, the RMSE was calculated and the atypical values were spatially determined using local Moran´s I.

3 Results

3.1 Evaluation of orthometric height with the MG EGM 96 of the MDTs

The distribution of differences was analyzed in the level heights captured in the field with respect to

the orthometric heights of the MDTs obtained with the MG EGM 96. Figure 3 and 4 and table 2 show the influence of spatial resolution on the distribution of differences for models that have been distributed with two resolutions. In the case of the MDT SRTM, it is observed that the differences obtained with the 30-meter resolution model are better than the differences obtained with the 90-meter model, since they present a better grouping of the data, a narrowing and better location of the box. In the case of the MDT ALOS PALSAR, the differences between the 30-meter model and the 12.5-meter model are hardly identifiable since their boxes have the same size and are in the same position.

Table 2. Values of orthometric height assessment box diagram with MG EGM 96.

MDTs	Max	Q3	Med	Q1	Min
SRTM 90	13.58	1.32	-1.96	-6.94	-19.30
SRTM 30	14.22	4.92	1.61	-1.33	-10.56
ASTER	24.03	7.24	1.88	-3.99	-20.83
ALOS 30	10.95	1.48	-1.45	-4.88	-14.41
ALOS 12.5	10.59	1.46	-1.48	-4.70	-13.94
IGM	10.26	0.98	-1.81	-5.26	-14.58

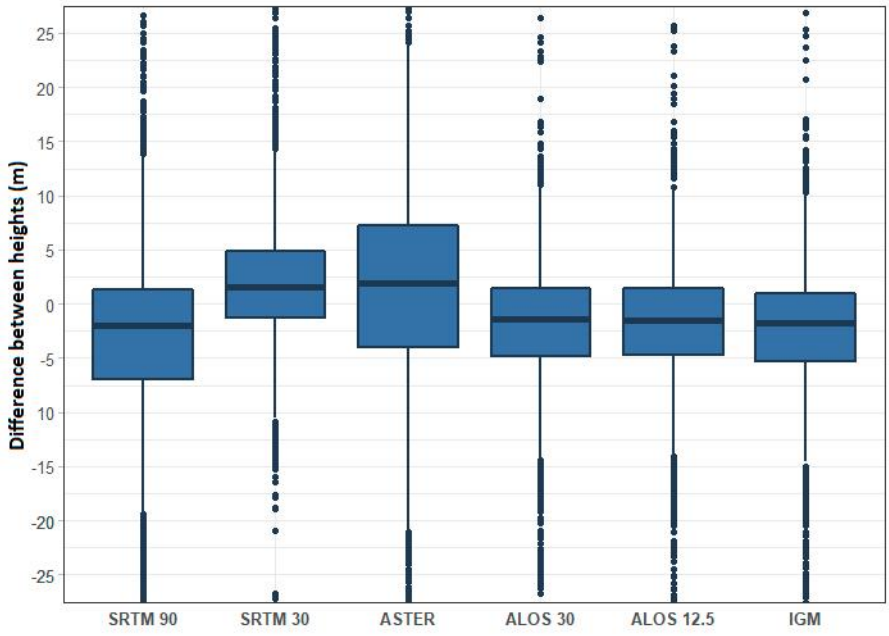

Figure 3. Orthometric Height Difference Box Diagram with MG EGM 96.

When comparing the box of all the MDTs, it is observed that the box of the MDT ASTER GDEM is the widest and therefore has the worst distribution of the differences; in turn, it is observed that the median of this model is similar to the median of the MDT SRTM of 30 meters. The ALOS PALSAR and IGM MDT boxes have similar statistical characteristics both in the width of the box and in its location. All boxes except the 90-meter SRTM model box show a similar data distribution both above and below the median value in the boxes, and to the right and left of the mean value, in the histogram (symmetry).

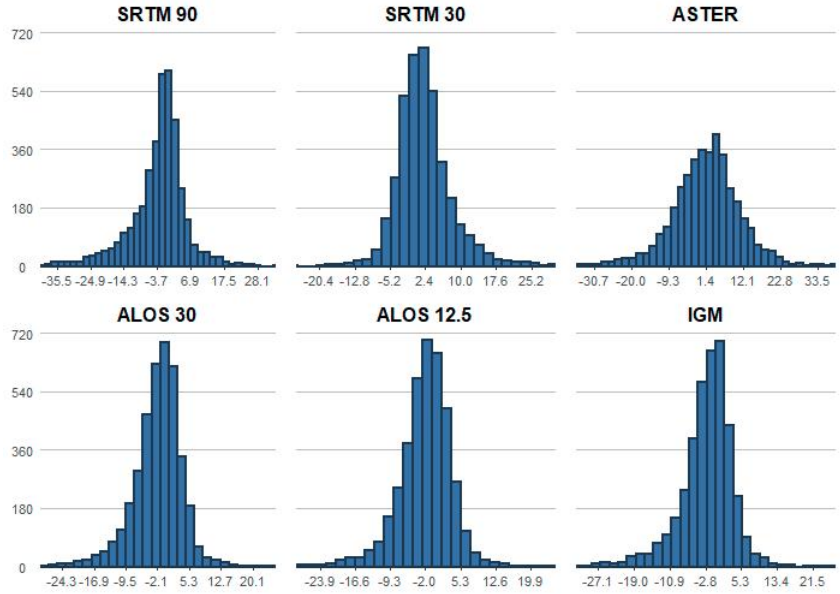

Figure 4. Histogram of orthometric height difference with MG EGM 96.

Figure 5 shows that the dispersion of differences regarding the height of the evaluation point is grouped around 0 meters, where MDTs SRTM 30 meters, ALOS PALSAR 30 and 12.5 meters and IGM tend to be better grouped than MDTs ASTER GDEM and SRTM 90 meters. It is observed that the 30-meter MDT SRTM has more positive differences, while the 90-meter MDTs SRTM ALOS PALSAR 30 and 12.5 meters and IGM tend to have more negative differences.

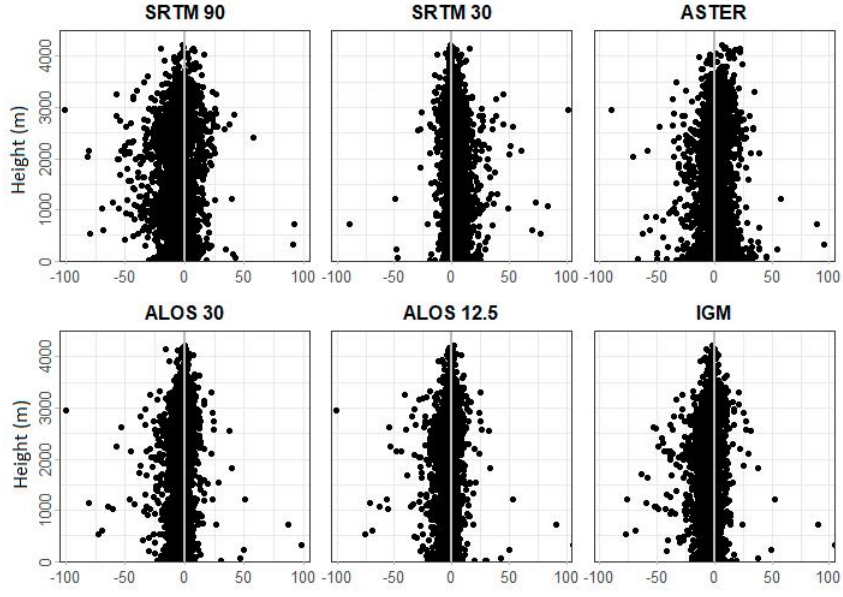

Figure 5. Dispersion of orthometric height difference with MG EGM 96.

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A uniform distribution of differences is observed in all TDMs as the assessment height increases. Based on observations in Figure 3, Figure 4 and Figure 5. In addition, it was decided to choose the 30-meter MDTs SRTM and 12.5- meter ALOS PALSAR for the next steps of the evaluation, as they presented better statistical results.

In the spatial analysis of outliers with the local Moran´s I observed in the outliers. can be seen a similar behavior in the typology and location of outliers in the MDTs SRTM, ALOS PALSAR and IGM, where most of the outliers are present in the Andes. The MDT ASTER GDEM is characterized by having a considerable amount of differences with a high value surrounded by differences with a low value in the northeast of Ecuador. All TDMs had 3% high-low outliers and 2% low-high outliers.

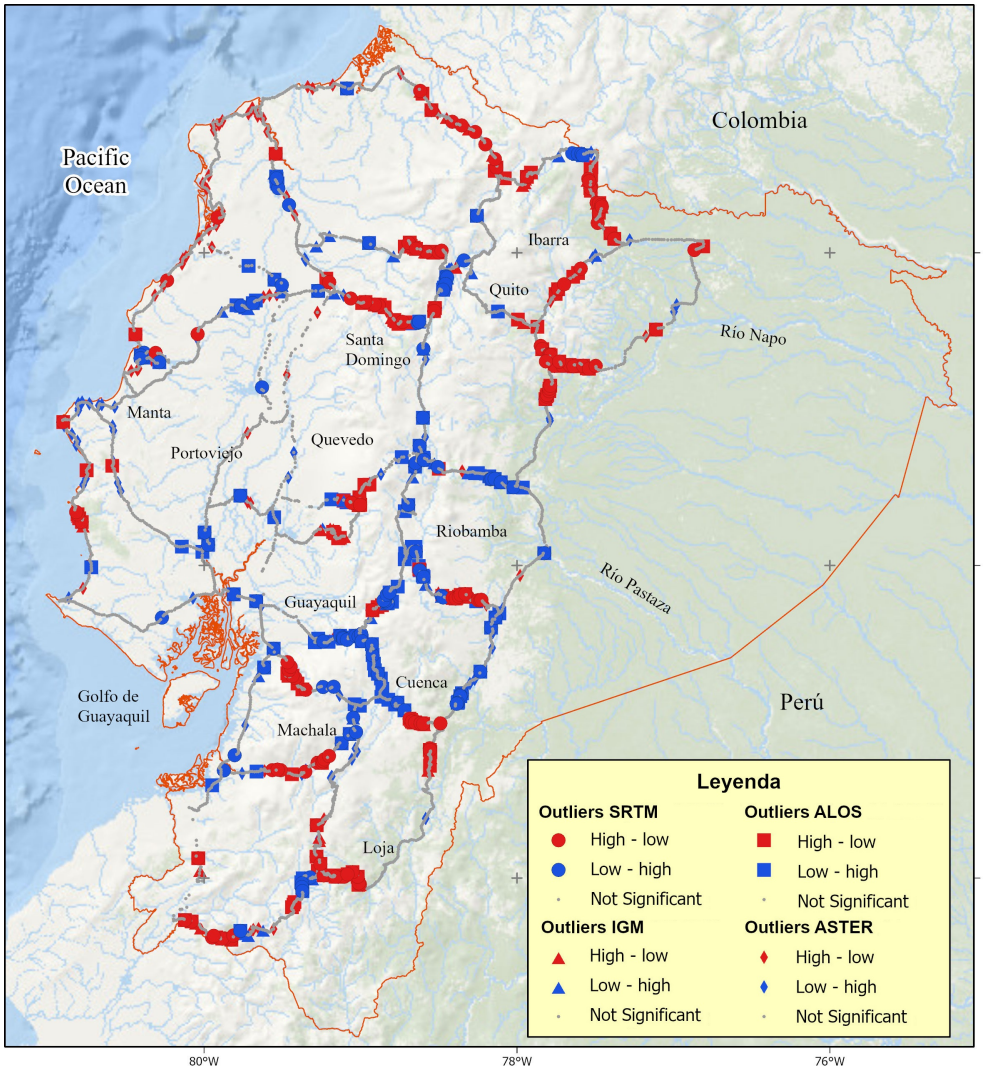

Figure 6. Outliers analysis of orthometric height differences with MG EGM 96.

The spatial distribution of the differences between the MDTs that had the original height at orthometric height was determined; for this reason, the MDT ALOS PALSAR was excluded from the analysis. The difference between IGM and SRTM MDTs is observed in Figure 7, where an area of high differences between the provinces of Sucumbíos and Orellana stands out. By analyzing the models separately, it was discovered that this difference is caused because the IGM MDT has zones that have a constant height value.

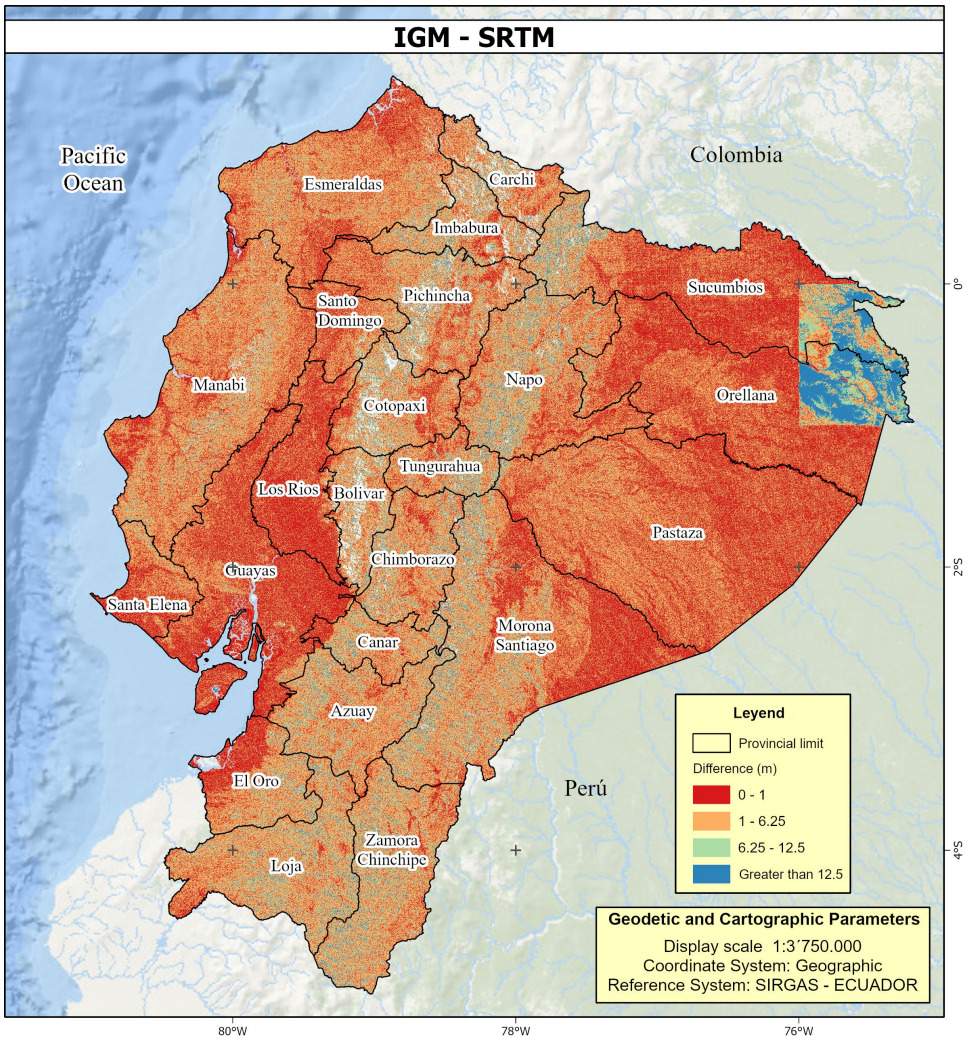

Figure 7. Difference between IGM and SRTM MDTs.

A bar chart was generated with the classification of differences between the MDTs to quantify what is observed in Figure 7. According to Figure 8, 96% of the differences are lower than the tolerance of the scale 1:50,000 (12.5 meters), so it can be considered that these models can be complemented to fill their shortcomings. For example, the deficiency of the SRTM model covers 93% of the continental territory, while the MDT IGM covers 100%.

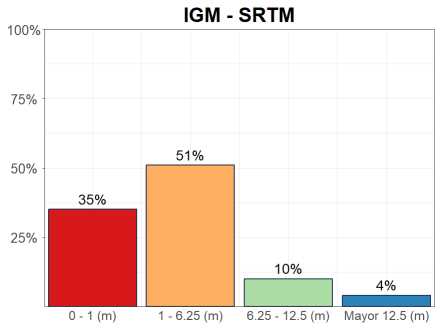

Figure 8. Difference bar diagram between IGM and SRTM MDTs.

Figure 9 shows that the tones that prevail in the

map of differences between IGM MDTs and ASTER GDEM are in the ranges between 1 and 12.5 meters. The bar diagram in Figure indicates that 63% of the differences are in the range between 1 and 12.5 meters. Even though there is a considerable reduction in the percentage of differences that are less than the tolerance of the scale 1:50,000, only 70% of the differences are smaller.

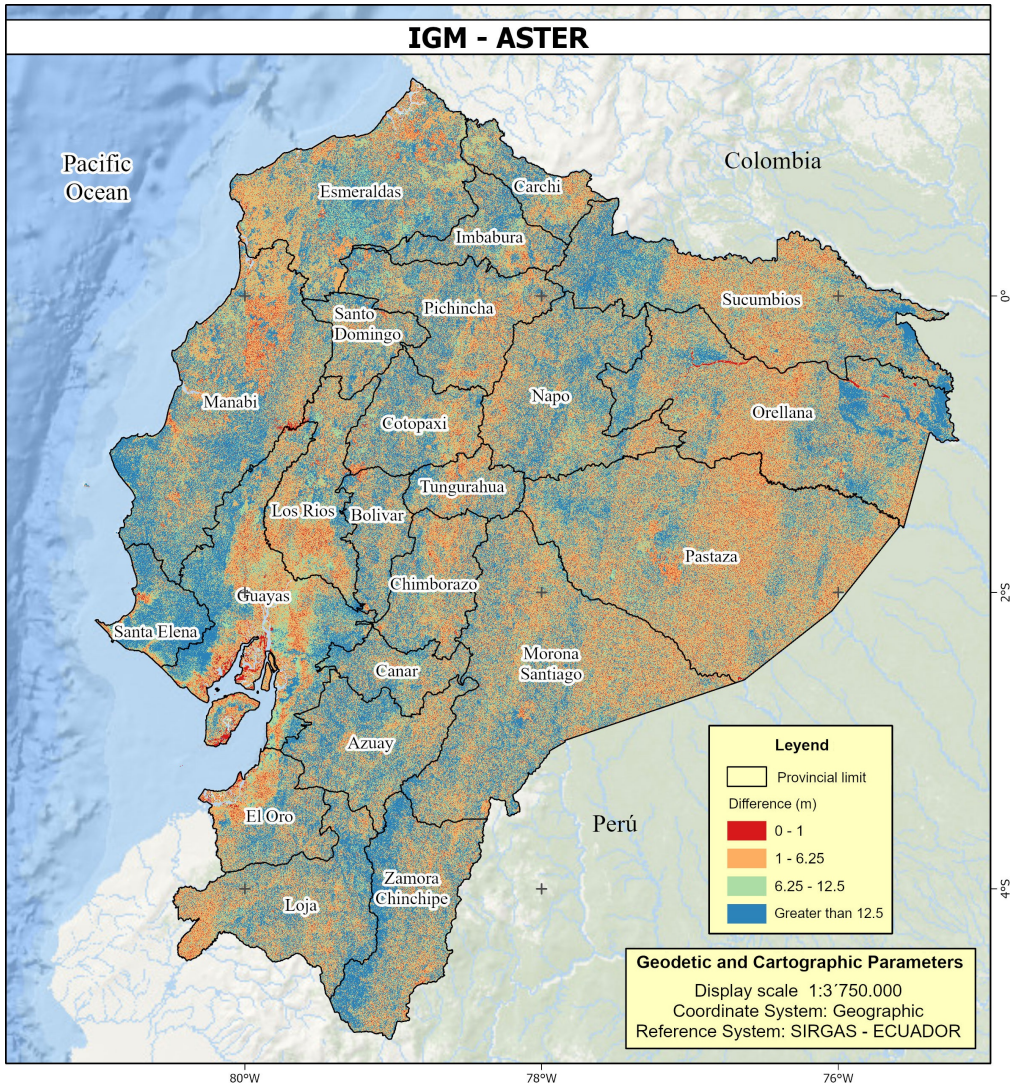

Figure 9. Difference between IGM and ASTER MDTs.

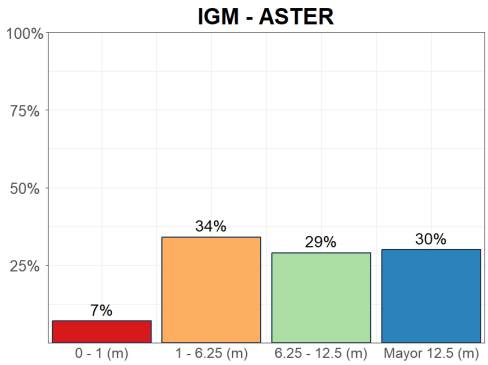

Figure 10. Difference bar diagram between IGM and ASTER MDTs.

A similar behavior is observed on the Difference Map of Figure and in the difference map of Figure . When analyzing the bar diagram of Figure , it is found that the behavior is the same, since the percentages of the differences ranges are equal to that of Figure. When the differences between IGM

and SRTM MDTs were analyzed, a zone of high differences between the provinces of Sucumbíos and Orellana was observed. In Figure traces of that area are seen, while in Figure this area disappears.

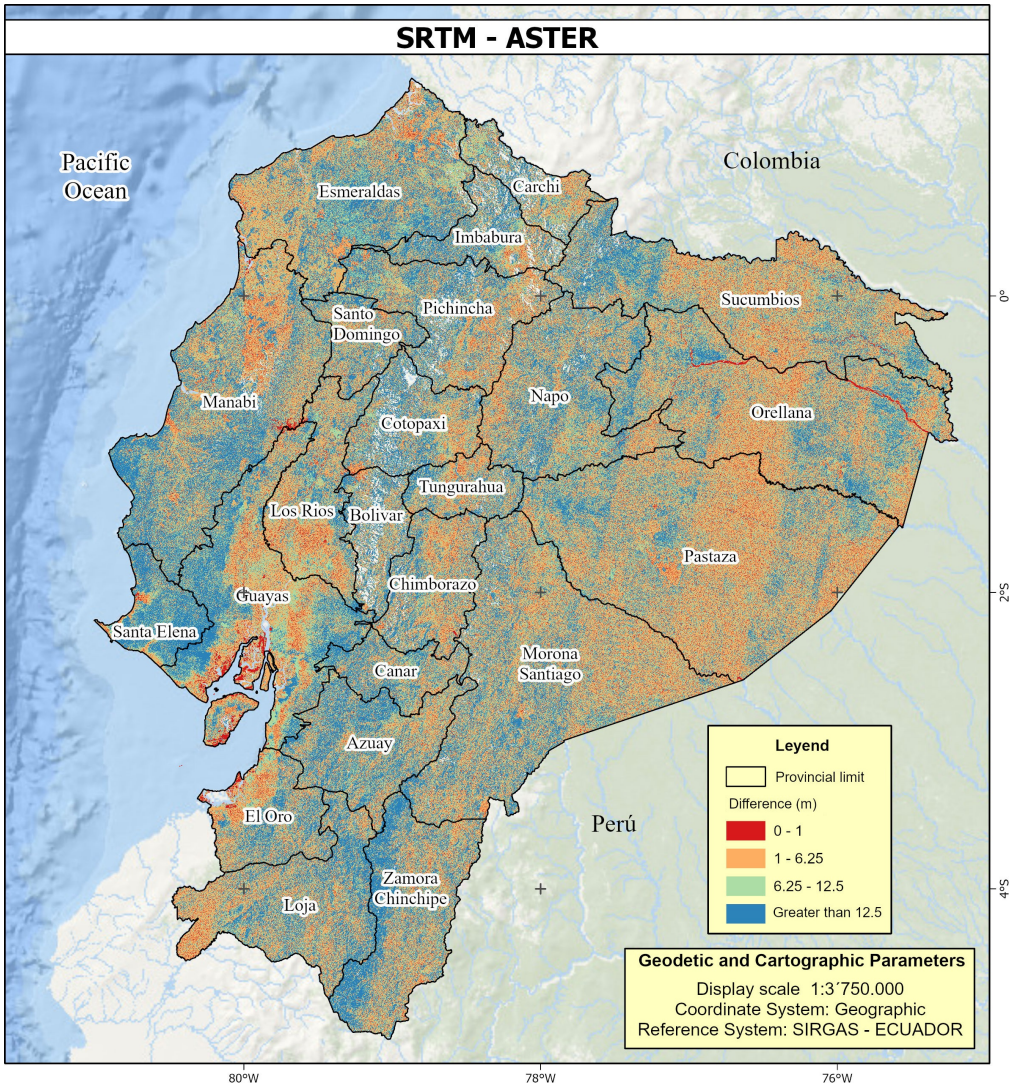

Figure 11. Difference between MDTs SRTM and ASTER.

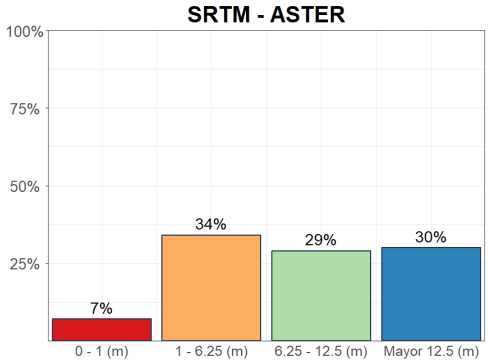

Figure 12. Difference bar diagram between SRTM and ASTER MDTs.

3.2 Evaluation of MGs

We analyzed the distribution of the differences between the geoidal pseudo-undulations calculated from the information captured in the field with respect to the geoidal undulations obtained from the MGs. Figures 13 and 14 and Table 3 show that the MGs box is symmetrical with respect to the median. MG EGM 96 has a similar median as MG EGM 08

and the MG IGM box has the smallest extent of all.

Table 3. Values of the MGs box diagram.

	EGM 08	EGM 96	IGM
Maximum	1.38	2.65	-0.12
Q3	0.29	0.59	-0.75
Median	-0.12	-0.12	-0.94
Q1	-0.47	-0.90	-1.17
Minimum	-1.48	-2.92	-1.81

Figure 15 shows the dispersion of the differences in the geoidal undulation with respect to the evaluation height, where the differences in the MGs EGM 08 and IGM tend to be better grouped around 0 meters. The MGs EGM 08 and IGM show a uniform distribution of differences as height increases, while the MG EGM 96 shows a high dispersion of difference between 500 and 2000 meters in height.

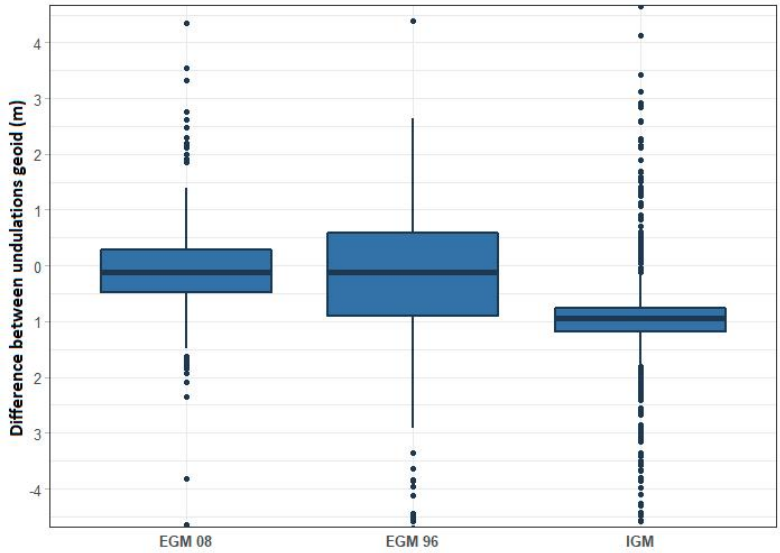

Figure 13. Boxplot diagram of the difference of MGs.

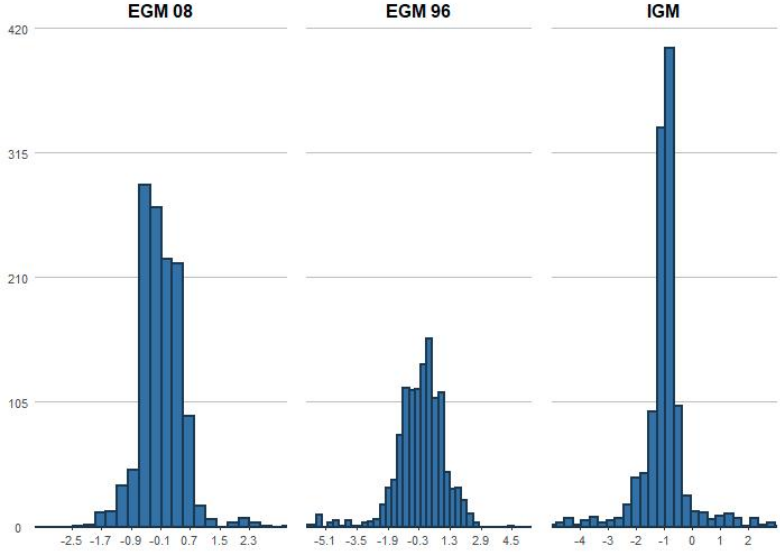

Figure 14. Histogram of MGs difference.

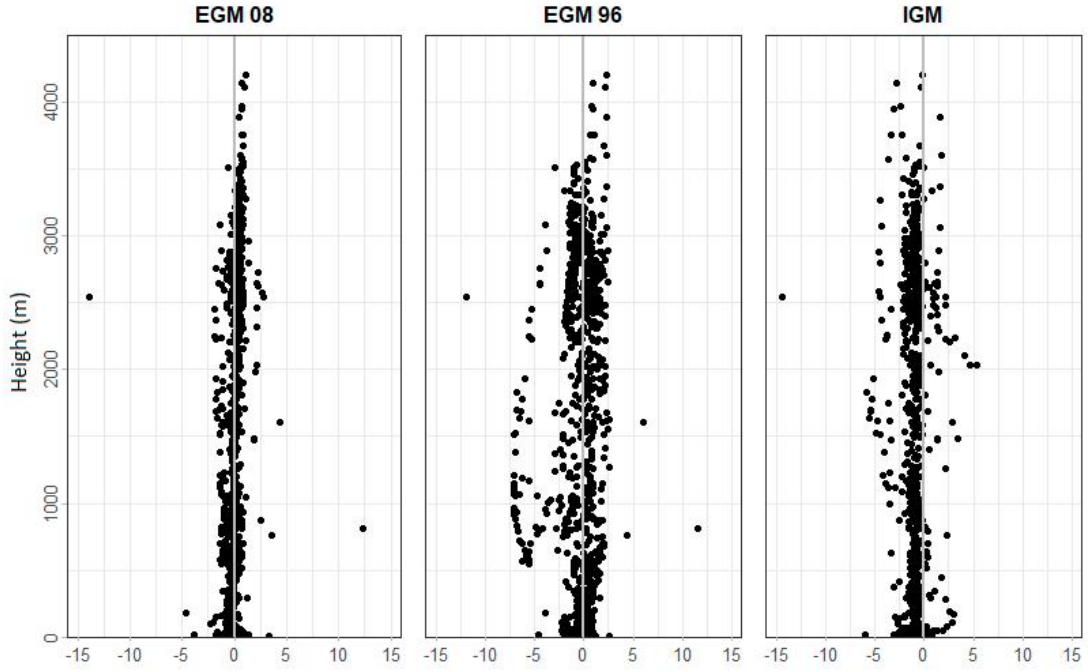

Figure 15. Dispersion of the difference in MGs.

Figure 16 highlights a low number of outliers in the three MGs. The spatial evaluation of the MG EGM 08 shows that there are differences with high values surrounded by differences with low values in the north, while the values in the south show there are differences with low values surrounded by differences with high values. Although the MG EGM 96 has a minimal amount of outliers, they maintain the behavior observed in the MG EGM 08. IGM MG has no pattern in the distribution of outliers.

The RMSE of the MGs is shown in Table 4, where it is verified that the MG EGM 08 has the best accuracy.

Table 4. RMSEs of MGs.

MGs	RMSE (m)
EGM 08	0.82
EGM 96	1.67
IGM	1.43

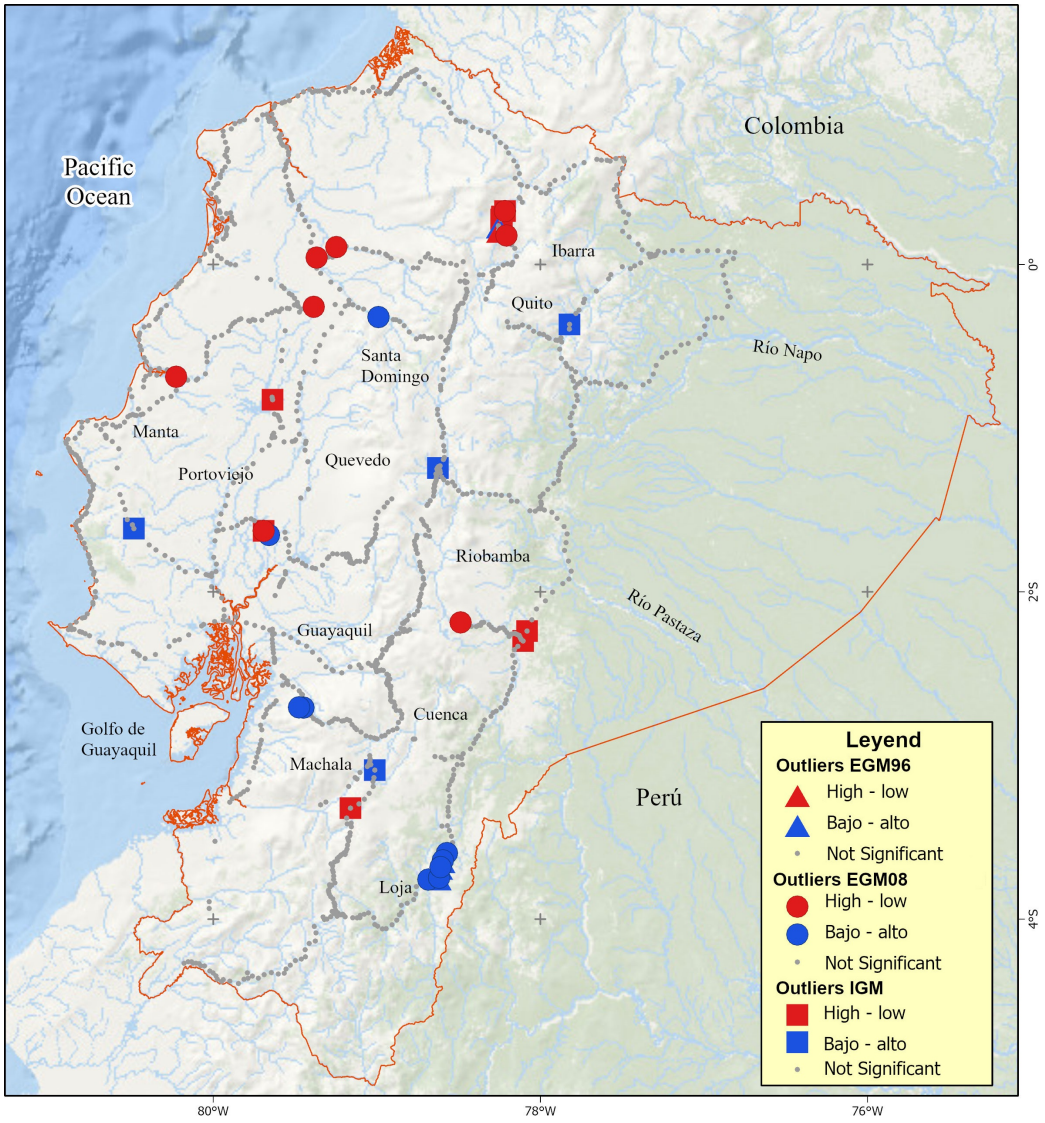

Figure 16. Analysis of atypical values for the differences in MGs.

3.3 Evaluation of ellipsoidal heights of MDTs

We analyzed the distribution of the differences between the ellipsoidal heights captured in the field with respect to the ellipsoidal heights calculated from those of MDTs, except for MDT ALOS PALSAR whose original heights are ellipsoidal heights. As shown in Figures 17 and 18 and Table 5, there is aError: no se encontró el origen de la referencia similar behavior of box diagrams and distribution of differences in orthometric heights with the MG EGM 96, where symmetry and similarity in size, location and statistical values of MDTs SRTM, ALOS PALSAR and IGM stand out.

Table 5. Ellipsoidal Height Box Diagram Values.

MDTs	Max	Q3	Med	Q1	Min
SRTM 90	8.95	1.02	-1.50	-4.62	-12.98
ASTER	23.51	7.01	1.91	-4.12	-20.80
ALOS 12.5	8.97	1.05	-1.43	-4.26	-11.94
IGM	8.76	0.66	-1.76	-4.85	-13.10

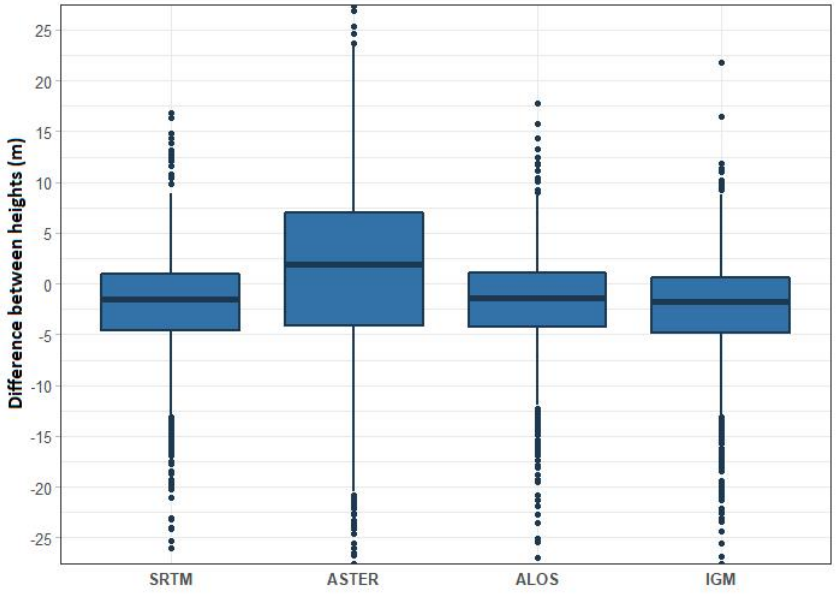

Figure 17. Ellipsoidal Height Difference Boxplot Diagram.

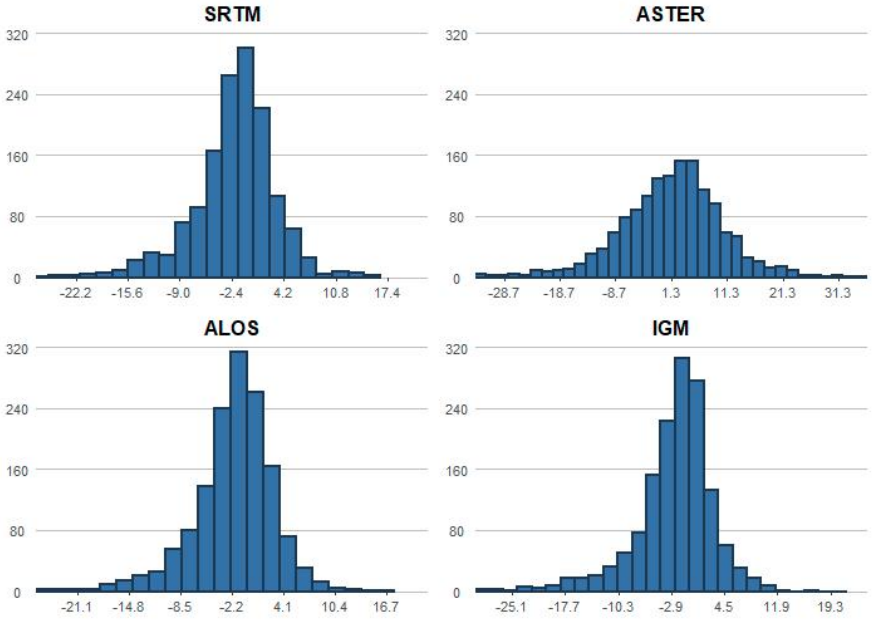

Figure 18. Difference histogram of ellipsoidal heights.

Figure 19 shows that the dispersion of the ellipsoidal heights exhibits the same behavior as the orthometric height differences of Figure 5; however, the differences have a lower dispersion range. In the case of orthometric heights, the differences reach 100 meters, while with the ellipsoidal heights, the differences reach 50 meters.

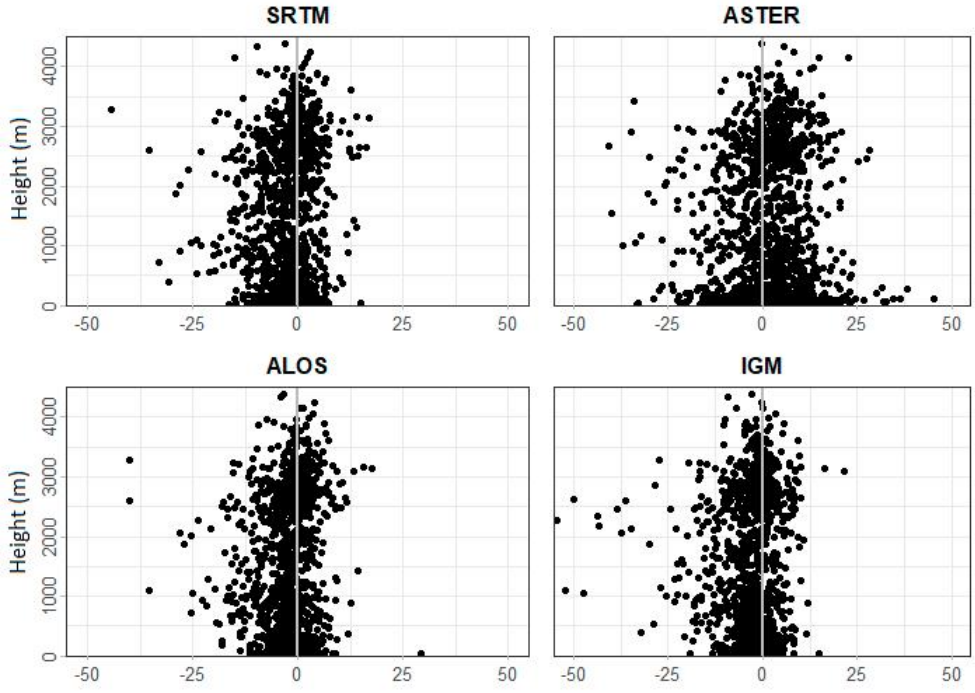

Figure 19. Difference Dispersion of the ellipsoidal heights.

A similar behavior is identified in Figure 20 in the typology and location of outliers in the MDTs SRTM, ALOS PALSAR and IGM, where the largest number of outliers of differences with high values surrounded by differences with low values are present in southern Ecuador, while there is a greater presence of outliers of differences with low values surrounded by differences with high values in central and northern Ecuador. The MDT ASTER GDEM does not present areas where there is a predominance of some type of atypical value. The percentages of outliers of ellipsoidal heights with respect to the original orthometric heights outliers of the MDTs show a slight reduction in the percentage of high-low outliers and a slight increase in the percentage of low-high outliers.

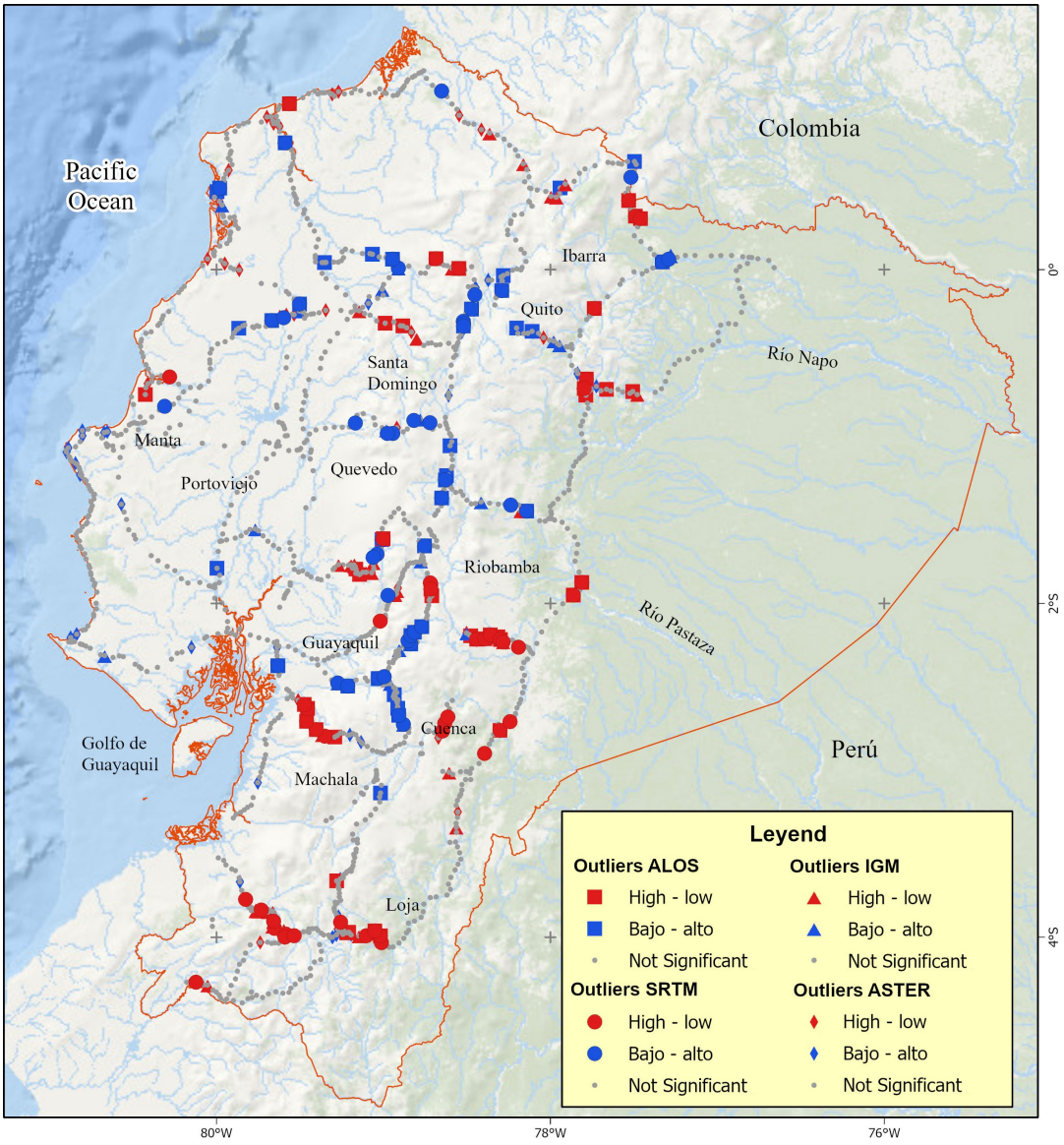

Figure 20. Outliers of differences in ellipsoidal heights.

3.4 Evaluation of orthometric height of the MDTs with MG EGM 08

New orthometric heights for the MDTs were calculated from the replacement of MG EGM 96 by MG EGM 08. In the case of MDT ALOS PALSAR, equation 1 and MG EGM 08 were used to obtain the new orthometric height. The box diagrams, the dispersion of heights regarding the evaluation height and the analysis of outliers did not vary visually with respect to the origin of the reference of the orthometric heights assessment of the MDTs with the MG EGM 96, but there was a slight improvement in the RMSE. Table 6 shows the results of the RMSE analysis of the MDTs with orthometric heights with MGs EGM 96, 08 and ellipsoidal heights.

Table 6. RMSE of the different height systems of the MDTs.

MDTs	Orthometric EGM 96 (m)	Orthometric EGM 08 (m)	Ellipsoidal (m)
SRTM 90	11.20	11.19	10.25
SRTM 30	7.97	7.92	7.06
ASTER	10.76	10.71	10.05
ALOS 30	7.75	7.67	6.87
ALOS 12.5	7.57	7.47	6.74
IGM	8.54	8.50	7.96

4 Discussion

Mancero et al. (2015) used 28 points to evaluate the 90-meter MDT SRTM in the areas of Carchi, Imbabura and Pichincha, located in the northern of Ecuador, determining that the model has an RMSE of 21 (m), and highlighting that the sites with high slope has an influence on vertical precision, data gaps and the sign of errors, while in the sites with low and medium slope, the errors are minor. The RMSE obtained in Mancero et al. (2015) differ from the ones obtained in this research, because the points used in this research were captured in the roads of Ecuador, hence the heights were better adapted to the shape of the terrain with respect to the heights captured in areas of high relief or where vegetation prevails.

Falorni et al. (2005) used 112 points to evaluate the MDT SRTM in the basins of the Washita and Tolt rivers in the United States. Washita characterizes by having a low relief topography except for the steeper hills located in the central part of the north of the basin. Tolt is characterized by a changing topography from the rugged mountains of the easternmost part of the basin, with a high relief and steep slopes to the lowland plains. The difference with the National Geodetic Survey (NGS) was 7.18 RMSE, while with GPS it was 8.94 RMSE. Hirt et al. (2010) determined that the 90-meter MDT SRTM for Australia has a 6-meter RMSE. With all the results presented, it is shown that the RMSE obtained from the MDT SRTM in both resolutions are within the expected range.

Hirt et al. (2010) determined that the MDT ASTER GDEM in Australia has an RMSE of 15 meters. Zhang et al. (2017) evaluated the vertical accuracy of the MDT ASTER GDEM in the northern margin of the Tibetan plateau, for this purpose 89 GPS control points were used and the normal heights were transformed using the MG EGM 96; thus, it was determined that the standard deviation between the MDT ASTER GDEM and the points was 9.3 meters. With all the results presented, it is shown that the RMSE obtained from the MDT ASTER GDEM is within the expected range.

Tierra (2009) used 144 points to evaluate the accuracy of global geopotential models (GGMs) EGM 96 and EGM 08 in continental Ecuador, determining that the MG EGM96 has a standard deviation of 1.35 meters, while the MG EGM08 has a standard deviation of 0.93 meters. The results obtained in Tierra (2009) agree with the results obtained in this research, confirming the improvement between the MG EGM 08 compared to the MG EGM 96, although in both cases geoidal pseudo-undulations were used to evaluate the MGs. Although the evaluation process of the IGM MG is not detailed in Tierra and Acurio (2014), it is difficult to define the reason for discrepancy with the result obtained, but this research maintains the obtained RMSE, since the statistics that support them were presented.

Kotsakis et al. (2010) used 1542 points with GPS data and the level of the Hellenic national triangulation network to evaluate the accuracy of the MG EGM 08, determining a deviation of 0.14 meters. Martínez and Bethencourt (2012) used the high-precision geometric 160-kilometer leveling line existing in Puerto Rico to determine the accuracy of the MGs EGM 96 and EGM 08, determining that the standard deviation of the MG EGM96 is 0.055 meters, while the EGM08 was 0.029 meters. Both studies demonstrate how the steep relief of Ecuador has influenced the loss of precision.

5 Conclusions

The MDT ALOS PALSAR showed the best statistical characteristics, both with orthometric and ellipsoidal heights. MDTs SRTM 30, ALOS PALSAR 30 and 12.5 meters, and IGM can be used in projects that require a vertical accuracy of less than 10 meters or generate maps at a scale of less than 1:50 000, in any height system, either ellipsoidal height or orthometric height.

Spatial resolution is a factor that directly influences the vertical accuracy of MDTs. The 30-meter MDT SRTM improved the RMSE by about 3 meters in all height systems over the 90-meter MDT SRTM, while the 12.5-meter MDT ALOS PALSAR improved the 20-centimeter RMSE over the 30-meter MDT ALOS PALSAR.

The evaluation of the MGs allows to determine that the MG EGM 08 can be used in projects that require orthometric heights with a vertical precision of less than 1.25 meters or a scale less than 1:5 000, since the ellipsoidal heights have a centimeter accuracy greater than 40 centimeters. The MGs EGM 96 and IGM can be used in projects that require an orthometric height with precision lower than 2.5 meters or a working scale of less than 1:10 000, since the ellipsoidal heights have centimeter accuracy greater than 80 centimeters.

Acknowledgment

The authors thank Geoint CÍA LTDA for providing access to the software used and the Military Geographic Institute for providing the information of the geometric leveling project and the digital elevation model that allowed conducting this research.

References

Abbondati, F., Oreto, C., Viscione, N., and Biancardo, S. A. (2020). Rural road reverse engineering using bim: an italian case study. In Vilnius Gediminas Technical University, D. o. C. E. . P., editor, *Environmental Engineering. Proceedings of the International Conference on Environmental Engineering*, volume 11, pages 1–7.

Alaska Satellite Facility (2021). Alos palsar – radiometric terrain correction. Alaska Satellite Facility0. Online:https://n9.cl/91h0u.

Anselin, L. (1995). Local indicators of spatial association-lisa. *Geographical analysis*, 27(2):93–115. Online:https://n9.cl/buf79h.

Cañizares, E. (2015). Análisis del sistema de alturas para la red de control básico vertical del ecuador. Master's thesis, Universidad de las Fuerzas Armadas ESPE.

Drewes, H., Sánchez, L., Blitzkow, D., and Freitas, S. (2002). Scientific foundations of the sirgas vertical reference system. In *Vertical Reference Systems: IAG Symposium Cartagena, Colombia, February 20-23, 2001*, pages 297–301.

ESRI (2020). Cómo funciona análisis de clúster y de valor atípico (i anselin local de moran). Esri. Online:https://n9.cl/1f7r2.

Falorni, G., Teles, V., Vivoni, E., Bras, R., and Amaratunga, K. (2005). Analysis and characterization of the vertical accuracy of digital elevation models from the shuttle radar topography mission. *Journal of Geophysical Research: Earth Surface*, 110(F2):Online:https://n9.cl/xtg90.

Federal Geographic Data Committee (1998). *Geospatial Positioning Accuracy Standards Part 3: National Standard for Spatial Data Accuracy*. U.S. Geological Survey.

Gil-Docampo, M., Peña Villasenín, S., Bettencourt, A. M., Ortiz-Sanz, J., and Peraleda-Vázquez, S. (2023). 3d geometric survey of cultural heritage by uav in inaccessible coastal or shallow aquatic environments. Technical report, Archaeological Prospection.

Hirt, C., Filmer, M., and Featherstone, W. (2010). Comparison and validation of the recent freely available aster-gdem ver1, srtm ver4. 1 and geodata dem-9s ver3 digital elevation models over australia. *Australian Journal of Earth Sciences*, 57(3):337–347. Online:https://n9.cl/2l9cp.

Kotsakis, C., Katsambalos, K., Ampatzidis, D., and Gianniou, M. (2010). *Gravity, Geoid and Earth Observation*, chapter Evaluation of EGM08 Using GPS and Leveling Heights in Greece, pages 481–488. International Association of Geodesy Symposia.

Lemoine, F., Kenyon, S.and Factor, J., Trimmer, R., Pavlis, N., Chinn, D., Cox, C., Klosko, S., Luthcke, S., Torrence, M., Wang, Y., Williamson, R., Pavlis, E., Rapp, R., and Olson, T. (1998). The development of the joint nasa gsfc and the national imagery and mapping agency (nima) geopotential model egm96. Technical report, National Aeronautics and Space Administration, Greenbelt.

Li, Z., Zhu, C., and Gold, C. (2004). *Digital terrain modeling: principles and methodology*. CRC press.

Luna, M. P., Staller, A., Toulkeridis, T., and Parra, H. (2017). Methodological approach for the estimation of a new velocity model for continental ecuador. *Open Geosciences*, 9(1):719–734.

Mancero, H., Toctaguano, D., Tacuri, C., Kirby, E., and Tierra, A. (2015). Evaluación de modelos digitales de elevación obtenidos por diferentes sensores remotos. In *Congreso de Ciencia y Tecnología ESPE*, pages 107–111.

Martínez, E. and Bethencourt, A. (2012). Comparación de la precisión de los modelos geopotenciales egm96 y egm08 en la zona del caribe. In *X Topcart 2012. I Congreso Iberoamericano de Geomática y Ciencias de la Tierra*.

McClean, F., Dawson, R., and Kilsby, C. (2020). Implications of using global digital elevation models for flood risk analysis in cities. *Water Resources Research*, 56(10):e2020WR028241.

Orejuela, I., González, C., Guerra, X., Mora, E., and Toulkeridis, T. (2021). Geoid undulation modeling through the cokriging method–a case study of guayaquil, ecuador. *Geodesy and Geodynamics*, 12(5):356–367.

Pavlis, N., Holmes, S., Kenyon, S., and Factor, J. (2012). The development and evaluation of the earth gravitational model 2008 (egm2008). *Journal of geophysical research: solid earth*, 117. Online:Online:https://n9.cl/f0ntr7.

Peña Villasenín, S., Gil-Docampo, M., and Ortiz-Sanz, J. (2017). 3-d modeling of historic façades using sfm photogrammetry metric documentation of different building types of a historic center. *International Journal of Architectural Heritage*, 11(6):871–890.

Rabus, B., Eineder, M., Roth, A., and Bamler, R. (2003). The shuttle radar topography mission-a new class of digital elevation models acquired by spaceborne radar. *ISPRS journal of photogrammetry and remote sensing*, 57(4):241–262. Online:https://n9.cl/hkgft.

Sánchez, L. (2015). Iag resolutions adopted by the iag council at the xxvith iugg general assembly. In *XXVIth IUGG General Assembly*, Praga. IAG.

Seeber, G. (1993). *Satellite Geodesy: foundations, methods, and applications*. Walter de Gruyter GmbH y Co. KG.

Sinde-González, I., Gil-Docampo, M., Arza-García, M., Grefa-Sánchez, J., Yánez-Simba, D., Pérez-Guerrero, P., and Abril-Porras, V. (2021). Biomass estimation of pasture plots with multitemporal uav-based photogrammetric surveys. *International Journal of Applied Earth Observation and Geoinformation*, 101:102355.

Tachikawa, T., Kaku, M., Iwasaki, A., Gesch, D., Oimoen, M., Zhang, Z., Danielson, J., Krieger, T., Curtis, B., and Haase, J. (2011). Aster global digital elevation model version 2-summary of validation results. Technical report, NASA.

Tierra, A. (2009). Evaluación del egm08 y egm96 en el ecuador a partir de datos de gps y nivelación geométrica. *Revista Geoespacial*, 6:72–80. Online:https://n9.cl/udlci.

Tierra, A. and Acurio, V. (2014). Modelo neuronal geoidal geométrico del ecuador continental - mngge14. In *Simposio SIRGAS 2014*.

Zafar, S. and Zaidi, A. (2019). Impact of urbanization on basin hydrology: a case study of the malir basin, karachi, pakistan. *Regional Environmental Change*, 19:1815–1827.

Zhang, G., Shen, W., Zhu, Y., Wang, Y., and She, Y. (2017). Evaluation of aster gdem in the northeastern margin of tibetan plateau in gravity reduction. *Geodesy and Geodynamics*, 8(5):335–341. Online:https://n9.cl/jztl4g.

La Granja:
Revista de
Ciencias de la Vida

pISSN:1390-3799; eISSN:1390-8596

http://doi.org/10.17163/lgr.n38.2023.06

Scientific paper/ Artículo científico

Biotechnology

First Report of Endophytic Bacteria Isolated from *Senecio glaucus* L., Egypt

Primer Informe de Bacterias Endofíticas Aisladas de *Senecio glaucus* L., Egipto

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Article received on February 15th, 2022. Accepted, after review, on June 28th, 2022. Published on September 1st, 2023.

Abstract

Microorganisms are naturally associated with plants in several ways. The study was conducted to isolate bacteria endophytes from the internal cells of roots, stems, leaves, and capitula of *Senecio glaucus* collected from 2 diverse (coastal and desert) habitats in Egypt. A total of 10 endophytic bacteria were obtained from the isolation; the highest diversity of bacterial endophytes was observed in desert samples roots and leaves. The isolates were recognized based on morphology, biochemical and 16S rRNA sequence genes. All isolates indicated the ability for enzyme production as amylase, cellulase, lipase, catalase, and protease in their biochemical descriptions; analyses also gave a significant indication of their potential to produce plant growth hormones, as their ability to dissolve Phosphate. In the world and Egypt, we are the first to report bacterial endophytes isolated from *Senecio glaucus*. This study could aid in determining the role of endophytic bacteria in severe habitats, as well as their potential applications in medicine, bioremediation, agriculture, and industry.

Keywords: Bacterial endophytes, Biochemical, 16S rRNA, Senecio, *Asteraceae*.

Resumen

Los microorganismos están naturalmente asociados con las plantas. El presente experimento se llevó a cabo para aislar bacterias endófitas de las células internas de raíces, tallos, hojas y Tejido capitulear de *Senecio glaucus* recolectadas en 2 hábitats diversos (costeros y desérticos) de Egipto. Del aislamiento se obtuvieron un total de 10 bacterias endófitas; la mayor diversidad de endófitos bacterianos se observó en raíces y hojas de muestras del desierto. Los aislamientos se reconocieron con base en la morfología, la bioquímica y los genes de la secuencia del ARNr 16S. Todos estos aislados indican la capacidad de producir enzimas como amilasa, celulasa, lipasa, catalasa y proteasa en sus descripciones bioquímicas; los análisis también mostraron una indicación significativa de su potencial para producir hormonas de

crecimiento vegetal; como su capacidad para disolver el fosfato. En el mundo y en Egipto, somos los primeros en reportar endófitos bacterianos aislados de *Senecio glaucus*. Este estudio podría ayudar a determinar el papel de las bacterias endófitas en hábitats severos, así como sus posibles aplicaciones en medicina, biorremediación, agricultura e industria.

Palabras clave: Endófitos bacterianos, Bioquímica, 16S rRNA, Senecio, *Asteraceae*.

Suggested citation: Sabry Sultan, M., Elsayed, A. and Ahmed El-Amier, Y. (2023). First Report of Endophytic Bacteria Isolated from *Senecio glaucus* L., Egypt. La Granja: Revista de Ciencias de la Vida. Vol. 38(2):80-93. http://doi.org/10.17163/lgr.n38.2023.06.

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1 Introduction

How to define an endophyte is a point of contention. It was suggested that bacteria that are isolated from the internal tissues of the plant and that do not cause any damage to their host are classified as endophytes. Other descriptions recommend that it is essential to establish that the bacterial occupation is of the inner tissues of the plant. Altruism, commensalisms, symbiosis, or passivity to pathogenicity have been used to describe this unique host endophyte interaction; so, on the specific relationships involved, internal plant colonization by bacteria constitutes a vast and, yet little mapped ecological niche (Kobayashi and Palumbo, 2000; Hallmann et al., 1997). The bacterial diversity that has been reported as endophytes spans a variety of important Gram-negative and -positive bacteria that contain genera of Alpha-, Beta- and Gammaproteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes (Bacon and Hinton, 2007; Lodewyckx et al., 2002).

Nearly 1250 Senecio species are widely distributed and comprise about 6 species that occur in Egypt including *S. glaucus, S. flavus, S. aegyptius, S. Vulgaris, S. hoggariensis*, and *S. belbeysius*. This genus is important due to its pharmacological, botanical, and toxicological properties (Singh et al., 2017a; Nori-Shargh et al., 2008). A survey on the phytochemical examination of Senecio extracts revealed antioxidant, antimicrobial, cytotoxic activity (Tundis et al., 2009), anti-inflammatory, insecticidal and antiviral properties (Sultan et al., 2022; El-Amier et al., 2014; Joshi et al., 2013; Kahriman et al., 2011).

Species of Senecio that inhabit sandy plains and desert wadies are used as a sedative of the central nervous system, diuretic, and emetic in Egypt (Eissa et al., 2014). *Senecio glaucus* L. (Morrar) is an annual herb that grows in Egypt and has two subspecies; *S. glaucus* subsp. *coronopifloius* (Maire) C. Alexander. Subsp. *coronopifloius* and *S. glaucus* L. subsp. *glaucus* grows in desert wadis, saline soils, coastal sandy, and cultivation edges and it is the most common in Egypt than subsp. *glaucus Boulos2002*.

Endophytes may benefit plants indirectly by improving the herbivore's infections or stress resistance, or by further unexplained processes (Schulz and Boyle, 2005). Endophytes have been found in several studies to be able to protect their plant hosts from drought (Clay and Schardl, 2002). Infected plants with endophytes showed salt and temperature tolerance, according to Waller et al. (2005). Endophytes function as a biological trigger to stimulate the stress response more quickly and robustly than non-symbiotic plants, promoting plant growth and protecting the plant to reduce diseases and insect pests, according to Redman et al. (2002).

Endophytic bacteria can solubilize phosphate and provide plants with assimilable nitrogen (Rosenblueth and Martínez-Romero, 2006). Furthermore, interactions between plants and endophytic bacteria may aid in ecosystem restoration processes, protecting plants from biotic and abiotic stress and promoting the production of important secondary metabolites (Mowafy et al., 2021; Cheng et al., 2019; Müller et al., 2015; Alavi et al., 2013).

The genetic background of plant host species, appropriateness, nutrients, and ecological niches (Jia et al., 2016); environmental circumstances, host genotypes, bacterial species (Chebotar et al., 2015); and host developmental stage and inoculum density (Dudeja and Giri, 2014), all have a significant impact on the endophytic bacteria population.

Some cold-resistant bacteria were discovered in the roots and leaves of *Senecio vulgaris* and defined as core bacterial operational taxonomic units and reported as having an apparent strong antibacterial effect and the ability to survive in extremely low temperatures, dry, and UV-contaminated settings (Gaspard and Rice, 1989; Koo et al., 2016; Vishnivetskaya et al., 2009).

Endophytes are advantageous to *S. vulgaris* (Cheng et al., 2019; Singh et al., 2016), and their application to rice resulted in a reduction of arsenic accumulation and generation of IAA, which aids in growth promotion; heavy metal resistance, particularly cadmium tolerance; and nitrogen fixation ability (Purchase et al., 1997); and maize and lettuce plant growth promotion (Gamel et al., 2017; Chabot et al., 1996).

Because of its long history of usage in traditional medicine and selection in a variety of climatic, edaphic, and biotic habitats in geographically different places, *S. glaucus* exhibits amazing diversity.

It was reported that 10^2 to 10^4 endophytic bacteria populations exist per plant tissue gram (Kobayashi and Palumbo, 2000). This study aims to assess the variety of bacterial endophytes communities isolated from *S. glaucus* in two different habitats in Egypt: Gamasa City (Mediterranean Coastal) and Wadi Araba (Eastern Desert).

2 Materials and Methods

2.1 Plant Material Collection

Healthy entire plants of *S. glaucus* in the flowering phases were randomly taken from two separate locations Wadi Araba (Eastern Desert, 29°4′23.72″N 32°25′38.49″E) and Gamesa City (Mediterranean coast, 31°26′58.78″N 31°28′36.14″E) for the isolation of bacterial endophytes. Samples were packed in clean plastic bags and transported to the Microbiology Laboratory for further testing as shown in Figure 1.

2.2 Isolation and Purification of Endophytes

The isolation and purification of endophytes were done according to the procedure by Bacon and Hinton (2002) using LB agar medium (1.25 gm yeast extract, 2.5 gm peptone, 2.5 gm sodium chloride, 3.75 agar, and 250 ml distilled water). The plant samples were first washed under tap water then separated into 4 parts including root, stem, leaf, and capitula, then surface sterilized resulting in the (Geris dos Santos et al., 2003) method.

Surface sterilization was achieved by rinsing the plant parts with 70% ethanol (C_2H_5OH) for 30 seconds, then 0.5 percent sodium hypochlorite (NaOCl) for 2-3 minutes, and finally sterile distilled water (Dil.H_2O) for 10 minutes (2-3 times). After that, the plant material was dried between the folds of sterile filter papers. With a flame sterilized scalpel, the cut ends of surface-sterilized segments were removed and placed in appropriate LB agar media, with the cut surface touching the agar. The maximum possible colonies of bacterial endophytes were determined by incubating plates at 35 °C for 48 hours.

2.3 Characterization of Endophytic Bacteria

2.3.1 Morphological characterization

Aneja et al. (2006); Cappuccino and Sherman (1992) used the standard gram staining procedure to determine cell shape, colony color, and texture were used to define the isolates to establish the morphology of the bacterial cells.

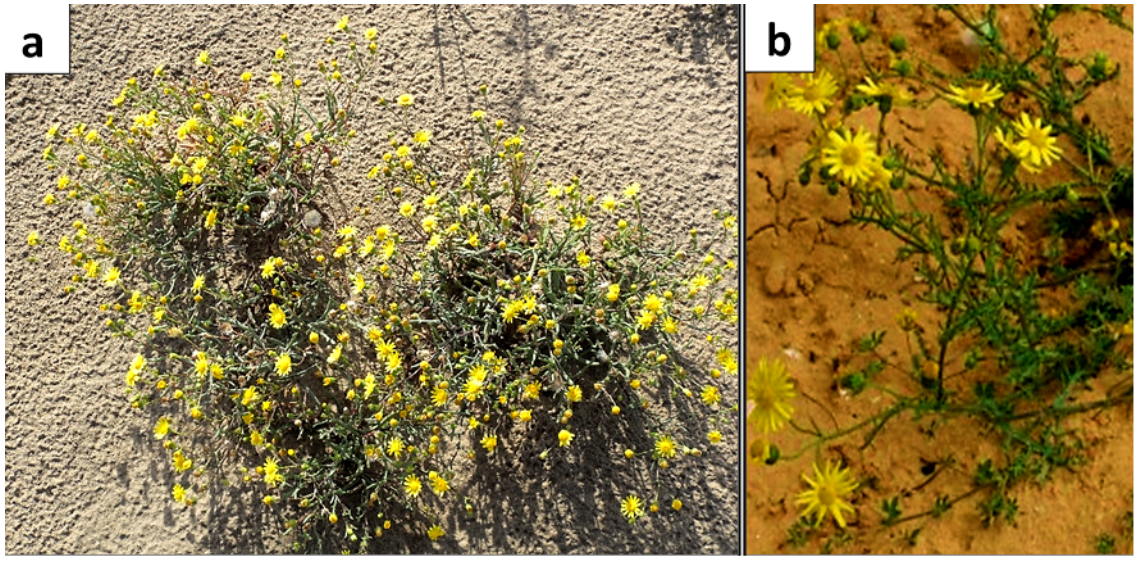

Figure 1. a) General views of *S. glaucus*, and b) Close-up views of *S. glaucus* in the study area.

2.3.2 16S rRNA gene sequencing

The isolated bacteria were molecularly identified using the MicroSeq® 500 16SrRNA Bacterial Identification Kits methodology. The sequencing reactions were carried out in the 9700 thermal cyclers with a total volume of 20 l (7 l purified PCR product and 13 l sequencing module) by setting the thermal cycler to 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 seconds (25 cycles). The Dye ExTM 2.0 Spin Kit was then used to remove the excess dye terminators and primers from the cycle sequencing reaction (Qiagen PN 63204). Finch TV (version1.4.0) and MEGA-X (version10.2.5) software were used to analyze the sequences, and Seaview software was used to create phylogenetic trees using the closest published type of strain sequences. The sequences of the isolates obtained in this investigation were submitted to the NCBI's GeneBank database.

2.4 Statistical Analysis

The trials were carried out in triplicates, with the mean standard deviation (MSD) calculated.

3 Results

In this study, 10 bacterial endophytes were isolated from different parts of *Senecio glaucus* plant collected from 2 different places (4 isolates from the Mediterranean coastal plant and 6 isolates from the desert plant) on L.B agar medium under aseptic conditions and according to the difference in morphology as shown in Figures 2 and 3, and have codes (SGC-R, SGC-S, SGC-L, SGC-C) for the coastal samples and (SGD-R, SGD-S, SGD-L, SGD-C) for desert samples.

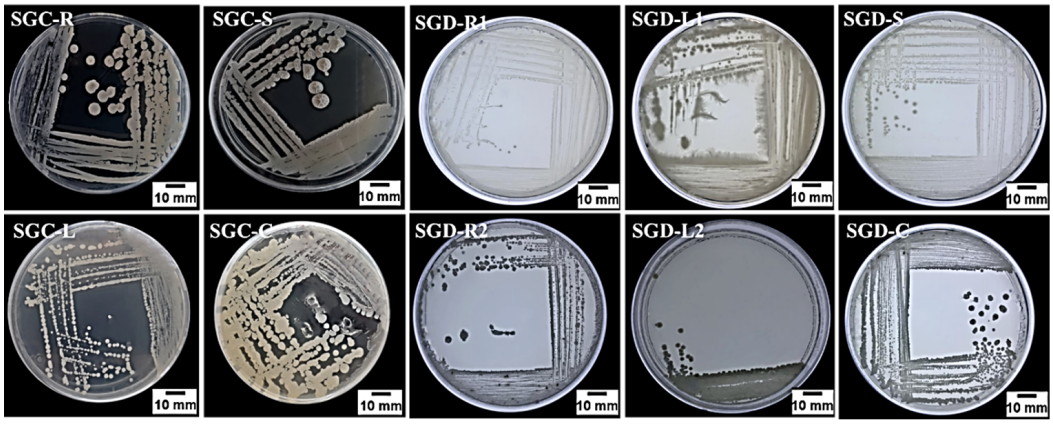

Figure 2. Bacterial endophytes isolated from *S. glaucus* SGC-R: *Senecio glaucus* Coastal-Root, SGC-S: -Stem, SGC-L: -Leaf, and SGC-C: -Capitula; SGD-R: *S. glaucus* Desert-Root, SGD-S: -Stem, SGD-L: -Leaf, and SGD-C: -Capitula.

The bacterial isolates were characterized morphologically according to colony shape, margin, elevation, texture, and pigmentation as shown in Table 1, and were scanned microscopically according to cell shape, whereas all isolates were rod shape and Gram stain, whereas the coastal sample showed 3 strains to be Gram-positive and 1 strain Gram-negative, by the other side the desert sample showed 3strains Gram-positive and 3 strains Gram-negative (Table 2).

The purified isolates were biochemically characterized according to enzymatic activity and function properties. The isolates showed an ability to produce a variety of enzymes (Tables 3 and 4).

Both coastal and desert bacterial isolates were able to produce indole with variable concentrations ranging from high to low compared with the control sample. The isolates SGC-L and SGC-C presented very weak results; while the isolates SGD-L2 and SGD-C did not indicate any positive results as shown in Table 4.

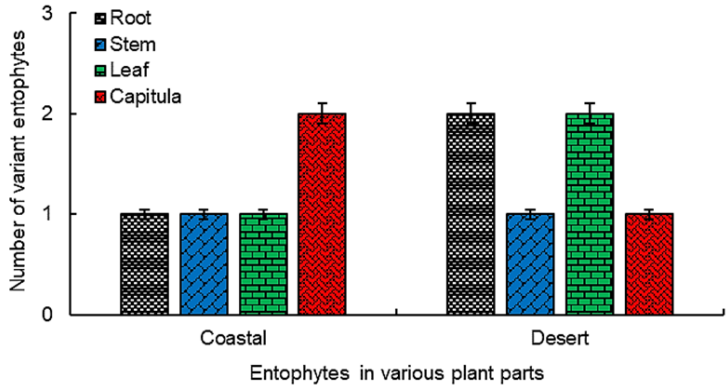

Figure 3. Number of the endophytic bacteria isolated from different tissues of the medicinal plant *S. glaucus* collected from coastal and desert habitats.

Table 1. Morphological characteristics of colonies of endophytic bacteria isolated from different tissues of the medicinal plant *S. glaucus* collected from coastal and desert habitats in Egypt.

Isolates	Tissue origin	Colony characterization					
		Size (mm)	Colony shape	Margin	Elevation	Texture	Pigmentation
Coastal sample							
SGC-R	Root	3.5	Irregular	Curled	Umbonate	Dry/Rough	Off-white
SGC-S	Stem	3.7	Irregular	Curled	Umbonate	Dry/Rough	Off-white
SGC-L	Leaf	1.5	Irregular	Lobate	Raised	Dry/Rough	Off-white
SGC-C	Capitula	2.5	Irregular	Curled	Umbonate	Dry/Rough	Off-white
Desert sample							
SGD-R1	Root	1.2	Circular	Entire	Raised	Creamy	Yellowish white
SGD-R2		1.4	Irregular	Lobate	Raised	Dry/Rough	Off-white
SGD-S	Stem	1.9	Circular	Entire	Raised	Creamy	Yellowish white
SGD-L1	Leaf	1.3	Irregular	Filamentous	Flat	Shiny Creamy	Pale Yellow
SGD-L2		2.1	Circular	Entire	Convex	Dry/Rough	Chalky White
SGD-C	Capitula	2.5	Irregular	Curled	Umbonate	Dry/Rough	Off-white

SGC-R: *Senecio glaucus* Coastal-Root, SGC-S: *S. glaucus* Coastal-Stem, SGC-L: *S. glaucus* Coastal-Leaf, and SGC-C: *S. glaucus* Coastal-Capitula; SGD-R: *S. glaucus* Desert-Root, SGD-S: *S. glaucus* Desert-Stem, SGD-L: *S. glaucus* Desert-Leaf, and SGD-C: *S. glaucus* Desert-Capitula.

Based on 16S rRNA gene sequence analysis, the isolated strains were identified as *Bacillus velezensis* strain CBMB205 (NR_116240.1), *Bacillus velezensis* strain CBMB205 (NR_116240.1), *Bacillus amyloliquefaciens* strain WS3-1 (MT579842.1), *Klebsiella aerogenes* strain ATCC13048 (NR_118556.1), *Enterobacter bugandensis* strain 247BMC (NR_148649.1), *Enterobacter hormaechei* subsp. *xiangfangensis* strain 10-17 (NR_126208.1), *Sphingobacterium faecium* strain DSM11690 (NR_025537.1), and *Kitasatospora aburaviensis* strain NBRC12830 (NR_112295.1); all the strains were correlated in the genetic distance as shown in Table 5 and Figure 4.

Table 2. Morphological characteristics of cells of endophytic bacteria isolated from different tissues of the medicinal plant *S. glaucus* collected from coastal and desert habitats in Egypt.

Isolates	Tissue origin	Cell Characteristics			
		Gram stain	Cell shape	Size (μm)	Motility
Coastal sample					
SGC-R	Root	Gram-positive	Rod	2.5	Motile
SGC-S	Stem	Gram-positive	Rod	2.5	Motile
SGC-L	Leaf	Gram-negative	Rod	1.2	Motile
SGC-C	Capitula	Gram positive	Rod	2.7	Motile
Desert sample					
SGD-R1	Root	Gram-negative	Rod	1.6	Motile
SGD-R2		Gram-negative	Rod	1.3	Motile
SGD-S	Stem	Gram-negative	Rod	1.7	Motile
SGD-L1	Leaf	Gram-positive	Rod	1.9	Motile
SGD-L2		Gram-positive	Rod	2.3	Motile
SGD-C	Capitula	Gram positive	Rod	1.8	Motile

SGC-R: *Senecio glaucus* Coastal-Root, SGC-S: *S. glaucus* Coastal-Stem, SGC-L: *S. glaucus* Coastal-Leaf, and SGC-C: *S. glaucus* Coastal-Capitula; SGD-R: *S. glaucus* Desert-Root, SGD-S: *S. glaucus* Desert-Stem, SGD-L: *S. glaucus* Desert-Leaf, and SGD-C: *S. glaucus* Desert-Capitula.

Table 3. Qualitative analysis of the biochemical characterization of the endophytic bacteria isolated from different tissues of the medicinal plant *S. glaucus* collected from different habitats in Egypt.

Isolates	Tissue organ	Biochemical Characterization					
		Catalase	Amylase	Cellulase	Protease	Lipase	H_2S
Coastal							
SGC-R	Root	+ve	+ve	+ve	+ve	+ve	-ve
SGC-S	Stem	+ve	+ve	+ve	+ve	+ve	-ve
SGC-L	Leaf	+ve	+ve	+ve	+ve	+ve	-ve
SGC-C	Capitula	+ve	+ve	+ve	+ve	+ve	-ve
Desert							
SGD-R1	Root	+ve	+ve	+ve	-ve	+ve	-ve
SGD-R2		+ve	+ve	+ve	+ve	+ve	-ve
SGD-S	Stem	+ve	+ve	+ve	-ve	+ve	-ve
SGD-L1	Leaf	+ve	+ve	+ve	-ve	+ve	-ve
SGD-L2		+ve	+ve	+ve	+ve	+ve	-ve
SGD-C	Capitula	+ve	+ve	+ve	+ve	+ve	-ve

+ve: positive reaction; -ve: negative reaction.

4 Discussion

Bacterial endophytes have long been known to be present in most healthy plant tissues (McInroy and Kloepper, 1995; Sturz, 1995; Frommel et al., 1993). Endophytic bacteria have been found in every plant species studied, according to Partida-Martínez and Heil (2011) as well as in this study. Several plant species have been found to have diverse endophytic bacterial communities that showed significant phenotypic and genotypic diversity (Santoyo et al., 2016; Miliute et al., 2015). The study of population diversity of bacterial endophytes isolated from the desert sample of *S. glaucus* showed more diverse species than the coastal sample (Figure 3). The plant host species, host specificity, and tissue types can strongly affect the type of endophytic community

(Ding and Melcher, 2016). Qualitative and quantitative variations between plant species in microbial colonization are mainly due to genotypic host-endophyte compatibility and ecological conditions (tropical versus temperate) (Rajan, 2012).

Table 4. Qualitative analysis of Plant growth promoting (PGP) parameters of the bacterial endophytes isolated from the medicinal plant *S. glaucus*.

Isolates	Tissue organ	Plant growth promoting			
		Phosphate solubilization	Nitrate reductase	IAA	GA3
Coastal					
SGC-R	Root	+ve	+ve	+ve	+ve
SGC-S	Stem	+ve	+ve	+ve	+ve
SGC-L	Leaf	+ve	+ve	+ve	+ve
SGC-C	Capitula	+ve	+ve	+ve	+ve
Desert					
SGD-R1	Root	+ve	+ve	+ve	+ve
SGD-R2		+ve	+ve	-ve	+ve
SGD-S	Stem	+ve	+ve	+ve	+ve
SGD-L1	Leaf	+ve	+ve	+ve	+ve
SGD-L2		+ve	+ve	+ve	+ve
SGD-C	Capitula	+ve	+ve	-ve	+ve

+ve: positive reaction; -ve: negative reaction.

The strains were isolated from roots, stems, leaves, and capitula tissues of *S. glaucus*. The highest population of endophytes was obtained from the internal tissues of the roots and leaves of the plant (Figure 3). The colonies' morphology indicated the endophytes variation. The tested isolates were chosen for their morphological variation as well as their dominance (Table 1). A large variety of both Gram-negative and Gram-positive bacteria are involved in the endophytic bacteria (Lodewyckx et al., 2002). Interestingly, Gram-positive was the most distributed population in the coastal sample than Gram-negative isolates of *S. glaucus*. On the other hand, the Gram-negative population was equal to the Gram-positive isolates of the desert sample (Table 2), as reported in several plants. An equal presence of Gram-negative and Gram-positive bacteria were identified (Zinniel et al., 2002). Literature has reported a predominance of Gram-negative bacteria in the tissues of various plants (Elbeltagy et al., 2000; Stoltzfus et al., 1997). These bacterial species could have coevolved with the plant to be adapted to a specific arid habitat that is nutrient-poor. In response to environmental conditions such as pH, temperature, and salinity, the Gram-positive bacteria from the isolated strains form spores, which may provide a survival advantage.

All morphology of the isolates cell shape showed to be bacilli/rod (Table 2). According to Jacobs et al. (1985), *Erwinia* sp., *Enterobacter* sp., *Bacillus* sp., *Pseudomonas* sp., *Micrococcus*, *Microbacterium*, *Stenotrophomonas*, *Pantoea*, *Burkholderia*, *Pseudomonas* and *Flavobacterium* sp. were the most common isolated bacterial genera of endophytic bacteria in several plants like tomato, cotton, soybean, rice, and maize (Chaturvedi et al., 2016; Hallmann et al., 1997).

Endophytic bacteria have been isolated from Senecio species tissues previously. Cheng et al. (2019); Singh et al. (2016) isolate the endophytes *Brevundimonas diminuta* and *Rhizobium leguminosarum* from *S. vulgaris; Sphingomonas aerolata, Sphingomonas faeni, Exiguobacterium sibiricum* and *Oxalobacteraceae* (OTU3) were characterized in leaves and roots of *S. vulgaris* (Gaspard and Rice, 1989; Koo et al., 2016; Vishnivetskaya et al., 2009).

In this study, the obtained isolates were biochemically characterized according to enzymatic acti-

vity and function properties (Table 3). The isolates showed a variety ability to produce a variety of enzymes such as catalase enzymes, amylolytic, cellulolytic, proteolytic, and lipolytic enzymes. However, no survey on these enzymes' secretion by endophytes has been conducted (Elbeltagy et al., 2000; Reinhold-Hurek and Hurek, 1998). Endophytic bacteria might act as virulence factors for plant pathogenic bacteria due to the cellulases and hydrolytic enzymes may play a role in the mechanisms which enter and persist in the host plant as reported for *Enterobacter asburiae* JM22 *Quadt-Hallmann1997* and *Azoarcus* sp. (Hurek et al., 1994).

Table 5. The 16S rRNA gene reference sequence of the strains in the GenBank database.

Serial	Plant parts	Similar Strain	Similarity %	NCBI sequence
Costal sample				
SGC-R	Root	*Bacillus velezensis* strain CBMB205 (NR_116240.1)	96.08%	MZ520618.1
SGC-S	Stem	*Bacillus amyloliquefaciens* strain WS3-1 (MT579842.1)	99.80%	OK148122.1
SGC-L	Leaf	*Klebsiella aerogenes* strain ATCC13048 (NR_118556.1)	94.31%	MZ520791.1
SGC-C	Capitula	*Bacillus velezensis* strain CBMB205 (NR_116240.1)	99.80%	MZ520618.1
Desert sample				
SGD-R1	Root	*Enterobacter bugandensis* strain 247BMC (NR_148649.1)	99.91%	OK147922.1
SGD-R2		*Klebsiella aerogenes* strain ATCC13048 (NR_118556.1)	94.31%	OK057209.1
SGD-S	Stem	*Enterobacter hormaechei* subsp. *xiangfangensis* strain 10-17 (NR_126208.1)	94.03%	OK044126.1
SGD-L1	Leaf	*Sphingobacterium faecium* strain DSM11690 (NR_025537.1)	87.64%	OK156473.1
SGD-L2		*Kitasatospora aburaviensis* strain NBRC12830 (NR_112295.1)	100%	MZ477009.1
SGD-C	Capitula	*Bacillus velezensis* strain CBMB205 (NR_116240.1)	98.06%	OK147924.1

Endophytic bacteria isolated from the coastal *S. glaucus* indicated the highest production of the studied enzymes than the desert isolates, despite the high diversity in the desert *S. glaucus* sample (Figures 4 and 5). All isolates secreted amylases, cellulases, protease, and lipase except *Enterobacter hormaechei* subsp. *Xiangfangensis* and *Sphingobacterium faecium* could not produce protease enzymes (Table

3). The cellulosic activity of these endophytes may give an advantage for intercellular entry and spreading of endophytes into the host plant, as the host plant's cell wall contains cellulose (Hallmann et al., 1997). Hydrolases, extracellular enzymes produced by endophytic bacteria, aid in the establishment of systemic resistance to pathogen invasion in plants (Singh et al., 2017b; Elbeltagy et al., 2000).

In addition, all isolates were biochemically characterized according to function properties (Table 4). The isolates showed ability to produce a variation of phytohormones that can help plants and can be used as PGPB indole acetic acid, and gibberellic acids as they also indicated their ability to solubilize phosphate and nitrate reductase. On the other hand, all isolates showed a negative result for hydrogen sulfide production. Likewise, the strains SGD-R2 and SGD-C isolated from the desert sample were negative for IAA.

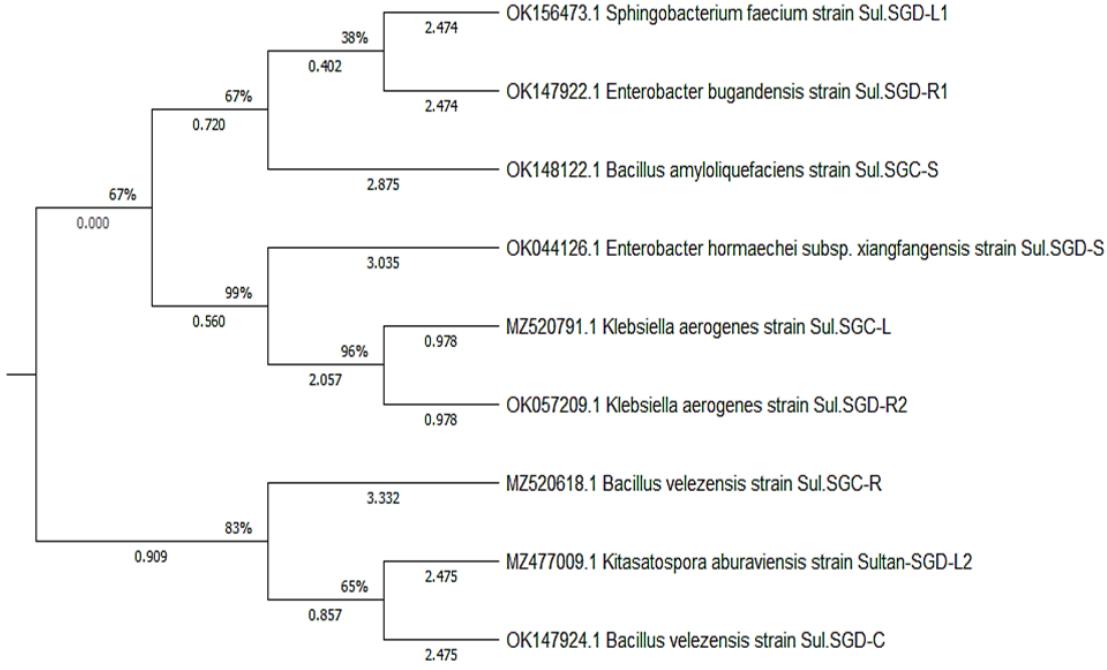

Figure 4. The phylogenetic tree derived from 16S rRNA gene sequences of the 10 bacterial endophytes strains.

Ten strains with various colony morphologies were isolated and their 16S rRNA gene sequences were analyzed for taxonomic relationships (Table 5 and Figure 6). Non *B. japonicum* bacteria were found in the isolates from surface-sterilized *S. glaucus* tissues studied, and most of them were morphologically unique. According to phylogenetic analysis, the isolates were shown to belong to four extremely different phyla already known to be plant-associated: Bacteroidetes, Proteobacteria, Actinobacteria, and Firmicutes (Reinhold-Hurek et al., 2015). Based on 16S rRNA gene sequence analysis, the isolated strains were identified as *Bacillus velezensis* strain CBMB205 and *Bacillus amyloliquefaciens* strain WS3-1 (Class: Bacilli), *Enterobacter bugandensis* strain 247BMC, and *Enterobacter hormaechei* subsp. *xiangfangensis* strain 10-17 (Class: Gamma Proteobacteria), *Sphingobacterium faecium* strain DSM 11690 (Class: Flavobacteria), *Klebsiella aerogenes* strain ATCC 13048 (Class: Gamma Proteobacteria), and *Kitasatospora aburaviensis* strain NBRC 12830 (*Streptomyces aburaviensis*) (Class: Actinomycetes). The sequence analysis revealed that the isolates may contain previously unknown bacterial species: strains from two phylotypes showed less than 98.7% identity to previously reported 16S rRNA genes of known species. They are likely to represent at least unique species, given this value it has recently

been proposed as a "gold standard" for distinguishing species (Stackebrandt, 2006). All the strains were correlated in genetic distance. The phylogenetic dendrogram illustrated the correlation among six isolates was conducted by MEGA-X program as shown in Figure 4.

5 Conclusion

This study confirmed the diversity and occurrence of bacterial endophytes in different parts of *Senecio glaucus* (Morrar) collected from different habitats in Egypt. These bacteria might be promising candidates for future applications. The isolation of Bacillus strains opens up biotechnological options for *S. glaucus* production and the prospective application of putatively unique species. Through the biochemical descriptions of these isolates, they show their ability to produce some decomposing enzymes such as cellulase, amylase, protease, catalase, and lipase. On the other hand, the descriptive analyzes showed a strong indication of their ability to produce some plant growth hormones that can increase growth and protect plants such as their ability to produce nitrate reductases, phosphate solubilization, indole, and gibberellins. The fact that these plants were successfully colonized by each microbe suggests that they could be used in many applications, such as bio-fertilizers, bioremediation, and biological control.

References

Alavi, P., Starcher, M., Zachow, C., Müller, H., and Berg, G. (2013). Root-microbe systems: the effect and mode of interaction of stress protecting agent (spa) *Stenotrophomonas rhizophila* dsm14405t. *Frontiers in Plant Science*, 4:141. Online:https://bit.ly/3lobBfA.

Aneja, M., Sharma, S., Fleischmann, F., Stich, S., Heller, W., Bahnweg, G., Munch, J., and Schloter, M. (2006). Microbial colonization of beech and spruce litter—influence of decomposition site and plant litter species on the diversity of microbial community. *Microbial Ecology*, 52:127–135. Online:https://bit.ly/3YqGLBz.

Bacon, C. and Hinton, D. (2002). Endophytic and biological control potential of *Bacillus mojavensis* and related species. *Biological Control*, 23(3):274–284. Online:https://bit.ly/3YLUaEh.

Bacon, C. and Hinton, D. (2007). *Plant-associated bacteria*, chapter Bacterial endophytes: the endophytic niche, its occupants, and its utility, pages 155–194. Online:https://bit.ly/414QjUo. Springer Dordrecht.

Cappuccino, J. and Sherman, N. (1992). *Microbiology: A Laboratory Manual*. Benjamin/Cummings Pub, 3 edition.

Chabot, R., Antoun, H., and Cescas, M. (1996). Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar. *phaseoli*. *Plant and Soil*, 184:311–321. Online:https://bit.ly/3UwY9DK.

Chaturvedi, H., Singh, V., and Gupta, G. (2016). Potential of bacterial endophytes as plant growth promoting factors. *J Plant Pathol Microbiol*, 7(9):1–6. Online:https://bit.ly/3UwYrdO.

Chebotar, V., Malfanova, N., Shcherbakov, A., Ahtemova, G., Borisov, A., Lugtenberg, B., and Tihonovich, I. (2015). Endophytic bacteria in microbial drugs that improve plant development (review). *Prikladnaia Biokhimiia i Mikrobiologiia*, 51(3):283–289. Online:https://bit.ly/3YOCojC.

Cheng, D., Tian, Z., Feng, L., Xu, L., and Wang, H. (2019). Diversity analysis of the rhizospheric and endophytic bacterial communities of *Senecio vulgaris* l.(asteraceae) in an invasive range. *PeerJ*, 6:e6162. Online:https://bit.ly/3YtFaLy.

Clay, K. and Schardl, C. (2002). Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *The American Naturalist*, 160(S4):S99–S127. Online:https://bit.ly/3I0qbl0.

Ding, T. and Melcher, U. (2016). Influences of plant species, season and location on leaf endophytic bacterial communities of non-cultivated plants. *PloS One*, 11(3):e0150895. Online:https://bit.ly/3K56zyR.

Dudeja, S. S. and Giri, R. (2014). Beneficial properties, colonization, establishment and molecular diversity of endophytic bacteria in legumes and non legumes. *African Journal of Microbiology Research*, 8(15):1562–1572. Online:https://bit.ly/3IkTzDY.

Eissa, T., Palomino, O., Carretero, M., and Gómez-Serranillos, M. (2014). Ethnopharmacological study of medicinal plants used in the treatment of cns disorders in sinai peninsula, egypt. *Journal of Ethnopharmacology*, 151(1):317–332. Online:https://bit.ly/3jRLWM7.

El-Amier, Y., Abdelghany, A., and Abed Zaid, A. (2014). Green synthesis and antimicrobial activity of senecio glaucus-mediated silver nanoparticles. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 5(5):631–642. Online:https://bit.ly/41oQHwM.

Elbeltagy, A., Nishioka, K., Suzuki, H., Sato, T., Sato, Y., Morisaki, H., Mitsui, H., and Minamisawa, K. (2000). Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. *Soil Science and Plant Nutrition*, 46(3):617–629. Online:https://bit.ly/3K4ua2D.

Frommel, M., Nowak, J., and Lazarovits, G. (1993). Treatment of potato tubers with a growth promoting *Pseudomonas* sp.: plant growth responses and bacterium distribution in the rhizosphere. *Plant and Soil*, 150:51–60. Online:https://bit.ly/3Xs4yzZ.

Gamel, R., Elsayed, A., Bashasha, J., Haroun, S., et al. (2017). Priming tomato cultivars in β-sitosterol or gibberellic acid improves tolerance for temperature stress. *International Journal of Botany*, 13(1):1–14. Online:https://bit.ly/3YtKbUo.

Gaspard, P. and Rice, S. (1989). Semiclassical quantization of the scattering from a classically chaotic repellor. *The Journal of Chemical Physics*, 90(4):2242–2254. Online:https://bit.ly/3lzH5zG.

Geris dos Santos, R., Rodrigues-Fo, E., Caldas, W., and Simas Teixeira, M. (2003). Endophytic fungi from *Melia azedarach*. *World Journal of Microbiology and Biotechnology*, 19:767–770. Online:https://bit.ly/3Yzmvhy.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W., and Kloepper, J. (1997). Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology*, 43(10):895–914. Online:https://bit.ly/3K4cPXV.

Hurek, T., Reinhold-Hurek, B., Van Montagu, M., and Kellenberger, E. (1994). Root colonization and systemic spreading of *Azoarcus* sp. strain bh72 in grasses. *Journal of Bacteriology*, 176(7):1913–1923. Online:https://bit.ly/3YkiYU1.

Jacobs, M., Bugbee, W., and Gabrielson, D. (1985). Enumeration, location, and characterization of endophytic bacteria within sugar beet roots. *Canadian Journal of Botany*, 63(7):1262–1265. Online:https://bit.ly/3K1lp9B.

Jia, M., Chen, L., Xin, H., Zheng, C., Rahman, K., Han, T., and Qin, L. (2016). A friendly relationship between endophytic fungi and medicinal plants: a systematic review. *Frontiers in Microbiology*, 7:906. Online:https://bit.ly/3jRQYbt.

Joshi, N., Sah, G., and Mishra, D. (2013). Gc-ms analysis and antimicrobial activity of essential oil of senecio pedunculatus. *IOSR Journal of Applied Chemistry*, 6(3):49–51. Online:https://bit.ly/3od3wf5.

Kahriman, N., Tosun, G., Terzioglu, S., Karaoglu, S., and Yayli, N. (2011). Chemical composition and antimicrobial activity of the essential oils from the flower, leaf, and stem of *Senecio pandurifolius*. *Records of Natural Products*, 5(2):82–91. Online:https://bit.ly/3K5rq5h.

Kobayashi, D. and Palumbo, J. (2000). *Microbial Endophytes*, chapter Bacterial endophytes and their effects on plants and uses in agriculture, pages 213–250. Online:https://bit.ly/3GGav6G. CRC Press, 1 edition.

Koo, H., Strope, B., Kim, E., Shabani, A., Kumar, R., Crowley, M., Andersen, D., and Bej, A. (2016). Draft genome sequence of *Janthinobacterium* sp. ant5-2-1, isolated from proglacial lake podprudnoye in the schirmacher oasis of east antarctica. *Genome Announcements*, 4(1):e01600–15. Online:https://bit.ly/3RXVhhL.

Lodewyckx, C., Vangronsveld, J., Porteous, F., Moore, E., Taghavi, S., Mezgeay, M., and der Lelie, D. (2002). Endophytic bacteria and their potential applications. *Critical Reviews in Plant Sciences*, 21(6):583–606. Online:https://bit.ly/3K4h4mj.

McInroy, J. and Kloepper, J. (1995). Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant and Soil*, 173:337–342. Online:https://bit.ly/3lyTCDt.

Miliute, I., Buzaite, O., Baniulis, D., and Stanys, V. (2015). Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. *Zemdirbyste-Agriculture*, 102(4):465–478. Online:https://bit.ly/4157PIa.

Mowafy, A., Fawzy, M., Gebreil, A., and Elsayed, A. (2021). Endophytic *Bacillus, Enterobacter*, and *Klebsiella* enhance the growth and yield of maize. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, 71(4):237–246. Online:https://bit.ly/3xjzZBT.

Müller, H., Berg, C., Landa, B., Auerbach, A., Moissl-Eichinger, C., and Berg, G. (2015). Plant genotype-specific archaeal and bacterial endophytes but similar *Bacillus* antagonists colonize mediterranean olive trees. *Frontiers in Microbiology*, 6:138. Online:https://bit.ly/3XmQPug.

Nori-Shargh, D., Raftari, S., and Deyhimi, F. (2008). Analysis of the essential oil of *Senecio vernalis* waldst. & kit. from iran. *Flavour and Fragrance Journal*, 23(5):357–359. Online:https://bit.ly/3HYHHGl.

Partida-Martínez, L. and Heil, M. (2011). The microbe-free plant: fact or artifact? *Frontiers in Plant Science*, 2:100. Online:https://bit.ly/40Qt54H.

Purchase, D., Miles, R., and Young, T. (1997). Cadmium uptake and nitrogen fixing ability in heavy-metal-resistant laboratory and field strains of *Rhizobium leguminosarum* biovar *trifolii*. *FEMS Microbiology Ecology*, 22(1):85–93. Online:https://bit.ly/3RUkolQ.

Rajan, A. (2012). *Microbial Endophytes of crop plants and their role in plant growth promotion*. PhD thesis, University of Agricultural Sciences, Bangalore. Online:https://bit.ly/3MB2cwU.

Redman, R., Sheehan, K., Stout, R., Rodriguez, R., and Henson, J. (2002). Thermotolerance generated by plant/fungal symbiosis. *Science*, 298(5598):1581–1581. Online:https://bit.ly/3Yr6bz3.

Reinhold-Hurek, B., Bünger, W., Burbano, C., Sabale, M., and Hurek, T. (2015). Roots shaping their microbiome: global hotspots for microbial activity. *Annual Review of Phytopathology*, 53:403–424. Online:https://bit.ly/3lBKMVh.

Reinhold-Hurek, B. and Hurek, T. (1998). Life in grasses: diazotrophic endophytes. *Trends in Microbiology*, 6(4):139–144. Online:https://bit.ly/40NdJOt.

Rosenblueth, M. and Martínez-Romero, E. (2006). Bacterial endophytes and their interactions with hosts. *Molecular Plant-Microbe Interactions*, 19(8):827–837. Online:https://bit.ly/40PZFDM.

Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M., and Glick, B. (2016). Plant growth-promoting bacterial endophytes. *Microbiological Research*, 183:92–99. Online:https://bit.ly/40Rkj6s.

Schulz, B. and Boyle, C. (2005). The endophytic continuum. *Mycological Research*, 109(6):661–686. Online:https://bit.ly/3K1F899.

Singh, D., Satish, C., and Manisha, D. (2017a). Chemical and potential biological perspectives of genus *Senecio* [asteraceae]. *Eur J Pharm Med Res*, 4(11):200–222. Online:https://bit.ly/3K92uK6.

Singh, M., Kumar, A., Singh, R., and Pandey, K. (2017b). Endophytic bacteria: a new source of bioactive compounds. *3 Biotech*, 7(315):1–14. Online:https://bit.ly/3xlzGXm.

Singh, N., Marwa, N., Mishra, J., Verma, P., Rathaur, S., and Singh, N. (2016). *Brevundimonas diminuta* mediated alleviation of arsenic toxicity and plant growth promotion in *Oryza sativa* l. *Ecotoxicology and Environmental Safety*, 125:25–34. Online:https://bit.ly/3RY6dfx.

Stackebrandt, E. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol. Today*, 33:152–155. Online:https://bit.ly/3YyEioT.

Stoltzfus, J., So, R., Malarvithi, P., Ladha, J., and De Bruijn, F. (1997). Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. *Plant and soil*, 194:25–36. Online:https://bit.ly/3IvoNIZ.

Sturz, A. (1995). The role of endophytic bacteria during seed piece decay and potato tuberization. *Plant and Soil*, 175:257–263. Online:https://bit.ly/3YuRBql.

Sultan, M., Elsayed, A., and El-Amir, Y. (2022). *In vitro* effect of plant parts extract of *Senecio glaucus* l. on pathogenic bacteria. *Biointerface Res. Appl. Chem*, 12(3):3800–3810. Online:https://bit.ly/3xhZgMM.

Tundis, R., Loizzo, M., Bonesi, M., Menichini, F., Dodaro, D., Passalacqua, N., Statti, G., and Menichini, F. (2009). *In vitro* cytotoxic effects of *Senecio stabianus* lacaita (asteraceae) on human cancer cell lines. *Natural Product Research*, 23(18):1707–1718. Online:https://bit.ly/3E3zzDv.

Vishnivetskaya, T., Kathariou, S., and Tiedje, J. (2009). The *Exiguobacterium* genus: biodiversity and biogeography. *Extremophiles*, 13:541–555. Online:https://bit.ly/3I1q1Kg.

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R., Neumann, C., and von Wettstein, D. (2005). The endophytic fungus piriformospora indica reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceedings of the National Academy of Sciences*, 102(38):13386–13391. Online:https://bit.ly/3YJbny8.

Zinniel, D., Lambrecht, P., Harris, N., Feng, Z., Kuczmarski, D., Higley, P., Ishimaru, C., Arunakumari, A., Barletta, R., and Vidaver, A. (2002). Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Applied and Environmental Microbiology*, 68(5):2198–2208. Online:https://bit.ly/3xh95L0.

La granja:
Revista de
Ciencias de la Vida

pISSN:1390-3799; eISSN:1390-8596

http://doi.org/10.17163/lgr.n38.2023.07

Scientific paper/ Artículo científico

Environmental Pollution

Fluvial loads of pesticides in the Pisque River (Ecuador) between June 2018 and May 2019

Cálculo de la carga fluvial de plaguicidas en el río Pisque (Ecuador) entre junio de 2018 y mayo de 2019

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Article received on September 06th, 2021. Accepted, after review, on April 28th, 2022. Published on September 1st, 2023.

Abstract

The Pisque river basin in Ecuador has a high presence of the floricultural industry, hence the aim of the research is to estimate the losses of pesticides that enter the river water through sources such as surface runoff, contact with the ground, permeate of a previous runoff or by infiltration, and that can be measured in the final channel of the Pisque river before its mouth. In order to know the pesticides used, surveys have been carried out with flower producers. The measurements were made in the Granobles and Guachalá rivers, the two tributaries of the Pisque river; and at two separate points on the same river Pisque, one immediately after the conjunction between the two tributaries and a point before their mouth to the next river. The flow gauges were monthly from June 2018 to May 2019. As a sampling method, SPMD and POCIS passive devices were used during the three dry months, from June to August 2018. To obtain the retention rates of the passive devices, a calibration with the pesticides was carried out in the laboratory through a hydrodynamic channel. Twenty-four main active ingredients were identified from the surveys, mostly compounds with Type III and Type IV toxicities. According to the results of the model, the fluvial load of pesticides in surface waters was 2,982.24 Kg between the months of June 2018 to May 2019, with environmental degradation of various compounds along the stretch of the river.

Keywords: Floriculture, passive SPMD and POCIS samplers, environmental degradation of pesticides.

Resumen

La cuenca del río Pisque en el Ecuador tiene alta presencia de industria florícola, desarrollándose aquí un estudio cuyo objetivo es la estimación de la magnitud de las pérdidas de plaguicidas que ingresan al agua fluvial por fuentes como escorrentía superficial, contacto con el suelo, permeado de una escorrentía previa o por infiltración, y que pueden ser medidas en el cauce final del río Pisque antes de su desembocadura. Para conocer los pesticidas utilizados se han realizado encuestas a los productores florícolas. Las mediciones se realizaron en los ríos Granobles y Guachalá, afluentes del río Pisque, y en dos puntos separados en el mismo río Pisque, uno inmediatamente después de la conjunción entre los dos afluentes y un punto antes de su desembocadura al siguiente río. Los aforos de caudal fueron mensuales desde junio 2018 hasta mayo 2019; cómo método de muestreo se usaron dispositivos pasivos *SPMD* y *POCIS* durante los tres meses secos, de junio a agosto de 2018. Para obtener las tasas de retención de los dispositivos pasivos se realizó una calibración con los plaguicidas en laboratorio mediante un canal hidrodinámico. De las encuestas se identificaron 24 ingredientes activos principales, en su mayoría compuestos con toxicidades Tipo III y Tipo IV. Según los resultados del modelo, la carga fluvial de pesticidas en aguas superficiales fue de 2982,24 *Kg* entre los meses de junio de 2018 a mayo de 2019, existiendo degradación ambiental de varios compuestos a lo largo del tramo del río.

Palabras clave: Floricultura, muestreadores pasivos SPMD y POCIS, degradación ambiental de pesticidas.

Suggested citation: Sánchez Proaño, R., Cerón Pánchig, C. and Landeta Jibaja, K. (2023). Fluvial loads of pesticides in the Pisque River (Ecuador) between June 2018 and May 2019. La Granja: Revista de Ciencias de la Vida. Vol. 38(2):94-103. http://doi.org/10.17163/lgr.n38.2023.07.

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1 Introduction

Pesticides are almost universally applied on agricultural crop lands, plots and in the floriculture industry, focusing this research on this economic activity, since it is one of the main economic activities within the Pisque river basin.

The Pisque river basin is a valley in which two main cities are located, Cayambe and Tabacundo, with a population of 152,153 inhabitants for the year 2018 (GAD Municipal de Pedro Moncayo, 2018; GADIP Cayambe, 2020), in addition to having 3,201.73 hectares of flowers grown in greenhouses in 2017 (Cachipuendo, 2018). It is located in the province of Pichincha and its waters flow into the Guayllabamba River, which subsequently flows into the Esmeraldas River and the Pacific Ocean. The greenhouses dedicated to flower production are more common in Cayambe and Tabacundo, as seen in the orthophoto in Figure 1.

In the basin, floricultural crops by the 1980s occupied 25 hectares (Bravo and Flores, 2006), and increased in the mid-1990s due to economic factors such as the elimination of exporting tariffs to the United States (Corrales, 2016) and environmental factors, such as the great sun exposure that occurs at 2800 and 2900 m.a.s.l, stable temperatures throughout the year (Bravo and Flores, 2006), and proximity to air ports; this increase in flower production led to an increase in the use of pesticides.

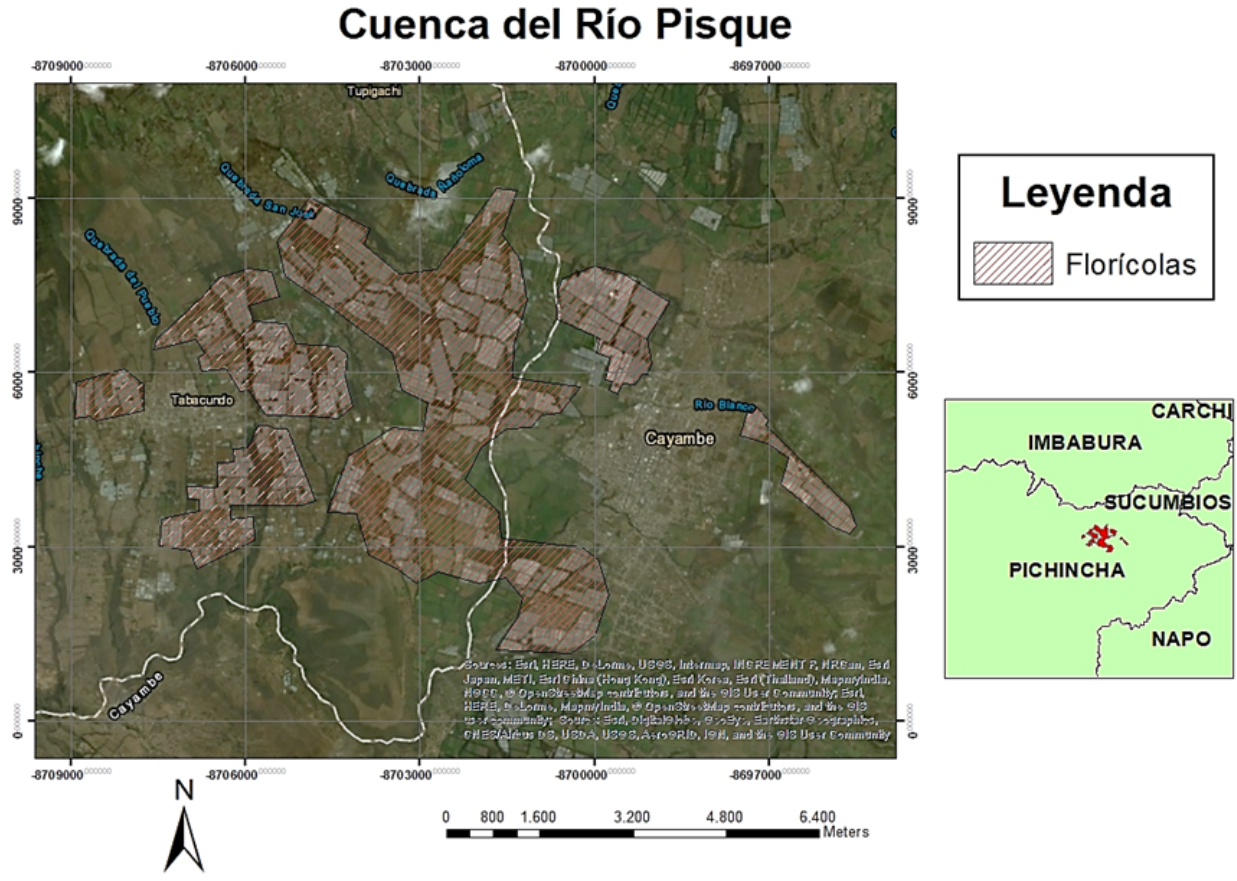

Figure 1. Area occupied by flower growers in the cities of Cayambe and Tabacundo in 2018.

It is estimated that less than 0.1% of the pesticides applied to crops reach their target, while the rest end up contaminating the air, soil and water (Arias et al., 2008). Much of this pesticide release is transported to water, affecting its quality and human health. Among the effects on water is the increase in toxicity, non-biodegradable organic carbon, electrical conductivity and solid matter (Calamari and Barg, 1993); while the main effects on human health are damage to the nervous system, hormonal alterations, cancer, damage to the immune system, reproductive damage, among others (Badr, 2020). It is therefore necessary to monitor pesticides, which is generally done through active sampling and only Persistent Organic Compounds (POPs) (Alvarez et al., 2014; Miège et al., 2012). Therefore, the aim is to sample the most commonly used chemicals in the flower industry to evaluate their permanence, and using passive sampling techniques to record continuous discharges, avoiding point discharges (Alvarez et al., 2007).

A useful technique for evaluating organic compounds in natural water bodies was used for sampling pesticides, such as passive sampling methods (Narváez et al., 2013). These can remain in the water for extended periods of time, passively adsorbing contaminants by diffusion and partitioning processes (Vrana et al., 2005). The use of a passive sampler to monitor contaminants in the aquatic environment is simpler and more practical than the measurement of bioaccumulated pesticides in living organisms (Alvarez et al., 2004; Vrana et al., 2005; Fedorova et al., 2014; Kot et al., 2000). However, its use in the environment requires a previous laboratory calibration to determine the value of the sampling rate of the specific compound (Morin et al., 2012), obtaining the entrainment or fluvial load that each chemical has in a surface water flow.

2 Materials and Methods

This section explains the protocols used for flow measurement, passive water sampling, laboratory analysis and calibration of sampling devices. First, the pesticides to be evaluated were defined through a survey of 20 floricultural producers.

For the gauging, a small section was sought in each river where the water flows continuously and unidirectionally, without interruption by rocks or obstacles; the cross-sectional area of the water body was measured by bathymetry (Swanson et al., 2009), and a Simtech micromoline, model FP111, was used to measure the velocity. This procedure was performed once a month for one year, starting in June 2018. There were three gauging points, one in the Granobles river, another in the Pisque river (point 1) after the junction with the Guachalá river and a last one in the Pisque river (point 2) before contributing its flow to the Guayllabamba river. To calculate the flow of the Guachalá river, the flow of the Granobles river was subtracted from the flow of the Pisque river (point 1), since there are no significant contributions within the section studied, as shown in Figure 2.

To measure the polar pesticides, SPMD (Semipermeable Membrane Devices) and for non-polar POCIS (Polar Organic Chemical Integrative Sampler) devices were used. The difference of chemical potentials of the analyte between the liquid and solid media of the samplers makes that these balance in the time in which the analysis is performed, obtaining in the passive sampler the average concentration of analyte that was in the water body (Górecki and Namieśnik, 2002). To calculate the mass of the accumulated analyte with respect to the concentration in the water the Equation 1 proposed by Vrana et al. (2005) was used.

$$M_s(t) = C_w R_s t \tag{1}$$

Where $M_s(t)$ is the mass of analyte accumulated in the sampler after the exposure time. R_s is the proportionality constant, C_w is the concentration of analyte in the aqueous environment and t is the exposure time. The devices used are those distributed by the company "EST-Lab" located in St. Joseph, Missouri, USA. The POCIS devices are the "Oasis HLB rectangular" model and the SPMD devices are the "99% purity 15cm with loops" model; both membranes were held by a metal structure and placed inside a plastic casing made of PVC pipe (Figure 3).

Figure 2. Sampling area and gauging points at the junction of the Granobles and Guachalá rivers to form the Pisque.

There were four passive sampling points, three located at the sites where the flow gauges were obtained, and a fourth in the Guachalá river. Two sampling points were carried out in the Pisque river in order to know which chemicals remain in the water and which chemicals are degraded. An SPMD sampler and a POCIS were placed in each of the four sampling points with a 28-day permanence in the water in each month during the three dry months, i.e., June to August. The objective of sampling during the dry months is to detect lower concentrations of pesticides, which is difficult if there are concentration dilutions due to precipitation.

As for the analysis of pesticides, an extraction procedure known as dialysis was performed, in which analytes are separated from membranes with different methods for SPMD and POCIS, according to the procedure proposed by Narváez et al. (2013). The sample preparation technique for measurement used was the one recommended by Aguilar (1998); López-Roldán et al. (2004) and Rodrigues et al. (2007), among others. The measurement was performed by the High Performance Liquid Chromatography (HPLC) method. For this, pesticide techniques developed by Kiso et al. (1996); Hernández et al. (2001) and Ferrer and Thurman (2007), among others, were considered. These techniques were applied both in the normal phase of polar compounds, for which a polar stationary phase and a non-polar mobile phase are used, and for non-polar compounds in which the stationary phase is non-polar and the mobile phase is polar.

A Waters HPLC equipment was used for the measurement, the models of its components are 1525 of the binary pump, 2998 of the photodiode array detector and the Empower 3 software developed by the same brand. A Restek brand C18 with code 9534565 was used as the column.

Figure 3. Passive samplers placed in water bodies.

According to Huckins et al. (1999); Luellen and Shea (2002) and Murdock et al. (2001), the calibration of exchange kinetics in passive sampling can be performed in the laboratory. Hence, the experimental method is the best to know the transfer coefficients, since the transfer rate depends on several hydrodynamic factors such as turbulence, environmental properties, shape and permeability of the casing, among others, which are simplified into a single factor (Yabuki et al., 2016). Experimentally, the obtaining of this single factor in the laboratory was performed in an Armfield model S16-11-b hydrodynamic channel. The degradation values of each analyte were obtained with the degradation of pesticides in the same channel. The ingredients

to be analyzed were placed in the hydrodynamic channel at a concentration of 1 ppm using commercial products; the initial concentration was verified and three SPMD devices and three POCIS devices were placed, and a pair was removed and analyzed every three days. Finally, quantifying the pesticides in the water bodies, the laboratory proportionality constants corrected by the data obtained from the SPMD devices and the field POCIS will be used, obtaining an average pesticide concentration in the water for each month, which when multiplied by the flow rate of the same month gives the value of the pesticide load in the moving water body in magnitudes of mass over time.

3 Results

The results in flow measurements in cubic meters per second (m^3/s) of the gauging for each month are shown in Table 1, with one measurement made in the Granobles river and two in the Pisque river, at two different points; in the case of the Guachará river, the flow value corresponds to the mathematical calculation explained above.

The surveys conducted with flower growers revealed the use of 24 pesticides in greater quantities, which are presented in Table 2. None of these ingredients were found to have Type I toxicity or compounds classified as POPs; out of those three had Type II toxicity, 13 Type III and 9 Type IV. However, it was decided to increase the measurement of DDT 4,4' in order to check the permanence of this chemical in the soil that presents contact with water and that was possibly used in previous decades.

The SPMD and POCIS passive samplers were calibrated with these pesticides. Table 2 shows the type of sampling device used for their analysis according to the polarity of the ingredient, the temporary concentrations measured directly in the water after mixing the chemical, and the measurements obtained from the passive devices at 3, 6 and 9 days.

The proportionality constants were obtained from the above values, and with these rates and the monthly flow rates, the river loads of pesticides transported per year at the four measurement points were obtained, which are presented in Table 3.

Table 1. Results of river gauging in m^3/s.

No.	Source	2019						2018					
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	Granobles river	1.2	3.4	2.8	3.6	5.4	2.1	1.6	1.4	0.8	2.1	2.4	3.6
2	Guachalá river	3.1	5.9	21.9	24.4	13.5	0.2	4.7	0.4	2	5.6	7.5	7.1
3	Pisque river (point 1)	4.3	9.3	24.7	28	18.9	2.3	6.3	1.8	2.8	7.7	9.9	10.7
4	Pisque river (point 2)	9.6	15.4	20.9	25.8	35.5	6.3	11.2	5.2	6.5	12.9	13.4	15

4 Conclusions and Discussion

Out of the 25 pesticides measured, all were detected by SPMD and POCIS devices excepting Cyproconazole, of these Thiabendazole was previously detected in the watershed by direct composite sampling in the Granobles River in the study conducted by Breilh et al. (2009); thus, 24 chemicals have not been characterized before in the watershed due to their commercial production of less than ten years (Securities and Exchange Commission, 2017).

The chemical with the highest concentration in the Granobles River was the acaricide Hexythiazox with a discharged amount of 1.2 T/year, also acaricide Clofentezine and the fungicide-bactericide Kasugamicin with concentrations higher than half a ton per year. The chemical with the highest con-

Table 2. Pesticides measured, devices used for this measurement and concentrations obtained at 0, 3, 6 and 9 days.

Ingredient	Tool used to measure	Water sample			
		From 0 days (μg/L)	At 3 days (μg/L)	At 6 days (μg/L)	At 9 days (μg/L)
Abamectina	SPMD	0.646695501	0.53849858	0.54167056	0.47745176
Bifenazato	SPMD	0.518783287	0.340566	0.273792	0.150416
Captan	SPMD	0.560243903	0.46804891	0.46794318	0.41566033
Carboxina	SPMD	0.693843997	0.68067431	0.49621795	0.42715263
Clofentezine	SPMD	0.563645605	0.47704974	0.45991542	0.38852853
Clorotalonil	POCIS	0.619721452	0.55072321	0.56884298	0.53318026
Clorfenapir	SPMD	0.562550269	0.51234852	0.43466815	0.40084613
Ciproconazol	SPMD	0.96418066	0.84196316	0.86543625	0.68150459
Dazomet	SPMD	0.712663906	0.57978667	0.59948895	0.59945474
DDT 4,4'	POCIS	0.80498488	0.7250131	0.7246846	0.7596978
Diafentiuron	SPMD	0.917674635	0.5788426	0.46744052	0.39246549
Difenoconazol	SPMD	0.760377939	0.6463111	0.62050694	0.53513305
Furalaxyl	SPMD	0.615093338	0.55017808	0.47513903	0.41824266
Hexythiazox	POCIS	0.886933592	0.60465611	0.46895995	0.38146892
Imidacloprid	POCIS	0.56145588	0.51898931	0.50362015	0.52374401
Isopyrazam	SPMD	1.079953092	0.96459406	0.96196111	0.97657145
Kasugamicina	SPMD	0.961088186	0.82968248	0.84669486	0.89287705
Mancozeb	SPMD	0.999337494	0.8777455	0.83864724	0.72522865
Mandipropamida	SPMD	0.727720428	0.68136426	0.64661057	0.64078673
Metalaxil-M	SPMD	0.70121361	0.55710826	0.51705397	0.52321987
Oxicarboxina	SPMD	0.6815315	0.56779358	0.51636684	0.54778815
Tiabendazol	SPMD	0.814691604	0.49154876	0.42893184	0.33380668
Tiametoxam	SPMD	0.536073681	0.35322476	0.29355718	0.23723684
Thiocyclam	SPMD	1.067956414	0.87624062	0.90435532	0.78547042
Thiram	SPMD	0.505084445	0.41808088	0.37347192	0.34748851

centration in the Guachalá river was the acaricide Hexythiazox with a measured discharge of 5.5 tons/year, while the insecticide-acaricide Abamectin has a discharge of half a ton per year. The chemical with the highest concentration at the mouth of the Pisque River was the acaricide Clofentezine with a permanence in the river of 0.605 T/year, while the fungicide Diafeconazole and the fungicide-bactericide Kasugamicin exceeded the permeance of entrainment in the river by more than 0.4 T/year. The results confirm that insecticides and fungicides in Ecuador are the most widely used pesticides (Valarezo and Muñoz, 2011).

The highest DDT carryover in surface water was in the Granobles River with 0.194 T/year, because this sub-basin is home to the floriculture industry. However, this carryover value is low compared to the rest of the agrotoxins, because since 2008 in Ecuador this persistent pesticide has not been imported; therefore, the contamination of soils and water with persistent chemicals and residues is the result of many years of its unrestricted application, finding metabolites such as DDT 4,4' still in water bodies (Cairns and Sherma, 1992; Kouzayha et al., 2013).

There is a decrease in pesticide concentrations downstream comparing points 1 and 2 of the Pisque River, maybe related with the decreases in concentrations found in laboratory (Table 2). In field analysis this may be due to environmental factors in addition to variable climatic conditions including drought, desertification and other factors present in the study area (Aisha et al., 2017). The acaricide Clofentezine has a longer permanence in water bodies despite having Type IV toxicity, with a decrease of only 37% between points 1 and 2 of the Pisque River. It is not possible to compare the presence and degradation of these chemicals with other basins or micro-basins in the country, since there are no similar studies.

Table 3. Riverine pesticide loads from June 2018 to May 2019 at the Granobles, Guachalá, Pisque (point 1) and Pisque (point 2) sampling points.

Agrotoxic	Mass transported to the river every year (Kg/year)			
	Granobles river	Guachalá river	Pisque river (Point 1)	Pisque river (Point 2)
Abamectin	6.653528	505.477019	629.176347	122.808668
Bifenazate	6.620319	160.148325	77.819682	64.863366
Captan	144.33157	0.360573	160.588781	28.004311
Carboxine	39.944796	18.24502	96.992282	370.995619
Clofentezine	852.05047	1.020008	953.89041	605.280247
Chlorothalonil	29.324674	117.808407	103.970527	0.051113
Chlorfenapyr	16.68012	0.833866	10.451968	12.665925
Ciproconazol	0	0	0	0
Dazomet	12.829044	10.348819	30.244102	14.955096
DDT 4,4'	194.122733	0	15.599694	0.533583
Diafenthiuron	17.144261	354.515585	264.258417	235.911596
Difeconazol	121.341225	0.244132	322.424488	430.743341
Furalaxyl	347.413055	4.556359	491.844465	253.193159
Hexythiazox	1207.15446	555.134675	935.364327	1.356934
Imidacloprid	467.290891	68.016049	965.451402	35.350273
Isopyrazam	68.172396	21.459916	129.310744	131.940685
Kasugamicin	644.610004	84.379842	956.951816	441.095821
Mancozeb	205.192694	0	78.173762	29.875791
Mandipropamid	2.579379	0	4.215016	2.875818
Metalaxyl-M	6.479502	0.240234	7.657236	1.748932
Oxycarboxine	463.488287	0.879675	178.184092	82.686197
Thiabendazole	7.520012	3.113556	10.797758	6.585262
Tiametoxam	21.313513	14.81764	49.24951	11.928538
Thiocyclan	11.820493	2.312543	37.89562	20.001449
Thiran	84.577268	6.421586	144.596888	76.786843

The chemical profile of the Pisque River is relatively similar to those observed in Lake Ziway in Ethiopia, which also has the presence of the flower industry in its watershed parenciteLamessa2021. Knowing the type and quantity of pesticides present in the basin allows evaluating their effect on human health and ecosystems by analyzing the complete life cycle of pesticides in the basin, analyzing their final destinations and the exposure of humans and other species through different media and pathways, such as food, evaporation into the air, transfer to soil or groundwater (Margni et al., 2002).

Acknowledgments

This project was financed by Universidad Politécnica Salesiana in Quito, Ecuador.

References

Aguilar, C. (1998). Comparison of automated on-line solid-phase extraction followed by liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization and particle beam mass spectrometry for the determination of a priority group of pesticides in environ. *Journal of Chromatography A*, 794:1–2. Online:https://bit.ly/3LD6Hq5.

Aisha, A., Hneine, W., Mokh, S., Devier, M., Budzinski, H., and Jaber, F. (2017). Monitoring of 45 pesticides in lebanese surface water using polar organic chemical integrative sampler (pocis). *Ocean Science Journal*, 52:455–466. Online:https://bit.ly/3JweeVg.

Alvarez, D., Huckins, J., Petty, J., Jones-Lepp, T., Stuer-Lauridsen, F., Getting, D., and Goddard, J.and Gravell, A. (2007). *Passive sampling techniques in environmental monitoring*, volume 48, chapter Tool for monitoring hydrophilic contaminants

in water: polar organic chemical integrative sampler (POCIS), pages 171–197. Comprehensive Analytical Chemistry.

Alvarez, D., Perkins, S., Nilsen, E., and Morace, J. (2014). Spatial and temporal trends in occurrence of emerging and legacy contaminants in the lower columbia river 2008-2010. *Science of the total environment*, 484:322–330. Online:https://bit.ly/3JqFjZQ.

Alvarez, D., Petty, J., Huckins, J., Jones-Lepp, T.and Getting, D., Goddard, J., and Manahan, S. (2004). Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. *Environmental Toxicology and Chemistry: An International Journal*, 23(7):1640–1648. Online:https://bit.ly/3FyOJBd.

Arias, M., López, E., Martínez, E., Simal, J., Mejuto, J., and García, L. (2008). The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agriculture, ecosystems & environment*, 123(4):247–260. Online:https://bit.ly/40mdh8J.

Badr, A. (2020). Organophosphate toxicity: Updates of malathion potential toxic effects in mammals and potential treatments. *Environmental Science and Pollution Research*, 27(21):26036–26057. Online:https://bit.ly/3LDjEAd.

Bravo, M. and Flores, S. (2006). Incidencia de la producción de rosas en el sector de cayambe período 2000–2005. Master's thesis, Guayaquil.

Cachipuendo, C. (2018). *Modelo dinâmico para a gestão e manejo sustentável de sistemas de irrigação comunitários, no marco do Bom Viver: estudo do caso na bacia do Rio Pisque*. PhD thesis, Universidade de São Paulo.

Cairns, T. and Sherma, J. (1992). *Emerging strategies for pesticide analysis*. CRC press.

Calamari, D. and Barg, U. (1993). *Prevention of Water Pollution by Agriculture and Related Activities*, chapter Hazard assessment of agricultural chemicals by simple simulation models, pages 207–222. Comprehensive Analytical Chemistry.

Corrales, J. (2016). Análisis del sector florícola ecuatoriano periodo 2005-2015 caso cayambe. Master's thesis, Pontificia Universidad Católica del Ecuador.

Fedorova, G., Randak, T., Golovko, O., Kodes, V., Grabicova, K., and Grabic, R. (2014). A passive sampling method for detecting analgesics, psycholeptics, antidepressants and illicit drugs in aquatic environments in the czech republic. *Science of the total environment*, 487:681–687. Online:https://bit.ly/3JZu384.

Ferrer, I. and Thurman, E. (2007). Multi-residue method for the analysis of 101 pesticides and their degradates in food and water samples by liquid chromatography/time-of-flight mass spectrometry. *Journal of Chromatography A*, 1175(1):24–37. Online:https://bit.ly/3LIifIF.

GAD Municipal de Pedro Moncayo (2018). Plan de desarrollo y ordenamiento territorial pdot actualización 2018 - 2025. Technical report, GAD Municipal de Pedro Moncayo.

GADIP Cayambe (2020). Plan de desarrollo y ordenamiento territorial del cantó cayambe 2020-2030. Technical report, GAD Municipal de Pedro Moncayo.

Górecki, T. and Namieśnik, J. (2002). Passive sampling. *TrAC Trends in Analytical Chemistry*, 21(4):276–291. Online:https://bit.ly/3nf6r6w.

Hernández, F., Sancho, J., Pozo, O., Lara, A., and Pitarch, E. (2001). Rapid direct determination of pesticides and metabolites in environmental water samples at sub-μg/l level by on-line solid-phase extraction-liquid chromatography-electrospray tandem mass spectrometry. *Journal of Chromatography A*, 939(1-2):1–11. Online:https://bit.ly/3JBjzdU.

Huckins, J., Petty, J., Orazio, C., Lebo, J., Clark, R., Gibson, V., Gala, W., and Echols, K. (1999). Determination of uptake kinetics (sampling rates) by lipid-containing semipermeable membrane devices (spmds) for polycyclic aromatic hydrocarbons (pahs) in water. *Environmental science & technology*, 33(21):3918–3923. Online:https://bit.ly/3FIHXca.

Kiso, Y., Li, H., Shigetoh, K., Kitao, T., and Jinno, K. (1996). Pesticide analysis by high-performance liquid chromatography using the direct injection method. *Journal of chromatography A*, 733(1-2):259–265. Online:https://bit.ly/3lwCazI.

Kot, A., Zabiegała, B., and Namieśnik, J. (2000). Passive sampling for long-term monitoring of organic pollutants in water. *TrAC Trends in Analytical Chemistry*, 19(7):446–459. Online:https://bit.ly/3JEJd18.

Kouzayha, A., Al Ashi, A., Al Akoum, R., Al Iskandarani, M., Budzinski, H., and Jaber, F. (2013). Occurrence of pesticide residues in lebanon's water resources. *Bulletin of environmental contamination and toxicology*, 91:503–509. Online:https://bit.ly/3LIG0R6.

López-Roldán, P., López de Alda, M., and Barceló, D. (2004). Simultaneous determination of selected endocrine disrupters (pesticides, phenols and phthalates) in water by in-field solid-phase extraction (spe) using the prototype profexs followed by on-line spe (prospekt) and analysis by liquid chromatography-atmospheric pressure chemical ionisation-mass spectrometry. *Analytical and bioanalytical chemistry*, 378:599–609. Online:https://bit.ly/40JYC7F.

Luellen, D. and Shea, D. (2002). Calibration and field verification of semipermeable membrane devices for measuring polycyclic aromatic hydrocarbons in water. *Environmental science & technology*, 36(8):1791–1797. Online:https://bit.ly/3naONkr.

Margni, M., Rossier, D., Crettaz, P., and Jolliet, O. (2002). Life cycle impact assessment of pesticides on human health and ecosystems. *Agriculture, ecosystems & environment*, 93(1-3):379–392. Online:https://bit.ly/3LIN6VK.

Miège, C., Schiavone, S., Dabrin, A., Coquery, M., Mazzella, N., Berho, C., Ghestem, J., Togola, A., Gonzalez, C., and Gonzalez, J. (2012). An in situ intercomparison exercise on passive samplers for monitoring metals, polycyclic aromatic hydrocarbons and pesticides in surface waters. *TrAC Trends in Analytical Chemistry*, 36:128–143. Online:https://bit.ly/3TBNIhB.

Morin, N., Miège, C., Coquery, M., and Randon, J. (2012). Chemical calibration, performance, validation and applications of the polar organic chemical integrative sampler (pocis) in aquatic environments. *TrAC Trends in Analytical Chemistry*, 36:144–175. Online:https://bit.ly/3FHx6zk.

Murdock, C., Kelly, M., Chang, L., Davison, W., and Zhang, H. (2001). Dgt as an in situ tool for measuring radiocesium in natural waters. *Environmental science & technology*, 35(22):4530–4535.

Narváez, J., López, C., and Molina, F. (2013). Passive sampling in the study of dynamic and environmental impact of pesticides in water. *Revista Facultad de Ingeniería Universidad de Antioquia*, (68):147–159. Online:https://n9.cl/59jyk.

Rodrigues, A., Ferreira, V., Cardoso, V., Ferreira, E., and Benoliel, M. (2007). Determination of several pesticides in water by solid-phase extraction, liquid chromatography and electrospray tandem mass spectrometry. *Journal of Chromatography A*, 1150(1-2):267–278. Online:https://bit.ly/42B1QMb.

Securities and Exchange Commission (2017). Annual report pursuant to section 13 or 15(d) of 1934.

Swanson, R., Brownawell, B., and O'Connell, C. (2009). The forge river, problem identification.

Valarezo, O. and Muñoz, X. (2011). Insecticidas de uso agrícola en el euador. Technical report, GAD Municipal de Pedro Moncayo.

Vrana, B., Mills, G., Greenwood, R., Knutsson, J., Svensson, K., and Morrison, G. (2005). Performance optimisation of a passive sampler for monitoring hydrophobic organic pollutants in water. *Journal of Environmental Monitoring*, 7(6):612–620. Online:https://rsc.li/3JJt0rU.

Yabuki, Y., Nagai, T., Inao, K., Ono, J., Aiko, N., Ohtsuka, N., Tanaka, H., and Tanimori, S. (2016). Temperature dependence on the pesticide sampling rate of polar organic chemical integrative samplers (pocis). *Bioscience, biotechnology, and biochemistry*, 80(10):2069–2075. Online:https://bit.ly/3nc78Oa.

La Granja:
Revista de
Ciencias de la Vida

pISSN:1390-3799; eISSN:1390-8596

http://doi.org/10.17163/lgr.n38.2023.08

Scientific paper/ Artículo científico

Environmental pollution

Effect of mining activity on biodiversity in a sector of the Paquisha parish, Province of Zamora Chinchipe-Ecuador

Efecto de la actividad minera sobre la biodiversidad en un sector del cantón Paquisha, Provincia de Zamora Chinchipe-Ecuador

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Article received on March 30th, 2021. Accepted, after review, on January 26th, 2022. Published on September 1st, 2023.

Abstract

The Ecuadorian Amazon region represents 45% of the national territory and constitutes one of the largest ecological reserves of humanity due to its biological wealth. In recent years, the forest area in the Latin American Amazon has been reduced by 4.5% (240,000 km^2). Ecuador is one of the countries with the highest deforestation in the region (2.4%). The objective of this study was to assess the effect of mining activity on biodiversity, hence possible changes in the ecosystem, fragmentation, abundance, richness, dominance and diversity of species were estimated. The study area was located in a mining area in the Province of Zamora Chinchipe, Cantón Paquisha-Ecuador. Through point estimators, 123 species of vascular plants divided into 43 families were identified, the highest abundance was presented by Asteraceae with 12%, followed by Araceae with 8.5% and Melastomataceae with 7.5%. Likewise, 42 species of birds were identified, 16 of mammals, 12 of amphibians and reptiles, and 36 macroinvertebrate individuals. It could be inferred that there is a marked deterioration of the ecosystem in the area, however an interesting diversity of species remains, mainly flora. In relation to fauna, the loss of certain species is evident, mainly due to agricultural expansion, hunting and mining activity. According to the Shannon index, the aquatic fauna is low, and according to the BMWP / Col index the water in the area is highly polluted.

Keywords: Biodiversity, abundance, ecosystem, dominance.

Resumen

La región amazónica ecuatoriana representa el 45% del territorio nacional y constituye una de las mayores reservas ecológicas de la humanidad debido a su riqueza biológica. En los últimos años el área de bosque en la amazonia latinoamericana se redujo en un 4,5% (240.000 km^2); en este orden, Ecuador es uno de los países con mayor deforestación en la región (2,4%). Por esta razón, el objetivo de este estudio es valorar el efecto de la actividad minera sobre la biodiversidad. Para el efecto se estimaron los posibles cambios en el ecosistema, la fragmentación, abundancia, riqueza, dominancia y diversidad de especies. El área de estudio se ubicó en una zona de explotación minera en la Provincia de Zamora Chinchipe, Cantón Paquisha-Ecuador. Mediante estimadores puntuales se identificaron 123 especies de plantas vasculares divididas en 43 familias. Asteraceae presentó mayor abundancia con el 12%, Araceae con el 8,5% y Melastomataceae el 7,5%. Asimismo, se identificaron 42 especies de aves, 16 de mamíferos, 12 entre anfibios y reptiles y 36 individuos macroinvertebrados. Los resultados permiten inferir que en la zona existe un deterioro marcado del ecosistema, empero se mantiene una diversidad interesante de especies, principalmente de flora. En lo referente a fauna la pérdida de ciertas especies es evidente, debido principalmente a la expansión agrícola, la caza y la actividad minera. La fauna acuática de acuerdo con el índice de Shannon es baja, y de acuerdo al índice BMWP/Col el agua en la zona es muy contaminada.

Palabras clave: Biodiversidad, abundancia, ecosistema, dominancia.

Suggested citation: Delgado Fernández, E., León Peralta, M., Cantos Guamán, C. and Guzmán Juárez, M. (2023). Effect of mining activity on biodiversity in a sector of the Paquisha parish, Province of Zamora Chinchipe-Ecuador. La Granja: Revista de Ciencias de la Vida. Vol. 38(2):104-121. http://doi.org/10.17163/lgr.n38.2023.08.

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1 Introduction

Ecuador is home to the highest number of ecosystems, biological species and biodiversity. The Ecuadorian Amazon region represents 45% of the national territory and constitutes one of the largest ecological reserves of humanity (Myers et al., 2000). According to the Amazon Cooperation Treaty Organization (OTCA), the Amazon region contains approximately 80% of the country's biodiversity, with 150 to 312 species of trees per hectare, 600 species of fish, and more than 250 species of amphibians and reptiles (Toro, 2006). The Amazon region has a unique biological importance because it has native microbial load of uncatalogued species of microorganisms. It is important to mention that the topographic and environmental conditions of the region, the sum of these and other interactions directly influence the biodiversity of the area (Alexa, 2010). However, the productive activities cause environmental impacts and the problem in the Amazon region is linked to mining activity and deforestation. According to the report of the Amazonian network of geo-referenced socio-environmental information (RAISG) between the years 2000 and 2010 the forest area of the Latin American Amazon was reduced by 4.5%, about 240 thousand square kilometers approximately, Ecuador is one of the countries in the region with the highest deforestation rate (2.4%); in this sense biodiversity is under increasing pressure and threat (MAE, 2015).

Mining activity particularly related to gold in the republic of Ecuador has been occurring for centuries (Equipo MMSD América del Sur, 2002); therefore, this research suggests that the expansion of mining activity in the area causes losses in biodiversity. The study of the ecosystem had the purpose of identifying possible changes in the ecosystem, fragmentation, abundance, richness, dominance and diversity of species, in short, the aim is to generate data that in the future can be used and compared with other research in order to develop conservation strategies.

2 Materials and methods

This study was carried out in the province of Zamora Chinchipe, Paquisha parish, Congüime community, UTM coordinates: WGS 84.17m 762056.24 m E; 9553263.64 m S; 838 m altitude (Figure 1). The site has elevations with steep slopes that vary between 700 m.a.s.l. and 2800 m.a.s.l. and that make up the Cordillera del Cóndor mountain range to the east and in the lower part the Nangaritza river valley that runs from south to north.

Five sampling points were randomly and strategically located and coded as: *COFA* for fauna and *COF* for flora (Table 1).

Table 1. Flora and fauna sampling points.

POINTS (**CODES**)	COORDINATES UTM (17S Zone) WGS84 X	Y	ALTITUDE (m)	AREA
COF-1/COFA-1	764002	9553233	901.3	Secondary forest and scrub mosaic
COF-2/COFA-2	763548	9552652	857	Scrub and grass mosaic
COF-3/COFA-3	763222	9553334	905.6	Scrub and grass mosaic
COF-4/COFA-4	762188	9552973	844.7	Mosaic scrubland, pasture and crops
COF-5/COFA-5	762042	9553272	842	Scrubland, pasture and anthropogenic actions

Coordinates and location, maximum altitude 905.6 m.a.s.l.

2.1 Flora in the study area

Plots of 50 m $\times$ 50 m were drawn in a total area of 0.25 ha; they were subsequently subdivided into areas of 0.25 $\times$ 0.25 m each (Arias et al., 2012). The study variables were basal area, relative density, relative dominance, importance value index, and Simpson's dominance index. When the range was (0 to 1) values closer to (1) were interpreted as dominance of one species over the other, according to Simpson's dominance index when values were (0 to 5); values close to zero were interpreted as low diversity and vice versa, according to Shannon's di-

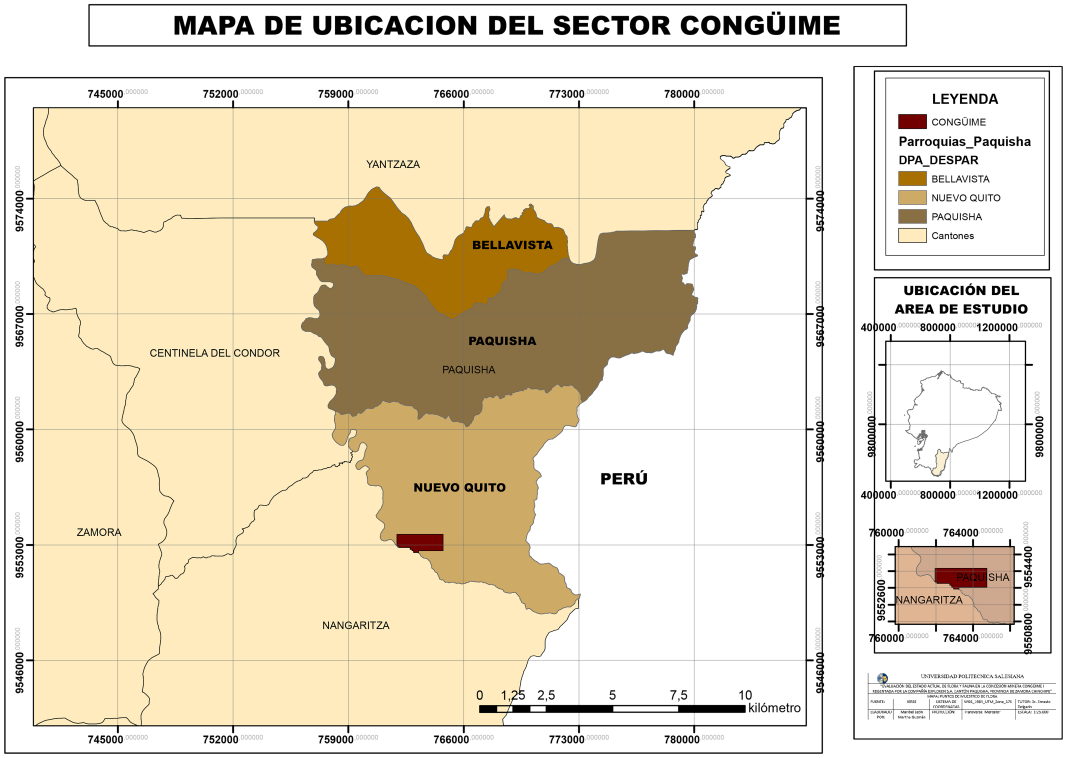

Figure 1. Cartographic map, location of the study area.

versity index (Zamora, 2015).

3 Avifauna

Sampling was done based on 3 criteria: census points, random walks and auditory records (Balderrama et al., 2005); diversity, abundance and geographic location were studied, and the references were taken from the list of birds of continental Ecuador (Ridgely et al., 1998). To estimate the relative abundance and diversity of species, we applied Simpson's dominance index in a range from 0 to 1. We inferred that values close to (1) indicate dominance of one species over the others (Campo and Duval, 2014a). The Shannon-Weaner index was used to estimate diversity (Zamora, 2015); ecological aspects such as trophic guild and sensitive and indicator species were also evaluated.

3.1 Mastofauna y herpetofauna

The study was carried out through transects, sightings, counts, indirect monitoring, trails, presence of excrement, burrows, diameter of holes and surveys of community dwellers (Arévalo, 2001). Three observation walks were made over 200, 500 and 1000 m. In addition, auditory records and visual encounters were used to identify frogs, toads, salamanders, etc (Yánez et al., 2007). The assessment was made using Shannon's index.

3.2 Macroinvertebrates

In the riverbed, sampling was done in the Chinapintza stream, Congüime river and at the junction of the two using a kick net (Carrera and Fierro, 2001). The data sets were compared to determine species richness and abundance. Biological diversity was determined using the indices: Shannon-Weaver (Shannon and Weaver, 1949); Simpson's dominance (Campo and Duval, 2014b); Margalef's richness and evenness index. Water quality was estimated through the EPT (*Ephemeroptera, Plecoptera, Trichoptera*) indexes (Bispo et al., 2006) and the BMWP/Col (Biological Monitoring Working Party/modified by Colombia) index (Zamora and Alba, 1996).

4 Results and Discussion

4.1 Flora of the study area

A total of 123 plant species and 43 families were recorded, showing more abundance *Araceae* and *Asteraceae* species. There was the greatest diversity of species at point (COF-3), while there was a greater number of families at point (COF-1), inferring that points 1 and 3 were areas of less intervention (Figure 2).

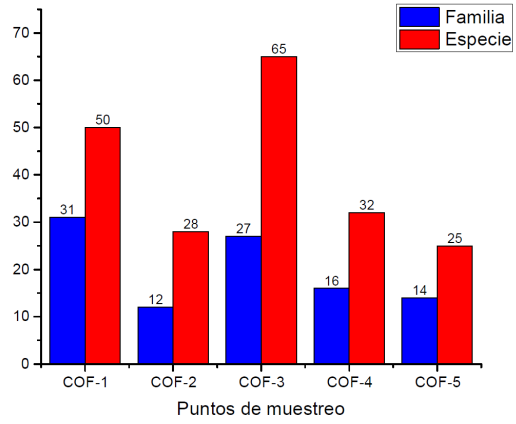

Figure 2. Bar diagram, families and species at sampling points.

4.1.1 Estimation of biological diversity

According to the Shannon-Weaner index, the values (3.708) for (COF-1) and (3.873) for (COF-3), would correspond to a high diversity secondary forest fraction. As for (COF-2) (3.00) (COF-4) (3.01) and (COF-5) (2.87), these are indicators of medium diversity according to Simpson's index (>0.9) (Table 2).

Table 2. Species diversity.

sampling areas	N° of species	N° of individuals	Shannon Index	Interpretation	Simpson Index	Interpretation
COF-1	50		3.708	High diversity	0.9713	High diversity
COF-2	28		3.005	Average diversity	0.936	High diversity
COF-3	65	200	3.873	High diversity	0.974	High diversity
COF-4	32		3.01	Average diversity	0.9318	High diversity
COF-5	25		2.877	Average diversity	0.9287	High diversity

4.1.2 Importance Value Index (IVI)

Due to the variety of tree species and the partially open canopy (aerial vegetation layer), was considered to make the assessment in (COF-3). Two *Urticaceae* and *Arecacea* families were observed. The species with the highest IVI were *Mauritia flexuosa L.f.* (27.44), *Pourouma bicolor Mart.* (20.76), *Ficus americana Aubl.* (19.45), *Cecropia ficifolia* (18.56) and *Astrocaryum chambira Burret* (14.91) (Table 3).

Table 3. Importance Value Index (IVI) of the (COF-3).

Species	ABU	AB (m^2)	DR	DM	IVI
Mauritia flexuosa	2	1.865	2.47	24.97	27.44
Pourouma bicolor Mart.	5	1.09	6.17	14.60	20.77
Ficus americano Aubl.	6	0.9	7.41	12.05	19.46
Cecropia ficifolia	12	0.28	14.81	3.75	18.56
Astrocaryum chambira Burret	11	0.1	13.58	1.34	14.92
Batocarpus orinocensis Karsten.	1	0.99	1.23	13.26	14.49
Himatanthus sucuuba Woodson	6	0.48	7.41	6.43	13.83
Pourouma cecropiifolia	9	0.2	11.11	2.68	13.79
Iriartea deltoidea Ruiz & Pav.	8	0.13	9.88	1.74	11.62
Aegiphila sellowiana Cham.	6	0.2663	7.41	3.57	10.97
Myriocarpa stipitata Benth	8	0.025	9.88	0.33	10.21
Perebea guianensis Aubl.	3	0.43	3.70	5.76	9.46
Urera baccifera (L.) Gaudich.	1	0.43	1.23	5.76	6.99
Bactris gasipaes Kunth	2	0.25	2.47	3.35	5.82
Inga thibaudiana DC.	1	0.032	1.23	0.43	1.66
TOTAL	81	74.683	100	100	200

AB= Basal Area, DnR= Relative Density,

DmR= Relative Dominance, IVI= Importance Value Index.

4.1.3 Habit of plant species

Herbaceous species, trees and shrubs were found with 36%, 29% and 22% respectively, the herbaceous species correspond to areas of greater intervention (Jørgensen and León-Yánez, 1999) (Figure 4).

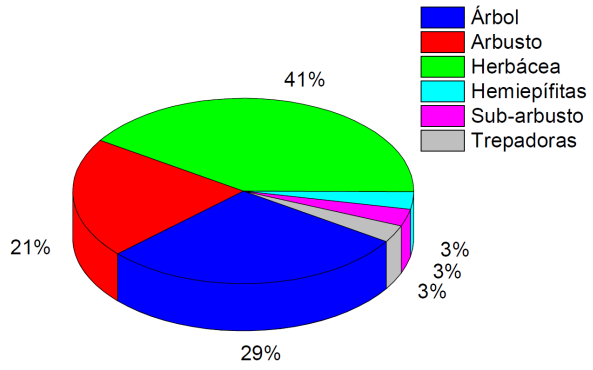

Figure 3. Distribution of plant species according to their development pattern (habit).

4.1.4 Conservation state

Seventy-four plant species were recorded as native (76.2%); 6 introduced species (6.2%); 7 introduced and cultivated (7.2%); 7 native and cultivated (7.2%); and 2 endemic species (2.05%) (Figure 5).

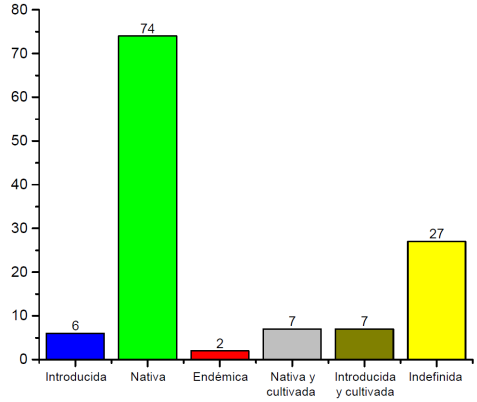

Figure 4. Biotic component (plant species) and their status.

The cultivated species sampled were: *Carica papaya L.* (papaya), *Inga edulis* Mart. (Guabilla), *Ipomoea batatas (L.)* Lam. (wild sweet potato), *Pourouma cecropiifolia* (Uvilla), *Renealmia alpinia* (Achira del monte), *Solanum quitoense* Lam. (Naranjilla) and *Bactris gasipaes* Kunth. (Palmito). In addition, the native species: *Paspalum saccharoides* Ness ex Trin (Yajoch irpa), *Sobralia rosea* Poepp. Endl (Orchid), *Pourouma minor Benoist* (Chumico) and *Piper obliquum* Ruiz Pav (Matico liso). Likewise, 13 introduced and cultivated plant species were observed, 2 endemic species *Anthurium jaramilloi* and *Miconia dodsonii*, species that are in a vulnerable and endangered category (León-Yánez et al., 2011; IUCN, 2017) (Table 5).

Table 4. Habit of species.

Species	Habit	Species	Habit	Species	Habit
Bactris sp.	Tree	*Ficus sp.*	Tree	*Phytolacca rivinoides kunth &CD.Bouché*	Herbaceus
Ipomoea ramosissima (Poir.)Choisy	Herbaceus	*Floscopa sp.*	Herbaceus	*Piper aduncum L.*	Shrubs
Acalypha macrostachya Jacq.	Shrubs	*Gynerium sagittatum (Aubl.) P Beauv.*	Herbaceus	*Piper obliquum Ruiz & Pav.*	Shrubs
Aciotis indecora	Herbaceus	*Hedychium coronarium J. Koenig*	Herbaceus	*Piper peltatum*	Herbaceus
Aegiphila sellowiana Cham.	Tree	*Hedyosmum racemosum (Ruiz & Pav.) G. Don*	Shrubs	*Piper sp.1*	Shrubs
Aeschynomene americana var. glandulosa (Poir. ex Lam.) Rudd	Sub-Shrubs	*Heliconia orthotricha L. Andersson*	Herbaceus	*Piper sp.2*	Shrubs
Andropogon bicornis L.	Herbaceus	*Heliconia sp.*	Herbaceus	*Piptocoma discolor*	Shrubs
Anthurium jaramilloi	Herbaceus	*Himatanthus sucuuba Woodson*	Tree	*Pourouma bicolor Mart.*	Tree
Aphelandra neillii	Herbaceus	*Hyptis brevipes Poit.*	Herbaceus	*Pourouma cecropiifolia*	Tree
Asteraceae indeterminada	Herbaceus	*Inga edulis Mart.*	Herbaceus	*Pourouma minor Benoist*	Tree
Astrocaryum chambira Burret	Tree	*Inga leiocalycina Benth.*	Tree	*Pourouma sp.*	Tree
Baccharis latifolia (Ruiz & Pav.) Pers.	Shrubs	*Inga thibaudiana DC.*	Tree	*Pteridium arachnoideum (Kaulf.) Maxon*	Herbaceus
Baccharis trinervis Pers.	Shrubs	*Ipomoea batatas (L.) Lam.*	Climber	*Renealmia alpinia*	Herbaceus
Batocarpus orinocensis Karsten.	Tree	*Ipomoea ramosissima (Poir.)Choisy*	Herbaceus	*Renealmia sp.*	Herbaceus
Besleria aff. barbata (Poepp.) Hanst	Sub-Shrubs	*Iriartea deltoidea Ruiz & Pav.*	Tree	*Rhodospatha latifolia Poepp.*	Hemiepiphitic
Caladium steudneriifolium Engl.	Herbaceus	*Leandra cf. Caquetá Spruce*	Shrubs	*Rubus niveus*	Shrubs
Carica papaya L.	Tree	*Macrothelypteris torresiana*	Herbaceus	*Sacharum officinarum L.*	Shrubs
Cecropia andina Cuatrec.	Tree	*Manihot esculenta Crantz*	Shrubs	*Sapium marmieri Huber*	Tree
Cecropia ficifolia	Tree	*Matricaria recutita*	Herbaceus	*Sicydium tamnifolium (Kunth) Cogn.*	Bushes
Chelonanthus acutangulus (Ruiz & Pav.) Gilg	Herbaceus	*Mauritia flexuosa L.f.*	Tree	*Sida poeppigiana (K. Schum.) Fryxell*	Sub-Shrubs
Chelonanthus acutangulus (Ruiz & Pav.) Gilg	Herbaceus	*Merremia quinquefolia*	Herbaceus	*Sobralia rosea Poepp. & Endl.*	Herbaceus
Cissus verticillata (L.) Nicolson & C.E. Jarvis	Climber	*Miconia dodsonii*	Tree	*Socratea exorrhiza (Mart.) H. Wendl.*	Tree
Citrus medica L.	Shrubs	*Miconia sp.*	Shrubs	*Solanum quitoense Lam.*	Shrubs
Clidemia hirta	Shrubs	*Mikania sp.*	Herbaceus	*Solanum sp.*	Shrubs
Colacasia esculenta (L.) Schott.	Herbaceus	*Munnozia hastifolia (Poepp.) H. Rob. & Brettell*	Herbaceus	*Sp.1*	Herbaceus
Columnea inaequilatera Poepp.	Herbaceus	*Musa x paradisiaca L. (pro sp.)*	Herbaceus	*Sp.3*	Tree
Costus lasius Loes.	Herbaceus	*Myriocarpa stipitata Benth*	Tree	*Tessaria integrifolia Ruiz & Pav.*	Shrubs
Costus sp.	Herbaceus	*Niphidium crassifolium*	Herbaceus	*Tibouchina ciliaris (Vent.) Cogn.*	Shrubs
Cyperus aggregatus	Herbaceus	*Opuntia ficus-indica*	Shrubs	*Tibouchina ciliaris (Vent.) Cogn.*	Shrubs
Desmodium aff. purpusii Brandegee	Herbaceus	*Paspalum saccharoides Ness ex Trin.*	Herbaceus	*Urera baccifera (L.) Gaudich. /*	Tree
Drymonia urceolata Wiehler	Herbaceus	*Pennisetum purpureum*	Herbaceus	*Vernonanthura patens (Kunth) H. Rob.*	Tree
Epidendrum bracteatum Barb. Rodr.	Herbaceus	*Perebea guianensis Aubl.*	Tree	*Xanthosoma aff. pubescens Poepp.*	Herbaceus
Epidendrum calanthum	Herbaceus	*Philodendron aff. asplundii Croat & M.L. Soares*	Herbaceus	*Zapoteca sp.*	Shrubs
Epidendrum sp.	Herbaceus	*Philodendron ernestii Engl.*	Hemiepiphitic	*Sp.4*	Tree
Erythrina peruviana Krukoff	Tree	*Philodendron pedatum (Hook.) Kunth*	Hemiepiphitic	*Sp.5*	Tree
Ficus aff. insipida Willd	Tree	*Philodendron sp.*	Herbaceus	*Sp.6*	Tree
Ficus americana Aubl.	Tree	*Physalis pubescens L*	Herbaceus		

5 Fauna of the study area

5.1 Avifauna

Forty-two bird species were observed, which were placed in 13 orders and 22 families (2.31%) of the total birds recorded in Ecuador (MAE-SUIA, 2015). *Tyrannidae* (20%) and *Thraupidae* (15%) were the most abundant (Figure 8).

6 Biogeographic location

Twenty bird species were recorded as common and 13 as uncommon (Ridgely et al., 1998). The dominance of common species over uncommon species is an indicator of the disturbed area (Velásquez et al., 2003).

Tyrannus melancholicus (tropical tyrant), *Sicalis flaveola* (coarse seedeater), *Ramphocelus carbo* (wine-shell tanager), *Oryzoborus angolensis* (lesser seedeater), *Myiozetetes similis* (social flycatcher), *Doliornis remseni* (ventricast cotinga), and *Crotophaga ani* (pied tickcatcher) were the most abundant (Figure 11). The total number of species recorded in the area was 42, and the majority belonged to the Tyrannidae family. A higher percentage of species was recorded in point COFA-3; 49 sp. and in smaller numbers in point COFA-5 19 sp. This is mainly due to the fact that the area is intervened by mining activities.

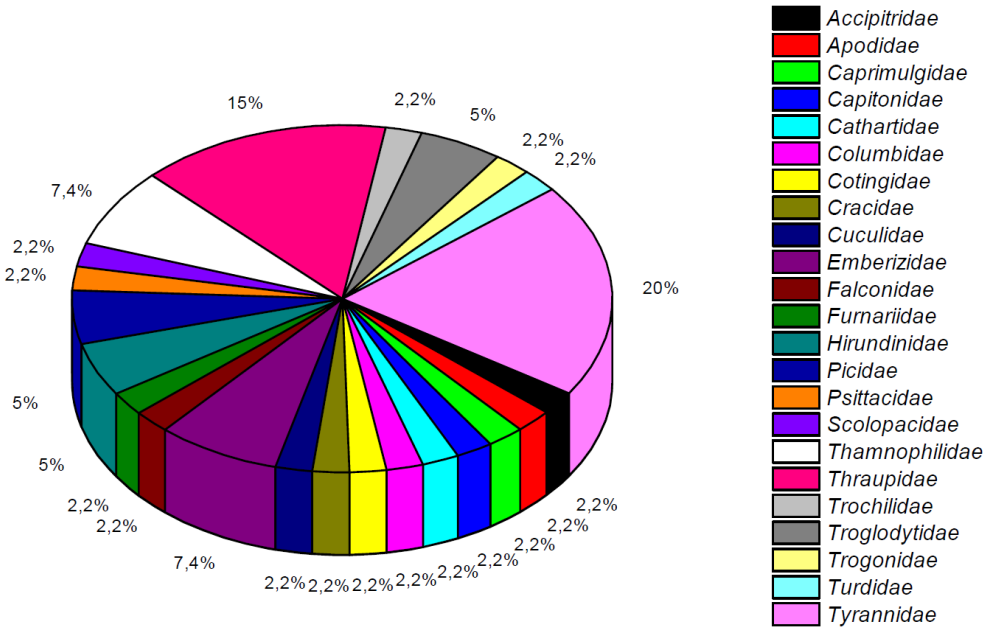

Figure 5. Percentages of birds present in the area.

The species accumulation curve for avifauna is an indicator of the rate at which new species can be found, and each unit of effort consists of sampling points carried out at strategic times and places. The negative exponential model was used to evaluate the quality of sampling and collection, obtaining a determination coefficient (R^2) of 0.9995, a slope of 0.0062 and a sampling effort of 87%, which indicates a good fit of the model and a complete and reliable sampling with a good inventory quality (Figure 6).

6.1 Biodiversity estimation

According to the Shannon-Weaner index, the value (3.1) for COFA-3 is an indicator of high diversity (Zamora, 2015). The biodiversity of the area was assessed by comparing observed and expected values (Pielou-J Index) (Moreno, 2001). The results are close to (1), so we infer that species are abundant in the different points (Table 6).

Table 5. Introduced species and conservation status according to IUCN.

Species	Status (IUCN) (IUCN)	Catalog: Vascular plants of Ecuador
Pennisetum purpureum	LC	Introduced
Citrus medica L.	NE	Introduced and cultivated
Colacasia esculenta (L.) Schott.	LC	Introduced and cultivated
Hedychium coronarium J. Koenig	-	Introduced
Macrothelypteris torresiana	NE	Introduced
Manihot esculenta Crantz	-	Introduced and cultivated
Matricaria recutita	LC	Introduced and cultivated
Musa x paradisiaca L. (pro sp.)	NE	Introduced and cultivated
Musa x paradisiaca L. (pro sp.)	-	Introduced and cultivated
Opuntia ficus-indica	DD	Introduced
Rubus niveus	LC	Introduced
Sacharum officinarum L.	LC	Introduced and cultivated
Urochloa aff. dictyoneura	NE	Introduced

Nomenclature: LC= Least Concern; NE =Not Evaluated; DD= Data Deficient.

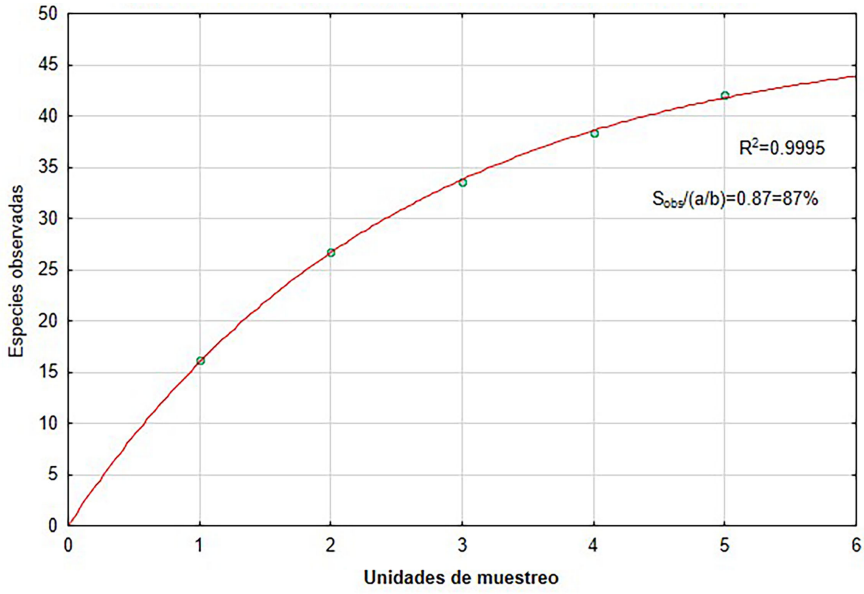

Figure 6. Bird species accumulation curve.

According to Simpson's index, there are no dominant species in the sampling points (Table 7).

6.2 Ecological aspects

The trophic guild was classified under 8 parameters according to the type of feeding or condition (Albuja, 2011). The trophic guild with the highest representation was the insectivore (23), the guild increased its f requency the further it was from the disturbed populations and areas (Canaday and Rivadeneyra, 2001) (Figure 7).

Table 6. Diversity index- avifauna.

Sampling Areas	N° of species	N° of individuals	Shannon Index (H)	Equity (j)	Location
COFA1	20		1,59535	0,8904	Average diversity
COFA2	37		2.36959	0.8979	Average diversity
COFA3	49	173	3.05037	0.9477	High diversity
COFA4	48		2.83833	0.9182	Average diversity
COFA5	19		2.47912	0.9394	Average diversity

Shannon-Weaner indexes and equity data sets.

Table 7. Calculation of Simpson's dominance index - Avifauna.

Sampling points	N° of species	Dominance	Diversity	Location	Location
COFA1	20	0.23	0.77	Low dominance	Average diversity
COFA2	37	0.12	0.88	Low dominance	High diversity
COFA3	49	0.06	0.94	Low dominance	High diversity
COFA4	48	0.07	0.93	Low dominance	High diversity
COFA5	19	0.10	0.90	Low dominance	High diversity

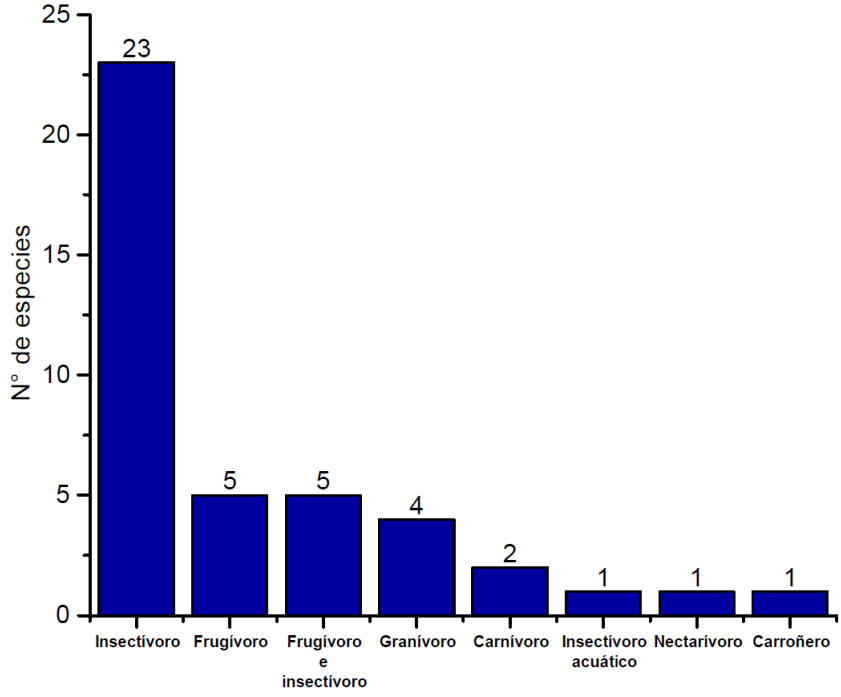

Figure 7. Trophic avifauna guild.

6.3 Sensitive and indicator species

Birds have different degrees of sensitivity to alterations in their environment (Stotz et al., 1996). Species with low sensitivity were recorded in greater numbers (28 sp.); medium sensitivity (13 sp.) and high sensitivity (1 sp.). The dominance of species with low sensitivity is an indicator of ecosystem disturbance.

6.4 Conservation state

Most species were placed in the "Least Concern" (LC) category (40 species); 2 species in the vulnerable category *Patagioenas subvinacea* (Red-breasted Pigeon) and *Doliornis remseni* (Ventricast Cotinga). The species *Buteo magnirostris* (Homing Sparrowhawk), *Thalurania furcata* (Hummingbird scissor-tailed nymph), *Amazona ochrocephala* (Yellow-crowned Amazon Parrot) and *Daptrius ater* (Black Caracara) are not endangered species, but their trade must be controlled (CITES, 2010).

In addition, 3 species of migratory birds, *Buteo magnirostris* (Sparrowhawk), *Coragyps atratusc* (Black Gallinule) and *Actitis macularius* (Sandpiper) (Appendix II), species with an unfavorable conservation status that require international agreements for their conservation (CMS, 2015) (Figure 8).

Figure 8. (A) Common Chickadee (*Crotophaga ani*) (B) Short-toed Sparrowhawk (*Buteo magnirostris*).

7 Presence of mammals

Through surveys, 16 mammal species were recorded (3.7% of the total number of mammals in Ecuador). 100% of those surveyed stated that they knew about it and had had sightings of *Dasypus novemcinctus* (9-banded armadillo), and *Bradypus variegatus* (sloth) and *Leopardus tigrinus* (small tigrillo) to a lesser extent (2.2%).

7.1 Records by indirect methods

The relative abundance index is the result of dividing the number of observations by the length of the route (Zapata et al., 2006). Eight species of mammals were recorded over a distance of 1.250 m (Table 8).

7.2 Estimation of diversity

Indirect sampling recorded 8 species of mammals. The relative abundance was determined through the number of tracks, and the results show a low diversity index (1.50) according to the Shannon-Wiener index.

7.3 Conservation state

Most of the species were found in the category of Least Concern (LC) except for *Cuniculus paca* (lowland guanta); *Tayassu pecari* (Wild Pig); *Mazama americana* (Red Deer) and *Leopardus tigrinus* (Small Tigrillo) which are in the categories: Near Threatened (NT), Endangered (EN), Near Threatened (NT) and Vulnerable (VU), respectively (Cuesta and Tirira, 2011). Most species are in the category of least concern, except *Tayassu pecari* (Wild Pig) and *Leopardus tigrinus* (Small Tigrillo) which are in the vulnerable

Table 8. Monitoring by indirect methods.

Specie	N	Abundance (n/km)	Distance by Specie (km)
Didelphis marsupialis	2	1.6	250
Marmosa murina	3	2.4	200
Dasypus novemcinctus	26	20.8	500
Sylvilagus brasiliensis	3	2.4	250
Cuniculus paca	4	3.2	250
Platyrrhinus brachycephalus	1	0.8	100
Carollia brevicauda	3	2.4	250
Dasyprocta fuliginosa	9	7.2	500
TOTAL	51	40.8	-

Data sets: Relative abundance of mammals in the area.

(VU) category (IUCN, 2017).

Three species were recorded in appendix III. *Nasua nasua* (Cuchucho); *Eira barbara* (Cabeza de mate) and *Cuniculus paca* (Guanta de tierras bajas); 2 species in appendix II, *Tayassu pecari* (Pecari de labio blanco) and *Bradypus variegatus* (Sloth); y *Leopardus tigrinus* (Small Tigrillo) in appendix I (Most threatened species) (CITES, 2017).

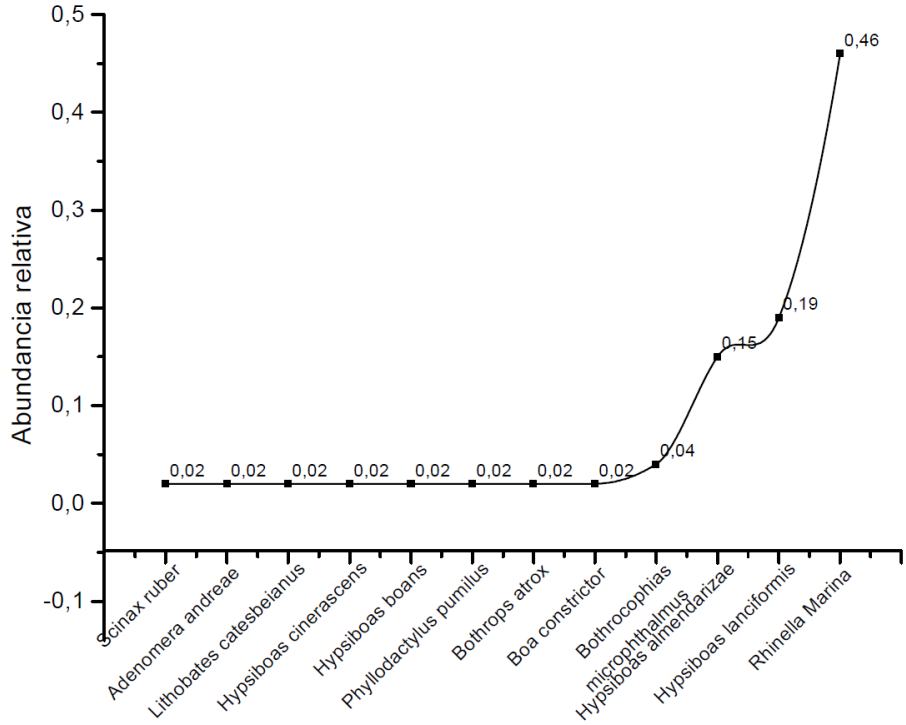

Figure 9. Abundance-diversity herpetofauna.

7.4 Trophic guild analysis of mammals

Changes in habitat and ecosystem were assessed, as well as the way in which species use their resources over time (Pérez-Irineo and Santos-Moreno, 2013). Six trophic groups were recorded at the site: carnivores (1); omnivores (5); insectivores (1); frugivores (6); folivores (1) and herbivores (2). (second trophic level) (secondary consumers).

8 Herpetofauna

There were 52 individuals (47 amphibians and 5 reptiles) 0.96% reptiles and 1.62% amphibians that were located in 7 families. The most abundant was Bufonidae with 46%, while Hylidae had 40%, and Leptodactylidae and Ranidae with 2%. Most reptiles belong to the Squamata family, with the highest abundance of Viperidae with 6%; while Gekkonidae and Boidae had 2%. As for amphibians, *Rhinella marina* (Giant Toad) was the most abundant (Figure 14) (0.46%), followed by *Hypsiboas lanciformis* (Tree Frog) (0.19%), *Hypsiboas almendarizae* (Tree Frog) (0.15%), *Bothrocophias microphthalmus* (Hoja podrida) (0.04%); the remaining species represent 0.02%. According to Shannon's index (1.70), the diversity in the study site is medium (Figure 13).

8.1 Conservation state

Hypsiboas almendarizae (tree frog) is in the Near Threatened (NT) category. *Bothrocophias microphthalmus* (Hoja podrida) and *Boa constrictor* (Boa Mata caballo) are in the vulnerable category (VU) (Carrillo et al., 2005; CITES, 2017) (Table 10). According to the IUCN most species are in the category "Least Concern" (LC). *Hypsiboas almendarizae* is an endemic species according to the red list of amphibians of Ecuador (Coloma, 2005).

Table 9. Mammalian guild analysis.

Specie	Trophic guild	Activity
Didelphis marsupialis	Om	Nocturnal terrestrial arboreal
Marmosa murina	Om	Arboreal nocturnal
Carollia brevicauda	Fr	Night forage
Platyrrhinus brachycephalus	Fr	Nocturnal
Dasypus novemcinctus	In	Terrestrial night
Sylvilagus brasiliensis	H	Terrestrial night
Sciurus granatensis	Fr	Diurnal arboreal
Cuniculus paca	Fr	Night foraging
Dasyprocta fuliginosa	Fr	Daytime
Nasua nasua	Om	Daytime
Eira barbara	Om	Diurnal-crepuscular arboreals
Mazama americana	H	Diurnal with more frequency at night
Ateles sp.	Om	Daytime
Tayassu pecari	Fr	Terrestrial and diurnal gregarious
Bradypus variegatus	Fo	Diurnal and nocturnal arboreals
Leopardus tigrinus	Cr	Nocturnal-crepuscular

Nomenclature: Carnivore (Cr), Frugivore (Fr), Insectivore (In), Omnivore (Om), Folivore (Fo), Herbivore (H).
Compiled from: Vallejo & Boada, (2014); Brito, Astua de Moraes, & Lew, (2015); Emmons y Feer, (1999); Tirira, (2007).

Table 10. Conservation status of Herpetofauna species.

Specie	UICN (2017)	CITES (2017)	Red lis of amphibians of Ecuador	Red list of reptiles of Ecuador
Rhinella Marina	LC	NA		
Scinax ruber	LC	NA		
Adenomera andreae	LC	NA		
Lithobates catesbeianus	LC	NA		
Hypsiboas almendarizae	NE	NA	NT	
Hypsiboas lanciformis	LC	NA		
Hypsiboas cinerascens	LC	NA	LC	
Hypsiboas boans	LC	NA	LC	
Phyllodactylus pumilus	DD			DD
Bothrops atrox	NE	NA		LC
Bothrocophias microphthalmus	NE	NA		VU
Boa constrictor	NE	Apendix I		VU

Nomenclature: DD =Data Deficient; LC = Least Concern; NT = Near Threatened; NE = Not Evaluated; VU = Vulnerable

Figure 10. (A) *Rhinella Marina* (Giant frog), (B) *Hypsiboas boans* (Tree frog).

8.2 Sensitive and indicator species

The "low sensitivity" condition is the most representative (8 species); medium sensitivity (3) and high sensitivity (1). The indicator species of disturbed environments are: *Rhinella marina* (Giant Toad), *Scinax ruber* (S/n), *Hypsiboas lanciformis* (Tree Frog), *Lithobates catesbeianus* (Bullfrog); *Bothrops atrox* (Equis); *Hypsiboas cinerascens* (Tree Frog) and *Hypsiboas boans* (Tree Frog) (IUCN, 2017).

9 Aquatic Fauna

Thirty-six aquatic macroinvertebrates were collected in 4 orders, 8 families and 10 genera (Figure 15). In the Chinapintza stream, 12 individuals were collected from 4 orders, 4 families and 6 genera. In the Congüime river, 13 individuals were collected from 3 orders, 5 families and 5 genera, and in the junction between these two rivers, 11 individuals were collected from 3 orders, 4 families and 4 genera (Figure 15).

Forty-two percent belonged to the order *Ephemeroptera* and 33% to the order *Diptera*, which in turn represents the largest number of genera at the sampling points (Figure 16). In the richness and abundance variables, there is no significant difference in the sampling points; the low abundance and richness is due to the evident contamination of the water due to mining activity in the area (Table 11).

Figure 11. Stereomicroscope photos: Macroinvertebrates (A) *Diptera* Order- Chironomidae family, the most abundant in the sampling points and tolerant to high levels of contamination; (B) *Coleoptera* Order- Elmidae family- *Cylloepus* genus; (C) *Diptera* Order- Empididae family; (D) *Coleoptera* Order- Hydrophilidae family; (E) *Coleoptera* Order- Elmidae family- *Neoelmis* genus, (F) *Ephemeroptera* Order- Baetidae family- *Paracloeodes* genus are tolerant to certain levels of pollution; (G) *Diptera* Order- Psychodidae family; (H) *Trichoptera* Order- Hydropsichydae family- *Smicridea genus*; (I) *Ephemeroptera* Order- Leptohyphidae family- *Trichorytodes* genus, considered as bioindicators of water quality like the *Trichoptera* order.

Table 11. Richness and abundance at each macroinvertebrate sampling point.

	Chinapintza Creek	Congüime River	Chinapintza and Congüime confluence
Wealth	4	5	4
Abundance	12	13	11

Wealth = Families; Abundance = Individuals

9.1 Diversity and abundance by family

Baetidae and Chironomidae, considered tolerant to certain levels of contamination (Mosquera, 2008), were dominant. The highest number was recorded in the Congüime river. It is important to mention that Chironomidae are associated with environments with low oxygen levels and high pollution levels (Hahn et al., 2009).

9.2 Estimation of diversity

According to the Shannon-Weaver index, the site is in a low diversity range, mainly due to water pollution. Simpson's dominance index shows medium values, with the presence of a certain number of dominant individuals in the community. According to the Margalef index, diversity in the area is low, as well as the evenness index, the values were medium and low (Table 12).

9.3 Water quality indices

The values of the BMWP/Col index in the 3 sampling points are 20%, 26% and 18%, respectively, and are located in class IV (very polluted waters with critical quality) (Zamora and Alba, 1996). In the ASPT calculation, values of 5, 5.2 and 4.5 were obtained, respectively, when compared with the BMWP/Col. indexes (Pérez, 1999) (Table 13).

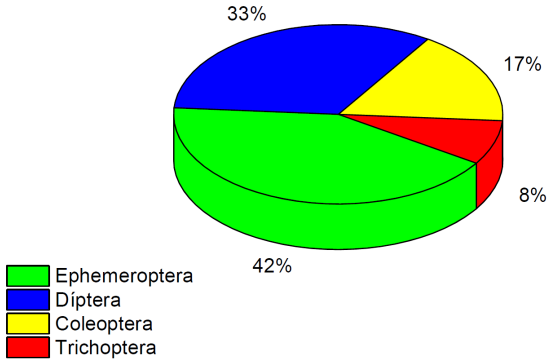

Figure 12. Total percentage of macro benthos orders present in the sampling points.

The calculation of the Andean Biotic Index (ABI) shows values of 16, 20 and 15, respectively, and according to these parameters we infer that the water quality is poor with a range between 11 and 26 (Loayza, 2016) (Table 13).

Table 12. Diversity indices- Macroinvertebrates.

Index	P1	P2	P3
Shannon-Weaver index	1.27	0.90	1.04
Simpson dominance	0.31	0.52	0.37
Margalef diversity	3.22	3.90	4.02
Uniformity index	0.35	0.24	0.30

Table 13. BMWP/Col., ASPT and ABI indices for each sampling point.

CHINAPINTZA RIVER				
Index	**Value**	**Range**	**Quality**	**Meaning**
BMWP/Col	20	16 - 35	Critical	Highly polluted waters
ASPT	5	0-10		
ABI	16	01/11/26	Bad	–
CONGÜIME RIVER				
BMWP/Col	26	16 - 35	Critical	Highly polluted waters
ASPT	5,2	0-10		
ABI	20	01/11/26	Bad	–
INTERSECTION OF THE RIVERS				
BMWP/Col	18	16 - 35	Critical	Highly polluted waters
ASPT	4,5	0-10		
ABI	15	01/11/26	Bad	–

BMWP/Col (Biological Monitoring Working Party/ modified by Colombia); ASPT (Average Score per Taxon); ABI (Andean Biotic Indexes).

10 Conclusions

The objective of this research was to evaluate the effect of mining activity on the biodiversity of the study area, estimating possible changes in the ecosystem in relation to fragmentation, abundance, richness, dominance and diversity of species. According to the results, we can conclude that the flora of the site has a medium level, although the level of species dominance is low in all sampling points. As for fauna, the level is medium for avifauna, except in point COFA-3 where diversity is high. A total of 42 species of birds were recorded in 22 families, with the highest abundance presented by *Tyrannidae* and *Thraupidae*, species considered with low sensitivity (28 sp.). Sixteen species of mammals were recorded; *Cuniculus paca*, *Tayassu pecari*, *Mazama americana* and *Leopardus tigrinus* are species considered highly threatened; the relative abundance of these species is low (1.50). In terms of herpetofauna *Bufonidae* (46%) and *Hylidae* (40%) were the most abundant. Most of the reptiles belong to the *Viperidae* family (6%). *Rhinella marina* (giant neotropical toad or marine toad) was the most common

amphibian species. In the riverbed, 36 macroinvertebrates were identified in 4 orders and 8 families, predominantly Baetidae and Chironomidae, species considered tolerant to water pollution.

Therefore, the study area showed a medium diversity and dominance of species with low sensitivity, most of them generalists, with more abundance of frugivorous and omnivorous guild and indicators of disturbed environments due to mining activity, agricultural and livestock expansion and deforestation.

References

Albuja, L. (2011). *Fauna de Guiyero, Parque Nacional Yasuní*. Escuela Politécnica Nacional-EcoFondo.

Alexa, I. (2010). Biodiversidad y factores que lo determinan. Blogspot. Online:https://bit.ly/3zOqJa3.

Arévalo, J. (2001). *Manual de campo para el monitoreo de mamíferos terrestres en áreas de conservación*. Asociación de conservacionistas de Monteverde.

Arias, R., Tapia, A., Tapia, A., Santacruz, L., Yasaca, R., and Miranda, N. (2012). Evaluación de la biodiversidad en cinco comunidades kichwa de la zona de colonización de la alta amazonía ecuatoriana. *Revista Amazónica Ciencia y Tecnología*, 1(3):157–172. Online:https://bit.ly/3GA9dub.

Balderrama, J., Aguirre, L., Aguayo, R., Alfaro, F., Rejas, D., and Zuñiga, L. (2005). *Técnicas de Colecta y Censo de Fauna*. Museo Americano de Historia Natural.

Bispo, P.and Oliveira, L., Bini, L., and Sousa, K. (2006). Ephemeroptera, plecoptera and trichoptera assemblages from riffles in mountain streams of central brazil: environmental factors influencing the distribution and abundance of immatures. *Brazilian Journal of Biology*, 66:611–622. Online:https://bit.ly/3KvzniC.

Campo, A. and Duval, V. (2014a). Diversidad y valor de importancia para la conservación de la vegetación natural: Parque nacional lihué calel (argentina). *Anales de Geografía*, 34(2):25–42. Online:https://bit.ly/3mX4IDn.

Campo, A. and Duval, V. (2014b). Diversidad y valor de importancia para la conservación de la vegetación natural: Parque nacional lihué calel (argentina). 34(2):25–42. Online:https://bit.ly/3nVhT7K.

Canaday, C. and Rivadeneyra, J. (2001). Initial effects of a petroleum operation on amazonian birds: terrestrial insectivores retreat. *Biodiversity & Conservation*, 10:567–595. Online:https://bit.ly/3oJV3AB.

Carrera, C. and Fierro, K. (2001). *Manual de monitoreo los macroinvertebrados acuáticos como indicadores de la calidad del agua*. EcoCiencia.

Carrillo, E., Altamirano, M., and Ayala, F. (2005). *Lista Roja de los Reptiles del Ecuador*. Fundación Novum Milenium, UICN-Sur, UICN-Comité Ecuatoriano, Ministerio de Educación y Cultura.

CITES (2010). *Listados actualizados de las especies de Fauna y Flora*. CITES.

CITES (2017). Convenciøn sobre el comercio internacional de especies amenazadas de fauna y flora silvestres. CITES. Online:https://bit.ly/404TYAg.

CMS (2015). Convención sobre la conservación de las especies migratorias de animales silvestres. Technical report, CMS. España.

Coloma, L. (2005). Anfibios de ecuador. quito: Museo de zoología, pontificia universidad católica del ecuador. Online. Información de los años 200-2008.

Cuesta, F. and Tirira, D. (2011). *Libro rojo de los Mamiferos del Ecuador*. Fundación Mamíferos y Conservación. Pontificia Universidad Católica del Ecuador y Ministerio del Ambiente del Ecuador, 2a edition.

Equipo MMSD América del Sur (2002). *Minería, minerales y desarrollo sustentable en América del Sur*. Equipo MMSD América del Sur.

Hahn, C., Toro, D., Grajales-Quintero, A., Duque, G., and Serna, L. (2009). Determinación de la calidad del agua mediante indicadores biológicos y fisicoquímicos, en la estación piscícola, universidad de caldas, municipio de palestina, colombia. *Boletín Científico. Centro de Museos. Museo de Historia Natural*, 13(2):89–105. Online:https://bit.ly/3Hef88e.

IUCN (2017). The iucn red list of threatened species. IUCN. Online:https://bit.ly/2Rxndc0.

Jørgensen, P. and León-Yánez, S. (1999). *Catalogue of the vascular plants of Ecuador*, volume 75. Missouri Botanical Garden Press St. Louis.

León-Yánez, S., Valencia, R., Pitman, N., Endara, L., Ulloa, C., and Navarrete, H. (2011). Libro rojo de las plantas endémicas del ecuador. *Pontificia Universidad Católica del Ecuador*.

Loayza, R. (2016). Macroinvertebrados como indicadores de la calidad de agua. Online:https://bit.ly/3Lspbt8.

MAE (2015). Programa de las naciones unidas para el desarrollo gobierno de ecuador. Web Page. Online: https://www.undp.org/es/ecuador.

MAE-SUIA (2015). Ministerio del ambiente-sistema único de información ambiental. MAE-SUIA. Online:https://bit.ly/43ZDvjT.

Moreno, C. E. (2001). *Métodos para medir la biodiversidad*. Manuales y tesis SEA.

Mosquera, D. (2008). Bioindicación de la calidad del agua del río cali, valle del cauca, colombia; usando macroinvertebrados acuáticos. *Revista de la Asociación Colombiana de Ciencias Biológicas*, 1(20. Online:https://bit.ly/3L9Jl9F).

Myers, N., Mittermeier, R., Mittermeier, C., and Da Fonseca, G.and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403(6772):853–858. Online:https://go.nature.com/2Gnztdw.

Pérez, G. (1999). Los macroinvertebrados y su valor como indicadores de la calidad del agua. *Academia Colombiana de Ciencia*, 23(88):375–387. Online:https://n9.cl/t5zuq.

Pérez-Irineo, G. and Santos-Moreno, A. (2013). Riqueza de especies y gremios tróficos de mamíferos carnívoros en una selva alta del sureste de méxico. *Therya*, 4(3):551–564. Online:https://n9.cl/lomj5.

Ridgely, R., Greenfield, P., and Guerrero, M. (1998). *Una lista anotada de las Aves del Ecuador Continental*. Fundacion Ornitologica del Ecuador, CECIA.

Shannon, C. and Weaver, W. (1949). *Amazonía ecuatoriana bajo presión*. University of Illinois Press.

Stotz, D., Fitzpatrick, J., Parker, T., and Moskovits, D. (1996). *Neotropical birds: ecology and conservation*. University of Chicago Press.

Toro, H. (2006). Organización del tratado de cooperación amazónica (otca). In *Reunión Subregional de Representantes y Directores de Centro del Cono Sur y Area Andina*, pages 1–20. OPS.

Velásquez, A., Ricaurte, L., Lara, F., Cruz, E., Tenorio, G., and Correa, M. (2003). Lista anotada de las aves de los humedales de la parte alta del departamento de caqueta. In *MEMORIAS: Manejo de Fauna Silvestre en Amazonía y Latinoamérica*, pages 320–329.

Yánez, M., Reyes, J., and Meza, P. (2007). Análisis de diversidad de la herpetofauna en el área de guayllabanba. distrito metropolitano de quito. Online:https://bit.ly/3AuZ4vg.

Zamora, C. and Alba, J. (1996). Bioassessment of organically polluted spanish rivers, using a biotic index and multivariate methods. *Journal of the North American Benthological Society*, 15(3):332–352. Online:https://bit.ly/3Lwodfa.

Zamora, H. (2015). Macroinvertebrados acuáticos registrados durante la época de lluvias en tres ríos del piedemonte llanero de colombia. *Revista Colombiana de Ciencia Animal*, 7(2):139–147. Online:https://n9.cl/553n7.

Zapata, G., Araguillin, E., and Jorgenson, J. (2006). Caracterización de la comunidad de mamíferos no voladores en las estribaciones orientales de la cordillera del kutukú, amazonia ecuatoriana. *Mastozoología neotropical*, 13(2):227–238. Online:https://n9.cl/k58v0.

La granja:
Revista de
Ciencias de la Vida

pISSN:1390-3799; eISSN:1390-8596

http://doi.org/10.17163/lgr.n38.2023.09

Review / Reseña bibliográfica

Veterinary Sciences

Reproductive parameters in the fingerling production of tilapia *Oreochromis niloticus*: review

Parámetros reproductivos en la producción de crías tilapia *Oreochromis niloticus*: revisión

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Article received on August 16th, 2022. Accepted, after review, on October 19th, 2022. Published on September 1st, 2023.

Abstract

Aquaculture contributes significantly to the food source for human consumption. Tilapia *Oreochromis niloticus* is one of the main fish in aquaculture production, so its reproduction phases are vital to ensure the quality and quantity of organisms available for production systems. The reproductive parameters in fish aquaculture activities, such as fertility, fecundity, egg diameter, gonadosomatic index, survival rate in larvae and fingerlings, among others, are relevant for an economically profitable and sustainable production, and these can vary according to the diet, environmental conditions, age, genetic characteristics of the fish or the quality of the water. Therefore, this document aims to identify the reproductive parameters that influence on the production of fingerlings tilapia to present a general description in a simple way of the different reproductive parameters involved in aquaculture production activities of fingerlings tilapia. A search of the information in the last 20 years incorporated in various specialized databases was carried out. In this research, information is compiled to date on the reproductive parameters in tilapia, the results indicate that there is no more important reproductive parameter than another, since it is a cluster of factors and synergy that intervene in reproduction, hence it is necessary to establish clear management plans and research in the production systems to improve and enhance their production. Knowing the reproductive parameters of tilapia can help reduce production costs, thus it is necessary to establish clear management plans and research in production systems to improve and

enhance their production.

Keywords: Reproduction, fingerlings production, fertility, fecundity, fingerlings.

Resumen

La acuicultura contribuye de manera importante a la fuente de alimento destinado al consumo humano. La tilapia *Oreochromis niloticus* es uno de los peces principales de la producción acuícola, por lo que las fases de su reproducción son vitales para asegurar la calidad y cantidad de organismos disponibles para los sistemas de producción. Los parámetros reproductivos en actividades acuícolas de peces, como la fertilidad, fecundidad, diámetro de huevos, índice gonadosomático, tasa de supervivencia en larvas y alevines, entre otros, son de relevancia para una producción económicamente rentable y sostenible, y estos pueden variar según la dieta, condiciones ambientales, edad, características genéticas de los peces o la calidad del agua. Por lo anterior, este documento tiene como objetivo identificar los parámetros reproductivos que tengan una influencia en la producción de crías de tilapia presentar una descripción general de forma sencilla los diferentes parámetros reproductivos involucrados y de consideración en actividades de producción acuícola de crías de tilapia. Se realizó una búsqueda de la información en los últimos 20 años incorporada en diversas bases de datos especializadas. En esta investigación se recopila la información a la actualidad sobre los parámetros reproductivos en tilapia, y los resultados indican que no existe un parámetro reproductivo más importante que otro, ya que es un cúmulo de factores y sinergia que intervienen en la reproducción, por lo que resulta necesario establecer planes de manejo claro e investigación particular en los sistemas de producción para mejorar y potencializar su producción. El conocimiento de los parámetros reproductivos de la tilapia puede ayudar a disminuir los costos de producción para establecer planes de manejo claro e investigación particular en los sistemas de producción para mejorar y potencializar su producción.

Palabras clave: reproducción, producción de crías, fertilidad, fecundidad, alevines.

Suggested citation: Reyes-Trigueros, L., Monroy-Dosta, M., Torres-Ochoa, E., Cortés-Sánchez, A. y Espinosa-Chaurand, L. (2023). Reproductive parameters in the fingerling production of tilapia *Oreochromis niloticus*: review. La Granja: Revista de Ciencias de la Vida. Vol. 38(2):122-134. http://doi.org/10.17163/lgr.n38.2023.09.

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1 Introduction

Tilapia (*Oreochomis niloticus*) is an economic important species in Mexico, with a production above 168 thousand tons live weight reported in 2018 (CONAPESCA, 2018). Tilapia is a favorable organism for aquaculture because of its characteristics, such as its wide range of tolerance to environmental variations, its easy reproduction and its potential for cultivation (El-Sayed, 2016). Tilapia cultivation has responded to the high-quality production of food at low cost in rural areas.

The reproductive behavior of tilapia differs from others. The reproductive activity of tilapias of the genus *Oreochromis* occurs throughout the year if the environmental conditions allow an early reproduction (Vega-Villasante et al., 2009), since sexual maturation occurs at sizes below the commercial size of 250g approximately, between 50 and 100g (El-Sayed, 2016). Differences in growth between males and females are observed, since males have a higher growth rate and greater efficiency in the feed conversion rate. For this reason, the application of hormonal treatments to freshly hatched fingerlings has allowed optimizing biomass yields obtained on a commercial scale.

It is not advisable to underestimate the importance of environmental stimuli on tilapia reproduction, such as photoperiod, temperature, water quality and good nutrition (Carrillo et al., 2009). Reproductive success in terms of fertility, percentage of larvae obtained, growth rate, survival, disease resistance and adult fish shape are determined by the genetic characteristics of the species (Perea-Ganchou et al., 2017). Therefore, it is very important to determine the reproductive parameters that may interfere with the production of tilapia *O. niloticus*.

Therefore, this document focuses on providing reproductive parameters that have influenced on the production of tilapia breeding, describing in a general way the parameters involved in production activities of tilapia breeding to contribute to a scientific basis orientation for aquaculture producers, academics, government agencies and the general public interested in aquaculture activities in reproductive phases, and to define controlled strategies for obtaining rearing of this aquaculture species.

2 Methodology

Descriptive bibliographic research was conducted using various specialized databases such as Web of Science, Redalyc, ELSEVIER, SciELO, ACS Publications, Dialnet, Scince.gov, as well as on the websites of government agencies and institutes within the aquaculture sector such as the National Commission for Fisheries (CONAPESCA) and the National Fisheries Institute (INAPESCA) of Mexico, and the World Organization for Agriculture and Food of the United Nations (FAO). The main search keywords used were tilapia, tilapia from the nile, *Oreochromis*, reproduction, reproductive parameters, reproduction in tilapia, tilapia characteristics, physiology of reproduction, etc., in Spanish and English. Approximately 300 documents were collected, from which a first filtering was performed, resulting 174 documents of related or complementary information, which were filtered a second time for the specific parameters of this review to the number within the selected years, resulting in 40 references.

3 Important characteristics of tilapia

Tilapia is a fish native to Africa, currently distributed in America, Southeast Asia, some countries in Europe and even Australia. It belongs to a group of fish from Jordan, Israel and Africa, and was dispersed, transported and adapted by almost all other regions of the world; its breeding was successful, so it was introduced into the tropical and subtropical regions of various countries (Zimmermann, 2005). In Mexico, tilapia was introduced in 1964 from the United States of America, which was reproduced in almost all areas of the country (INAPESCA, 2018).

It has become one of the most cultivated species worldwide due to its tolerance to environmental variations, which has allowed it to develop in poorly oxygenated waters, sweet or salt, has a rapid growth, high reproductive capacity and adaptation to live in captivity conditions, as well as high crop densities, besides providing nutritional food with good taste, little spine and affordable price (Oso et al., 2006; Vega-Villasante et al., 2009).

The ideal temperature for its breeding oscillates between 31 to 36°C (INAPESCA, 2018; FAO, 2022).

It is possible to grow its crop at intervals of 20 to 30°C, but it may not develop or may even die at temperatures of less than 15°C or above 40°C (Saavedra Martínez, 2006; FAO, 2022). Another factor influencing survival is pH, which can be optimal for the species if it is in a value range of seven and eight, if this value is at pH conditions equal to or less than five it generates a harmful environment for fish (INAPESCA, 2018).

For oxygen dissolved in water, the optimal values are in the range of five to six mg/L, while there could be serious growth damage between two to three mg/L; and values equal to or less than one are critical, which can lead to death. As for turbidity, it is recommended to maintain a visibility of 30 cm depth (Saavedra Martínez, 2006).

Its growth is accelerated, reaching 500-680g in six to nine months according to the cultivation system used (Noriega-Salazar et al., 2020; FAO, 2022). Globally, it is one of the most studied fish, both in its life cycle as well as in its type of nutrition, eating habits, type of reproduction, disease resistance and handling, which facilitates its proliferation and handling in farming systems (Saavedra Martínez, 2006; FAO, 2022). Tilapias belonging to the genus *Oreochromis* have an omnivorous diet, making it possible for them to ingest algae, small aquatic organisms, roots, zooplankton, insects, bacteria, among other things (Vega-Villasante et al., 2009).

They also facilitate the filtering process with branchiospins, which allows them to eat the food (Nasrin et al., 2021). When swallowing the food it must pass through the pharynx of the fish, where it is crushed in the pharyngeal teeth, so that later it can continue with the digestion process (Yem et al., 2020). The type of food in crops can vary, reaching annual yields of between 5 tons/ha or more (FAO, 2022).

4 Reproductive features

4.1 Reproductive features of *O. niloticus* tilapia

As for sexual dimorphism, males have two orifices located in the caudal area, which are composed of the anus and the urogenital orifice, while the female has three orifices near the caudal area, these would be the anus, the genital pore and the urine excretory orifice or urinary orifice (Hussain, 2004; Saavedra Martínez, 2006). Urogenital orifice in males is a tiny point, and its urinary orifice in females is microscopic; a good way to differentiate the sexual genera of tilapias consists in identifying the genital pore in the female, which is located in a slit, perpendicular to the axis of the body (Saavedra Martínez, 2006) (Figure 1).

Sexual maturity occurs from five to six months, and although this is also related to weight it is known that sexual maturity in tilapias can be reached from 30g from two to four months if environmental conditions are favorable. Once females reach size for maturity, they can spawn eight to 12 times per year, depending on the temperature and other conditions (INAPESCA, 2018).

Spawning usually begins when the temperature reaches about 24°C, however, reproduction in natural environments takes place when the male first establishes an area and creates a hole for a nest. Later, he dedicates to watch over the nest until the female comes to spawn, so that the male fertilizes the eggs laid by the female, and once these have been fertilized by the male, the female places the eggs inside her mouth (oral incubation) and then she leaves with them (Vega-Villasante et al., 2009).

The female keeps the eggs in her mouth for hatching for one or two weeks (INAPESCA, 2018). Once pups are released from the eggs they can leave the mother's mouth, but if they are threatened by some danger, they tend to re-enter (INAPESCA, 2018; FAO, 2022).

The number, volume, and size of eggs a female can spawn depends on the mother's size. It is estimated that a female weighing up to 100 g may spawn approximately 100 eggs, but if her weight increases over a range of 600 to 1000g, the number of eggs she can spawn will increase from 1000 to 1500 (Perdomo et al., 2020; FAO, 2022). The same occurs under culture conditions, i.e., males can reach sexual maturity between four to six months, while females between three and five months.

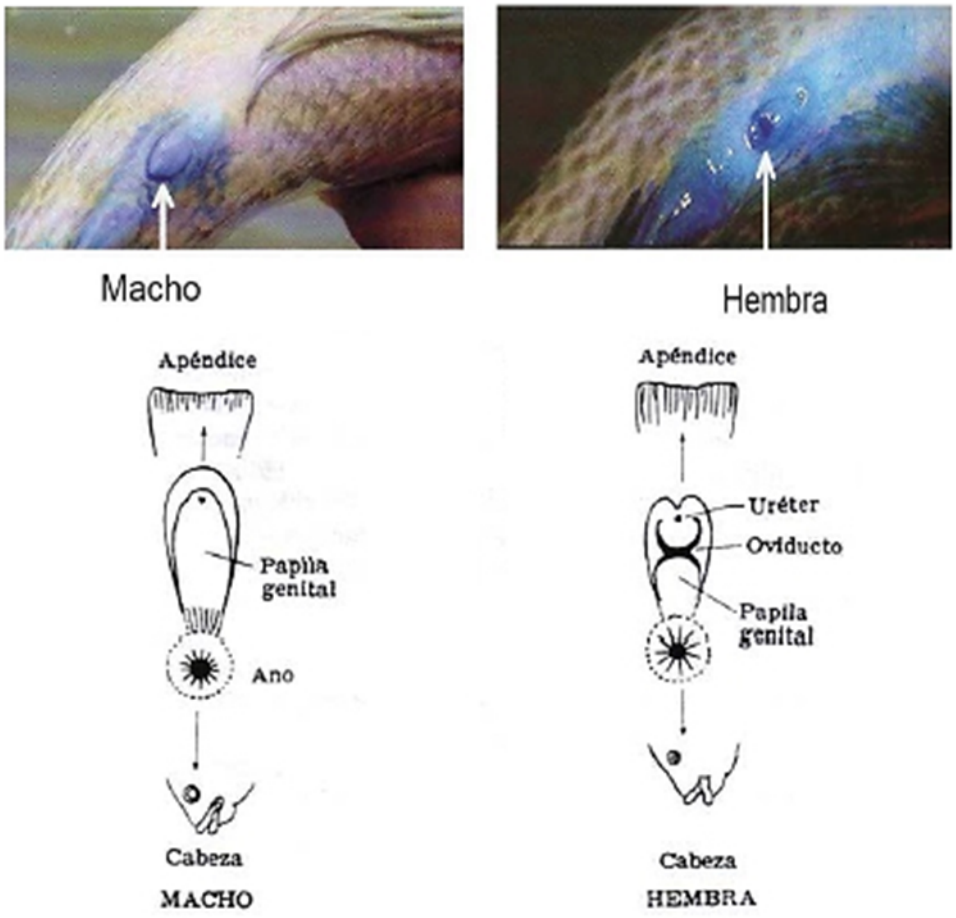

Figure 1. External morphological characteristics of sexual dimorphism of *O. niloticus* (Huet, 1998; Saavedra Martínez, 2006).

4.2 Reproductive evaluation

Reproduction is a mechanism used by living beings to maintain the prevalence of their species. Each organism has its reproduction strategies, and its success depends on the reproductive traits of each individual, which are stipulated by the genetics. In the case of farmed fish, reproduction is primarily defined by local environmental conditions and available infrastructure (FAO, 2022).

Fertility in fish is one of the most important data for reproduction in captivity, since it is possible to determine with this information the amount of eggs that a female can produce, i.e., it is used to calculate the reproductive potential of a fish population and survival from egg to recruitment, while fertility helps to know how many of the eggs produced will survive, therefore, fertility is the ability of females and males to conceive viable offspring (FAO, 2022; Zimmermann, 2005).

Another important data in the reproduction of fish is the hatching rate, making it possible to know the potential spawners have to produce viable eggs. The hatching rate refers to how many of the eggs produced and fertilized can hatch, since eggs can often be affected by various variables, compromising their viability and preventing hatching (Anene and Okorie, 2008; Abarike and Ampofo-Yeboah, 2016).

Table 1. Studies of reproductive parameters of tilapia (*O. niloticus*) and its relationship with environmental variables, fish age, absolute fecundity (Fa), relative fecundity (Rf), dissolved oxygen (DO), Chlorophyll a (Chl a), weight of the gonads (Pg), total body weight (Tbw) and gonadosomatic index (GSI).

Author(s):	Reproductive parameters studied	Method	Contribution
Costa and Carvalho (2012)	1-GSI	1-Effect of physicochemical and environmental variables. 2.-Spearman correlation to detect relationships with physicochemical variables measured	1-GSI values were positively related to pH, DO, conductivity, water transparency and Chl a. GSI values showed a response to the hydrological cycle, with a two-month delay for increases and decreases in values.
Massako et al. (2015)	1-Spawning frequency 2-Number of eggs per spawning.	1-In situ verification once a week and statistical model of eggs/female, according to the ages of females (1, 2 and 3 years). 2-Volume.	1-The spawning frequency was higher in 3-year-old females, with a total of 3.49, while 2-year-old females only had a value of 0.80 at site 2, and 1.53 at site 1. 2- One-year-old females with fewer eggs (13 435 and 16 105 eggs) than those of 2 years (43 395 and 24 650 eggs).
Abarike and Ampofo-Yeboah (2016)	1-GSI 2-Development of gonads 3-Fertility	1-The following formula was performed: GSI=Gw/Ptc*100. 2-Direct observation. 3- Fertility vs. body length was calculated using the Fertility-Total Length ratio	1-The highest GSI was recorded at higher temperature. 2-Only 20% of females were loaded, and the following stages were identified in their ovate: -Immature (white) = 7.14% January 2007.-Maturing (yellow) = 7.14% March 2007.-Mature (deep green) = 50% April 2007-Spent (Red, floppy) = 35.7% March 2007. 3-0% fertility in the cold months (nov-feb). 4.-The correlation between fecundity vs. body length was higher than the correlation between fecundity vs. body weight. The average fecundity was 173 oocytes.
Sièfo et al. (2018)	1-Fertility 2-GSI	1-Fa: Number of oocytes in ovaries. 1-Fr: Ratio of absolute fecundity to total body weight of the sample. 2-GSI: Ratio of gonad weight to gutted body weight.	1-Average absolute fecundity was 412 and ranged between 174 and 593. Relative fecundity ranged from 3 to 9 oocytes per gram of body weight, with an average of 6 oocytes/g. 2-The highest percentage of GSI for females occurred between the warmest months (April and July).
Teame et al. (2018)	1-Size of first maturity 2-GSI 3-Fertility	1-Direct observation 2-It was calculated as the percentage of the weight of the gonads with respect to the total weight of the fish 3-Direct counting and statistical analysis	1-The smallest sexually mature male measured 14cm, and the female measured 12.5cm. Sexual maturity in males (50%) was 15cm, and females 14cm. 2-Males and females followed almost the same trend. Males had higher average values in July, and females in August. Two peaks of GSI values were recorded in females during February and August, indicating that females can reproduce more than once a year3-It was estimated for 30 females a total size of 14cm up to 37cm, and with a weight of 78.8 to 711g. The total number of eggs ranged from 399 to 2129, the fecundity ranged from 104 to 709 eggs corresponding to fish with sizes from 12.5 to 20.9cm. Fertility ranged from 243 to 847 eggs per fish
Tessema et al. (2019)	1-Fertility 2-GSI	1-Fa was determined gravimetrically and statistically with 2-way ANOVA 2-Size-weight ratio of fish	1-The average Fa was 217 eggs/fish and was positively correlated with the total length, total weight and weight of the gonads. 2-GSI values of males and females were highest in April and lowest in February. The highest average value in females was 2.6 in April, and 0.7 for males in the same month

Table 2. Studies of reproductive parameters and their relationship with diets implemented in tilapia (*O. niloticus*) and their relationship with genetic characteristics, Relative Number of Eggs Being (NRH), Spawning Index (ID), Absolute Fertility (Fa) and Relative Fertility (Rf).

Author(s):	Reproductive parameters studied	Method	Results
Quốc et al. (2013)	1-Fr 2-Fertilization rate	1-Females belonging to generation 12 of the tilapia GIFT strain were used in the Vietnam Mekong delta, where they were divided into 4 experiments named as "FAM" (Family), "MM" (Multiple Males and Multiple Females), "SM-1" (Single Male and Multiple Females) with a repetition called "SM-2" 2-Fr was calculated by the total number of eggs per female/Body weight of spawned females 3-Fertilization rate was calculated as 100*total number of eggs fertilized per female/total number of eggs per female	1-Females in MM and SM-1 on average had the highest values of Fr2. In general, the fertilization rate was good, ranging between 77 and 87%.
Bizarro et al. (2019)	1-GSI	1-150 fingerlings of *O. niloticus* GIFT were exposed to different light intensities. 1-GSI calculated by gonad weight/body weight*100	1-GSI did not vary much with respect to the photoperiod
Silva et al. (2020)	1-Fa and Fr 2-ID 3-Hatching rate 4-NRH	1-Tilapias of the variation "Aqua America" and "GIFT" tilapias were used, making a cross between them to create 4 experiments with the following tilapias: Aqua America non-inbred, Aqua America inbred, GIFT and Aqua America×GIFT 2-Fa was calculated with the number of eggs per spawning, while Fr was calculated with the number of eggs per spawning/g female weight 3-The ID was calculated with the total weight of eggs per spawning in g / female weight in g*100 -% 4-The hatching rate was calculated with number of hatched larvae in the sample/ total number of eggs and larvae in the sample] * 100 -% 5-The NRH was calculated with the number of eggs/g of eggs	1-The highest values of Fa were found in the Aqua America×GIFT genetic group, having a value of 7084.3, while the genetic group with the lowest value was found in GIFT, having a value of 2581.1. The highest Fr values were found in the Aqua America×GIFT genetic group, having a value of 5.4, and the lowest value was found in the GIFT genetic group; obtaining a value of 2.2 2-The highest and lowest valuesof ID were 3.0 and 0.9 respectively, and were found in the genetic groups of Aqua America×GIFT and Aqua America endogamous, respectively 3-The highest and lowest Hatching Rate values were 99.2 and 93.0 respectively, belonging to the non-inbred Aqua América and Aqua América×GIFT genetic groups, respectively 4- The highest and lowest valuesof NRH were 247.6 and 168.8 respectively, and were found in the genetic groups of non-endogamous Aqua America and Aqua America×GIFT. 5-The Fa, Fr, ID and hatching rate did not differ significantly between the genetic groups

One of the great challenges in aquaculture is to be able to control reproduction effectively, since this physiological process has a marked seasonal characteristic, which is interpreted by specific sensory systems that culminate in a hormonal cascade mediated by the endocrine system (Carrillo et al., 2009). The goal is that reproduction occurs in the most favorable place and time for the survival of the progeny, but in almost all cases it is random due to the lack of control of some components (Navas, 2009). The main disadvantages faced in this process are the changes in the natural environmental conditions, reason for which they are programmed in production systems.

Table 3. Studies of reproductive parameters and their relationship with the diets implemented in tilapia (*O. niloticus*), Total Egg Weight per Female (PTHH), Number of Eggs per Gram (NHG), Spawning Index (ID), Survival During the Lecitotrophic Period (SPDL), Body Weight (BW), Absolute Fertility (Fa) and Relative Fertility (Rf).

Author(s):	Reproductive parameters studied	Diet	Method	Contribution
Moraes et al. (2014)	1-Fa and Fr 2-Diameter egg 3-Fasting larval survival capacity	5 servings of crude protein at 32, 34, 36, 38 and 40%, one for each treatment	1- The number of eggs per spawning was counted to calculate the Fa, and the total number of eggs per gram of body weight of the female was counted to calculate the Fr. 2-100 eggs were measured for each treatment, using a stereoscopic microscope with micrometric ocular microscope. 3-2000 larvae were collected per treatment for 3 days to evaluate the effect of the rations provided to the fingerlings on the survival time of the fasting.	1-The highest values of Fa and Fr were found in the diet of 38% crude protein, and the lowest in the diet of 32%. 2-The highest values of egg diameter were found in the diet of 38% crude protein, and the lowest in the diet of 32%. 3-The highest value of the larval survival capacity in fasting was in the diet with 38% crude protein, while the lowest value was found in the diet with 32% crude protein.
Mehrim et al. (2015)	1-Fa and Fr 2-Number of eggs	8 treatments with a brand name hydroyeast with 0, 5, 10 and 15g of hydroyeast/kg diet, male and female.	1-Fa was calculated using the following equation: Fa= PTHH (g)*NHG, and Fr was calculated using the following equation: Fr= Fa/PC (g). 2- The number of eggs was counted per gram of eggs and then related to the weight of the ovary or the body weight of the fish.	1-The Fa and Fr was higher in the treatment with basal ration + 10 g of hydroyeast / kg diet for females, having a value of 3,416.6±97.95 and 20.6±1.13 respectively. 2-The number of eggs was also higher in the treatment with basal ration + 10g of hydroyeast/kg diet with a value of 325.00±14.43, while the lowest value was 250.00±28.86 in the treatment of basal ration + 15g of hydroyeast/kg diet.

One of the alternatives to know the conditions in which reproduction in fish takes place are reproductive evaluations, which refer to trying to understand the environmental and biological requirements of fish through studies and experiments to guarantee their healthy reproduction, and thus generate an optimal number of viable gametes that promote fertilization, so that embryogenesis can be achieved, culminating in the hatching of eggs that will give origin to progeny (Carrillo et al., 2009). These have many applications that must consider not only reproduction but also the nutritional part to avoid diseases or malformations that could result from a nutritional unleveling that participates directly or indirectly in the processes of reproduction, development and survival (Navas, 2009).

Reproductive evaluations consider physical and chemical factors that limit their development, such as feeding, the number of individuals that should be in a population to avoid stress caused by a high sowing density, water quality and crop safety. Biological factors should also be considered such as the physiology of the fish, its behavior in relation to individuals of the same species or different species, among others (Elgaml et al., 2019).

As for the reproduction of species, its commercial or conservation value should also be considered because meeting the requirements that tilapia need to achieve reproduction can cause a decrease in profit and / or can permit having a projection of the investment needed and ensure the profitability of the crop. This can be estimated by relating the qualities of the fish such as fertility, fecundity, survival rate of larvae, as well as the number of eggs per spawning, which can give an estimate of the number of pups after the reproduction of the fish, as well as the estimated size that they could reach, guaranteeing an optimal production level according to the economic objectives estimated for the crop, which could drive to increased profits.

Regarding conservation, the reproductive eva-

luation could impact positively to rescue endangered species or under special conservation category (Mair et al., 1997; Anene and Okorie, 2008; Peña et al., 2010; Ramos de Alvarenga et al., 2017).

4.3 Overview of evaluations and studies of reproductive parameters in tilapia

Reproductive research has been conducted, including environmental variables or age (Table 1), genetic qualities (Table 2), diet (Tables 3 and 4), and water quality (Table 5), including, but not limited to, the fertility rate, survival rate, number and size of eggs per lay.

Table 4. Table 3 continued. Studies of reproductive parameters and their relationship with the diets implemented in tilapia (*O. niloticus*).

Author(s):	Reproductive parameters studied	Diet	Method	Contribution
Orlando et al. (2017)	1-Fa and Fr 2-ID	5 diets with different levels of digestible energy (3200, 3400, 3600, 3800 and 4000 kcal/kg)	1-Fr was calculated with the number of eggs per gram of female weight, and Fa was calculated with the total number of eggs per spawning. 2-The ID was calculated with the spawning weight per gram of the female.	1-The values of Fa were higher with the diet of 4000 kcal / kg having a value of 449.32±13.48. The lowest value was found in the diet of 3400kcal / kg with a value of 348.50±10.00. In Fr the highest value was in the diet with 4000kcal/kg with a value of 6.57±0.23, while the lowest value was found in the diet with 3400kcal/kg, and had a value of 5.10±0.15. 2-The highest value of DI was found in the diet with 4000kcal/kg and the lowest in the diet with 3400kcal/kg, the values were 9.43±1.23 and 5.48±0.35, respectively.
Sarmento et al. (2018)	1-Fa and Fr 2-SDPL 3-Hatching rate 4-Average egg production per female	Diets were implemented with vitamin C supplementation concentrations at 0, 261, 599 and 942 mg/kg of diet.	1-Fa was calculated with the number of eggs in the spawning. Fr was calculated with the number of eggs/weight of the female(g). 2-The SDPL was calculated with the total number of larvae after 120 h/number of larvae after hatching*100 3-The hatching rate was calculated with the number of hatched larvae/total number of eggs*100 4- The average egg production per female was calculated with the number of eggs per lot/number of spawned females	1-The highest value of Fa was found in the diet with 942 mg/kg of diet and had a value of 892.7±352.19, while the lowest value was found in the control (0 mg/kg of diet) with a value of 622.6±192.18. The highest value of Fr was found in the diet with 599 mg/kg of diet, and had a value of 10.09±2.93, while the lowest value was found in the control, and had a value of 3.61±1.56. 2-SPDL had the highest value in the diet with 599 mg/kg of diet 3-The highest hatching rate was 942 mg/kg diet 4-The average egg production per female had a higher value in the concentration of 599 mg/kg of diet.

It is necessary to know the reproductive functions of fish to guarantee a good tilapia production, reason for which it is essential to know the physicochemical variables and biology that affect their reproduction. Aquaculture producers in Mexico and the world are limited in technology and knowledge to be able to regulate these variables, and thus be able to optimize reproduction (Ramos de Alvarenga et al., 2017).

The reproductive parameters of *O. niloticus* change depending on the variables they are exposed to, and the reproductive parameters can be affected in a negative or positive way depending on the variable. An example is age, where according to Massako et al. (2015), 3-year-old females had a higher frequency of spawning than 2-year-old, differing somewhat by Tsadik Getinet (2008), who mentions that the half-life of spawners in tilapias can be up to 3 years, so it would be expected that the older spawners the lower reproductive parameters, but it is not the case.

Regarding its relationship with nutrition, reports indicate that a nutritious and well-balanced diet greatly favors the reproductive qualities in tilapia and in turn improves health, preventing some diseases, although an excess of nutrients in the diets could reduce reproductive parameters. Chong et al. (2004) mention that diets with high protein content

can be beneficial for the processes related to the reproduction of fish, as long as it is well balanced.

In turn, many eggs do not always mean a larger number of fish, as fertility and the hatching rate also depend on genetic characteristics (Sun et al., 2009). Artificial selection has proven to be a key component for improving reproduction in fish, as well as aspects related to aquaculture production, but as mentioned by Camero-Escobar and Calderón-Calderón (2018), the implementation of new technologies without supervision, such as artificial selection, could have negative effects on fish and in the production systems.

Having a good water quality is an essential part in the reproduction of tilapia, more often the sources where water is obtained for fish farming are compromised by pollutants that affect both health and reproduction, so it is important to have a good quality of water free of pollutants (Elgaml et al., 2019), since this is one of the main resources in aquaculture. Therefore, a good supply of clean water can prevent the loss of fish by pollutants or any other substance that could contain water (Gerbron et al., 2014).

Table 5. Studies on reproductive parameters in tilapia (*O. niloticus* and its relationship with water quality, absolute fertility (Fa), relative fertility (Rf) and gonadosomatic index (ID).

Author(s):	Reproductive parameters studied	Method	Results
Zulfahmi et al. (2018)	1-GSI 2-Fa and Fr	1-Exposure to concentrations of palm oil manufacturing effluent (Control= 0 mg/L, A= 1.565 mg/L, B= 2.347 mg/L and C= 3.130 mg/L). 2-GSI calculated with gonad weight/total body weight*100. 3-Fa was calculated with the partial number of eggs in gonads/partial weight of the gonads*weight of the gonads. Fr was calculated with body weight/total number of eggs in the gonads	1-The highest mean values of GSI were found in treatment B (6,007±2,78 %). GSI values tended to increase in treatments A and B compared to control treatment, then decreased in treatment C (2.446±0.46 %). There were no significant differences between GSI values for treatment control, treatment A and treatment B. 2-The highest average value of Fa was obtained in the treatment A (520±254 eggs), while the lowest value was in the treatment B (307±57 eggs). Although the Fa value for treatment C was higher than that of treatment B. However, treatment C has a lower Fr than treatment B. Exposure to palm oil production effluent did not reveal significant differences in Fa and Fr.

5 Conclusions

Knowing the reproductive parameters of tilapia can help reduce production costs and although there is not a reproductive parameter more important than another, there are factors and synergy involved in the reproduction. It is necessary to establish clear management plans and research on production systems to improve and enhance production. The evaluation of the reproductive parameters of *O. niloticus* tilapia can help reducing the production costs at the time of investing in a crop, since knowing the requirements of the species can help obtaining a better result in this phase. It is suggested to conduct more research to evaluate the reproductive parameters, but even though there is enough information in the topic, there is not enough support, hard data and the interaction of the variables involved in the process.

References

Abarike, E. and Ampofo-Yeboah, A. (2016). Reproductive potential of nile tilapia (*Oreochromis niloticus* linnaeus, 1757) in the golinga reservoir in ghana. *International Journal of Fisheries and Aquatic Studies*, 4(5):279–283. Online:https://bit.ly/41xMmI2.

Anene, A. and Okorie, P. (2008). Some aspects of the reproductive biology of tilapia mariae (boulenger 1901) in a small lake in southeastern nigeria. *African Journal of Biotechnology*, 7(14):2478–2482. Online:https://acortar.link/YKQqCt.

Bizarro, Y. S., Navarro, F., Ribeiro, O., and Navarro, R. (2019). Photoperiodic effects in blood glucose, cortisol, hematological parameters and reproductive indexes of gift lineage reversed male tilapia. *Bioscience Journal*, 35(6):1915–1922. Online:https://acortar.link/SNd54J.

Camero-Escobar, G. and Calderón-Calderón, H. (2018). Vigilancia tecnológica e inteligencia competitiva para la producción de tilapia roja (*Oreochromis mossambicus*) en el departamento del huila, colombia. *Revista de Investigación, Desarrollo e Innovación*, 9(1):19–31. Online:https://acortar.link/yAYwr9.

Carrillo, M., Zanuy, S., and Bayarri, M. (2009). *El control ambiental de la reproducción de los peces con especial referencia al control del ciclo sexual, de la pubertad y de la precocidad*, chapter La reproducción de los peces: aspectos básicos y sus aplicaciones en acuicultura, pages 173–233. Publicaciones científicas y tecnológicas de la Fundación Observatorio Espanol de Acuicultura.

Chong, A., Ishak, S.and Osman, Z., and Hashim, R. (2004). Effect of dietary protein level on the reproductive performance of female swordtails xiphophorus helleri (poeciliidae). *Aquaculture*, 234(1-4):381–392. Online:https://bit.ly/3V7qAsm.

CONAPESCA (2018). Anuario estadístico de acuacultura y pesca 2018. Technical report, Comisión Nacional de Acuacultura y Pesca.

Costa, J. and Carvalho, E. (2012). Reproduction, food dynamics and exploitation level of *Oreochromis niloticus* (perciformes: Cichlidae) from artisanal fisheries in barra bonita reservoir, brazil. *Revista de biologia tropical*, 60(2):721–734. Online:https://acortar.link/B9VSQX.

El-Sayed, A. (2016). *Tilapia culture*. CAB Int. Oxfordshire, UK. Online: https://acortar.link/zinHtL.

Elgaml, S., Saad, T., Hamed, M., and Zaki, V. (2019). Effects of heavy metal pollutants on the reproduction of nile tilapia. *Int j Fish Aquat*, 7(5):542–547. Online:https://bit.ly/3oDKsqI.

FAO (2022). *Oreochromis niloticus*. FAO0. Online:https://acortar.link/1GN9Ck 0.

Gerbron, M., Geraudie, P., Fernandes, D., Rotchell, J., Porte, C., and Minier, C. (2014). Evidence of altered fertility in female roach (rutilus rutilus) from the river seine (france). *Environmental pollution*, 191:58–62. Online:https://acortar.link/WVWajE.

Huet, M. (1998). *Tratado de piscicultura*. Mundi-Prensa.

Hussain, M. (2004). Farming of tilapia, breeding plans, mass seed production and aquaculture techniques. Technical report, Bangladesh Fisheries Research Institute, Bangladesh, India. Online: https://acortar.link/eZnleQ.

INAPESCA (2018). Acuacultura tilapia. Instituto Nacional de Pesca0. Online:https://acortar.link/hNiQHS0.

Mair, G., Abucay, J., Abella, T., Beardmore, J., and Skibinski, D. (1997). Genetic manipulation of sex ratio for the large-scale production of all-male tilapia *Oreochromis niloticus*. *Canadian Journal of Fisheries and Aquatic Sciences*, 54(2):396–404. Online:https://acortar.link/SeNd0c.

Massako, G., Lopes, C., Miwa, N., Soriani, G., and Pereira, R. (2015). Reproduction performance of female nile tilapia under different environments and age classes. *Acta Scientiarum. Animal Sciences*, 37:221–226. Online:https://acortar.link/ja9Iud.

Mehrim, A., Khalil, F., and Hassan, M. (2015). Hydroyeast aquaculture® as a reproductive enhancer agent for the adult nile tilapia (*Oreochromis niloticus* linnaeus, 1758). *Fish Physiology and Biochemistry*, 41:371–381. Online:https://acortar.link/WSJY9O.

Moraes, M., Ribeiro, T., Maria, T., Garcia, D., Martins, M., Fonseca, R., and Vieira, P. (2014). Effects crude protein levels on female nile tilapia (*Oreochromis niloticus*) reproductive performance parameters. *Animal Reproduction Science*, 150(1-2):62–69. Online:https://acortar.link/Sz8g5H.

Nasrin, S.and Rahman, M., Awal, M., Das, M., Hossain, M., and Sarker, F. (2021). Effect of feeding frequency on the growth of gift (*Oreochromis niloticus*). *International Journal of Fisheries and Aquatic Studies*, 9(2):98–107. Online:https://acortar.link/v7ruUM.

Navas, J. (2009). *La reproducción de los peces: aspectos básicos y sus aplicaciones en acuicultura*, chapter Capitulo 8. Los perturbadores endocrinos y sus posibles efectos en la reproducción de peces cultivados, pages 531–580. Publicaciones científicas y tecnológicas de la Fundación Observatorio Espanol de Acuicultura.

Noriega-Salazar, A., Rivas-Salazar, D., Silva-Acuña, R., and Hurtado, E. (2020). Crecimiento y sobrevivencia de juveniles de tilapia roja con dietas suplementadas de vitaminas c y e. *Revista Ciencia UNEMI*, 13(34):16–27. Online:https://acortar.link/lkyePL.

Orlando, T., de Oliveira Moraes, M., Paulino, R., Costa, A., Allaman, I., and Rosa, P. (2017). Reproductive performance of female nile tilapia (*Oreochromis niloticus*) fed diets with different digestible energy levels. *Revista Brasileira de Zootecnia*, 46:1–7. Online:https://acortar.link/EfIWUC.

Oso, J., Ayodele, I., and Fagbuaro, O. (2006). Food and feeding habits of *Oreochromis niloticus* (l.) and sarotherodon galilaeus (l.) in a tropical reservoir. *World Journal of Zoology*, 1(2):118–121. Online:https://acortar.link/Ap2Zpl.

Peña, E., Tapia, R., Velázquez, J., Orbe, A., and Ruiz Velazco, J. (2010). Growth, mortality and reproduction of the blue tilapia *Oreochromis aureus* (perciformes: Cichlidae) in the aguamilpa reservoir, mexico. *Revista de biología tropical*, 58(4):1577–1586. Online:https://acortar.link/6vYvD6.

Perdomo, D., Corredor, Z., Reyna, Y., González, M., Moratinos, P., and Perea, F. (2020). Influencia del tamaño, la variedad y la proporción de sexos en la producción de huevos de tilapia (*Oreochromis* spp) en un sistema tropical intensivo al aire libre. 31(4):e19037. Online:https://acortar.link/vZr0nP.

Perea-Ganchou, F., Perdomo-Carrillo, D., Corredor-Zambrano, Z., Moreno-Torre, R., Pereira-Morales, M., and González-Estopiñán, M. (2017). Factores que afectan el desempeño reproductivo de tilapias del género *Oreochromis* en la zona baja del estado trujillo, venezuela. *Revista Científica*, 27(2):78–86. Online:https://acortar.link/tIxIQP.

Quốc, T., Van-Arendonk, J., , and Komen, H. (2013). Genetic parameters for reproductive traits in female nile tilapia (*Oreochromis niloticus*): Ii. fecundity and fertility. *Aquaculture*, 416–417:72–77. Online:https://acortar.link/x4lds4.

Ramos de Alvarenga, É., Moreira de Sales, S., Soares de Brito, T., Santos, C., Dias Serafim Corrêa, R., de Oliveira Alves, G., Guimarães Manduca, L., and Maldonado Turra, E. (2017). Effects of biofloc technology on reproduction and ovarian recrudescence in nile tilapia. *Aquaculture Research*, 48(12):5965–5972. Online:https://bit.ly/3Lm47TZ.

Saavedra Martínez, M. (2006). *Manejo del cultivo de tilapia*. USAID-CIDEA. Online:https://acortar.link/gac8mw0.

Sarmento, N., Martins, E., Costa, D., Mattioli, C.and da Costa Julio, G., Figueiredo, L., Luz, M., and Luz, R. (2018). Reproductive efficiency and egg and larvae quality of nile tilapia fed different levels of vitamin c. *Aquaculture*, 482:96–102. Online:https://acortar.link/zD8Dqr.

Sièfo, G., Adama, O., Awa, N., Awa, G., Wendengoudi, G., Papa, N., and Gustave, K. (2018). Some reproductive aspects of *Oreochromis niloticus* (linnaeus, 1758) at peele reservoir, nakanbé river basin, burkina faso. *International Journal of Fisheries and Aquatic Studies*, 6(4):124–130. Online:https://acortar.link/UEDLWs.

Silva, A., Corrêa, R., Ventura, A., Nunes, A., Laice, L., Ribeiro, R., Oliveira, C., Almeida, L., Barbosa, P., and Povh, J. (2020). Reproductive traits in different nile tilapia genetic groups. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 72:1797–1804. Online:https://acortar.link/qT5A95.

Sun, C., Madsen, P., Nielsen, U., Zhang, Y., Lund, M., and Su, G. (2009). Comparison between a sire model and an animal model for genetic evaluation of fertility traits in danish holstein population. *Journal of Dairy Science*, 92(8):4063–4071. Online:https://acortar.link/v6pJ3q.

Teame, T., Zebib, H., and Meresa, T. (2018). Observations on the biology of nile tilapia, *Oreochromis niloticus* l., in tekeze reservoir, northern ethiopia. *International Journal of Fisheries and Aquaculture*, 10(7):86–94. Online:https://acortar.link/InIM0K.

Tessema, A., Getahun, A., Mengistou, S., Fetahi, T., and Dejen, E. (2019). Length-weight relationship, condition factor and some reproductive aspects of nile tilapia (*Oreochromis niloticus*) in lake hayq, ethiopia. *International Journal of Fisheries and Aquatic Studies*, 7(5):555–561. Online:https://acortar.link/ak7Tga.

Tsadik Getinet, G. (2008). Effects of maternal age on fecundity, spawning interval, and egg quality of nile tilapia, *Oreochromis niloticus* (l.). *Journal of the World Aquaculture Society*, 39(5):671–677. Online:https://acortar.link/zEylZE.

Vega-Villasante, F., Jaime-Ceballos, B., Cupul-Magaña, A., and Galindo-López, J. y Cupul-Magaña, F. (2009). *Acuicultura de tilapia a pequeña escala para autoconsumo de familias rurales y periurbanas de la costa del Pacífico*. Universidad de Guadalajara.

Yem, I., Bankole, N., Umar, R., Ibrahim, A., and Ewutanure, S. (2020). Food habit and growth pattern of nile tilapia *Oreochromis niloticus* in wase dam, nigeria. *Int. J. Fish. Aqua. Stud.*, (4):257–260. Online:https://acortar.link/1EqatL.

Zimmermann, S. (2005). *Reproducción de los peces en el trópico*, chapter Reproducción de la tilapia, pages 147–164. Ministerio de Agricultura y Desarrollo Rural, Instituto Colombiano de Desarrollo Rural.

Zulfahmi, I., Muliari, M., Akmal, Y., and Batubara, A. (2018). Reproductive performance and gonad histopathology of female nile tilapia (*Oreochromis niloticus* linnaeus 1758) exposed to palm oil mill effluent. *The Egyptian Journal of Aquatic Research*, 44(4):327–332. Online:https://acortar.link/nOZhYm.

La granjA:
Revista de
Ciencias de la Vida
pISSN:1390-3799; eISSN:1390-8596
http://doi.org/10.17163/lgr.n38.2023.10

Review / Reseña bibliográfica

Veterinary Sciences

Prevalence and risk factors associated with bovine brucellosis in dairy farms in the province of Azuay-Ecuador

Prevalencia y factores de riesgo asociados a brucelosis bovina en ganaderías lecheras de la provincia del Azuay-Ecuador

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Article received on September 22nd, 2021. Accepted, after review, on April 11th, 2022. Published on September 1st, 2023.

Abstract

The health of herds that are not within the official Brucellosis control program in the province of Azuay is unknown, and there may be areas with a higher frequency of seropositive herds. This paper aims to determine the prevalence and risk factors associated with bovine brucellosis in dairy farms. An epidemiological study was carried out in 436 farms, for which milk samples were taken from producers in collection centers, collecting trucks and herds. A georeferenced survey was used to collect information on the management of the herds. The milk was analyzed by indirect-ELISA, and thirty-seven farms were seropositive, obtaining a prevalence of 8,5%. The percentages of seropositivity were: Cuenca (14.84%), Girón (23.07%), Nabón (8.21%), Oña (11.53%), San Fernando (33.33%), Sevilla de Oro (7.14%), Sigsig (4.16%). The Rose Bengal and competitive ELISA tests were performed on bovines that contributed to the milk pool in 34 herds, establishing a 100% concordance of indirect ELISA to detect seronegative farms. In the logistic regression analysis, a significant association ($P < 0,05$) was determined between seropositivity and factors such as: geographic location, extension of the farm, exploitation system, presence of other domestic species, elimination of placental remains, reproduction system, having a higher probability of seropositivity in herds that presented abortions ($OR = 2,71$), estrus problems ($OR = 2,09$), birth of weak calves ($OR = 3,24$) and extensive management ($OR = 3,67$). These findings constitute serological evidence that *Brucella spp.* circulates in farms in the area.

Keywords: Prevalence, Brucellosis, Enzyme Linked Immunoadsorbent Assay, Risk factors.

Resumen

Se desconoce el estatus sanitario de ganaderías que no están dentro del programa oficial de control de Brucelosis en la provincia del Azuay, pudiendo existir zonas con mayor frecuencia de rebaños seropositivos. Este trabajo pretende determinar la prevalencia y factores de riesgo asociados a brucelosis bovina en predios lecheros. Se llevó a cabo un estudio epidemiológico en 436 fincas, para lo cual se tomaron muestras de leche de productores en centros de acopio, camiones recolectores y hatos. Se usó una encuesta georeferenciada a fin de recopilar información del manejo de las ganaderías. La leche se analizó mediante ELISA-indirecto, 37 fincas resultaron seropositivas, obteniendo una prevalencia de 8,5%. Los porcentajes de seropositividad fueron: Cuenca (14,84%), Girón (23,07%), Nabón (8,21%), Oña (11,53%), San Fernando (33,33%), Sevilla de Oro (7,14%), Sigsig (4,16%). Se realizaron las pruebas Rosa de Bengala y ELISA-competitivo a bovinos que aportaron al pool de leche en 34 ganaderías, estableciéndose una concordancia del 100% de ELISA-indirecto para detectar fincas seronegativas. En el análisis de regresión logística se determinó una asociación significativa ($P < 0,05$) entre la seropositividad y factores como: ubicación geográfica, extensión de la finca, sistema de explotación, presencia de otras especies domésticas, eliminación de restos placentarios, sistema de reproducción, teniendo una mayor probabilidad de seropositividad las ganaderías que presentaron abortos ($OR = 2,71$), problemas de celo ($OR = 2,09$), nacimiento de terneros débiles (OR=3,24) y manejo extensivo ($OR = 3,67$). Estos hallazgos constituyen evidencia serológica que *Brucella spp.* circula en ganaderías de la zona.

Palabras clave: Prevalencia, Brucelosis, Ensayo Inmunoadsorvente Ligado a Enzimas, Factores de riesgo.

Suggested citation: Andrade Guzmán, O., Vintimilla Rojas, A., López Espinoza, M., Guevara Riera, G. and Rivera Pirela, S. (2023). Prevalence and risk factors associated with bovine brucellosis in dairy farms in the province of Azuay-Ecuador. La Granja: Revista de Ciencias de la Vida. Vol. 38(2):135-148. http://doi.org/10.17163/lgr.n38.2023.10.

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1 Introduction

Brucellosis is a zoonotic bacterial disease caused by several species of the genus *Brucella spp.* that infects domestic and wildlife animals (Ledwaba et al., 2019), affecting the reproductive system and causing abortions, weak offspring and producing economic losses due to the slaughter of infected animals and the limitation to trade (Assenga et al., 2015). Symptoms of brucellosis in humans are fever, fatigue, arthralgia, muscle pain and sweating, sometimes producing physical disability (Zheng et al., 2018).

Twelve species are known of which *B. abortus* affects cattle, *B. mellitensis* causes abortions in goats, *B. suis* infects pigs, *B. canis* is specific in canines, *B. ovis* infects sheep, *B. neotomae* has been reported in rats (Suárez-Esquivel et al., 2017); two species *B. pinnipedialis* and *B. ceti* were isolated in marine mammals (Kroese et al., 2018); *B. microti* has been identified in a variety of animals such as voles, wild boars; *B. papionis* has been described as host to baboons, *B. vulpis* in red foxes; *B. inopatia* has been isolated in humans although the animal reservoir has not been identified (Leclercq et al., 2020). Zoonotic capacity is more expressed in *B. mellitensis*, but *B.abortus* is also responsible for brucellosis in humans (Awah-Ndukum et al., 2018).

Transmission to humans occurs by the consumption of milk, infected dairy products, inhalation of aerosolized particles, and direct contact with tissues of diseased animals (Dal et al., 2019). Sources of infection for animals include aborted materials, vaginal secretions, milk, semen, water consumption, contaminated feed, and infection in calves can occur through the uterus and by colostrum (Ogugua et al., 2018). A close relationship between wildlife and cattle would provide potential opportunities for the transmission and persistence of brucellosis (Godfroid et al., 2013). Likewise, some studies suggest that the bacterium may circulate among several susceptible wildlife species, thus remaining permanently in ecosystems (Aruho et al., 2021).

Infections declared by the World Organization for Animal Health (OIE) as zoonotic diseases require prevention, diagnosis and control measures. For this reason, it is essential to identify risk factors associated with the pathogenesis of *Brucella spp.* infection in the different livestock management systems responsible for the spread of the disease, thus allowing effective management for its management and control (OIE, 2018).

Brucellosis is one of the most important zoonoses with more prevalence in Latin America. Argentina reports a prevalence of 19.7% in herd (Aznar et al., 2015); Uruguay 0.02% (Baruch et al., 2020); Colombia 22% (Cárdenas et al., 2018). It is difficult to establish official prevalence data in Ecuador because it has been under-reported to the OIE. However, studies on the presence of antibodies against *Brucella spp.* have been reported, varying between regions, even within regions. A nationwide study in 1979 reported a seroprevalence in the north Highland of 1.97 to 10.62%, in the Cost of 4.2 to 10.62% and in the south Highland of 1.3 to 2.6%. Another study reports a prevalence of 6% (Salguero, 2011; Román-Cárdenas and Luna-Herrera, 2017). In recent years, some research allow updating the seroprevalence level of this disease, with a significant variability ranging from 1.80-12% throughout the country (Zambrano et al., 2016).

It is necessary to understand the epidemiology of brucellosis in other regions of the country where serological surveillance is not performed as a requirement, prior to implementing control programs and determining the areas with the highest prevalence of the disease. There are several tests for diagnosing it in blood or milk. Currently, the tests prescribed for international cattle trade are Rose Bengal (RBT), Buffered Plate Agglutination (BPAT), ELISA-I (ELISA-indirect), ELISA-C (ELISA-competitive), Complement Fixation (CFT), and Polarized Fluorescence (PF) (Vhoko et al., 2018).

An initial step to set appropriate brucellosis control programs at the local level would be the georeferencing of the infection of some dairy areas that would allow measuring the disease at the farm level and generating epidemiological evidence of the endemicity of the bacterium.
Hence, the aim of the paper was to estimate the prevalence of bovine brucellosis in cattle farms in the province of Azuay, using the ELISA-I technique in milk samples. Likewise, the associated factors that could cause the appearance of the disease will be evaluated, such as presence of abortions, increased calving intervals, birth of weak calves, veterinary

assistance, absence of vaccination, herd size, among others, related to the pathogenesis and signs of brucellosis (Akinseye et al., 2016; Mugizi et al., 2015).

2 Materials and methods

2.1 Study area

This research was conducted in Cuenca, Santa Isabel, Gualaceo, Paute, Sigsig, Sevilla de Oro, Girón, San Fernando, Pucará, Oña, Nabón, El Pan and Chordeleg, belonging to the province of Azuay, located in the southern region of Ecuador, with an approximate area of 8.639 km^2. There are two distinctive zones: the east, with eastern Andes, and the west, with the coastal region. The climate varies from warm to cold due to the altitude, the presence of the Andes massif and subtropical vegetation. To the west, the province is climatologically divided in different sectors. In addition, due to its location, each climatic zone has only two defined seasons: wet and dry. In the West, the temperature ranges between 20°C and 33°C, while in the Andean zone, it is usually between 10°C and 28°C (Cárdenas and Murillo, 2018).

2.2 Study population

It consisted of agricultural production units (PU) dedicated to milk production regardless of size, which included lactating cows during the research period. The Holstein Friesen breed predominated (Instituto Nacional de Estadísticas y Censos, 2019). Herd ranged from 5 to 120 animals; the management system covered a wide range, from extensive technified herds larger than 50 ha, medium-sized herds between 5 to 50 ha, and small farms with traditional extensive management with little technology, smaller than 5 ha. For this research, dairy production zones were defined by the highest concentration of farms that supply this raw material (Ortega et al., 2017).

2.3 Study design

A descriptive cross-sectional study was conducted between 2019 and 2020. The unit of analysis consisted of milk samples obtained from collection centers, collection vehicles and directly from farms. The Win Epi epidemiological program (De Blas et al., 2006) was used to calculate the number of farms to be sampled. The total population was taken as the 15,784 production units (PU) included in the program for the control and eradication of Foot and Mouth Disease in Azuay (Agrocalidad, 2019). Since there were no previous studies in this area on the prevalence of brucellosis, we assumed an expected prevalence of 50%, an estimated error of 5% and a confidence level of 95%. The program yielded a figure of 376 farms to be sampled; however, a total of 436 cattle farms were assessed.

Proportional sampling was used to determine the number of PUs to be studied in each parish. The farms were selected randomly, according to accessibility to the area, distance, time to reach the farms, availability of resources, willingness of producers, collection centers and transporters with the greatest feasibility to participate in this research. A georeferenced survey was conducted with each owner using Survey 123 ArcGis software installed on mobile devices. None of the farms reported having a vaccination program against brucellosis.

2.4 Georeferenced survey

A geo-referenced survey was conducted with questions designed to obtain information on animal health status, and farm management based on existing literature (Cárdenas et al., 2019) with the objective of determining the possible risk factors for suffering brucellosis considering: reproductive management, animal replacement, origin of drinking water, presence of susceptible domestic animals, farming system, knowledge of the disease, reproductive problems, presence of abortions, management of waste after parturition or abortions (Cárdenas et al., 2019). Informed consent for questionnaire administration and sample collection was obtained verbally from owners prior to sampling and interview.

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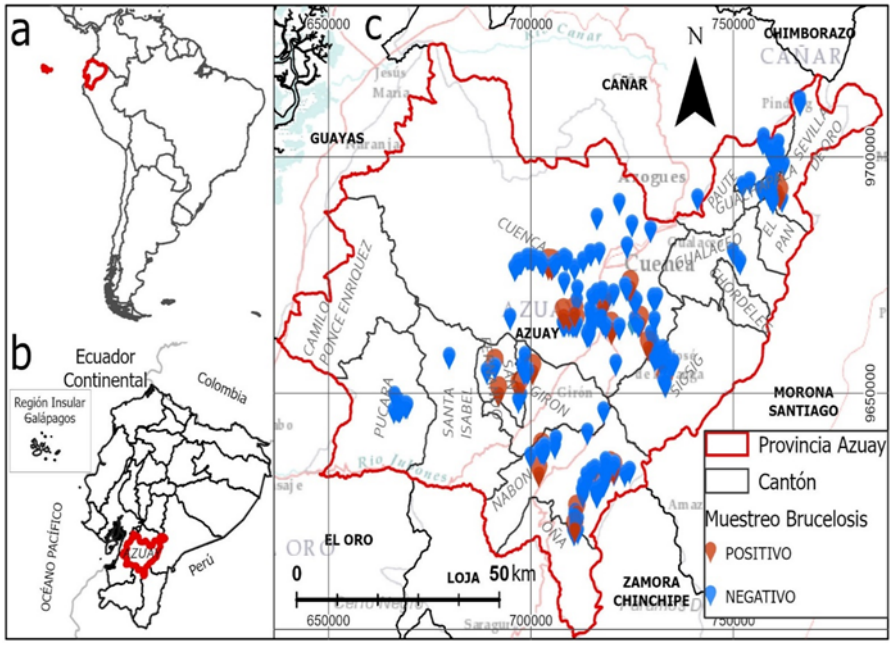

Figure 1. a) Location of Ecuador in South America b) Location of the project at the national level c) Distribution of parishes with seropositive cattle in the province of Azuay.

2.5 Analysis of milk samples by indirect ELISA

The samples were collected in sterile containers in a quantity of 100 ml. The containers were transported refrigerated to the Microbiology Laboratory of the Faculty of Agricultural Sciences of the University of Cuenca, where they were stored at $-20°C$. The ELISA-I kit (Innovate Diagnostic, France) was used to identify the presence of antibodies to *Brucella spp.*, for which the milk samples were previously centrifuged at 8 000 rpm for 10 minutes to separate the lacto-serum from the fat. A 96-well plate impregnated with *Brucella abortus* LPS was used; 100 μl of negative control and positive control were distributed in duplicate and then 100 μl of the samples were added to the remaining wells. The plate was sealed and incubated at $21°C$ for 45 min, then each well was rinsed with 300 μl of wash solution three times. 100 μl of conjugate (peroxidase-labeled ruminant IgG) was added, incubated at $21°C$ for 30 minutes, the washing process was repeated and then 100 μl of developer solution (tetramethylbenzidine) was added to all wells. The plate was incubated again for 15 minutes at $21°C$ and finally 100 μl of stop solution was added to stop the reaction.

Optical density (OD) values of samples (m) and controls were read at 450 nm (wavelength), using an ELISA plate reader (Biotek 800TS, USA). Positive controls (cp) and negative controls (cn) were used to validate the assay. Percent inhibition (PI) was calculated using Equation 1. A sample was considered positive when its PI was higher than 50 %.

$$PI = \frac{OD_m - OD_{cn}}{OD_{cp} - OD_{cn}} \times 100 \quad (1)$$

2.6 Serology for identifying seropositive animals

We had access to 34 cattle farms to perform RBT and ELISA-C tests on all the cows that contributed to the milk pool to individually confirm the presence of seropositive animals. For this, 9 ml of blood were taken from the coccygeal region in vacuum tubes without anticoagulant, which were transported to the laboratory at a temperature of $8°C$. Centrifugation was performed at 8 000 rpm (Dynac, Clay Adams, USA), for 10 minutes, to extract the blood serum to be stored in eppendorf tubes and frozen at $-20°C$.

2.7 Rose Bengal

Sera extracted from peripheral blood obtained without anticoagulant were subjected to the RBT test (Innovate Diagnostics, France), according to the manual of the World Organization for Animal Health (OIE). A gridded glass plate was used; 40 ul of the reagent were mixed with the same amount of serum to be analyzed, and the plate was lightly shaken for 4 minutes. The agglutination appearance within one minute was scored as 4+ (++++), between 1 and 4 min was scored 1+ to 3+ (+, + + + and + + + + + +) according to the different agglutination degrees. The absence of agglutination within 4 minutes was considered negative.

2.8 ELISA-C as confirmatory test

The ELISA-C kit (Svanova, Sweden) was used to confirm the presence of animals that were seropositive to *Brucella spp.* The assay was performed by adding 45 μl of dilution solution in all wells and then adding 5 μl of positive, weak and negative controls in duplicate, as well as 5 μl of dilution solution as a conjugate control; 5 ul of the samples were added afterwards. Next, 50 μl of the pre-diluted mouse monoclonal antibodies (mAb) solution, specific for a common epitope of the smooth O-polysaccharide of LPS molecule, were added to both control and sample wells. The plate was sealed and shaken for 5 minutes, then incubated for 30 minutes at $20°C$. After incubation, the plate was rinsed 4 times with PBS-Tween Buffer solution, 100μl of conjugate solution (goat anti-mouse IgG antibody bound to horseradish peroxidase, HRP) was immediately added to each well and incubated at $20°C$ for 30 minutes.

The washing process was repeated and then 100 μl of substrate (hydrogen peroxidase and ABTS chromogen) was added. It was incubated at $20°C$ for 10 minutes and the reaction was stopped by adding 50 μl of the stop solution (H2SO4) (Viveros, 2019). The microplate was read at 450 nm with a spectrophotometer (Biotek 800TS, USA), calculating the percentage inhibition (PI) for each sample using Equation 2.

$$PI = \frac{OD_m - OD_{cn}}{OD_{cp} - OD_{cn}} \times 10 \quad (2)$$

Where, OD_m, OD_{cp}, OD_{cn} are the optical density readings for the samples, positive control and negative control, respectively. Samples were classified as positive if the antibody titers recorded a $PI \geq 30\%$, defined by the supplier. In addition, the fact that $OD_{cp} > 0,350$ and $OD_{cp}/OD_{cn} > 3$, confirmed that the test worked correctly.

2.9 Statistical analysis

The analyses were performed using Infostat software version 2020 (Di Rienzo et al., 2020). The absolute and relative frequency of antibody seropositive farms in milk samples against *Brucella spp*. infection was calculated. The Chi-square test was used to analyze if there was a relation between each of the risk factors and seropositivity. The influence of the factors was investigated using the logistic regression model. Double-entry tables were made to perform Odds Ratio calculations to estimate the relative risk of an event. The confidence interval was 95% for the logarithm of the odds ratio as 1.96 standard errors on both sides of the estimate, in addition to the *P* value in each case, stablishing statistical significance when $P \leq 0,05$

3 Results

3.1 Seroprevalence of bovine brucellosis at the farm level

Antibodies to *Brucella spp.* were found in 37 milk samples from a total of 436 farms analyzed (Figure 1), with a prevalence of 8.5%. The lowest seropositivity percentage was found in Sigsig parish, with 4.16%, and the highest in San Fernando with 33.33%. No seropositivity was found in the samples from the other six parishes, so the confidence intervals (–) are recorded without values (Table 1).

3.2 Confirmation of seropositive animals on ELISA-I positive and negative farms in milk

Serological tests with RBT and ELISA-C were performed in 34 farms on the cows that contributed to the milk pool to check the presence of seropositive animals, with a 100% negative diagnosis with ELISA-I in milk samples from 20 farms, as no seropositive animals were detected, and in 14 farms whose milk samples were positive, only 12 of them had cows with antibodies (Table 2).

4 Risk factors for the presence of infection

Logistic regression revealed that abortions, geographic location, farm extension, farming system, presence of other domestic species on the farm, estrus problems, elimination of placental remains, birth of weak calves and the reproduction system were significantly related with brucellosis seropositivity ($P < 0,05$), being the herds that presented abortions the ones that showed higher risks of contracting the disease ($OR = 2,71$). Likewise, herds whose animals presented problems of repeated estrus were more likely to be infected ($OR = 2,09$). The birth of weak calves was also a factor associated with more predisposition ($OR = 3,24$).

The association with factors such as veterinary assistance, calving area, water sources, replacement animals, breed, and the presence of retained placenta did not show significant differences ($P < 0,05$) (Table 3).

Table 1. Percentage of brucellosis seropositive herds according to parishes.

Parish	Analyzed farms	Seropositive farms	% Positivity	95 % IC Inferior limit	95 % IC Superior limit
Cuenca	128	19.00	14.84	8.65	20.95
El Pan	49	0.00	0.00	–	–
Girón	13	3.00	23.07	0.19	46.00
Guachapala	15	0.00	0.00	–	–
Gualaceo	4.00	0.00	0.00	–	–
Nabón	73	6.00	8.21	1.90	14.50
Oña	26	3.00	11.53	0.00	23.00
Paute	19	0.00	0.00	–	–
Pucará	26	0.00	0.00	–	–
San Fernando	6.00	2.00	33.33	0.00	71.00
Santa Isabel	1.00	0.00	0.00	–	–
Sevilla de Oro	28	2.00	7.14	0.00	16.00
Sigsig	48	2.00	4.16	0.00	9.00
TOTAL	**436**	**37.00**	**8.50**	**5.00**	**10.00**

5 Discussion

The presence of antibodies to *Brucella spp.* in milk samples by ELISA-I and confirmed with the existence of seropositive animals in RBT and ELISA-C suggests a high exposure to the bacterium of cattle herds in Azuay, which has been previously described by the identification of Brucella abortus strains in cattle, as well as in humans, in several regions of Ecuador (Ron-Román et al., 2014; Rodríguez-Hidalgo et al., 2015).

The correlation between ELISA-I in milk, RBT and ELISA-C in blood serum, indicate a high sensitivity of ELISA-I to diagnose brucellosis-positive farms with 100 % specificity. Only in two farms positive in milk with ELISA-I, no animals were found positive to RBT or ELISA-C, possibly due to the movement of these animals to the drying pen or to reproductive problems at the time of individual sampling or to the refusal of some owners to take samples in pregnant females. An antigenic cross-reaction with other bacterial infections (*Yersinia spp, Salmonella spp, Streptococcos spp, E. coli*) could lead to false positive results in serological diagnosis (Bonfini et al., 2018), although, according to Nielsen et al. (2004), this is unlikely, due to the high specificity of serological tests for brucellosis in milk.

Table 2. Results with ELISA-I, RBT, and ELISA-C in 34 cattle farms according to geographical location.

Parish	Farms positive in milk	Farms with seropositive animals	Seropositive animals			Farms negative in milk	Farms with seropistive animals	Seropositive animals		
			Sampled animals	RBT	ELISA-C			Sampled animals	RBT	ELISA-C
Cuenca	6	6	236	29	28	10	0	239	0	0
Santa Isabel	–	–	–	–	–	1	0	24	0	0
Girón	2	2	145	31	31	2	0	41	0	0
Sevilla de Oro	–	–	–	–	–	2	0	36	0	0
Oña	1	1	31	1	1	3	0	64	0	0
San Fernando	2	2	50	3	3	–	–	–	–	–
Nabón	1	–	18	0	0	1	0	14	0	0
Sigsig	2	1	16	1	1	–	–	–	–	–
TOTAL	14	12	496	65	64	20	0	418	0	0

The prevalence of brucellosis may vary depending on the study zones, influenced by different management practices, the origin of replacement animals, the farming system, and the greater permanence of the bacteria due to variations in climate, among other factors. *Brucella spp.* is very susceptible to sunlight and heat, surviving a few hours in hot and dry months, while in summer it can survive in humid soil for approximately 7 days (Matope et al., 2010), prevailing in endemic areas, due to the wide range of susceptible hosts, capable of transmitting the disease (Ducrotoy et al., 2017; Musallam et al., 2019).

In parishes with higher prevalence of affected farms, such as San Fernando, Girón, Cuenca, brucellosis could be due to the management system, mainly due to the mixing of animals from different herds within the same geographical area (Craighead et al., 2018), because these are places with a higher number of dairy herds and have an important cattle trade. In the epidemiological surveys, most producers stated that they were unaware of the symptoms of the disease, there was an absence of serological monitoring by laboratory analysis and no discarding of infected animals that are commonly traded, thus spreading the disease. In areas detected with low prevalence, such as the parishes of Paute, El Pan, Guachapala, Gualaceo, Pucará and Santa Isabel, there are low transmission rates, possibly due to agro-ecological factors that limit contact between herds.

The prevalence values obtained in this study (8.5%) are lower than those found by Mainato and Vallecillo (2017), in the neighboring province of Cañar (13.63%) where they report a higher presence of seropositive farms in the parishes of Biblián and Cañar. An epidemiological study of Brucellosis at national level (Carbonero et al., 2018) includes the province of Azuay with a herd level prevalence of less than 10%; Pichincha 37.5%; Santo Domingo 26.8%; Tungurahua 25.3% and Zamora with 4.8%. On the other hand, Poulsen et al. (2014), in a study to determine the prevalence in two provinces of Northern Ecuador, refer a value of 7.2%. These variations at the country level could be due to sampling techniques, test interpretation, reagents used, and number of animals sampled.

Among the risk factors, farm size was a significant factor associated with a higher brucellosis seroprevalence, probably due to hygiene problems resulting from high animal density in extensive production systems. Berhe et al. (2007) reported a seropositivity risk of 8.5 and 4.3 times higher in large and medium herds, respectively, compared to small herds, since the risk of contact with animals from other herds decreases in these herds. Similarly, McDermott and Arimi (2002), state that the size of herds in extensive systems, common crossbreeding with other animals and meeting at common grazing and watering points increase the risk of contagion of the disease.

A history of abortions or stillbirths was associated with brucellosis seropositivity. Aborted fetuses and uterine secretions provide a constant supply of the bacterium, maintaining the transmission of new infections (Sanchez et al., 2020). A relationship with estrus problems in animals was also observed, which is in line with other research (Asgedom et al., 2016), that also identified an increase in the number of services per calving when cattle presented reproductive problems due to brucellosis, which affects the genital tract, leading to uterine infection and poor conception rate.

Table 3. Risk factors associated with bovine brucellosis.

Factor	Variable	N° positive farms	Seropositivity ELISA-I%	Odd ratio	95% IC	P value
Abortions	Yes	58(10)	17.24	2.71	1.25 - 5.86	0.001
	No	378(27)	7.14	0.37	0.17 - 0.80	
Parish	Cuenca	127(19)	14.96	–	–	0.001
	El Pan	49(0)	0			
	Girón	13(3)	23.07			
	Guachapala	15(0)	0			
	Gualaceo	4(0)	0			
	Nabón	73(6)	8.21			
	Oña	26(3)	11.53			
	Paute	19(0)	0			
	Pucará	26(0)	0			
	San Fernando	6(2)	33.33			
	Santa Isabel	1(0)	0			
	Sevilla de Oro	28(2)	7.14			
	Sigsig	46(2)	4.34			
Farm extension	Big	208(25)	12.01	–	–	0.0043
	Big 2	44(6)	13.64			
	Medium	78(0)	0			
	Small	106(6)	5.66			
Exploitation system	Extensive	196(27)	13.78	3.67	1.76 -7.69	0.0003
	Rope attained	240(10)	4.17	0.27	0.13 - 0.57	
Presence of domestic species	Can, OVI, EQUI	256(17)	6.64	–	–	0.01
	POR, EQUI, OVI	19(2)	11.11			
	CAN, POR, EQUI, OVI	48(3)	6.25			
	Others	49(7)	14.29			
	Do not have other species	64(7)	10.9			
Estrus problems	Yes	95(13)	13.68	2.09	1.03 – 4.25	0.03
	No	341(24)	7.04	0.48	0.24 – 0.97	
Elimination of placenta remains	Buries, trash, burns	103(15)	14.56	–	–	0.03
	Eaten by the animal / others animals	164(12)	7.32			
	Leave in the area	169(10)	5.92			
Birth of weak calves	Yes	45(9)	20	3.24	1.44 – 7.28	0.0034
	No	391(28)	7.16	0.31	0.14 – 0.69	
Reproduction system	MN (own bull/borrowed)	333(20)	6	–	–	0.0001
	AI	78(16)	20.51			
	MN / AI	25(1)	4			
Veterinary assistance	Yes	140(10)	7.14	1.3	0.62 – 2.74	0.48
	No	296(27)	9.12	0.77	0.37 – 1.61	
Calving areas	Paddocks	13(0)	7.69	–	–	0.26
	Pens	423(37)	8.74			
Water Sources	Ditch, river, well	398(37)	9.3	–	–	0.14
	Drinking water	27(0)	0			
	AP, river, ditches, well	11(0)	0			
Replacement animals	Of the farm	352(25)	7.1	–	–	0.18
	PR and nearby farms	60(9)	15			
	Out of State	6(1)	16.67			
	PR and outside the state	18(2)	11.11			
Breed	Holstein	321(24)	7.48	–	–	0.5
	Half-Breed	69(7)	10.14			
	Brown Swiss	20(2)	10			
	Jersey	26(4)	15.38			
Retained placenta	Yes	74(8)	10.81	1.39	0.62 – 3.12	0.43
	No	362(29)	8.01	0.72	0.32 – 1.61	

CAN= canines; OVI= sheep; POR= porcine; EQUI= equine; MN= natural mating; AI= artificial insemination; AP= drinking water; PR= of the farm.

In relation to the farming system, there are similar results to those reported in this work, where traditional management would facilitate the spread of the disease due to poor animal movement control (Fero et al., 2020). However, Kumar et al. (2016) mention that horizontal transmission of the disease in organized farms would be related to overcrowding, high animal density and poor hygienic practices, such as inadequate disposal of aborted fetuses, fetal membranes and vaginal secretions, which help to spread the infection.

Logistic regression associated inadequate disposal of placenta and fetuses as a predisposing factor for the transmission of infection, because millions of Brucellae are excreted during normal parturition or abortions of infected cows, which can maintain infectivity for several months, given a suitable environment of temperature, sunlight and pH (Sussex, 2016). Similarly, John et al. (2010) indicate that herd owners who improperly disposed biological waste after calving, abortions or placental retentions were more likely to have at least one seropositive animal when compared to those who properly disposed these materials.

Although in this work, the association with the introduction of animals of unknown health status into the herd did not have a significant effect, the percentage of positive animals increases when animals from other farms are introduced. Kanouté et al. (2017) determine a higher probability of observing Brucella positive herds when untested cattle enter endemic areas. They emphasize the need to monitor cattle before entering the farm, and also to promote replacement with animals from brucellosis-free farms.

According to the epidemiological survey, most of the evaluated areas were unaware of the existence of preventive immunization programs against brucellosis, so we can infer that the presence of seropositive animals was due to contact with Brucella in the field and not to post-vaccination reactions. Dorneles et al. (2015) point out that vaccination is a determining strategy for brucellosis control and eradication programs. Likewise, Pascual et al. (2018) state that eradication programs should include diagnostic tests, discarding infected animals and the incorporation of vaccination, which has been shown to reduce infections and abortions in animals.

Olsen and Stoffregen (2005) have proven that the percentage of reactors in infected herds is lower in vaccinated animals compared to non-vaccinated animals. According to their data when using full doses of strain 19 in calves and evaluating protection in cattle up to 9 years old, they estimated that approximately 65-75% of all vaccinated animals were completely protected during their productive life. Undoubtedly, the high prevalence of brucellosis detected in the area is also related to the absence of vaccination.

The breeding system can also influence *Brucella spp.* infection especially through sexual contact with neighboring herds or through the exchange of bulls coming from infected farms (Nardi et al., 2017). Breed and water sources were not shown in this study to be predisposing factors for infection. Although other management factors not considered could influence, our findings are in line with the general epidemiology of bovine brucellosis observed in other parts of the world (Franc et al., 2018; Hull and Schumaker, 2018), understanding that the high prevalence of this disease, represents an important public and animal health problem in Ecuador.

6 Conclusions

This study provides serological evidence of the presence of brucellosis in dairy herds with different seropositivity levels in the province of Azuay, with a high prevalence (8.5%), associated with risk factors involved in the pathogenesis of the disease and responsible for its spread. The ELISA-I test in milk is a useful diagnostic tool to identify brucellosis-positive farms with very high specificity, reducing sampling time, cost, and effectively analyze a larger number of farms at the same time. It is necessary to carry out sero-surveillance in cattle farms to understand the spatial distribution of the disease in the country, prior to implementing control programs and raising public awareness of the zoonotic transmission of brucellosis.

Ethical approval

All procedures were carried out in accordance with experimental practice and international standards

for animal welfare.

Acknowledgments

This study was financed thanks to the XVIII call for research projects, carried out by the Research Department of the University of Cuenca, Ecuador.

References

Agrocalidad (2019). Programa de erradicación de fiebre aftosa, I Fase. techreport, Jefatura de Sanidad Agropecuaria Azuay.

Akinseye, V., Adesokan, H., Ogugua, A., Adedoyin, F., Otu, P., Kolawole, N., Awosanya, E., Cadmus, E., Cadmus, S., and Kwaghe, A. (2016). Seroepidemiological survey and risk factors associated with bovine brucellosis among slaughtered cattle in nigeria. *Onderstepoort Journal of Veterinary Research*, 83(1):1–7. Online:https://bit.ly/3LQAZG2.

Aruho, R., MacLeod, E., Manirakiza, L., and Rwego, I. (2021). A serological survey of brucellosis in wildlife in four major national parks of uganda. *BMC Veterinary Research*, 17(1):1–10. Online:https://bit.ly/40bOWTr.

Asgedom, H., Damena, D., and Duguma, R. (2016). Seroprevalence of bovine brucellosis and associated risk factors in and around alage district, ethiopia. *Springerplus*, 5:1–8. Online:https://bit.ly/3FOELvP.

Assenga, J., Matemba, L., Muller, S., Malakalinga, J., and Kazwala, R. (2015). Epidemiology of brucella infection in the human, livestock and wildlife interface in the katavi-rukwa ecosystem, tanzania. *BMC veterinary research*, 11:1–11. Online:https://bit.ly/3nlZRLu.

Awah-Ndukum, J., Mouiche, M., Bayang, H., Ngu Ngwa, V., Assana, E., Feussom, K., Manchang, T., and Zoli, P. (2018). Seroprevalence and associated risk factors of brucellosis among indigenous cattle in the adamawa and north regions of cameroon. *Veterinary Medicine International*.

Aznar, M., Linares, F., Cosentino, B., Sago, A., La Sala, L., Leon, E., Duffy, S., and Perez, A. (2015). Prevalence and spatial distribution of bovine brucellosis in san luis and la pampa, argentina. *BMC Veterinary Research*, 11(1):1–7. Online:https://bit.ly/40bRa5f.

Baruch, J., Suanes, A., Piaggio, J., and Gil, A. (2020). Analytic sensitivity of an elisa test on pooled sera samples for detection of bovine brucellosis in eradication stages in uruguay. *Frontiers in Veterinary Science*, 7:178. Online:https://bit.ly/3FIQpIM.

Berhe, G., Belihu, K., and Asfaw, Y. (2007). Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of tigray region of ethiopia. *International Journal of Applied Research in Veterinary Medicine*, 5(2):65. Online:https://bit.ly/40cJyzq.

Bonfini, B., Chiarenza, G., Paci, V., Sacchini, F., Salini, R., Vesco, G., Villari, S., Zilli, K., and Tittarelli, M. (2018). Cross-reactivity in serological tests for brucellosis: a comparison of immune response of escherichia coli o157: H7 and yersinia enterocolitica o: 9 vs brucella spp. *Vet Ital*, 54(2):107–114. Online:https://bit.ly/3G5RKcK.

Carbonero, A., Guzmán, L., García-Bocanegra, I., Borge, C., Adaszek, L., Arenas, A., and Saa, L. (2018). Seroprevalence and risk factors associated with brucella seropositivity in dairy and mixed cattle herds from ecuador. *Tropical animal health and production*, 50:197–203. Online:https://bit.ly/40vkZ0C.

Cárdenas, C. and Murillo, M. (2018). Calidad bacteriológica de la leche cruda en ganaderías de la provincia del azuay. Master's thesis, Universidad de Cuenca.

Cárdenas, L., Melo, O., and Casal, J. (2018). Evolution of bovine brucellosis in colombia over a 7-year period (2006-2012). *Tropical animal health and production*, 50:19–27. Online:https://bit.ly/3G42KXT.

Cárdenas, L., Peña, M., Melo, O., and Casal, J. (2019). Risk factors for new bovine brucellosis infections in colombian herds. *BMC Veterinary Research*, 15:1–8. Online:https://bit.ly/3Ky3QOd.

Craighead, L., Meyer, A., Chengat, B., Musallam, I., Akakpo, J., Kone, P., Guitian, J., and Häsler, B. (2018). Brucellosis in west and central africa: A review of the current situation in a changing landscape of dairy cattle systems. *Acta tropica*, 179:96–108. Online:https://bit.ly/40F6RC1.

Dal, T., Kara, S., Cikman, A., Balkan, C., Acıkgoz, Z., Zeybek, H., Uslu, H., and Durmaz, R. (2019). Comparison of multiplex real-time polymerase chain reaction with serological tests and culture for diagnosing human brucellosis. *Journal of Infection and Public Health*, 12(3):337–342. Online:https://bit.ly/3UcbvFt.

De Blas, I., Ruiz-Zarzuela, I., and Vallejo, A. (2006). Winepi: Working in epidemiology. an online epidemiological tool. In *ISVEE 11: Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics*.

Di Rienzo, J., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M., and Robledo, C. (2020). Infostat versión 2020. techreport, Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Online: http://www.infostat.com.ar.

Dorneles, E., Sriranganathan, N., and Lage, A. (2015). Recent advances in brucella abortus vaccines. *Veterinary research*, 46(1):1–10. Online:https://bit.ly/3U6W6Gl.

Ducrotoy, M., Bertu, W., Matope, G., Cadmus, S., Conde-Álvarez, R., Gusi, A., Welburn, S., Ocholi, R., Blasco, J., and Moriyón, I. (2017). Brucellosis in sub-saharan africa: Current challenges for management, diagnosis and control. *Acta tropica*, 165:179–193. Online:https://bit.ly/3MhYWGx.

Fero, E., Juma, A., Koni, A., Boci, J., Kirandjiski, T., Connor, R., Wareth, G., and Koleci, X. (2020). The seroprevalence of brucellosis and molecular characterization of brucella species circulating in the beef cattle herds in albania. *PloS one*, 15(3):e0229741. Online:https://bit.ly/40IYntH.

Franc, K.and Krecek, R., Häsler, B., and Arenas-Gamboa, A. (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC public health*, 18(1):1–9. Online:https://bit.ly/40NZ19y.

Godfroid, J., Garin-Bastuji, B., Saegerman, C., and Blasco, J. (2013). Brucellosis in terrestrial wildlife. *Revue Scientifique et Technique. Office International des Epizooties*, 32(1):27–42. Online:https://bit.ly/3UbHCoC.

Hull, N. and Schumaker, B. (2018). Comparisons of brucellosis between human and veterinary medicine. *Infection ecology & epidemiology*, 8(1):1500846. Online:https://bit.ly/3ZIbytu.

Instituto Nacional de Estadísticas y Censos (2019). Encuesta de superficie y producción agropecuaria continua espac-2019. INEC. Online:https://bit.ly/3K3sg0A.

John, K., Fitzpatrick, J., French, N., Kazwala, R., Kambarage, D., Mfinanga, G., MacMillan, A., and Cleaveland, S. (2010). Quantifying risk factors for human brucellosis in rural northern tanzania. *PloS one*, 5(4):e9968. Online:https://bit.ly/3UdPwxU.

Kanouté, Y., Gragnon, B., Schindler, C., Bonfoh, B., and Schelling, E. (2017). Epidemiology of brucellosis, q fever and rift valley fever at the human and livestock interface in northern côte d'ivoire. *Acta tropica*, 165:66–75. Online:https://bit.ly/435rxVy.

Kroese, M., Beckers, L., Bisselink, Y., Brasseur, S., van Tulden, P., Koene, M., Roest, H., Ruuls, R., and Backer, J.and IJzer, J. (2018). Brucella pinnipedialis in grey seals (halichoerus grypus) and harbor seals (phoca vitulina) in the netherlands. *Journal of wildlife diseases*, 54(3):439–449. Online:https://bit.ly/411lDTr.

Kumar, A., Gupta, V., Singhi, A., and Reddy, N. (2016). Seroprevalence and risk factors associated with bovine brucellosis in western uttar pradesh, india. *Indian journal of animal sciences*, 86(2):131–135. Online:https://bit.ly/3zxcMx9.

Leclercq, S., Cloeckaert, A., and Zygmunt, M. (2020). Taxonomic organization of the family brucellaceae based on a phylogenomic approach. *Frontiers in Microbiology*, 10:3083. Online:https://bit.ly/3GiJaXY.

Ledwaba, M., Gomo, C., Lekota, K., Le Flèche, P., Hassim, A., Vergnaud, G., and Van Heerden, H. (2019). Molecular characterization of brucella species from zimbabwe. *PLOS Neglected Tropical Diseases*, 13(5):e0007311. Online:https://bit.ly/3MsDoXE.

Mainato, S. and Vallecillo, A. (2017). Seroprevalencia de la brucelosis bovina en la provincia del cañar, ecuador. *Maskana*, 8:25–28. Online:https://bit.ly/3KdWuye.

Matope, G., Bhebhe, E., Muma, J., Lund, A., and Skjerve, E. (2010). Herd-level factors for brucella seropositivity in cattle reared in smallholder

dairy farms of zimbabwe. *Preventive Veterinary Medicine*, 94(3-4):213–221. Online:https://bit.ly/3KyFrIy.

McDermott, J. and Arimi, S. (2002). Brucellosis in sub-saharan africa: epidemiology, control and impact. *Veterinary microbiology*, 90(1-4):111–134. Online:https://bit.ly/3zFy63q.

Mugizi, D.and Boqvist, S., Nasinyama, G., Waiswa, C., Ikwap, K., Rock, K., Lindahl, E., Magnusson, U., and Erume, J. (2015). Prevalence of and factors associated with brucella sero-positivity in cattle in urban and peri-urban gulu and soroti towns of uganda. *Journal of Veterinary Medical Science*, 77(5):557–564. Online:https://bit.ly/40LTbVU.

Musallam, I., Ndour, A., Yempabou, D., Ngong, C., Dzousse, M., Mouiche-Mouliom, M., Feussom, J., Ntirandekura, J., Ntakirutimana, D., and Fane, A. (2019). Brucellosis in dairy herds: A public health concern in the milk supply chains of west and central africa. *Acta tropica*, 197:105042. Online:https://bit.ly/3KCjfNN.

Nardi, G., Megid, J., Mathias, L., Paulin, L., Vicente, A., Cortez, A., Listoni, F., Lara, G., Motta, R., and Chacur, M. (2017). Performance of microbiological, serological, molecular, and modified seminal plasma methods in the diagnosis of brucella abortus in semen and serum of bovine bulls. *Biologicals*, 48:6–9. Online:https://bit.ly/40IGM5i.

Nielsen, K., Smith, P., Widdison, J., Gall, D., Kelly, L., Kelly, W., and Nicoletti, P. (2004). Serological relationship between cattle exposed to brucella abortus, yersinia enterocolitica o: 9 and escherichia coli o157: H7. *Veterinary microbiology*, 100(1-2):25–30. Online:https://bit.ly/418YWwR.

Ogugua, A., Akinseye, V., Cadmus, E., Jolaoluwa Awosanya, E., Alabi, P., Idowu, O., Akinade, S., Dale, E.and Perrett, L., and Taylor, A. (2018). Prevalence and risk factors associated with bovine brucellosis in herds under extensive production system in southwestern nigeria. *Tropical animal health and production*, 50:1573–1582. Online:https://bit.ly/416zU1a.

OIE (2018). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. World Organization for Animal Health.

Olsen, S. and Stoffregen, W. (2005). Essential role of vaccines in brucellosis control and eradication programs for livestock. *Expert review of vaccines*, 4(6):915–928. Online:https://bit.ly/3MjqATs.

Ortega, V., Dután, J., Ayala, L., Rodas, R., Nieto, P., Vázquez, J., Pesántez, J., Andrade, O., Pesántez, M., and Guevara, R. (2017). Caracterización productiva de las ganaderías en los cantones occidentales de la provincia del azuay. *Maskana*, 8:145–147. Online:https://bit.ly/4182Gic.

Pascual, D., Yang, X., Wang, H., Goodwin, Z., Hoffman, C., and Clapp, B. (2018). Alternative strategies for vaccination to brucellosis. *Microbes and infection*, 20(9-10):599–605. Online:https://bit.ly/3Ke4XS1.

Poulsen, K., Hutchins, F., McNulty, C., Tremblay, M., Zabala, C., Barragan, V., Lopez, L., Trueba, G., and Bethel, J. (2014). Brucellosis in dairy cattle and goats in northern ecuador. *The American Journal of Tropical Medicine and Hygiene*, 90(4):712. Online:https://bit.ly/414gha4.

Rodríguez-Hidalgo, R., Contreras-Zamora, J., Benitez Ortiz, W., Guerrero-Viracocha, K., Salcan-Guaman, H., Minda, E., and Ron Garrido, L. (2015). Circulating strains of brucella abortus in cattle in santo domingo de los tsáchilas province–ecuador. *Frontiers in Public Health*, 3:45. Online:https://bit.ly/3m8noQb.

Román-Cárdenas, F. and Luna-Herrera, J. (2017). Revisión actualizada de la epidemiología de brucelosis (brucella abortus, brucella mellitensis, brucella suis, brucella canis) en el ecuador y el mundo. *Centro de Biotecnología*, 6(1):82–93. Online:https://bit.ly/3ZGYWTy.

Ron-Román, J., Ron-Garrido, L., Abatih, E., Celi-Erazo, M.and Vizcaíno-Ordóñez, L., Calva-Pacheco, J., González-Andrade, P., Berkvens, D., Benitez-Ortiz, W., and Brandt, J. (2014). Human brucellosis in northwest ecuador: typifying brucella spp., seroprevalence, and associated risk factors. *Vector-Borne and Zoonotic Diseases*, 14(2):124–133. Online:https://bit.ly/3KjgKyf.

Salguero, A. (2011). Determinación de la prevalencia serológica de brucelosis en bovinos de las provincias de carchi , esmeraldas e imbabura y análisis de factores de riesgo. Master's thesis, Universidad Central del Ecuador.

Sanchez, J., Jimenez, M., Gutierrez, J., Cruz, J., and Nahet, J. (2020). Seroprevalence of abortus diseases that commit the bovine reproductive efficiency in two dairy areas of chiapas. *ESPACIO I+D, INOVACIÓN MÁS DESARROLLO*, (10).

Sussex, W. (2016). *Veterinary Microbiology*. World Organization for Animal Health.

Vhoko, K., Iannetti, S., Burumu, J., Ippoliti, C., Bhebhe, B., and De Massis, F. (2018). Estimating the prevalence of brucellosis in cattle in zimbabwe from samples submitted to the central veterinary laboratory between 2010 and 2014. *Vet Ital*, 54(1):21–27. Online:https://n9.cl/q8pak.

Viveros, J. (2019). Prevalencia y factores de riesgo de la brucelosis bovina en ganaderías de imbabura que proveen leche a floralp s.a. Master's thesis, Pontificia Universidad Católica del Ecuador.

Zambrano, M., Pérez, M., and Rodríguez, X. (2016). Brucelosis bovina en la provincia manabí, ecuador: Estudio de los factores de riesgo. *Revista de Investigaciones Veterinarias del Perú*, 27(3):607–617. Online:https://n9.cl/3wzbe.

Zheng, R., Xie, S., Lu, X., Sun, L., Zhou, Y., Zhang, Y., and Wang, K. (2018). A systematic review and meta-analysis of epidemiology and clinical manifestations of human brucellosis in china. *BioMed research international*, 2018. Online:https://bit.ly/3KGWeJG.