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PRESENTACIÓN

En el marco del 70 Aniversario de la Facultad de Agronomía de la Universidad Autónoma de Nuevo León (UANL), tenemos el honor de presentar el primer número de la revista científica "Scientia Agricolis Vita". En este primer número es presentada una selección de artículos de investigación que abordan una amplia gama de temas relevantes y emergentes en el ámbito agrícola, con un enfoque multidisciplinario.

Esta publicación marca un hito significativo en nuestro compromiso continuo con la excelencia académica y la investigación multidisciplinaria en el ámbito de las ciencias agrícolas de nuestra Facultad. Extendemos una cordial invitación a investigadores, académicos y profesionales a explorar los contenidos de esta revista y a contribuir con sus propias investigaciones y reflexiones. *Scientia Agricolis Vita* se compromete a fomentar el intercambio de ideas y el avance del conocimiento en beneficio de la comunidad académica y del sector agrícola en general.

Dra. Guadalupe Gutiérrez Soto
Editora en Jefe

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Artículo

Actividad Antiparasitaria *In-vitro* del Extracto Metanólico de *Kalanchoe daigremontiana* (*Crassulaceae*) en Contra de *Entamoeba histolytica* (Amoebida: *Entamoebidae*) y *Trichomonas vaginalis* (Trichomonadida: *Trichomonadidae*)

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Resumen: Introducción: Las infecciones parasitarias como la amebosis y la tricomonosis representan un desafío significativo para la salud pública a nivel global. A lo largo de décadas, el metronidazol ha sido considerado como el fármaco principal para su tratamiento. Sin embargo, el uso descontrolado de este medicamento ha propiciado la aparición de cepas resistentes. Esta realidad ha generado una urgente necesidad de descubrir y desarrollar nuevos tratamientos eficaces contra las parasitosis. **Objetivo**: Evaluar la actividad antiparasitaria de *Kalanchoe daigremontiana* sobre *Entamoeba histolytica* y *Trichomonas vaginalis*. **Metodología**: Se preparó un extracto metanólico de K. *daigremontiana*. El extracto se caracterizó fitoquímicamente de manera cualitativa. Se determinó el efecto del extracto sobre trofozoítos de E. *histolytica* y T. *vaginalis*; finalmente se determinó su toxicidad en eritrocitos humanos. **Resultados**: El análisis fitoquímico del extracto de K. *daigremontiana* indicó que los flavonoides son los compuestos más abundantes. El extracto presentó la capacidad de inhibir el desarrollo de E. *histolytica* y T. *vaginalis* con una DL50 de 71 y 105 μg/mL, respectivamente y presento baja toxicidad en eritrocitos. **Conclusiones**: El extracto de metanolico de las hojas de K. *daigremontiana* posee actividad en contra de E. *histolytica* y T. *vaginalis*. Sin afectar significativamente los hematíes humanos en concentraciones efectivas frente a los parásitos evaluados.

Palabras Clave: Amebosis; Hemolisis; Parasitosis; Tricomonosis; Trofozoítos.

In vitro Antiparasitic Activity of Methanolic Extract of *Kalanchoe daigremontiana* (*Crassulaceae*) Against *Entamoeba histolytica* (Amoebida: *Entamoebidae*) and *Trichomonas vaginalis* (Trichomonadida: *Trichomonadidae*)

Abstract: Introduction: Parasitic infections such as amoebiasis and trichomoniasis represent a significant global public health challenge. Metronidazole as the drug of choice for decades, and its uncontrolled management has led to the emergence of resistant strains. Thus, the need to find new treatments against parasitosis has arisen. **Objective:** To evaluate the *In-vitro* antiparasitic activity of the plant *Kalanchoe daigremontiana* on *Entamoeba histolytica* and *Trichomonas vaginalis*. **Methods:** A methanolic extract of *K. daigremontiana* was prepared. The extract was qualitatively characterized phytochemically. The effect of the extract on the log-phase trophozoites of *E. histolytica* and *T. vaginalis* was determined; finally, its toxicity in human erythrocytes was determined. **Results:** Phytochemical analysis of *K. daigremontiana* extract indicated that flavonoids were the most abundant compounds. The extract presented the ability to inhibit the growth of *E. histolytica* and *T. vaginalis* with an LD_{50} of 71 and 105 μg/mL, respectively, and presented low toxicity in erythrocytes. **Conclusions:** *K. daigremontiana* extract possesses antiparasitic activity against *E. histolytica* and *T. vaginalis* trophozoites. It does not significantly affect red blood cells at effective concentrations against the parasites evaluated.

Keywords: Amebosis; Hemolysis; Parasitosis; Trichomonosis; Trophozoites.

1. Introducción

América Latina y México enfrentan una serie de enfermedades parasitarias que afectan significativamente la salud pública (Trejos-Suárez and Castaño-Osorio, 2009; Mitra and Mawson, 2017). La Organización Mundial de la Salud (OMS) identifica una serie de factores clave que contribuyen a la propagación y persistencia de las enfermedades parasitarias como lo son pobreza y condiciones socioeconómicas desfavorables así como condiciones de higiene y saneamiento inadecuadas (James et al., 2018). Existen diversos protozoarios de importancia clínica en el humano entre ellos *Entamoeba histolytica* que puede afectar el intestino humano (Pozio, 2019) y *Trichomonas vaginalis* agente etiológico de la tricomoniasis, la enfermedad de transmisión sexual no viral más prevalente en todo el mundo (Edwards *et* al., 2014).

El Metronidazol es un fármaco ampliamente utilizado para tratar diversas infecciones parasitarias efectivo contra una variedad de organismos protozoarios anaeróbicos, como T. *vaginalis*, *Giardia lamblia* y E. *histolytica* (Dingsdag and Hunter, 2018). A pesar de ser un tratamiento común, el uso prolongado o inadecuado de metronidazol ha generado resistencia por parte de los parásitos, lo que reduce la eficacia del fármaco y dificulta su capacidad para eliminar las infecciones (Pal *et* al., 2009). El uso prolongado del metronidazol puede ocasionar efectos adversos que incluyen malestar estomacal, náuseas, vómitos, diarrea, dolor de cabeza, mareos, sequedad bucal y un sabor metálico en boca, también se ha asociado con efectos adversos en el sistema nervioso central, como convulsiones (Hernández Ceruelos *et* al., 2019). Esto ha llevado a la necesidad constante de buscar alternativas terapéuticas y estrategias para combatir las infecciones parasitarias y fármacos más efectivos.

Por lo cual las plantas representan una fuente para el descubrimiento y desarrollo de tratamientos frente a las enfermedades parasitarias (Rodríguez-Garza *et* al., 2019) esto debido a que contienen diversos metabolitos con propiedades biológicas (Patel, Patel and Patel, 2011). Las plantas del género *Kalanchoe* son valiosas por que poseen una amplia variedad de propiedades biológicas, las que incluyen actividad frente enfermedades como infecciones virales y bacterianas (Aisyah et al., 2016). Hasta el momento los resultados disponibles en la literatura sugieren que las actividades terapéuticas derivadas de *Kalanchoe daigremontiana* pueden depender en parte de la presencia de los flavonoides como la quercetina (Kolodziejczyk-Czepas and Stochmal, 2017). Por lo anterior este estudio está enfocado en la determinación de la actividad antiparasitaria del extracto metanólico de hojas de K. *daigremontiana*.

2. Materiales y Métodos

Extracción: Se utilizaron hojas frescas de K. *daigremontiana* colectadas en Monterrey, N.L., México en el año del 2019. La identificación taxonómica de la planta se realizó en el Laboratorio de Botánica de la Facultad de Ciencias Biológicas de la Universidad Autónoma de Nuevo León (No. Reg. 029130). La taxonomía de la planta fue cotejada en el sitio web The World Flora Online (WFO) Plant List (https://wfoplantlist.org/plant-list/; accesado el 08/01/2024).

Para obtener el extracto metanólico de K. *daigremontiana*, se usaron 100 g de hojas secas y molidas a temperatura ambiente y se sometieron a extracción por maceración con 300 mL de metanol (MeOH) absoluto en matraz de 1 L el cual fue colocado en un agitador orbital a 250 rpm a temperatura ambiente y recambio de disolvente cada 24 horas durante 3 días consecutivos. El extracto se filtró con papel filtro Whatman N°1, se concentró a presión reducida a 40.0 ± 2.0 °C con un rotavapor. Finalmente, el extracto se pesó para calcular rendimiento de extracción, se etiquetó como KalH y se almacenó en obscuridad a 4.0 ± 1.0 ºC hasta su uso. Además, al extracto se le realizaron pruebas fitoquímicas cualitativas (Rodríguez-Garza et al., 2023).. Para la determinación del rendimiento, se utilizó la siguiente formula (1), en donde PE = Peso del extracto y PI = Peso inicial de planta seca.

$$\text{Rendimiento } (\% \, p/p) = \frac{P_f}{P_I} \text{ x } 100 \qquad (1)$$

Actividad Antiparasitaria: La efectividad de KalH contra trofozoítos de *E. histolytica* cepa HM1-IMSS (2 x 10^4 trofozoítos/mL) y *T. vaginalis* cepa GT15 IMSS:0989 (1 x 10^5 trofozoítos/mL) en fase logarítmica y se evaluó en medio PEHPS. Este medio consiste en peptona de caseína, extracto de hígado/páncreas, y suero bovino al 10%. La evaluación se realizó utilizando la técnica de microensayo descrita previamente en una publicación anterior (Elizondo-Luévano *et al.*, 2020). *E. histolytica* fue incubada a 36 °C/72 h y *T. vaginalis* fue incubada a 37 ˚C/24 h. Las concentraciones de los extractos evaluados fueron de 15.63 a 1000 µg/mL (Elizondo-Luevano *et al.*, 2020); se utilizó dimetilsulfóxido (DMSO) absoluto para disolver el extracto (solución stock) de esta solución se tomaron alícuotas para preparar las soluciones de

trabajo y las concentraciones finales nunca superaron el 5% de DMSO. La viabilidad se determinó por conteo en cámara de Neubauer. Los trofozoítos de *T. vaginalis*, se fijaron con formalina 1:10 previo al conteo. Para ambos parásitos, el control positivo (C+) consistió en metronidazol (1.0 µg/mL). Los resultados del porcentaje (%) de inhibición para cada parasito, se utilizaron para determinar la dosis letal media (DL_{50}).

Prueba de hemólisis: La toxicidad de KalH se determinó por la prueba de hemólisis en eritrocitos humanos para lo cual se analizaron distintas concentraciones de KalH (100 a 1000 µg/mL) a 37°C durante 30 minutos. Como C-, se utilizaron eritrocitos sin agregar extracto, se empleó agua destilada como C+, ya que induce hemólisis de células rojas. La evaluación de la hemólisis se realizó espectrofotométricamente a 540 nm, registrando las lecturas como absorbancia para cada tratamiento (Abs Tr) (Elizondo-Luevano *et al.*, 2023). El porcentaje de hemólisis se determinó utilizando la fórmula siguiente (2):

$$\% \text{ Hemólisis} = \frac{\text{Abs}_{\text{Tr}} - \text{Abs}_{\text{C}-}}{\text{Abs}_{\text{C}+} - \text{Abs}_{\text{C}-}} \text{ x } 100 \qquad (2)$$

Actividad anti-hemolisis: El método empleado en este ensayo fue el descrito por Quintanilla-Licea *et al*., 2023. Para evaluar el efecto protector sobre los eritrocitos, el extracto se incubó en concentraciones variables de 100 a 1000 µg/mL junto al radical AAPH (2,2'-azo-bis (2-amidino-propano) dihidrocloruro), aplicando agitación de 200 rpm/37°C por 5 horas a en una incubadora de rotación. Estos tratamientos se designaron como (Tr) (Quintanilla-Licea *et al*., 2023). Como C- de la hemólisis, se empleó PBS (pH 7.4) con eritrocitos, excluyendo el AAPH, como C+ se utilizó AAPH. Tras la incubación, todos los tratamientos se centrifugaron a 13,000 rpm / 4 °C durante 3 minutos. Se extrajeron 200 µL de sobrenadante y se transfirieron a una microplaca de 96 pozos transaprente. La hemólisis se determinó siguiendo el mismo procedimiento descrito en el método de hemólisis. El % de protección se determinó con la fórmula (3):

$$\% \text{ Protección} = [1 - \frac{\text{Abs}_{\text{Tr}} - \text{Abs}_{\text{C}-}}{\text{Abs}_{\text{C}+} - \text{Abs}_{\text{C}-}}] \text{ x } 100 \qquad (3)$$

Análisis estadístico: Cada ensayo se llevó a cabo en tres repeticiones y se empleó la prueba de ANOVA de una vía para identificar diferencias significativas entre los tratamientos. La determinación de la DL_{50} se efectuó mediante la prueba estadística de Probit (IC = 95%). Se consideraron diferencias significativas cuando $p < 0.05$. Los análisis estadísticos se determinaron en el software SPSS, ver. 24.

3. Resultados

Análisis fitoquímico: El rendimiento de extracción por maceración del extracto fue del 13 %. Los resultados del estudio fitoquímico básico en cual el extracto mostró respuesta positiva (+) para insaturaciones, quinonas, triterpenos, esteroles, saponinas, carbohidratos y flavonoides siendo estos últimos los más abundantes se muestran en la tabla 1.

Tabla 1. Análisis fitoquímico cualitativo del extracto de K. *daigremontiana*.

Prueba Fitoquímica												
Ins	**Gp. Car**	**Cu**	**SqL**	**Qn**	**Est**	**Gp. Cxo**	**Gp. Fn**	**Spo**	**Fla**	**Carb**	**Alc**	**Tri**
	+	-	-	-	+	+	-	+	+	++	+	-

Ausente: -, Presencia: +. Alc: Alcaloides; Carb: Carbohidratos; Cu: Cumarinas; Est: Esteroles; Fla: Flavonoides; Ins: Insaturaciones; Gp. Car: Grupo carbonilo; Gp. Cxo: Grupo carboxilo; Gp. Fn: Grupos fenólicos; Qn: Quinonas; Spo: Saponinas; SqL: Sesquiterpenlactonas; Tri: Triterpenos.

Actividad contra *E. histolytica* y *T. vaginalis*: Como puede observarse, el extracto posee la capacidad de inhibir el crecimiento de los trofozoítos, existiendo una relación dosis respuesta ya que el porcentaje de inhibición disminuye con la disminución de la concentración del extracto. La letalidad fue total a una concentración de 1000 µg/mL con un resultado similar a 500 µg/mL. El control positivo, mostró un 100 % de mortalidad, mientras que el control negativo y

el tratamiento blanco, revelaron una inhibición casi nula siendo del 2 y de 1 % para *E. histolytica* y *T. vaginalis,* respectivamente (Figura 1). Los resultados referentes a la DL_{50} determinada para el metronidazol fueron 0.3 y 0.2 µg/mL contra *E. histolytica* y *T. vaginalis,* respectivamente (tabla 2).

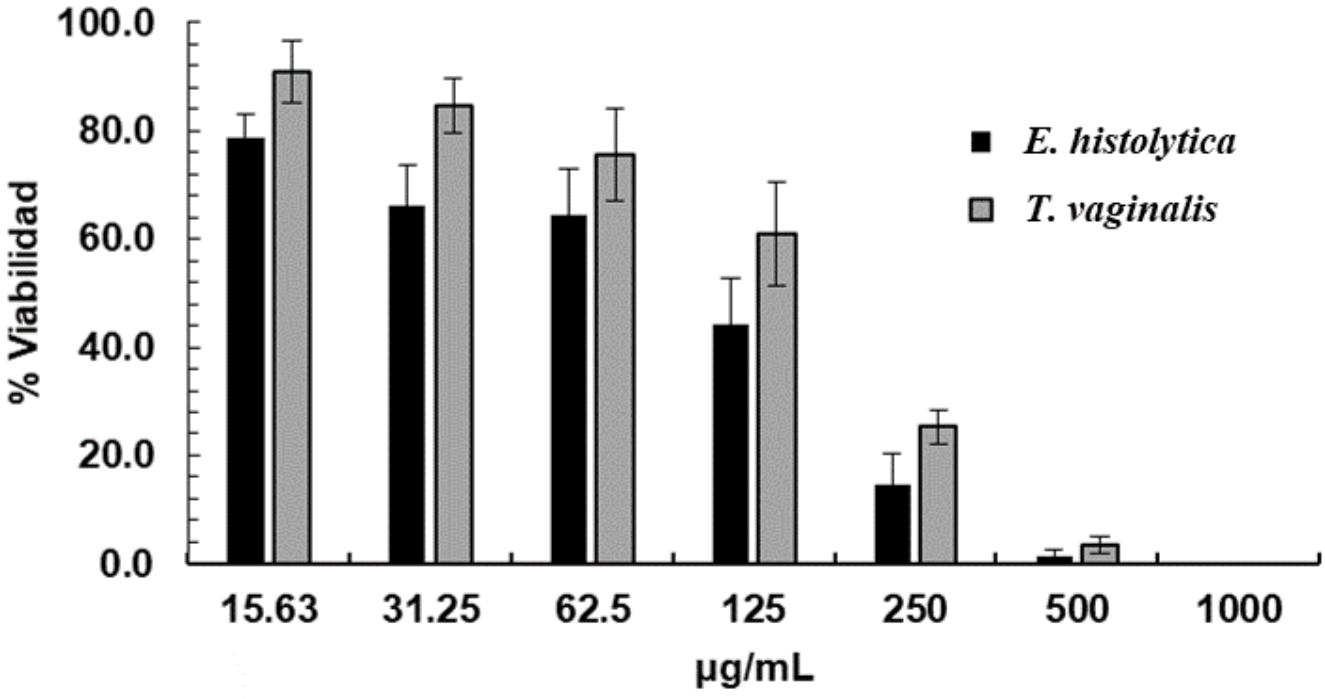

Gráfico 1. % de viabilidad de los parasitos *E. histolytica* y *T. vaginalis* en presencia de KalH. Datos expresados como la Media ± DE. Se utilizó como Tr testigo medio PEHPS sin inóculo, como C- se utilizó DMSO al 5% más inóculo de trofozoítos.

Tabla 2. DL50 de KalH contra E. histolytica y T. vaginalis

	DL_{50} en µg/mL
Parasito	**KalH**
E. histolytica	71.0 ± 5.4
T. vaginalis	105.3 ± 12.5

Datos expresados como la Media ± DE.

Toxicidad del extracto mediante la prueba de hemólisis: En la figura 2 se observa que el extracto crudo de hoja de *K. daigremontiana* a 1000 µg/mL tuvo una actividad hemolítica de 7.18 % la cual fue descendiendo conforme disminuye la concentración del extracto. En 100 µg/mL (concentración más baja evaluada) la hemólisis en los eritrocitos fue del 0.49 %. El C+ mostró un 100 % de hemólisis mientras que el C- no presentó actividad aparente (datos no mostrados en el gráfico).

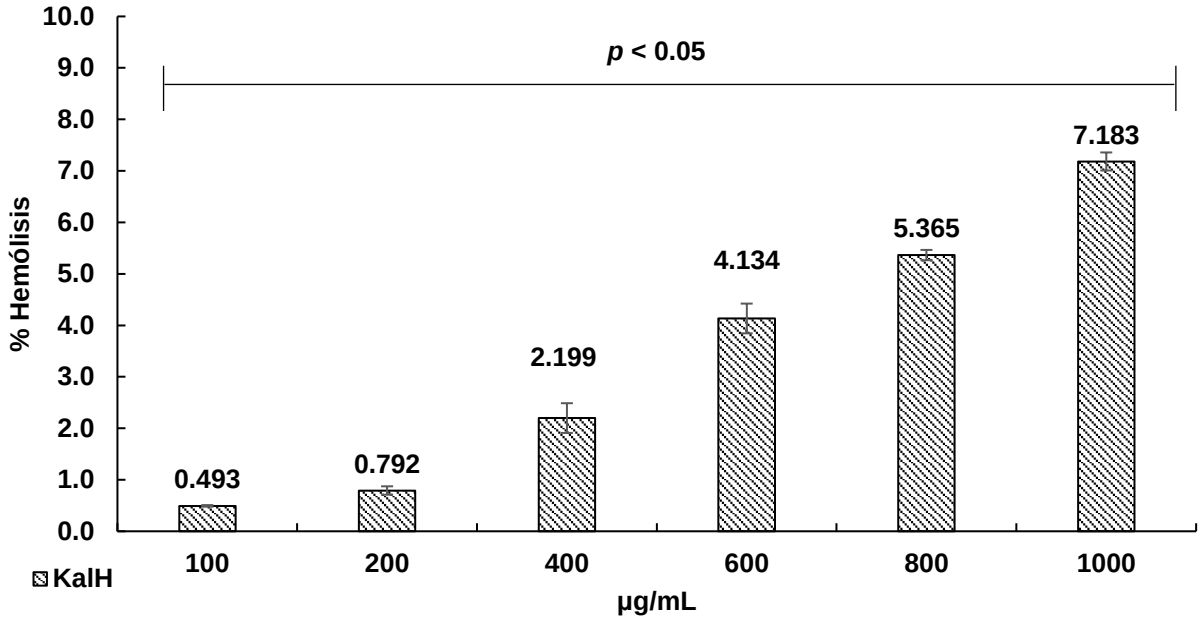

Gráfico 2. Evaluación del efecto hemolítico de KalH mediante la prueba de hemólisis. Valores presentados como la Media ± DE ($p < 0.05$).

Citoprotección del extracto: El extracto KalH, el cual no presentó hemólisis significativa en concentraciones de hasta 1000 µg/mL fue analizado mediante el método de AAPH (Quintanilla-Licea *et al.*, 2023). El extracto presentó citoprotección de manera inversamente proporcional a la concentración, siendo la concentración de 100 µg/mL la más efectiva y la de 1000 µg/mL la menos efectiva con 3.46 y 0.34 % de citoprotección frente a la hemolisis inducida por el AAPH, respectivamente (Fig. 3). El C- no presentó hemolisis detectable y el C+ presentó 100 % de hemólisis (datos no mostrados en el gráfico).

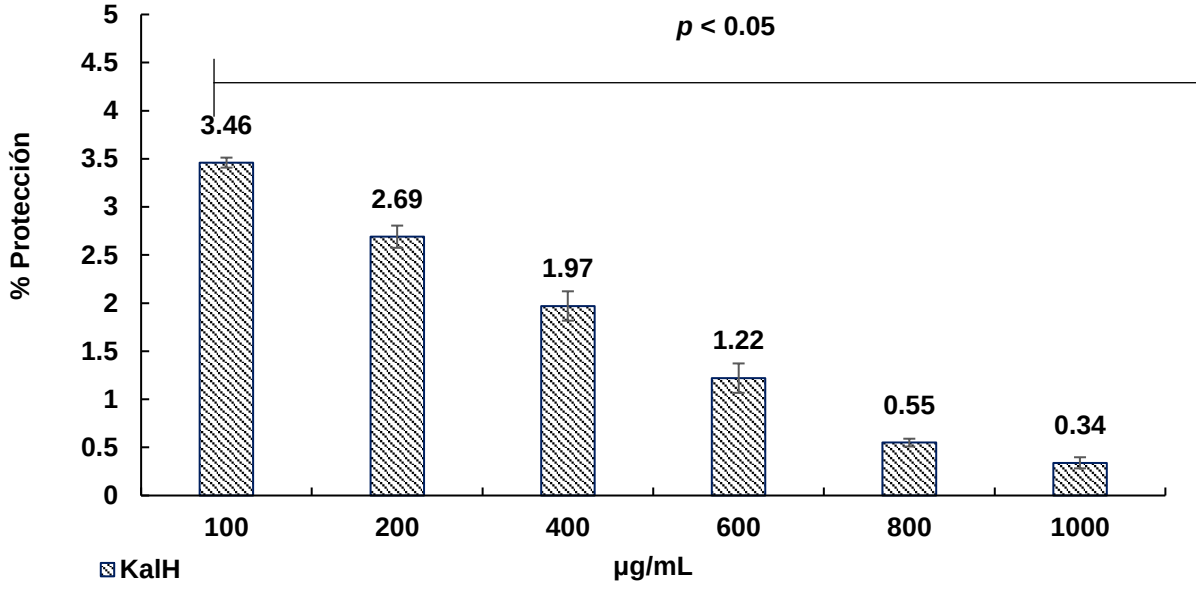

Gráfico 3. Evaluación de la protección de KalH mediante el método de hemolisis inducida por AAPH. Valores presentados como la Media ± DE ($p < 0.05$).

4. Discusión

Hoy en día, se han realizado varios estudios para encontrar nuevas fuentes de compuestos para tratar enfermedades que afectan al hombre; sin embargo, las parasitosis se encuentran entre las enfermedades más olvidadas ya que el desarrollo de nuevas terapias y medicamentos ha recibido muy poca atención. En este estudio, el papel de las hojas *K. daigremontiana* contra trofozoítos de *E. histolytica* y *T. vaginalis* mostró que los extractos exhiben efecto en contra de la viabilidad de ambos parásitos. En este estudio, el papel de las hojas *K. daigremontiana* sobre la viavulidad de *E. histolytica* y *T. vaginalis* mostró que el extracto exhibe un efecto en contra de la viabilidad de los parásitos de forma dependiente del tiempo de exposición y de la dosis evaluada (Fig. 1), además de una baja citotoxicidad (Fig. 2) según el criterio reportado por López-Villarreal *et al*., en 2022 para productos naturales, lo que le brinda un valor agregado al uso de esta planta (López-Villarreal *et al*., 2022). Estos resultados eran los esperados debido a los diferentes componentes en la planta detectados en el análisis fitoquímico, como lo son compuestos polifenólicos, saponinas y flavonoides entre otros (Tabla 1), pueden potencializar el efecto en contra de distintos agentes etiológicos (Boyko, Kabar and Brygadyrenko, 2020).

Los presentes hallazgos están en la misma línea de estudios previos que muestran que el grupo de las plantas pertenecientes al género *Kalanchoe* poseen diferentes actividades biológicas tanto *in-vitro* como *in-vivo* (Costa *et al*., 2008; Elizondo-Luévano *et al*., 2021). Lo anterior respecto a los diferentes metabolitos presentes en extractos de plantas medicinales en contra de algunos protozoarios, debido a que existen casos en los cuales se puede presentar resistencia por parte de los protozoos o reacciones de hipersensibilidad a los nitroimidazoles, siendo las plantas y sus metabolitos como alternativa en el tratamiento de las parasitosis (Mehriardestani *et al*., 2017).

En este estudio, se preparó un extracto metanólico a partir de hojas secas trituradas con el objetivo de obtener la mayor variedad de moléculas con diferentes polaridades. Este proceso se llevó a cabo mediante maceración para preservar la integridad de las moléculas, evitando la exposición al calor y la luz (Velázquez-Domínguez *et al*., 2013). Se logró un rendimiento del 13 % (Tabla 1). El extracto mostró respuestas positivas para Ins, Qn, Tri, Gp. Fn, Spo, Fla y Carb. Estos hallazgos coinciden con estudios anteriores que han informado sobre la presencia de triterpenos, fenoles, carbohidratos y flavonoides en plantas del mismo género, destacando la presencia de flavonoides del grupo de los quercetinoides (Fürer *et al*., 2016). Sin embargo, es importante tener en consideración que estos metabolitos pueden variar dependiendo de la zona de cosecha o zona geográfica (Escalada, Brumovsky and Hartwig, 2011).

Para tener los parásitos metabólicamente estables (Pires-Santos, Santana-Anjos and Vannier-Santos, 2012); las evaluaciones biológicas se llevaron a cabo en la fase logarítmica. Los bioensayos revelaron la capacidad del extracto metanólico KalH para inhibir el crecimiento de los trofozoítos de ambos parásitos *in vitro*. Como se ilustra en la figura 1, el porcentaje de inhibición aumenta con la concentración del extracto, mostrando un comportamiento dosis-respuesta. A una concentración de 500 μg/mL, la viabilidad fue inferior al 4 % y disminuyó progresivamente en ambos casos. La determinación de la DL50 reveló que KalH tiene una DL50 de 71 y 105.3 μg/mL, respectivamente (Tabla 2), mientras que el metronidazol muestra una DL50 de 0.17 y 0.09 μg/mL, respectivamente. A pesar de la falta de reportes sobre el uso de extractos de K. *daigremontiana* contra los parásitos estudiados, existen investigaciones que evalúan extractos de esta familia de plantas frente a otros microorganismos y virus, incluyendo parásitos como *Bombyx mori*, *Leishmania amazonenis* y *L. chagasi* (Costa *et al*., 2008; El Abdellaoui et al., 2010). Estos estudios informan sobre extractos crudos con concentraciones letales de 500 μg/mL, 400 μg/mL y 16 μg/mL, respectivamente.

La evaluación de la hemólisis causada por extractos se ha utilizado para examinar la posible actividad tóxica de materiales vegetales en los glóbulos rojos; en la presente investigación, se determinó la toxicidad de KalH en concentraciones de 100 a 1000 μg/mL. Como se muestra en la figura 2, el extracto no demostró una toxicidad elevada, registrando alrededor del 7.4 % a 1000 μg/mL, lo que sugiere que KalH posee una toxicidad mínima o nula (López-Villarreal *et al*., 2022). El radical AAPH, al reaccionar con el oxígeno molecular, genera radicales peroxílo que, al entrar en contacto con los eritrocitos, afectan sus membranas plasmáticas desencadenando la hemólisis (Shiva Shankar Reddy *et al*., 2007; Chisté *et al*., 2014). Esta prueba se ha convertido en un modelo ampliamente empleado para estudiar el comportamiento de las membranas eritrocitarias. Además, este modelo se ha utilizado para determinar la capacidad inhibitoria de radicales libres de ciertos antioxidantes (Pieroni *et al*., 2011).

En estudios *in vitro* e *in vivo* se ha demostrado que los polifenoles juegan un rol en la resistencia al estrés oxidativo (Sandner, Heckmann and Weghuber, 2020). Además, se ha sugerido que polifenoles y flavonoides, pueden distribuirse en las membranas celulares debido a su naturaleza anfipática. Esta distribución conlleva a una restricción en la fluidez

de dichas membranas, lo que obstaculiza la difusión estérica de los radicales libres, disminuyendo así la cinética de las reacciones de oxidación (Chaudhuri *et al*., 2007).

Por ello, si se considera por un lado que el extracto de *K. daigremontiana* no provocó hemolisis significativa y por otro, que no presentó protección sobre la membrana plasmática de estas células eucariotas, aunado al hecho de que el extracto provoca la inhibición del crecimiento de protozoarios dependiente de la dosis, se pude sugerir que dicha acción biológica no se ejerce a nivel de la membrana plasmática y que el principio activo debe ser absorbido y dentro de la célula pudiera presentar un receptor (Halliwell and Gutteridge, 1990). Se ha sugerido que ciertos polifenoles interaccionan con el ADN por intercalación impidiendo la correcta replicación provocando la muerte celular (Alam, Bristi and Rafiquzzaman, 2013).

Los productos naturales son una fuente prometedora de moléculas activas (Tienda-Vázquez *et al*., 2023). Este estudio presenta resultados preliminares sobre la actividad del extracto metanólico de *K. daigremontiana* contra los parasitos *E. histolytica* y *T. vaginalis*. Se sugiere realizar más ensayos para evaluar los fitometabolitos principales de *K. daigremontiana*, ya que la evaluación dirigida de extractos particionados puede revelar una mayor actividad biológica (Bazaldúa-Rodríguez *et al*., 2021). Además, considerando el comportamiento dosis-respuesta obtenido, se presume la presencia de metabolitos en *K. daigremontiana* que podrían inhibir el crecimiento de varios protozoarios, tales como la quercetina y quercitrina presentes en el género *Kalanchoe*, que han mostrado actividad leishmanicida (Muzitano *et al*., 2006).

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6. Conflictos de intereses

Los autores respaldan plenamente este trabajo y han contribuido de manera significativa que justifica su autoría. No existe conflicto de interés y se han seguido todos los procedimientos éticos y requisitos necesarios.

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Artículo

Effect of *Trametes maxima* CU1 supernatants on the bread physical properties

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Abstract: Enzymes are of great interest to the food industry because of their economic benefits in process optimization and the advantages in functional and rheological properties that positively influence foods. Therefore, in the present work, we evaluated the effect of *Trametes maxima* CU1 supernatants on the physical parameters of bread. For this, the supernatants of the 18-day cultures were recovered in a mineral medium supplemented with $CuSO_4$, orange peel, or peanut in combination with wheat straw, in addition to the presence of a co-culture with *Pycnoporus sanguineus* CS2. The titers of laccase, amylases, cellulases, and xylanases were quantified prior to their addition to the bread dough. Once the loaves were obtained, height, hardness, weight loss (%), pores per mm2, and color analysis were determined. The results of the enzyme titers showed a statistically significant difference ($p \leq 0.05$) between the treatments, with the medium with 350 µM $CuSO_4$ highlighting the production of laccase, while the medium supplemented with 5% orange peel presented the four types of activities. In general, the breads showed differences ($p \leq 0.05$) in the parameters evaluated. It should be noted that the bread treated with the supernatant with orange peel had the highest height and color, like the control, in addition to presenting the lowest hardness among the enzymatic treatments. Therefore, these results demonstrate the effect of the culture medium on the enzymatic profiles of the same fungus and its potential application in the baking industry.

Keywords: amylase; cellulases; laccase; *Pycnoporus sanguineus* CS2; xylanases.

Efecto de los sobrenadantes de *Trametes maxima* CU1 en las propiedades físicas del pan

Resumen: Las enzimas son de gran interés para la industria alimentaria debido a sus beneficios económicos en la optimización de procesos y a las ventajas en las propiedades funcionales y reológicas que influyen positivamente en los alimentos. Por ello, en el presente trabajo se evaluó el efecto de los sobrenadantes de *Trametes maxima* CU1 sobre los parámetros físicos del pan. Para ello, se recuperaron los sobrenadantes de los cultivos de 18 días en un medio mineral suplementado con $CuSO_4$, cáscara de naranja o cacahuete en combinación con paja de trigo, además de la presencia de un co-cultivo con *Pycnoporus sanguineus* CS2. Los títulos de lacasa, amilasas, celulasas y xilanasas se cuantificaron antes de su adición a la masa de pan. Una vez obtenidos los panes, se determinaron la altura, la dureza, la pérdida de peso (%), los poros por mm2 y el análisis del color. Los resultados de los títulos enzimáticos mostraron una diferencia estadísticamente significativa ($p \leq 0,05$) entre los tratamientos, destacando en el medio con 350 µM $CuSO_4$ la producción de lacasa, mientras que el medio suplementado con un 5% de cáscara de naranja presentó los cuatro tipos de actividades. En general, los panes presentaron diferencias ($p \leq 0.05$) en los parámetros evaluados. Cabe destacar que el pan tratado con el sobrenadante con cáscara de naranja tuvo la mayor altura y color, al igual que el control, además de presentar la menor dureza entre los tratamientos enzimáticos. Por lo tanto, estos resultados demuestran el efecto del medio de cultivo sobre los perfiles enzimáticos del mismo hongo y su potencial aplicación en la industria panadera.

Palabras clave: amilasa; celulasas; lacasa; *Pycnoporus sanguineus* CS2; xilanasas.

1. Introduction

The baking industry has an excellent social and strategic importance, as it provides essential food products to the population and contributes to food security (Rosell & Dura, 2015). It plays an important role in the stability of society and aims to meet the needs of all segments of the population (Ponte *et al*., 2000). However, negative processes affect the industry in terms of product quality, technical and economic performance, and the availability of raw materials (Rosell & Dura, 2015). In this sense, chemical additives such as emulsifiers, calcium phosphate, and L-ascorbic acid, among others, are known to positively affect the rheological properties of the dough, including water absorption capacity, stability, and energy (Abdullahi *et al*., 2022). However, some additives can cause flavor changes, and some may raise biosafety concerns (Olivieri *et al*., 2020).

In recent years, enzymes have emerged as powerful tools for bakers seeking to improve product quality, optimize processes, and address sustainability concerns. Among the most commonly, laccase, xylanase, and cellulose are particularly promising to revolutionize the baking landscape, highlighting their impact on texture, flavor, shelf life, and nutritional value (Lončar *et al*., 2016). Laccase (a multicopper oxidase) possesses oxidizing capabilities with benefits for baked goods. Thanks to its ability to attack phenolic compounds, it cross-links arabinoxylans (AX) in wheat flour, strengthening the structure of the dough and giving rise to a softer crumb and reduced elasticity, providing better texture (Selinheimo *et al*., 2007). In whole wheat bread, a reduction in the aging rate of bread, better gluten cross-linking, and flavor have been observed (Wang et al., 2023). For their part, xylanases are enzymes that catalyze the random hydrolysis of the β-(1 → 4) glycosidic bonds of heteroxylan and convert them into xylooligosaccharides, weakening the structure of xylan (Sharma *et al*., 2019). They are characterized by reducing the dryness and rigidity of the dough, as well as greater elasticity, extensibility, and coherence, as well as an increase in volume and a decrease in the density of the bread, resulting in greater moisture retention and better sensory attributes of the bread (Ahmad *et al*., 2014). Therefore, prospecting studies are essential to find enzymes with robust operational and functional properties that improve bakery products' quality, texture, and shelf life. Thus, the objective of this research was to evaluate the effect of the supernatants of *Trametes maxima* CU1 cultured in a mineral medium supplemented with inducers of inorganic and organic origin, in addition to the effect of a co-culture with *Pycnoporus sanquineus* CS2, on the physical parameters of the bread.

2. Materials and Methods

Strains

The ones used were Trametes maxima CU1 and Pycnoporus sanguineus CS2, which are preserved in the Laboratorio de Ciencias Naturales of the Facultad de Agronomía and preserved by periodic replanting every three months in medium potato dextrose agar (PDA).

Culture medium

The production of enzymes was carried out in modified Kirk medium (KM) whose composition was glucose (10 g/l), yeast extract (0.5 g/l), peptone (10 g/l), NH_4SO_3 (0.72 g/l), KH_2PO_4 (1 g/l), $MgSO_4 \cdot 7H_2O$ (1 g/l), KCl (0.5 g/l) and 10 ml of 100X trace element solution: $FeSO_4 \cdot 7H_2O$ (0.01 g/l), $ZnSO_4 \cdot 7H_2O$ (0.028 g/l) and $CaCl_2 \cdot 2H_2O$ (0.033 g/l). $CuSO_4$ (350 M) was used as an inorganic inducer, and wheat straw and orange peel were used as natural inducers. Table 1 shows the treatment and production conditions used.

Table 1. Inducers used in the production of the enzymes.

Tt	Inductors	Strain
T1	Orange peel 5%	*Trametes maxima* CU1
T2	Peanut shell 3% + wheat straw 0.5%	*Trametes maxima* CU1
T3	$CuSO_4$ 350 mM	*Trametes maxima* CU1
T4	Orange peel 5% + wheat straw 0.5 %	**Trametes maxima* CU1 + *Pycnoporus sanguineus* CS2

Tt= Treatment, * Growth in co-culture. The base medium used was modified Kirk (Pozdnyakova et al., 2004)

All media were inoculated with three 0.5 cm diameter cylinders from the periphery of a culture with 5 days of growth on PDA. Subsequently, they were incubated at 28 oC, under shaking conditions at 150 rpm in an orbital shaker for 18 days. The supernatants were recovered by filtration, using Whatman No. 1 paper and frozen at -20 oC until use.

Laccase assay

Laccase activity was determined by measuring the oxidation of A BTS [2,2'-Azinobis(3-Ethylbenzothiazoline-6-Sulphonic Acid)] at 405 nm [ε405 (molar extinction coefficient) = 36,000 M−1 cm−1]. The reaction mixture was prepared in sodium acetate buffer (100 mM), adjusted to pH 3.5 with 2 mM ABTS (Heinzkill et al., 1988). Enzymatic activities were expressed in units (U) defined as the amount of enzyme required to produce 1 μmol of product per minute. Enzymatic reactions were carried out in triplicate at 25 °C on a Shimadzu UV-Vis 1800 spectrophotometer (Japan).

Carbohydrate active enzymes (CAZyme)

Cellulases, xylanases and amylases were determined by the reducing sugar quantification method established by Miller et al. (1959). The reaction mixtures consisted of 0.5 ml of sodium citrate buffer solution (50 mM), adjusted to pH 5.0, 0.3 ml of 1% (w/v) substrate depending on the activity to be determined (CM-cellulose, D-xylan or starch) and 0.2 ml of sample. The reactions were incubated for 15 min at 60 °C. For the quantification of reducing sugars, 0.1 ml of the reaction mixture was added to 0.1 ml of dinitrosalicylic acid (DNS). The mixtures were boiled for 5 min at 100 °C and subsequently immersed in an ice bath at -4 °C. Afterwards, to photometrically determine the absorbance of the samples, 1 ml of double-distilled water was added, and it was read at a wavelength of 540 nm in a Shimadzu UV-Vis 1800 spectrophotometer. For the quantification of cellulases and amylases, a glucose curve in a range of 0 to 1 mg, for xylanases a xylan curve in the same range was used. One enzyme unit (U) was defined as the amount of enzyme required to release 1 μmol of glucose or xylan per minute. Enzymatic reactions were carried out in triplicate at 25 °C.

Preparation and characterization of bread

In the preparation of the bread, wheat flour, distilled water, salt and sugar, extra virgin olive oil, yeast and enzyme extracts selected from the quantification of the enzymatic activity present (lacase, amylase, cellulase and xylanase) at 5% were used. (Table 2).

Table 2. Effect of *Trametes maxima* CU1 supernatants on breads

Ingredients	Control	Treatments		
		T1	T2	T3
Wheat flour (g)	310.00	310.00	310.00	310.00
Warm water (ml)	175.00	145.00	145.00	145.00
Salt (g)	8.00	8.00	8.00	8.00
Sugar (g)	12.00	12.00	12.00	12.00
Olive oil (ml)	20.00	20.00	20.00	20.00
Yeast (g)	5.50	5.50	5.50	5.50
Supernatants (ml)	0.00	30.00	30.00	30.00

Tt=Treatment;, CON: water, T1= supernatants of the medium supplemented with 5% orange peel, T2= supernatant of the medium with 3% peanut shell + 0.5% wheat straw, T3= supernatant of the medium with orange peel 5 % + wheat straw 0.5% in co-culture with *Pycnoporus sanguineus* CS2. a-c Means (μ) ± standard deviation (σ) in columns with different superscripts indicate statistical difference (P≤0.05).

To make the bread, 5.5 g of yeast was hydrated with 12 g of sugar (Table 2). 30 ml of the enzyme extract, 20 ml of olive oil, and 310 g of wheat flour were added. The ingredients were homogenized in a Hamilton pedestal mixer (USA)

at low speed for 5 minutes, after which water was added and mixed for 7 minutes at maximum speed. The dough was left to rest for 30 minutes at room temperature (25 °C), and 6 g dough balls were made, on which elasticity tests were carried out. Before cooking, they were distributed randomly in silicone molds and placed in a convection oven heated to 185 °C for 30 minutes. After cooking, the hardness, weight loss, number of pores per mm2, height of the bread, and colorimetric characteristics were evaluated.

The height of the bread was measured with a digital Vernier Caliper with a precision of 0.01 mm (Mexico) after cooling the bread, taking the bread in half to have a standardized measurement in all the breads and thus be able to compare them. The texture analysis of the bread was carried out using a previously calibrated TA-XT2i texturometer (RHEO Stable Micro Systems). A 100 mm compression disc was used, and the parameters used were 5 mm/s. Likewise, a compression in millimeters (mm) corresponding to 50% of the average height of the bread was used for each treatment, and a force of 0.0493 N was applied. Weight loss was expressed as a percentage term and was determined by weighing three loaves of each treatment on an analytical balance of 0.1 mg sensitivity. Weight loss was reported as the difference found between the initial weight of the dough before baking and the weight after baking using the following formula:

$$Weightloss\ \ (\%) = (1 - (\frac{Final\ weight\ (g)}{Starting\ weight\ (g)})) * 100$$

To estimate the pores per mm2, the bread was divided in half, and the number of pores that existed in 1 mm2 was counted with the help of a digital Vernier Caliper with a precision of 0.01 mm (Mexico). Color analysis was previously calibrated with a CR 400 Minolta Camera Co. Ltd (Osaka, Japan) colorimeter. The data obtained after the measurement were L*, a, and b as luminosity, tendency to red color, and tendency to yellow color, respectively.

Statistic analysis

The data collected were expressed as means of five samples ± standard deviation, so the samples were analyzed in the Minitab software under the statistical significance of the analysis of variance (ANOVA), as well as with the comparison of means through the Tukey test, to be able to test the hypothesis at a 5% ($p \leq 0.05$), all under the Experimental Design of Randomized Complete Blocks (DBCA).

3. Results and Discussion

Enzymatic content of the supernatants

The different conditions studied were established to evaluate the effect of natural inducers (provided by agroindustrial waste), as well as the presence of another fungus (co-culture), in comparison with the enzyme levels in the medium supplemented with copper sulfate (inorganic inducer). Figure 1 shows the laccase titers after 18 days of culture, where the medium supplemented with the combination of peanut shell and wheat straw (T2) had the highest levels with 3757 U/l, while the rest of the treatments had a production of less than 1000 U/l. The chemical nature of the inducers can explain these differences since it has been reported that the addition of wheat straw and copper sulfate as inducers can significantly increase the volumetric activity of laccase in *Pycnoporus sanguineus* (Eugenio *et al*., 2010). Copper sulfate can induce laccase production in basidiomycete fungi, although its effectiveness may vary depending on the specific fungal species and growth conditions (Rodriguez *et al*., 2019). *Trametes versicolor, Trametes suaveolens, Daedaleopsis confragosa, Fomes fosterius* and *Trametes gibbosa* showed maximum production between day 15 and 18 in the presence of inducers based on covers (Vrsanska *et al*., 2016), with titers similar to those observed in the supernatants used in this research. On the other hand, when using agricultural waste as inducers of laccase activity, it has been reported that its effect depends on the type of waste used as a substrate, the fermentation parameters, and the addition of carbon and nitrogen sources (Pinheiro *et al*., 2020). In this regard, wastes such as orange peel, pumpkin peel, and rice straw have been described as effective substrates for laccase production (Wang et al., 2019; Zhao et al., 2017). It is important to mention that the mechanism of action of natural inducers obtained from plant materials in the production of laccase involves the activation of metabolic pathways and regulation of gene expression in fungi that produce this enzyme. According to a study conducted by Elisashvili *et al*. (2009), natural inducers derived from plant materials can stimulate laccase synthesis by interacting with fungal signaling systems, leading to an increase in the expression of genes related

to the production of this enzyme. Janusz *et al*. (2017) suggests that natural inducers of plant origin can trigger physiological responses in fungi, promoting the synthesis of ligninolytic enzymes such as laccase. For its part, the co-culture of basidiomycetes is considered a promising strategy to increase the production of individual enzymes. However, there needs to be more information on the production of lignin-modifying enzymes in the co-culture of basidiomycetes, mainly in media based on plant raw materials (Ijoma *et al*., 2021). However, the co-culture evaluated in the present investigation presented the lowest laccase titers. This may be due to the interspecies interactions that they establish in the culture medium, causing a negative effect as has been reported for other species (Kachlishvili *et al*., 2021).

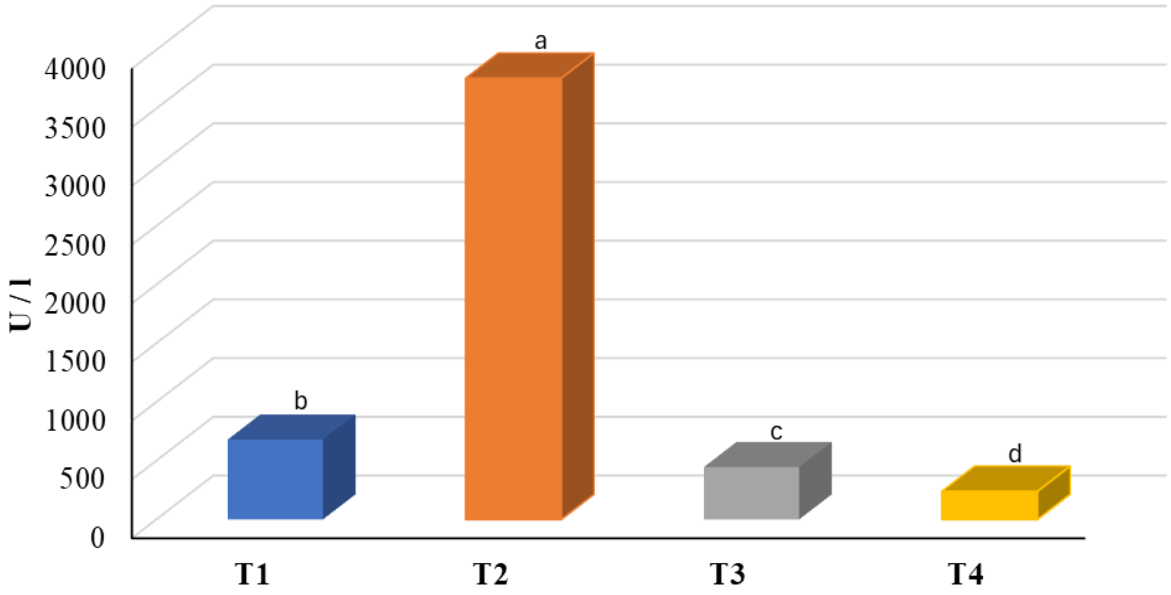

Figure 1. Laccase titers in 18-day supernatants. Where T1 corresponds to the medium supplemented with 5% orange peel, T2 to the medium with 3% peanut shell and 0.5% wheat straw, T3 with 350 mM $CuSO_4$ as an inorganic inducer, and T4 medium with 5% orange peel and 0.5% wheat straw, in co-culture *with Pycnoporus sanguineus* CS2.

The production of amylases, xylanases, and cellulase is shown in Figure 2. The co-culture *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2 (growing in the medium supplemented with orange peel and wheat straw; T4) had the highest activity of amylases and xylanase (46.7 and 41.9 mmol/min/l, respectively), although no cellulase activity was detected. At the same time, T1 showed the highest cellulase activity and the second highest amylase and xylanase activity (42.7 and 35.6 9 mmol/min/l). These values are similar to those reported for Lentinus edodes and Pleurotus spp., grown in different plant materials (Elisashvili et al., 2008). It is worth mentioning that, in the work above, it was observed that different strains of the same genus have different titers and combinations of enzymes produced, depending on the composition of the medium and fermentation conditions (Elisashvili et al., 2008). The same behavior was observed in *Trametes maxima* CU1 in the different fermentation media and conditions, which in this case included the presence of a co-culture with *Pycnoporus sanguineus* CS2. Therefore, in future studies, the optimal conditions for maximum enzyme production will be studied.

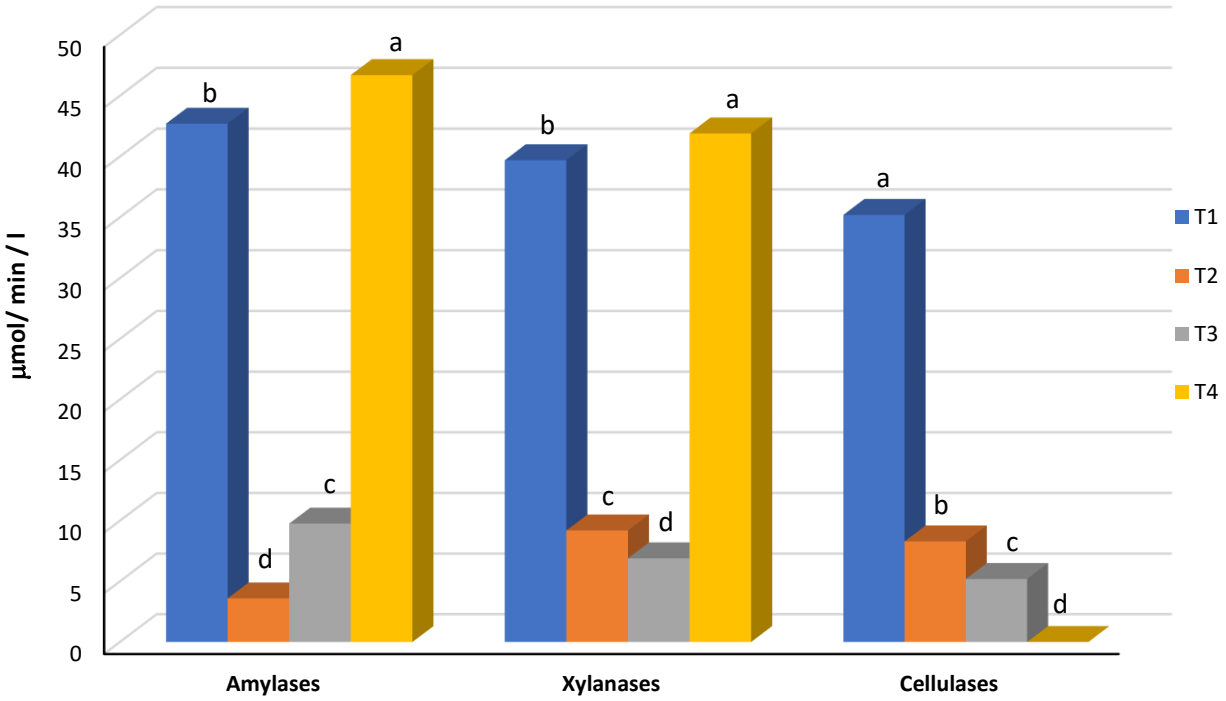

Figure 2. CAZymes titers in 18-day supernatants. Where T1 corresponds to the medium supplemented with 5% orange peel, T2 to the medium with 3% peanut shell and 0.5% wheat straw, T3 with 350 mM $CuSO_4$ as an inorganic inducer, and T4 medium with 5% orange peel and 0.5% wheat straw, in co-culture with *Pycnoporus sanguineus* CS2.

For the preparation of the bread, the supernatants of the medium supplemented with 5% orange peel, 3% peanut shell + 0.5% wheat straw, and the supernatant from co-culture with *Pycnoporus sanguineus* CS2 in medium with 5% orange peel + 0.5% wheat straw due to the combination of enzymes they presented, discarding the KM supernatant. Since it presented low values of the four enzymes, like co-culture and given the inorganic nature of the inducer.

Physical characterization of bread

Table 3 shows the results of height, hardness, weight loss (%), and the number of pores per mm2. The control and the T1 treatment presented the highest bread height ($p \leq 0.05$). The results obtained had a behavior similar to those reported by Benejam *et al*. (2009), who evaluated the effect of adding enzymes in panettone samples and observed no differences between the control and treatments with amylase and xylanase. For their part, Hernández (2014) mentions that the excessive addition of this type of enzyme can cause a dough that is too soft or sticky, which generates a deterioration in the quality of the bread. It is also important to mention that baking time is a critical factor in this parameter because a longer baking time results in a greater volume and height of the bread. The effect of laccase, xylanase, and amylase on bread height depends on the specific enzyme used and the dough formulation (Salinas-Sánchez *et al*., 2022). Thus, using xylanases can influence the degradation of hemicellulose present in flour, which can affect gas retention during fermentation and, consequently, the final height of the bread (Sheikholeslami *et al*., 2021). Similarly, amylases can influence the degradation of starch, which affects the dough's viscosity and, therefore, the final structure of the bread. As for laccase, its promising potential in the baking industry could be related to its ability to modulate the network of polysaccharides present in flour, which could influence bread's texture and height (Niño-Medina et al.., 2017).

Table 3. Effect of *Trametes maxima* CU1 supernatants on breads

Tt	Height (mm)	Hardness (N)	Weight loss (%)	Pores by mm^2
Control	27.0 ± 1.00^{a}	58.21 ± 7.72^{c}	1.03 ± 0.50^{a}	14.0 ± 2.64^{ab}
T1	28.7 ± 0.58^{a}	67.16 ± 9.06^{b}	0.93 ± 0.45^{ab}	19.3 ± 3.21^{a}
T2	24.3 ± 1.15^{b}	77.16 ± 4.77^{a}	0.00 ± 0.00^{b}	7.0 ± 1.00^{b}
T3	22.7 ± 0.58^{c}	68.05 ± 11.71^{b}	0.00 ± 0.00^{b}	9.3 ± 2.08^{b}

Tt=Treatment, WITH: water, T1= supernatants of the medium supplemented with 5% orange peel, T2= supernatant of the medium with 3% peanut shell + 0.5% wheat straw, T3= supernatant of the medium with orange peel 5 % + wheat straw 0.5% in co-culture with Pycnoporus sanguineus CS2. a-cMeans (μ) ± standard deviation (σ) in columns with different superscripts indicate statistical difference (P≤0.05).

The treatments had a statistically significant difference ($p \leq 0.05$), with the control having the lowest hardness, reflected in the best weight loss and the more significant number of pores per mm2. This behavior was similar to what Hernández (2014) observed, who observed no differences between the hardness of the control and the treatments associated with starch gelation. However, in bread made with the purified laccase of *Tramete maxima* CU1, the hardness of the bread was reduced by 17.71%, contrary to what was observed with the supernatants (Niño-Medina *et al*., 2017). This could be explained by the combination of enzymes and their activity titers.

Regarding the weight loss results, the treatments had a statistically significant difference ($p \leq 0.05$), with the control having the highest percentage (1%). However, the values were lower than those reported in other investigations (Niño-Medina *et al*., 2017). It is worth mentioning that the behavior of the treatments was also different from what was observed in breads treated with laccase, xylanase, and lipase (Vega Castro et al., 2015). This could be explained by the fact that despite the different levels of laccase activity in the supernatants, this is the main enzyme and could act on the phenolic compounds and proteins present in the dough, coupled with the activity of the enzymes active on carbohydrates, affecting the absorption and distribution of water in the dough, therefore, also the porosity. In future research, the optimal temperature and cooking time conditions for doughs treated with the supernatants of *Trametes maxima* CU1 will be evaluated.

For its part, the color analysis showed a statistically significant difference ($p \leq 0.05$) in the values of L* and a* between the treatments, except the results of b* (Table 4). It is worth mentioning that T1 and the control did not show a statistically significant difference ($p > 0.05$), which can be explained by the levels and activities in the supernatants. The supernatant obtained from the medium with orange peel showed amylase, cellulase, xylanases, and laccase activity, while the supernatant from the medium supplemented with peanut shell and straw had the highest laccase activity but the lowest CAZyme activity. As for the supernatant obtained from co-culture with *Pycnoporus sanguineus*, CS2 showed the maximum concentration of amylases and xylanases with the lowest production of laccase. This suggests that high and low titers of laccase activity result in hard bread and different colors in correlation with CAZyme levels. Thus, it has been reported that the combination of laccase and xylanase gives bread a darker color and a crispier crust (Niño-Medina *et al*., 2017). In the case of laccase, it is an enzyme with great potential to improve the properties of wheat flour dough and the quality of bread, due to its ability to improve the structure, resistance, and stability of the dough, in addition to improving the volume and texture of the bread (Rosell & Dura, 2015). The above is due to its mechanism of action, as it catalyzes the oxidation of various aromatic compounds, which can produce semiquinones associated with the reduction of molecular oxygen to water, while the free radicals generated can lead to the polymerization of semiquinones to form brown or black pigments (Figueroa-Espinoza and Rouau, 1998). However, its synergy with xylanase improves the color of the bread since it action on the xylan fibers in fractions weakens the structure of bread and allows browning in less time (Rosell & Dura, 2015). In the case of amylase, it is an enzyme that breaks down starch into simpler sugars, which can increase the number of sugars available for the Maillard and caramelization reactions during bread baking, contributing to the color of the bread, in addition to increasing the volume of the bread and crumb texture. (Lončar *et al*., 2016). Therefore, the effect of the enzymatic cocktail of laccase, amylase, xylanase, and cellulase on the color of wheat flour bread may vary depending on several factors, such as the concentration of enzymes used, the

fermentation, and cooking time, among others. In addition to influencing the quality and nutritional value of bread, it offers alternatives to the use of chemical additives and the development of biofunctional foods.

Table 4. Color analysis of breads

Tt	L*	a*	b*	View
Control	39.01±5.9855^{b}	27.25±1.9126^{a}	42.29±3.6220	
T1	47.50±10.0986^{b}	22.43±3.84^{a}	42.32±4.4571	
T2	67.25±4.3486^{a}	10.63±1.6127^{b}	43.15±2.2315	
T3	72.04±1.7225^{a}	5.22 ±0.5729^{c}	37.98±1.8278	

Tt= Treatment, CON: water, T1= supernatants of the medium supplemented with 5% orange peel, T2= supernatant of the medium with 3% peanut shell + 0.5% wheat straw, T3= supernatant of the medium with orange peel 5 % + wheat straw 0.5% in co-culture with Pycnoporus sanguineus CS2. a-cMeans (μ) ± standard deviation (σ) in columns with different superscripts indicate statistical difference (P≤0.05). L*: Luminosity; a*: the tendency to red; b*: the tendency to yellow.

4. Conclusions

Different profiles (combinations) and titers of laccase, amylases, xylanases, and cellulases were observed in the culture media evaluated, with $CuSO_4$ being the best laccase inducer and orange peel at 5% for enzymes active on carbohydrates (amylases, xylanases, and cellulases) and laccase. At the same time, the bread obtained with the orange peel supernatant had the highest height and color, like the control, in addition to presenting the lowest hardness among the enzymatic treatments. Therefore, these results demonstrate the effect of the culture medium on the enzymatic profiles of the same fungus and its potential application in the baking industry.

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7. Conflicts of Interest

The authors declare no conflict of interest.

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Artículo

Potential applications of microalgae bacteria consortia for waste treatment and valuable bioproducts

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Abstract: The application of microalgae and bacteria in wastewater treatment has attracted interest due to the greater environmental adaptability and stability resulting from their interactions, exceed those obtained with microalgae al sustainability and economic competitiveness. This manuscript aims to support existing and relevant literature on the use of microalgae and bacteria. As a result, numerous scholars and authors have been emphasizing recent research on the biotechnology of algae and bacteria, so this revision will be useful to advance and facilitate the technological development of biological processes.

Keywords: microalgae; bacteria; valuable bioproducts.

Posibles aplicaciones de los consorcios de bacterias de microalgas para el tratamiento de residuos y bioproductos valiosos

Resumen: La aplicación de microalgas y bacterias en el tratamiento de aguas residuales ha despertado interés debido a la mayor adaptabilidad y estabilidad ambiental resultante de sus interacciones, superando las obtenidas con microalgas a las de sostenibilidad y competitividad económica. Este manuscrito pretende apoyar la literatura existente y relevante sobre el uso de microalgas y bacterias. Como resultado, numerosos estudiosos y autores han hecho hincapié en las investigaciones recientes sobre la biotecnología de algas y bacterias, por lo que esta revisión será útil para avanzar y facilitar el desarrollo tecnológico de los procesos biológicos.
Palabras clave: microalgas; bacterias; bioproductos valiosos.

1. Introduction

Anthropogenic activities continue to produce a significant amount of wastewater discharge with society's rapid development. This wastewater typically contains organic pollutants, acids, alkalis, salts, nutrients, heavy metals, and other environmental contaminants that can degrade the environment (Mhedhbi et al., 2020). Physical processes such as filtration, adsorption, and reverse osmosis. Physical processes like filtration, adsorption, and reverse osmosis; chemical processes like coagulation, advanced oxidation, and ion-exchange; biological processes like activated sludge processes and microalgae-based methods; and hybrid processes are the conventional methods for treating wastewater. While chemical processes can result in secondary contamination, physical methods are energy-intensive and not cost-effective (Khan et al., 2023). Biological techniques, however, are environmentally and economically beneficial (Goh et al., 2023).

Through several studies, the use of bacteria and microalgae as essential elements in environmentally friendly biological techniques is becoming a growing trend. Through the processes of nitrification, adsorption, denitrification, anaerobic ammonia oxidation, and integration, bacteria eliminate pollutants. However, slow processes, ongoing maintenance requirements, limited applicability, and performance degradation due to abiotic conditions limit their use (Sátiro et al., 2022).

On the other hand, because of their high photon conversion efficiency, huge capacity for absorbing carbon dioxide (CO2), quick growth rates, and high productivity, microalgae are important biological resources with ecological significance. An eco-friendly method for the green remediation of the environment is offered by phycoremediation (Ishizaki et al., 2020).

Microalgae–bacteria consortia have been utilized for treating wastewater since the 1950s. Microalgae–bacteria consortia offer unique benefits for wastewater treatment, and an increasing amount of research has explored dual-species

cultures of microalgae and bacteria for overall ecological improvement (Zhuang et al., 2023). Microalgae can offer a habitat that protects bacteria from unfavorable environmental conditions, increases bacterial growth rate, and lowers aeration requirements and energy costs; concurrently, bacteria not only promote the growth of microalgae and the productivity of their bioproducts but also increase the consortium's sedimentation rates, which lowers the costs associated with harvesting biomass (Lauritano et al., 2020). Furthermore, compared to the impacts of each component alone in wastewater treatment, microalgae–bacteria consortia can more successfully encourage the transformation of pollutants and enhance their environmental adaptability and stability (Ríos et al., 2023). It is economically feasible to treat environmental pollutants utilizing symbiotic microalgae–bacteria consortia because of these important benefits. One of the most important aspects of this technique, which has major implications for environmental management, is the interaction between microalgae and bacteria. Additionally, consortiums of bacteria and algae can create biofuels and other bioproducts.

Microalgae–bacteria consortia have great economic application potential in the circular bioeconomy as an efficient option for wastewater treatment and bioproduct manufacturing. Therefore, this article aims to provide a comprehensive overview of the microalgae-bacterial consortium interaction process. This review also focuses on providing a bibliometric analysis of the microalgae-bacterial consortium and showing the trend in the use of microalgae biotechnology. The study offered here is a significant contribution to bibliometric technique and can help improve understanding of the field of use of microalgae-bacterium consortium by offering recommendations to researchers.

2. Materials and Methods

2.1. Bibliometric Analysis

The terms "microalgae", "bacteria" and "wastewater" were used to search the title, abstract and keywords of the publication. The aim was to exclude products with a broad reach over microalgae and to give priority to those that focus on the use of consortia with batteries to obtain valuable products. A search was carried out between 2014 and 2023, with 936 documents retrieved from the Web of Science database (Clarivate). Bibliometric data were analyzed using the R bibliometric package and the open-source software RStudio (www.rstudio.com) (Silva *et al*., 2020). The VOSviewer software, available at www.vosviewer.com, was used to build and present the keyword co-occurrence network, as described by (Verasoundarapandian et al., 2022).

3. Results

3.1. Bibliometric analysis for microalgae harvesting with EC.

Microalgae are increasingly used by a variety of companies, so it is critical to investigate the trend of the use of microalga-bacteria consortium, Using the search terms "microalgae", "bacteria" and "wastewater", a methodical search was carried out in the Web of Science scientific database for this purpose. Data was collected from 936 publications, including research and review articles. The bibliometric characteristics, including the total number of cites, the average number of citations per article, the rankings of the most cited publications and the evolution of the subject of study were calculated using the Bibliometrix package of the R Commander software (x64 4.1.0). In addition, bibliometric maps were produced using co-occurrence analysis with VOSviewer. The Web of Science database was used to gather research data on microalgae-bacterial consortium use published between 2014 and 2023. Countries that had articles written on the subject were ranked (Figure 1), with China having the most cited (6560), which also has the most publications (1248) followed by the United States (1835). Consequently, the impact of these publications on the field and their potential value as references for future research are obvious. The information extracted from the database was classified according to the author's associated affiliation address. Reviews (131) and research articles (819) constitute most published papers.

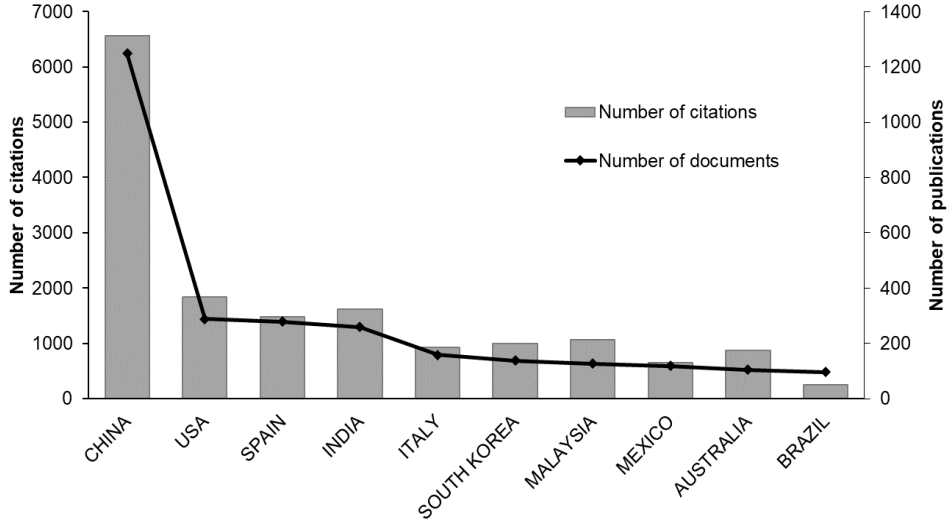

Figure 1. Key nations based on microalgae harvest data using microalga-bacteria consortium.

Table 1 presents a summary of the descriptive analysis performed on the data obtained from 2014 to 2023, utilizing the Web of Science database. 936 documents and 3122 authors were located during this search. Authors of single-authored documents were 7. The author/document relationship was 3.33 and the co-author/document ratio was 5.28.

Table 1. Descriptive analysis of retrieved data.

Description	
Documents	936
Period	2014-2023
Annual percentage growth rate	6.33
Average citations per documents	24.15
Authors	3122
Authors of single-authored documents	7
Documents per author	3.33
Coauthors per documents	5.28

Table 2 presents the most frequently cited papers pertaining to microalga-bacteria consortium. Notably, the papers pertinent to the review articles are categorized as significant reference papers. Additionally, the most influential contribution was made by the authors of the Portugal research institutions, who included 426 citations and 169.85 annual citations. To aid in the development and expansion of microalga-bacteria consortium, this review investigated the fundaments of this process.

Table 2. Works that have received the most quotations in worldwide studies on microalga-bacteria consortium.

Title	Journals	Authors Affiliation Countries	Number of Citations	Number of Citations per Year	References
A review on the use of microalgal consortia for wastewater treatment	Algal research-biomass biofuels and bioproducts	Portugal	426	60.85	(Gonçalves et al., 2016)
Interaction between Chlorella vulgaris and nitrifying-enriched activated sludge in the treatment of wastewater with low C/N ratio	Journal of cleaner production	USA	383	95.75	(Sepehri et al., 2020)
Perspectives on the feasibility of using microalgae for industrial wastewater treatment	Bioresource technology	Taiwan	281	35.12	(Wang et al., 2016)
Advanced nutrient removal from surface water by a consortium of attached microalgae and bacteria: A review	Bioresource technology	China	220	31.42	(Liu et al., 2017)
Use of hydrodynamic cavitation in (waste)water treatment	Ultrasonics sonochemistry	Slovenia	217	27.12	(Dular et al., 2016)
Removal of pharmaceutical and personal care products (PPCPs) from wastewater using microalgae: A review	Journal of hazardous materials	Australia	213	71	(Hena et al., 2021)
Effects of photoperiod on nutrient removal, biomass production, and algal-bacterial population dynamics in lab-scale photobioreactors treating municipal wastewater	Water research	South Korea	206	22.88	(Lee et al., 2015)
Trends and novel strategies for enhancing lipid accumulation and quality in microalgae	Renewable & sustainable energy reviews	South Africa	205	25.62	(Singh et al., 2016)
Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges	Biotechnology advances	South Africa	204	34	(Renuka et al., 2018)

Anaerobic digestate as substrate for microalgae culture: The role of ammonium concentration on the microalgae productivity enzymatic hydrolysis	Bioresource Technology	France	203	25.55	(Uggetti et al., 2014)

In addition, a thematic map was generated using Bibliometrix to illustrate the frequency of use of keywords in microalgae-bacteria consortium collection studies. (Table 2). The map, which considered references, authors, and the categories "microalgae" and "wastewater treatment" among others, revealed that these terms are most frequently used in China and Spain, respectively. An analysis of the research topics associated with this study's terms was done using keyword combination analysis. Figure 3 illustrates the results obtained. Keywords that appear at least twenty times are represented by a circle, with the diameter of each circle corresponding to the frequency of occurrence of the keyword. The keyword circle increases in size as it appears more often. The occurrence attribute of a keyword indicates the number of documents containing the key word. The clusters, marked by different colors on the map, function to distinguish sets of comparable elements. After a keyword that matches the network analysis, 936 were considered relevant and analyzed. As the results show, 11,529 link forces comprise 1212 links, while 52 elements are classified into five categories. The keywords (listed in Table 3) were used to generate five main categories, which correspond to the main fields of study associated with the collection of microalgae by the EC.

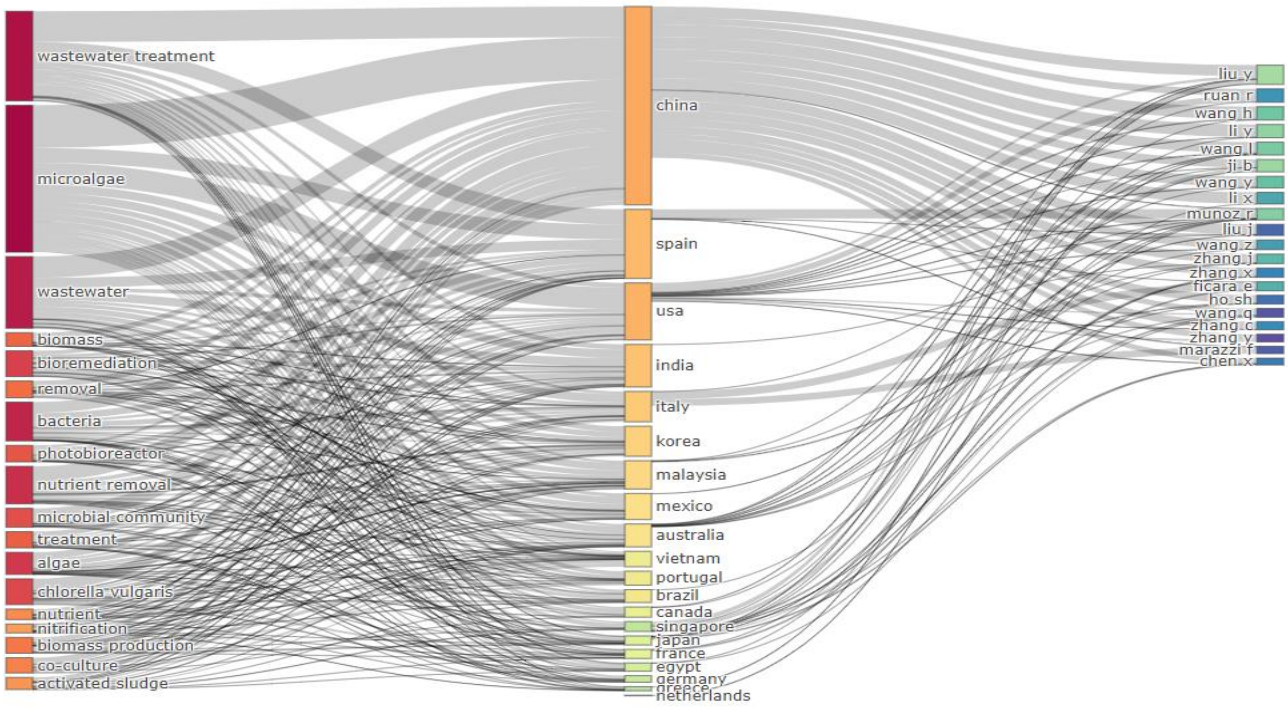

Figure 2. Thematic map of terms using microalgae bacteria (Bibliometrix).

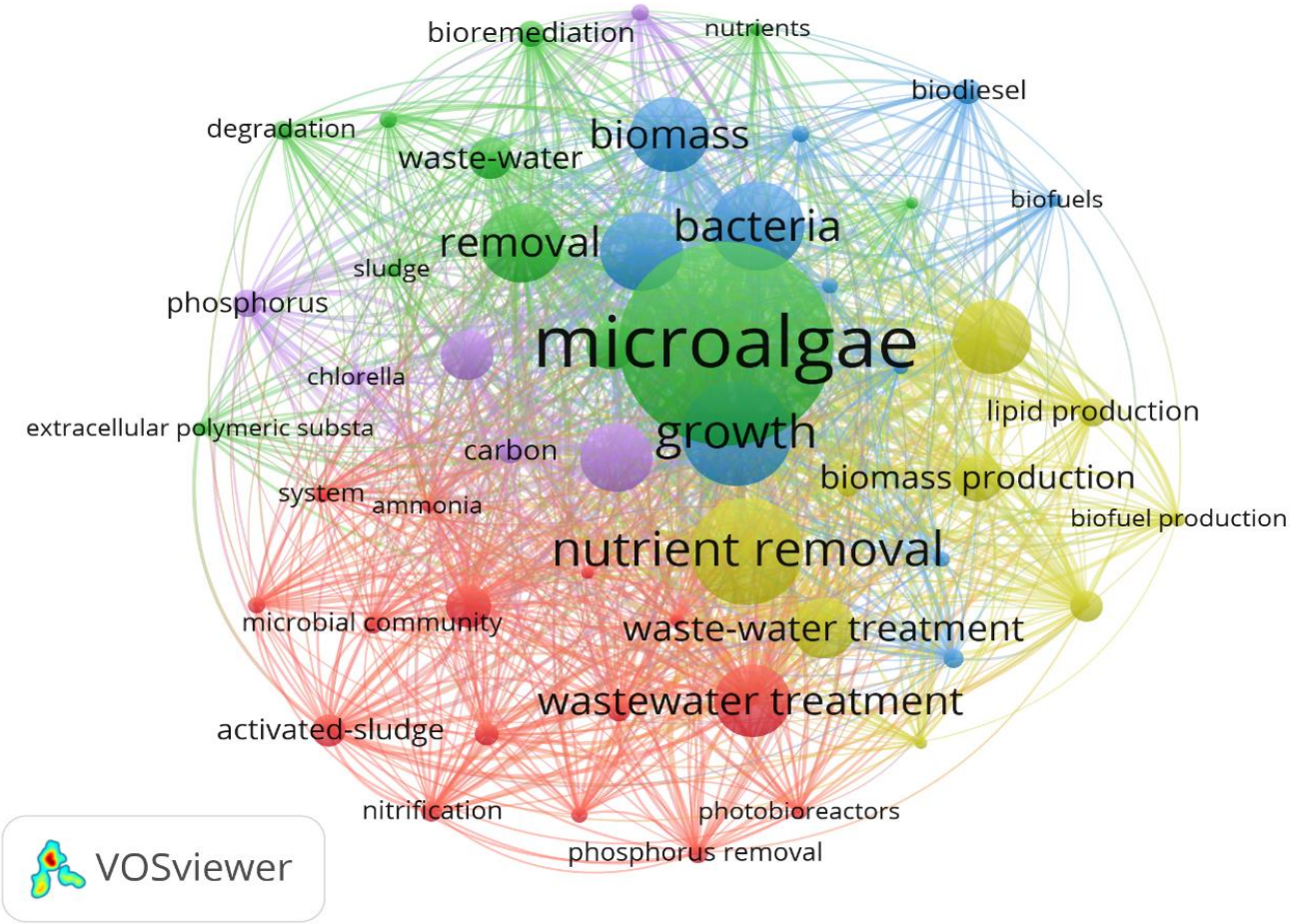

Figure 3. Keyword co-occurrence network analysis and emerging clusters (Cluster I: 15 words; Cluster II: 11 words; Cluster III: 11 words; Cluster IV: 9 words, Cluster V: 6 words).

Table 3. Words and clusters derived from co-occurrence network analysis of keywords.

Cluster	Words
Cluster I	15 words: activated-sludge, ammonia, consortium, culture, microbial community, nitrification, nitrogen removal, nutrients removal, performance, phosphorus removal, photobioreactor, photo-bioreactors, system, wastewater treatment and wastewaters.
Cluster II	11 words: biodegradation, bioremediation, cyanobacteria, degradation, extracellular polymerics substances, microalgae, nutrients, removal, sludge, wastewater and wastewater.
Cluster III	11 words: accumulation, bacteria, biodiesel, biofuels, biomass, chlorella vulgaris, cultivation, growth, light, recovery and temperature.
Cluster IV	9 words: Anaerobic-digestion, biodiesel production, biomass production, chlorella-vulgaris, light-intensity, lipid production, nutrient removal and waste-water treatment.
Cluster V	6 words: Algae, carbon, chlorella, effluent, nitrogen and phosphorus.

The main topics frequently found in scientific literature worldwide are illustrated in Figure 4. Most of the research topics concerned the use of microalgae and bacteria for water treatment.

Figure 4. Compilation of 52 topics shared in the academic papers on microalgae-bacteria.

The main journals are presented in Table 4. With 143, 68, and 47 articles, respectively, the main journals in which the authors studying microalgae-bacteria are Bioresource Technology (IF = 11.889), Algal Research-Biomass Biofuels and Bioproducts (IF = 5.276), and Journal of Science of the total environment (IF = 10.753)

Table 4. The main research journals on microalgae-bacteria, their impact factors, and the number of publications.

Journals	Impact Factor	Number of Publications
Bioresource technology	11.889	143
Algal research-biomass biofuels and bioproducts	5.276	68
Science of the total environment	10.753	47
Water research	7.481	39
Journal of water process engineering	7.34	32
Chemosphere	8.943	25
Journal of cleaner production	11.072	24
Journal of environmental management	3.644	24
Water science and technology	2.430	24
Environmental science and ecotechnology	11.357	22

3.2. Microalgae–bacteria consortia.

Wastewater presents a viable and economic growth medium for microalgae and bacteria due to its substantial nutrient content (e.g., carbon, nitrogen, phosphorus, and sulfur). Moreover, it enables the integration of microalgae

cultivation and biorefining processes into the pre-existing wastewater treatment infrastructure (Microalgae, 2024). Multiple mechanisms, including bioadsorption, assimilation, biodegradation, bioaccumulation, biotransformation, nitrification, anaerobic ammonia oxidation, denitrification, and sulfur oxidation, enable microalgae–bacterium consortia to remove hazardous contaminants from diverse types of wastewaters (La Bella et al., 2022). The consortia of microalgae and bacteria exhibited greater stability and adaptability to complex environmental conditions than their constituent species. Utilizing microalgae–bacterium consortiums as a biological strategy for holistic ecological improvement has garnered considerable interest, specifically in the treatment of effluent from agricultural, industrial, and municipal origins.

3.3. Microalgae bacteria consortia for valuable bioproducts.

By comprehending the dynamic between microalgae and bacteria, it is possible to discern or form consortia of microalgae and bacteria that operate in a mutualistic manner, thereby optimizing the treatment of effluent resources and producing biomass simultaneously for biorefining objectives. Based on microalgae bacteria group technology, this closed-loop circular bioeconomy integrates clean water recovery, co-culture of microalgae bacteria, effluent resource treatment, biomass production, and derivative bioproduct generation. Microalgae bacteria consortia serve as an ideal feedstock for biorefineries, facilitating the generation of an extensive array of bio-products including but not limited to food, feed, biochemicals, biomaterials, high-value bioproducts, and diverse forms of bioenergy including biofuels, electricity, and heat (Gao et al., 2016).

4. Conclusions

This review offered a bibliometric analysis that contributed to the understanding of interactions involving microalgae bacteria consortia, with the goal of expanding their usefulness within the circular bioeconomy. The coexistence of bacterial species and microalgae is an inexorable consequence of evolution, giving them greater environmental stability and adaptability compared to their individual states. Recent research with an increase of 6.33% annually indicates that the microalgae bacteria consortia system presents considerable potential and benefits in the fields of effluent treatment and biorefineries in order to increase the likelihood that modern biotechnological implementations will succeed and advance in ecological development.

5. Conflicts of Interest

The authors declare no conflict of interest.

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Artícle

Effect of agricultural production system on soil microbial populations

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Abstract: The physicochemical properties of soil and microbial populations are important factors that influence soil quality, nutrient cycling, and ecosystem functioning. These properties play a crucial role in regulating microbial activity and interactions, as well as the biogeochemical processes in soil. This document presents the partial results of the physicochemical and microbiological characterization of the soil from samples taken at 15 and 45 cm depth prior to the establishment of the different tillage and fertilizer treatments. Concerning the values observed in the physical and chemical parameters, no statistically significant differences ($p > 0.05$) were observed between samples from both depths. The microbiological analysis was conducted with soil samples taken at 15 cm depth from three tillage systems, considering the control treatment and the treatment with organic fertilization with poultry manure. Aerobic bacteria and actinomycetes counts did not show statistically significant differences ($p > 0.05$). Anaerobic, coliform, and fungal counts were statistically different ($p > 0.05$) between treatments due to the presence of organic fertilizer. However, no statistically significant difference ($p > 0.05$) was observed between the three tillage systems. In a second culture cycle, the effect of different concentrations of organic matter was evaluated, where T1(N: 60, P: 65.5, K: 74.4) showed the higher CFU g^{-1} (Log) of aerobic and anaerobic bacteria, actinomycetes, and fungi, while T3 had the highest CFU of coliforms. These results are important to understand soil dynamics better and inform agricultural and soil management practices. In addition, they highlight the importance of proper use of organic fertilizers and the need for further research to determine how they affect long-term soil health and quality.

Keywords: Agricultural machinery; fertilizer; microbiological; physicochemical; tillage.

Efecto del sistema de producción agrícola en las poblaciones microbianas del suelo

Resumen: Las propiedades fisicoquímicas del suelo y las poblaciones microbianas son factores importantes que influyen en la calidad del suelo, el ciclo de los nutrientes y el funcionamiento de los ecosistemas. Estas propiedades desempeñan un papel crucial en la regulación de la actividad y las interacciones microbianas, así como en los procesos biogeoquímicos del suelo. En este documento se presentan los resultados parciales de la carac-terización fisicoquímica y microbiológica del suelo a partir de muestras tomadas a 15 y 45 cm de profundidad antes del establecimiento de los diferentes tratamientos de laboreo y fertilización. En cuanto a los valores observados en los parámetros físico-químicos, no se observaron diferencias estadísticamente significativas ($p > 0,05$) entre las muestras de ambas profundidades. El análisis microbiológico se realizó con muestras de suelo tomadas a 15 cm de profundidad de tres sistemas de labranza, considerando el tratamiento control y el tratamiento con fertilización orgánica con gallinaza. Los recuentos de bacterias aerobias y actinomicetos no mostraron diferencias estadísticamente significativas ($p > 0,05$). Los recuentos de bacterias anaerobias, coliformes y hongos fueron estadísticamente diferentes ($p > 0,05$) entre los tratamientos debido a la presencia de abono orgánico. Sin embargo, no se observaron diferencias estadísticamente significativas ($p > 0,05$) entre los tres sistemas de laboreo. En un segundo ciclo de cultivo, se evaluó el efecto de diferentes concentraciones de materia orgánica, donde T1(N: 60, P: 65,5, K: 74,4) mostró la mayor UFC g-1 (Log) de bacterias aerobias y anaerobias, actinomicetos y hongos, mientras que T3 tuvo la mayor UFC de coliformes. Estos resultados son importantes para comprender mejor la dinámica del suelo e informar sobre las prácticas agrícolas y de gestión del suelo. Además, ponen de relieve la importancia del uso adecuado de fertilizantes orgánicos y la necesidad de seguir investigando para determinar cómo afectan a la salud y la calidad del suelo a largo plazo.

Palabras clave: Maquinaria agrícola; fertilizante; microbiológico; fisicoquímico; labranza.

1. Introduction

Microorganisms in soil play a crucial role in nutrient cycling, soil health, and crop productivity (Nabi et al.,2023). They are involved in various activities such as nutrient mobility and absorption, plant growth stimulation, and disease management (Beigmohammadi et al., 2023). They contribute to nutrient cycling, stimulate plant growth, and reduce diseases. Microbes such as mycorrhizal fungi and plant growth-promoting bacteria enhance nutrient mobility and absorption, hormone regulation, and disease management. Beneficial soil microorganisms improve plant health, nutritional status, and soil quality, making them essential for sustainable agriculture. The microbial population in the rhizosphere interacts with plants and influences bacterial activities, which can lead to better plant development. Microorganisms also play a significant role in the decomposition of agricultural waste, aiding in the production of organic compounds and minerals that are beneficial for crop growth (Prisa, 2023; Kaur & Rani, 2022). However, agricultural practices have a significant impact on soil health and soil quality, as conventional systems (such as the use of chemical fertilizers and mechanized tillage) can lead to soil erosion, loss of biodiversity, and declines in soil structure and organic matter (Aseeva et al., 2021). In this context, irregular and irresponsible practices, such as excessive tillage and inadequate fertilization, can lead to the loss of soil carbon and an increase in carbon dioxide emissions (Alori et al., 2020). It is worth mentioning that long-term agricultural use of soil can negatively affect the availability of chemical elements for plants and the number of microorganisms in the soil (Galic et al., 2019), causing a significant loss of biodiversity (Barros-Rodríguez et al., 2021). However, strategies can be implemented to increase crop production without endangering soil health, such as the use of organic fertilizers and conservation tillage techniques (Burcea, 2018). In the case of organic fertilization, fermentation processes have been found to impact soil microbial populations. For example, the use of forest wastes can increase the presence of bacteria and decrease the fungal population (Marois et al., 2022). On the other hand, bacterial communities in soils treated with spent microbial biomass from industrial fermentation processes have been compared with conventional agricultural systems, where a statistically significant increase in the diversity of the microbial population was observed, mainly in the populations of Proteobacteria and Actinobacteria (Halter et al., 2020). In addition, long-term application of soil conditioners derived from fast fermentation altered the distribution of the main functional bacterial communities in the soil, with *Proteobacteria*, *Actinobacteria*, *Acidobacteria* and *Firmicutes* becoming the dominant phyla (Jain and Saxena, 2019).

Regarding the influence of the tillage system on microbial populations, conventional methods can damage soil structure, whereas no-tillage can improve soil quality by maintaining structure and increasing microbial biodiversity (Angon et al., 2023). In turn, crop rotation and tillage also influence the soil environment by altering key soil properties such as pH and organic matter (Behnke et al., 2021). The use of alternative agricultural methods, such as reduced tillage, conservation tillage, no-tillage and organic farming can help preserve the richness and diversity of soil bacterial communities (Khmelevtsova et al., 2022). In addition, different tillage management and crop residue incorporation have been shown to affect soil bacterial community structure, and certain treatments lead to higher levels of specific fatty acids and bacterial populations (Tang et al., 2022). In general, the choice of tillage and fertilization system can have important implications for agricultural soil microbial populations. Therefore, the present research aims to evaluate the effect of nine agricultural production systems on soil microbial populations.

2. Materiales y Métodos

Experiment Location

The experiment was established in the spring-summer 2018 cycle, in the Marín experimental field of the Faculty of Agronomy belonging to the UANL, located in Marín Nuevo León, with geographical location 25° 52' 13.5'' north latitude and 100° 02' 22.56'' west longitude, at an altitude of 355 masl. The climate corresponds to a BSI (h) w (e), described as a dry warm steppe climate with rainfall in summer, an average annual precipitation of 595 mm and an average annual temperature of 22 °C. The predominant soil type is calcareous-clay with a pH between 7.5 and 8.5 and low organic matter content.

Genetic material

Sweet sorghum [Sorghum bicolor (L.) Moench] of the Roger genotype variety from the Sorghum Program of the Agronomy Faculty of the Universidad Autónoma de Nuevo León was used.

Life Cycle Assessment (LCA)

This method of analysis was performed according to Wanga et al. (2014) where he states that a product system is a collection of unit processes connected by intermediate product flows that perform one or more defined functions.

Experimental design

The research comprises a data collection period of two crop cycles comprising the fall-winter cycle of 2017-2018 and 2018-2019. The experiment was comprised of nine 40x24 m experimental plots, with a total area of 9 000 m^2. Three soil tillage systems in combination with two fertilization and no fertilization systems were evaluated. A split-plot design was used for data collection and its model is as follows:

$$Yijk = \mu + \beta i + Fj + eij + eij\ (a) + Vk + (FV)jk + eijk(b)$$

$$i = 1,2,\ldots,\ r\ j = 1,2,\ldots,a\ k = 1,2,\ldots,b$$

Yijk is the observation at tillage j, at moisture content k, in block i.
μ is the overall true mean.
βi is the effect of block i.
Fj is the effect of date level j.
eij(a) is the experimental error for the i j-th large plot for dates.
Vk is the effect of the level k of varieties.
FVjk is theeffect of the interaction of tillage j and moisture k.
eijk(b) is the experimental error of the i j k-th subplot.

Soil tillage systems:

- Tillage system 1 consists of clearing and harrowing labors and sowing.
- Tillage system 2 is composed of clearing, plowing, and harrowing labors and sowing.
- Tillage system 3 is composed of clearing, subsoil, plowing, and harrowing labors, and sowing; in this technology, subsoiling and plowing are done in November and when the sowing date approaches, harrowing is done a day or two days before planting.

Fertilization systems:

- Organic fertilization, as a base source poultry manure.
- Inorganic fertilization, commercial chemicals (100, 50, 0).
- Without fertilization.

Table 1 shows the systems.

Table 1. Treatment specifications.

Treatment	Description
T1	L 1 + F1
T2	L1 + F2
T3	L 1 + F3
T4	L 2 + F1
T5	L 2 + F2
T6	L 2 + F3
T7	L 3 + F1
T8	L 3 + F2
T9	L 3 + F3

L1: Minimum tillage (clearing and harrowing); L2: Traditional tillage (clearing, plowing, and harrowing); L3: Traditional tillage with breaking of the plow layer (clearing, subsoil, plowing, and harrowing); F1: Organic fertilizers; F2: Inorganic fertilizers; F3: No fertilizers.

A second crop cycle was established with traditional tillage with breaking of the plow layer and three concentrations of organic fertilizer (poultry manure): Summer-Winter Cycle 2019

Treatments

- T1= Organic fertilization N: 60, P: 65.5, K: 74.4
- T2= Organic fertilization N: 90, P: 98, K: 112
- T3= Organic fertilization N: 120, P: 131, K: 149.4

Soil preparation

Data collection for the determination of the physicochemical properties of the soil were carried out according to the methodology described by the Mexican Official Standard NOM-021-RECNAT-(2000), which establishes the specifications for fertility, salinity, and soil classification.

Physical and chemical properties

Soil sampling and determination of soil physical-chemical properties were carried out according to the methodology described in the Mexican Official Standard NOM-021-RECNAT-(2000), which establishes the specifications for soil fertility, salinity, and classification. Samples were taken at 0.15 m and 0.45 m depth to compare the initial soil conditions with the changes derived from the establishment of tillage and fertilization treatments.

Partial microbiological characterization of the soil.

Soil samples were diluted 1:10 and homogenized in 0.1% sterile peptone water. The dilutions were sown on a plate, taking 1 ml of each one and adding approximately 15 ml of the corresponding medium. Mesophilic and anaerobic counts were performed on standard count agar, varying the presence of O_2 and CO_2, respectively. Bile and red violet agar (RVBA) were used for coliform quantification. Fungal counts were carried out on potato dextrose agar (PDA)

medium, modified with 10% tartaric acid. Quantification of actinomycetes was performed on selective medium for isolation. All the plates were incubated at 37 °C for 24 h. All assays were performed in triplicate per treatment. The culture media were purchased from Laboratorios CONDA (Spain).

Statistical analysis

The data obtained from the variables evaluated were analyzed with the SPSS Statistics computer statistical package. Analysis of variance and correlation between variables were applied. Mean differences were determined by the Tukey method for a significance level of 95 % ($p \leq 0.05$).

3. Results and discussion

The composition of microbial communities in agricultural production systems is influenced by several factors, including the tillage and fertilization system. It is worth mentioning that the source of water used to produce agricultural products is also a factor that influences populations, as it can introduce foodborne pathogens. In general, agricultural practices, fertilizer applications, water sources and soil management practices play a role in shaping the composition of microbial communities in agricultural production systems.

Soil physicochemical characterization

The chemical characterization of the soil was carried out prior to soil preparation with the different production systems. This included quantification of soil organic matter (OM), nitrogen, phosphorus, potassium, zinc, and copper content (Table 2). In the initial soil condition, the organic matter content at 15 and 45 cm showed statistically significant differences ($p \leq 0.05$). The contents of N, P, K, Cu and Zn showed no difference ($p > 0.05$) at both depths. With respect to the nutrient contents observed, they were similar to those reported for other saline soils (Corwin et al., 2003).

The pH results at two depths in the initial soil condition prior to treatment and crop establishment showed no statistically significant difference ($p \leq 0.05$) at 0.15 and 0.45 cm depth. Electrical conductivity values observed at 0.15 and 0.45 m (5.40 ± 0.666 and 2.65 ± 0.667 mS/cm at 25 °C, respectively) had statistically significant difference ($p \leq 0.05$). All these results are like those reported by previous works such as Escoto (2014) for soils from the Marin Experimental Campus and similar to those reported for other saline soils (Corwin et al., 2003).

Table 2. Chemical characterization of the soil.

Parameter	Initial condition		Sig.
	15 cm	45 cm	
M.O (%)	2.19 ± 0.122	1.84 ± 0.164	0.000
N (ppm)	8.073 ± 0.205	8.096 ± 0.224	0.947
P (ppm)	40.415 ± 6.682	46.647 ± 6.074	0.184
K (ppm)	2.086 ± 0.565	1.694 ± 0.338	0.129
Zn (ppm)	0.227 ± 0.048	0.231 ± 0.332	0.537
Cu (ppm)	0.114 ± 0.026	0.112 ± 0.022	0.460

Identification of Microbial Populations

Aerobic bacteria and actinomycetes counts did not show statistically significant differences ($p \leq 0.05$) (Table 3). Anaerobic, coliform, and fungal counts were statistically different ($p \leq 0.05$) between treatments.

Table 3. Microbiological analysis completed for the Fall-Winter 2018 Cycle

Tt	Aer	Anr	Col	Acn	Fun
	CFU g^{-1} (Log)				
T1	8.69 ±0.93 [a]	8.82 ±0.08 [ab]	8.76 ±0.04 [b]	8.94 ±0.08 [a]	4.00 ±0.00 [a]
T2	9.28 ±0.27 [a]	9.09 ±0.12 [ab]	8.88 ±0.02 [ab]	9.18 ±0.16 [a]	4.08 ±0.15[c]
T3	9.30 ±0.30 [a]	9.07 ±0.41 [a]	8.83 ±0.05 [b]	7.41 ±3.52 [a]	4.46 ±0.53 [c]
T4	9.46 ±0.06 [a]	9.29 ±0.10 [ab]	8.87 ±0.03 [a]	9.05 ±0.07 [a]	4.12 ±0.24 [c]
T5	9.14 ±0.26 [a]	9.20 ±0.13 [b]	9.02 ±0.23 [ab]	9.09 ±0.01 [a]	4.00 ±0.00 [c]
T6	9.24 ±0.24 [a]	9.12 ±0.03 [a]	8.90 ±0.02 [b]	9.12 ±0.02 [a]	4.00 ±0.00 [c]
T7	9.18 ±0.25 [a]	9.27 ±0.09 [ab]	8.98 ±0.07 [ab]	9.00 ±0.09 [a]	4.81 ±0.13 [c]
T8	8.93 ±0.63 [a]	9.09 ±0.08 [a]	8.88 ±0.10 [ab]	9.17 ±0.04 [a]	4.07 ±0.15[b]
T9	9.37 ±0.17 [a]	9.15 ±0.03 [ab]	8.92 ±0.09 [ab]	9.17 ±0.17 [a]	4.07 ±0.15 [c]
Sig.	0.314	0.020	0.034	0.515	0.000

Tt=treatment; Aer=aerobes; Anae=anaerobes; Colif=coliforms; Act=actinomycetes; Fun=fungi. [a-c]Different literals in the same column denote statistically significant difference (Tukey, $p \leq 0.05$).

Differences ($p \leq 0.05$) in anaerobic, coliform, and fungal counts were also observed among the poultry manure treatments (Table 4), which could be explained by the different tillage systems. However, no statistically significant difference ($p > 0.05$) was observed among the control treatments of three tillage systems (Table 5). This suggests that the presence of poultry manure and tillage systems influence the microbial populations present.

Table 4. Microbiological analysis of treatments with organic fertilization

Tt	Aer	Anr	Col	Acn	Fun
	UFC (Log)				
T1	8.68 ±0.93[a]	8.8 2 ±0.08[b]	8.76 ±0.04[c]	8.94 ±0.07[a]	4.00 ±0.02[b]
T4	9.460 ±0.06 [a]	9.28 ±0.09[a]	8.87 ±0.03[b]	9.05 ±0.07[a]	4.12 ±0.24[b]
T7	9.18 ±0.26 [a]	9.27 ±0.09[a]	8.98 ±0.07[a]	9.00 ±0.09[a]	4.81 ±0.13[a]
Sig.	0.187	0.000	0.000	0.204	0.000

Tt=treatment; Aer=aerobes; Anae=anaerobes; Colif=Coliforms; Act=actinomycetes; Fun=fungi. a-cDifferent literals in the same column denote statistically significant difference (Tukey, $p \leq 0.05$).

Table 5. Microbiological analysis by tillage systems

Tt	Aer	Anr	Col	Acn	Fun
	CFU g^{-1} (Log)				
T3	9.30 ±0.30[a]	9.07 ±0.41[a]	8.87 ±0.05[a]	9.11 ±3.52[a]	4.16 ±0.53[a]
T6	9.24 ±0.24[a]	9.12 ±0.03[a]	8.90 ±0.02[a]	9.12 ±0.02[a]	4.00 ±0.00[a]
T9	9.37 ±0.17[a]	9.15 ±0.03[a]	8.92 ±0.09[a]	9.17 ±0.17[a]	4.07±0.15[a]
Sig.	0.767	0.833	0.144	0.177	0.146

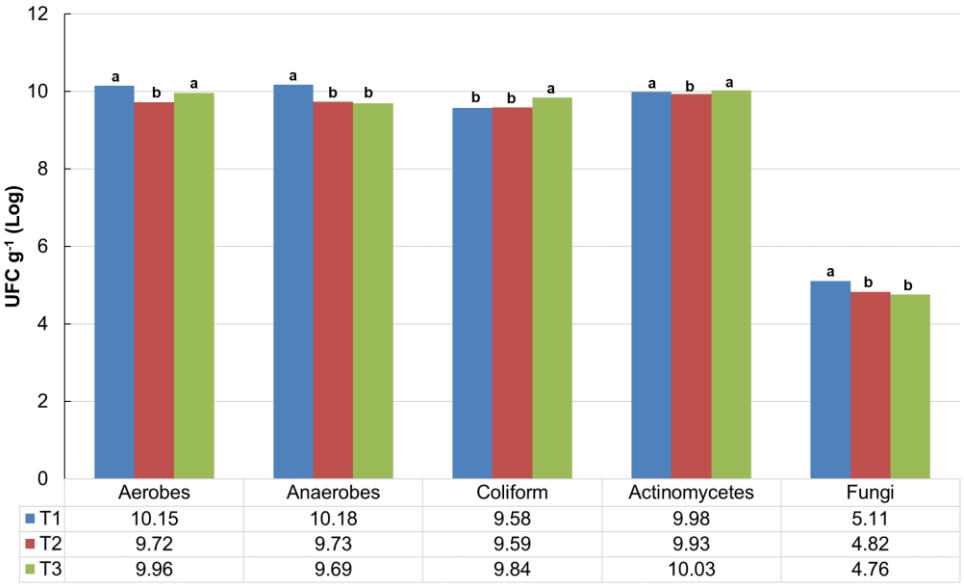
Tt=treatment; Aer=aerobes; Anae=anaerobes; Colif=coliforms; Act=actinomycetes; Fun= fungi. [a] Different literal in the same column denote statistically significant difference (Tukey, $p \leq 0.05$).

On the other hand, the overall results of CFU g^{-1} fungi were similar to those reported by Escoto-González (2014), but the number of CFU g^{-1} of aerobic bacteria reported in the present paper is higher than those mentioned by the same author for the nursery area of the Campo Agrícola Experimental Marín. These differences can be explained as a function

of microclimate variants. This would also explain the differences observed in the poultry manure treatments in the different tillage systems.

Likewise, the results reported here are higher than those reported for saline soils of intensive vegetable production (Crecchio et al., 2004). In comparison with acid soils, a lower number of bacteria has been reported (Álvarez et al., 2004) and similar counts of actinomycetes and fungi to those shown in the present work.

On the other hand, at the end of the second crop cycle, the analysis of the microbial populations of the soil fertilized with different doses of organic matter showed statistically significant differences ($p \leq 0.05$) in the groups of bacteria analyzed (Figure 1). T1(N: 60, P: 65.5, K: 74.4) showed the higher CFU g^{-1} (Log) of aerobic and anaerobic bacteria, actinomycetes, and fungi, while T3 had the highest CFU of coliforms. Population counts increased after the second cycle, which can be explained by incorporating organic matter through chicken manure fertilization (Zou et al., 2022). Although it is known that the efficiency of organic fertilization and the dynamics of soil microbial populations can be affected by salinity, negatively impacting soil health and crop quality (da Costa et al., 2023). It is worth highlighting the importance of actinomycetes in the growth and development of plants as biocontrol agents against phytopathogens, solubilizing phosphate, fixing nitrogen, producing phytohormones, antibiotics, and high-value enzymes, in addition to contributing to the recovery of saline soils (AbdElgawad et al., 2020). Therefore, the results obtained by the different doses of organic matter can counteract, to a certain extent, the negative effects of salinity, explaining the increase in microbial populations from one cycle to another.



	Aerobes	Anaerobes	Coliform	Actinomycetes	Fungi
T1	10.15	10.18	9.58	9.98	5.11
T2	9.72	9.73	9.59	9.93	4.82
T3	9.96	9.69	9.84	10.03	4.76

Figure 1. Initial microbiological analysis Fall-Winter 2019 Cycle. T1 = Fertilización orgánica N: 60, P: 65.5, K: 74.4; T2 = Fertilización orgánica N: 90, P: 98, K: 112; T3 = Fertilización orgánica N: 120, P: 131, K: 149.4.

The CFU contents of aerobic and anaerobic bacteria in agricultural soils may vary depending on the type of fertilization used. In general, it is considered that organic fertilization can contribute to increasing the levels of aerobic and anaerobic bacteria in soils, while inorganic fertilization can contribute to reducing them. In organically fertilized soils, it has been observed that the microbial community composition shifted from aerobic to anaerobic degradation of soil organic carbon, leading to increased carbon accumulation (Li et al., 2021). In contrast, inorganically fertilized soils showed an increase in the relative abundance of Proteobacteria and Firmicutes, which are associated with organic carbon accumulation (Zhang et al., 2014). However, under the conditions of the present study, similar values of aerobic and anaerobic bacteria were observed, which could be due to soil salinity. It is known that the ratio of these bacteria populations can vary in saline agricultural soils fertilized organically or inorganically, depending on the type and origin of the fertilizer used, as well as environmental conditions (Dong et al., 2022).

Concerning the presence of coliform, it is worth mentioning that the isolated colonies did not present the typical dark red color, generally surrounded by a halo of light red or pink precipitation due to bile salts. The above suggests that the total coliform obtained in the count could be from the water, although it should be noted that they are a relatively harmless set of microorganisms (Vahith & Sirajudeen, 2016).

Similar values were observed in all production systems for the actinomycetes count, so the biological implication of this group of bacteria in promoting plant growth and soil fertility is noteworthy (Shivlata & Satyanarayana, 2017).

Finally, concerning the effect of tillage, conservation tillage systems, such as no-till and strip tillage, can positively affect soil microbial diversity and its function in agricultural soils (West et al., 2023). These practices may maintain soil microbial health better than conventional tillage, which alters microbial communities and processes (Mackay et al., 2023), although no statistically significant differences were observed between the populations evaluated and the tillage treatments used. Therefore, to know the populations and their dynamics according to the different production systems, studies at the metagenomic level are required.

4. Conclusions

The results show no statistically significant differences in the physical and chemical parameters between the different depths of the soil. Furthermore, the counts of aerobic bacteria and actinomycetes did not show significant differences. However, statistically significant differences were observed in the counts of anaerobic bacteria, coliforms, and fungi, which are attributable to using organic fertilizers. Likewise, no significant differences were found between the three tillage systems studied. These findings suggest that, although the type of fertilizer can influence the microbiological composition of the soil, the different tillage methods do not significantly impact the parameters evaluated in this study. Regarding the effect of incorporating organic matter, treatment T1 (N: 60, P: 65.5, K: 74.4) showed the higher CFU values of the studied populations, except for the coliform count. These results are important to understand soil dynamics better and inform agricultural and soil management practices. In addition, they highlight the importance of proper use of organic fertilizers and the need for further research to determine how they affect long-term soil health and quality.

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6. Conflicts of Interest

The authors declare no conflict of interest.

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Letter to the Editor

The Affective Domain in the Teaching-Learning Process of Mathematics

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Abstract: This article presents a general overview of a problem that arises in our daily teaching work, the Teaching-Learning Process of the exact sciences, especially Mathematics. In the first section, a historical context is presented about the origin of the area of Didactics of Mathematics called Affective Domain, and then in the second section we introduce ourselves to the Socio-Epistemological Theory of Mathematics, analyzing the feelings and states of mind on which The theory of the Affective Domain is founded as well as its relationship with Mathematics and in the third section, we finally propose some research problems that are still open that can be addressed during our presentations in the classroom and deserve to be the subject of future research..

Keywords: Didactics of Mathematics; Teaching-Learning Process; Affective Domain; Constructs.

El ámbito afectivo en el proceso de enseñanza-aprendizaje de las matemáticas

Resumen: En este artículo se presenta una visión general de un problema que se plantea en nuestra labor docente diaria, el Proceso de Enseñanza-Aprendizaje de las ciencias exactas, especialmente de las Matemáticas. En el primer apartado se presenta un contexto histórico sobre el origen del área de Didáctica de la Matemática denominada Dominio Afectivo, luego en el segundo apartado nos introducimos a la Teoría Socio-Epistemológica de la Matemática, analizando los sentimientos y estados de ánimo en los que se fundamenta La teoría del Dominio Afectivo así como su relación con la Matemática y en el tercer apartado, finalmente se proponen algunos problemas de investigación aún abiertos que pueden ser abordados durante nuestras presentaciones en el aula y merecen ser objeto de futuras investigaciones.

Palabras clave: Didáctica de las Matemáticas; Proceso de Enseñanza-Aprendizaje; Dominio Afectivo; Constructos.

1. Introduction

Origin of the Affective Domain

Since Greek times, attempts have been made to explain people's behavior. Empedocles (495 - 425 BC) begins to formulate a theory, which Hippocrates (460 - 336 BC) later completes, based on bodily humours. Empedocles believed that the human body was made up of four elements. He related these four elements to the four bodily humors. Changes in these moods were considered the cause of certain mood states and character predispositions (Carrillo García, 2006).

Empedocles established the foundations of what would later be known as the Theory of the Four Temperaments, which would be expanded during the pre-Renaissance period by Robert Burton with his work The Anatomy of Melancholy. He and his contemporaries maintained that the bodily humor and, consequently, people's emotional states were susceptible to external influences such as age, diet, and passions. For a long time, psychology was dedicated to the observation, measurement, classification, and therapy of human behavior, but in isolation from the physiological point of view and, in turn, from the neurological one. Despite this, there have been significant contributions to psychology with works such as that of the neurologist Antonio Damasio (1994), who, based on his research, proposes the idea that reason is not so pure, that emotions and feelings are not They are intruders in the bastion of reason: they can be found entangled in its networks (Carrillo García, 2006). For this reason, today, it is established that the historical origin of the Affective Domain Theory has its origin in psychology, which focuses on cases of anxiety and how these affects academic performance. However, it was not until the late 1980s that Mcleod (1989) referred to the Affective Domain as "an exten-

sive range of feelings and moods (mood states), which are generally considered to be something other than pure cognition, and include as specific components of this domain, attitudes, beliefs, and emotions," this definition being the most accepted and still valid to this day even though there have been new proposals for this definition to cite some authors: according to Bloom et al. (1977), appreciations, preferences, beliefs, emotions, attitudes, values and feelings, and according to Lafortune and Saint-Pierre (cited in Gómez Chacón, 2000) attitudes, values, moral and ethical behavior, emotions, feelings, attributions, motivation and personal development and social (Martínez Padrón, 2005). As is known, Mathematics has been present in almost all the tasks of humanity, and, according to Galileo (cited in Barrow, 1997), it is the language in which the book of nature seems to be written. Perhaps that is why finding any phenomenon capable of escaping its descriptive power is complex. As an area of study, Mathematics has been considered the formal foundation of most disciplines, being present in many of the curricular structures that outline the academic training of children, adolescents, and adults (Martínez Padrón, 2005).

This combination of the omnipresence of Mathematics in all areas of knowledge, in addition to its complexity and abstraction in conjunction with the feelings and emotions of human beings, has caused it to be stereotyped throughout history (only studied by "intelligent" people."), causing animosity in learning them by most people, including students.

Socio-Epistemological Theory of Mathematics

There are different definitions of Mathematics Didactics, among which we can mention:

• Discipline that studies different aspects that intervene in the Teaching-Learning Process. (Royal Spanish Academy, 2014).

• Scientific discipline whose object of study is the relationship between knowledge, teaching, and learning of the contents of mathematics (Baldor, 2004).

The Didactics of Mathematics, as such, then focuses on purely cognitive issues, to the point where its research has focused exclusively on Teaching - Teacher - Student Learning Processes. Suppose at this moment, we begin to analyze issues that go beyond the cognitive aspects. In that case, we must introduce ourselves to the epistemological factors, the Socio-Epistemological Theory of Mathematics. Cantoral (2013, 2014) defines this theory as the part of epistemology that deals with the study of didactic phenomena linked to mathematical knowledge, assuming the legitimacy of all forms of knowledge, whether popular, technical, or cultured, since he considers that they, in Together, they constitute human wisdom. From this context, the Socio-Epistemological Theory of Mathematics addresses the metacognition of Mathematics from two guidelines:

• Systematizes sociocultural positions of education.

• Theoretical lines that allow us to understand the influences that affect, and attitudes have on mathematical learning.

Therefore, according to Cantoral (2013):

• Mathematics is a social construction; analyzing the history of Mathematics, each civilization proposed its own Mathematics according to its needs, feelings, and, most importantly, its beliefs.

• When considered socially, elements of culture intervene, for example, the mathematical writings in cuneiform writing of the Babylonian Civilization or the Egyptian hieroglyphs found on papyrus papers. Both are examples of the elements specific to each culture: the clay from the Tigris and Euphrates rivers in Mesopotamia and the plants from which papyrus paper was obtained on the banks of the Nile River.

• As we mentioned in the previous section, the history of humanity tells us, from a psychological point of view, that affects dominate the sociocultural characteristics of human beings and, therefore, their understanding and construction of the Teaching-Learning Processes. of Mathematics.

Going deeper into the understanding and construction of the Teaching - Learning Processes of Mathematics, there is an inverse correlation between the age of human beings and the level of acceptance of Mathematics; again, we quote

Mcleod (1992), who establishes that students express a negative relationship as the years go by. In other words, as we advance from kindergarten to higher education, most human beings experience feelings of denial, stress, anxiety, and helplessness when studying subjects related to Mathematics, a series of emotions.

Gómez Chacón (2000) tells us that some of these effects are firmly rooted in the students, so they are not easily displaceable from the Teaching-Learning Processes. In this sense, effects are not only inherent to students but also to teachers; therefore, when, as teachers, we identify cases of blockage in Mathematics or any other exact science, it is advisable to channel the student to an appropriate instance, such as a specialist in the Affective Domain or psychological care. The Affective Domain Theory of Mathematics is based on three basic constructs (Mcleod, 1992), although currently, even more constructs that have not been fully defined have been proposed, such as resilience. Each of these three constructs is defined below (Marban Prieto, 2016):

• Emotions. Rapid changes of feelings and vigorous intensity. This leads students to a fear of Mathematics or a satisfaction in solving a problem.

• Beliefs. Cognitive domain about Mathematics and oneself about the teaching of Mathematics and the social context. Here, questions arise: Do I could solve a problem? Is Mathematics useful?

• Attitudes. Evaluative predisposition (positive or negative) that determines personal intentions. For example, a student may say, "I like Mathematics," or a teacher: "I like teaching Mathematics."

Leder and Grootenboer (2005) establish the following for these constructs:

• That their descriptors can be exchanged.

• Each of them is a hypothetical constructor.

• They are acquired through the socialization process.

• They are transferable.

• They play a triggering role in the acquisition of knowledge.

Finally, regarding attitudes, these are not defined in educational programs (curriculum frameworks) as defined by Marban Prieto (2016), but rather as a series of desirable qualities of any student considering only purely academic aspects, underestimating the Affective Domain. (Casis, 2021).

Areas of opportunity in the Affective Domain in Mathematics.

The European Society for Research in Educational Mathematics (CERME) established at the CERME 12 Congress the following lines of research that must be addressed in the classroom (Gargonza, 2021):

• Definitions and inclusion of affective constructs and their relationship, particularly resilience and self-efficacy.

• Development of instruments for measuring constructs such as rubrics, questionnaires, or scales, to name a few examples.

• Inquire into the role of emotions, attitudes, values, and beliefs in Teaching-Learning Processes when considering problem-solving, problem creation, theorem demonstration, creation of teaching materials, or the inclusion of information technologies.

It is essential to mention that the return to face-to-face modalities with students is very favorable for developing these investigations, which, practically with online models, is much more challenging.

2. Conclusions

In conclusion, among the factors that influence the appearance of affection towards Mathematics, the following can be mentioned:

- The abstract and impersonal character of mathematics.
- The attitude of teachers towards students, especially those with learning difficulties.
- The methodology used in teaching Mathematics subjects.
- The family and social environment influence the stereotypical image of Mathematics.
- The same society is responsible for promoting and disseminating that Mathematics is difficult, complicated, and intended for "intelligent" people.

Without a doubt, the study of the Affective Domain not only of Mathematics but of any area of scientific knowledge is an area of opportunity that should not be omitted since, if treated adequately by students and teachers, it would potentialize better academic performance, a lower index of failure, lower student dropout and an educational program with even greater recognition. Finally, it is important to provide follow-up in our teaching practice for those students who have learning problems and channel them to the corresponding instance. It is also important that teachers feel comfortable with the class they teach (that is, a pleasure in teaching it and a mastery of it) since, if this is not met, they can transmit stress to the group and, therefore, a drop in the academic performance of the students.

3. Conflicts of Interest

The authors declare no conflict of interest.

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