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Journal of Experimental Systems

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Presentation of the Content

As a first article we present *Reassessment of wind resources using air density adjusted to specific atmospheric conditions in the Mapimí Biosphere Reserve*, by Meraz-Becerra, Fernando, Juárez-Ortiz, Cinthia Andrea and Martínez-Marroquín, Juan, based at the Universidad Tecnológica de Durango, as next article we present *Aquaponic culture with application of biostimulants in floating root recirculation systems*, by Chávez-Rangel Moisés, Arellano-Rodríguez, Luis Javier, Neri-Luna, Cecilia and Rodríguez William David with adscription in UdeG, University of Guadalajara, as next article we present *Assessment of lung function using spirometry in brick factory workers: impact of air pollution in Tlaquepaque, Jalisco [2023-2024]*, by Vargas-Chi, Melisa del Carmen, Figueroa-Montaño, Arturo, Orozco-Medina, Martha Georgina and Martínez-Abarca, Javier Omar, with secondment to the Universidad de Guadalajara, as next article we present *Complex thinking to transcomplexity: artificial intelligence teachers perceptions in neurodidactic activities design with educational neuroengineering* by Valladares-Ríos, Luis, Duarte-Reaño, Jhonny and Lino-Gamiño, Juan Alfredo, with secondment to the ISENCO and Universidad de Colima, as next article we present *Expanded polystyrene and whey goat as substrates for Tenebrio molitor breeding*, by Alonso-Segura, Diana, Hernández-Ballejo, Pedro D., González-Huerta, Jenifer and Mandujano-González, Virginia, with secondment to the Universidad Tecnológica de Corregidora, Academia de Ingeniería en Biotecnología, as the nextt article we present, *Implementation of a Reforestation Restorative Plan in Jilotzingo, Estado de Mexico*, by Rangel-Ruiz Karelia Liliana, Granados Olvera Jorge Alberto, Cureño-Jiménez, José Domingo Agustín and Calvillo-Beltrán, Sofía Valentin, with secondment to the Universidad Politécnica de Cuautitlán Izcalli and Subdirección de Desarrollo Regional de la Secretaria del Bienestar del Gobierno del Estado de México, as the last article we present, *Synthesis of Chitosan from Shrimp Exoskeleton an its Characterization by SEM and DRX*, by Fuentes-Romero, María Teresa, Bermúdez, Jesús Nicolás, Medina-Mendoza, Manuel and Maldonado-Mondragón, Erick Antonio, with secondment to the Universidad Tecnológica Fidel Velázquez.

Content

Journal of Experimental Systems 12[31]1-11: e11231111
Article

Reassessment of wind resources using air density adjusted to specific atmospheric conditions in the Mapimí Biosphere Reserve

Reevaluación del recurso eólico mediante densidad del aire ajustada a condiciones atmosféricas específicas en la reserva de la biósfera de Mapimí

Meraz-Becerra, Fernando * [a], Juárez-Ortiz, Cinthia Andrea [b] and Martínez-Marroquín, Juan [c]

[a] Universidad Tecnológica de Durango • NOF-5796-2025 • 0009-0006-1773-0036• 740821
[b] Universidad Tecnológica de Durango • NQF-5994-2025 • 0009-0007-8720-3247• 2136872
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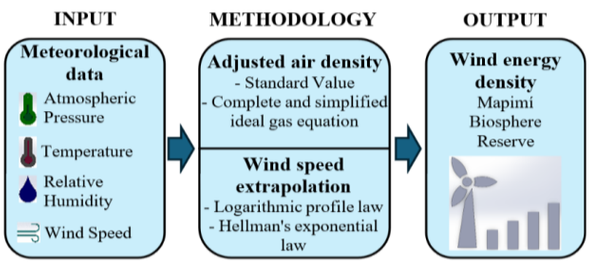
* ✉ [merazbecerra@gmail.com]

Abstract

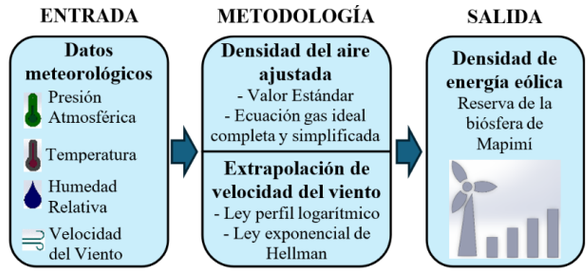
Accurate wind resource assessment requires proper characterization of local atmospheric conditions. Previous studies conducted in the Mapimí Biosphere Reserve used a standard air density value [1.225 kg/m³], which corresponds to dry air at sea level, 15 °C, and 101.325 kPa. However, the Automatic Weather Station at Ejido La Flor, which provides the data for this study, is located at an altitude where average pressure is 709 hPa, annual mean temperature is 22 °C, and relative humidity is approximately 44 %, making the use of the standard value inadequate. This article evaluates the impact of adjusting air density based on actual atmospheric conditions using both the simplified and full formulations of the CIPM-2007 model, with data from the year 2024. Using the standard density value, the average wind power density was estimated at 36.5462 Wh/m² at 10 meters height. In contrast, the simplified and full CIPM-2007 equations yielded values of 28.2551 Wh/m² [0.89861 kg/m³] and 28.0325 Wh/m² [0.89217 kg/m³], respectively. The results show that local parameters are decisive in wind resource estimation and in the feasibility of wind turbines in the area of interest.



Wind resource assessment, Air density, Mapimí Biosphere Reserve

Resumen

La estimación precisa del recurso eólico requiere una adecuada caracterización de las condiciones atmosféricas locales. En estudios previos en la Reserva de la Biósfera de Mapimí se utilizó un valor estándar para la densidad del aire [1.225 kg/m³], correspondiente a condiciones de aire seco a nivel del mar y 15 °C. Sin embargo, la Estación Meteorológica del ejido La Flor, fuente de datos del presente estudio, se localiza en una altitud con presión promedio de 709 hPa, temperatura media anual de 22 °C y humedad relativa del 44 %, lo que limita la validez del valor estándar. Este artículo evalúa el impacto de ajustar la densidad del aire a partir de condiciones atmosféricas reales mediante dos formulaciones del CIPM-2007, utilizando datos del año 2024. Con la densidad estándar se estimó una densidad de energía promedio en el viento de 36.5462 Wh/m² a 10 metros de altura, mientras que los valores obtenidos con las fórmulas simplificada y completa fueron de 28.2551 Wh/m² [0.89861 kg/m³] y 28.0325 Wh/m² [0.89217 kg/m³], respectivamente. Los resultados muestran que los parámetros locales son determinantes en la estimación del recurso eólico y la viabilidad de aerogeneradores en la zona de interés.



Estimación del recurso eólico, Densidad del aire, Reserva de la Biósfera de Mapimí.

Area: Advocacy and attention to national problems

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I. Introduction

The Mapimí Biosphere Reserve, located in the Chihuahuan Desert, is a priority region for ecological conservation and sustainable development. Its remote location and irregular terrain make it difficult to connect to the national electricity grid, which poses an energy challenge for the environmental monitoring and surveillance activities carried out by the National Commission for Protected Natural Areas [CONANP].

A previous study analysed the technical feasibility of installing low-power wind turbines by estimating local wind resources based on the extrapolation of wind speed at different heights and the calculation of average energy density using two exact methods. The results indicated that, from a height of 30 metres, the installation of these systems is technically feasible in the region [Meraz-Becerra & Carrillo-Martínez, 2024].

However, this study assumed a constant value for air density of 1,225 kg/m³, corresponding to standard conditions: dry air, sea level [101,325 kPa] and temperature of 288.15 K [15 °C] [Patel, 2006]. This approximation is inadequate for regions with different atmospheric conditions, such as the Mapimí Reserve, where the average pressure is 709 hPa, the average annual temperature is 22 °C and the relative humidity is 44%, which significantly affects the calculation of wind energy density.

The aim of this study is to reassess the wind resource in the region by comparing three methods for calculating air density: [1] the standard value mentioned above, [2] the formula for determining air density based on the ideal gas equation for dry air, and [3] the complete equation proposed by the International Committee for Weights and Measures [Picard et al., 2008], which incorporates pressure, temperature and relative humidity to obtain a more accurate estimate of humid air.

In addition, the exact methods previously applied to estimate the average wind energy density will be used again: the integration of the instantaneous power curve and the method based on the average cubic velocity.

Likewise, the results will be extrapolated to different heights using Hellmann's models, the logarithmic profile and the Monin–Obukhov method, using meteorological records from 2024 from the Automatic Meteorological Station [EMA] of the La Flor ejido [CONAGUA, 2024].

The acquisition, processing and visualisation of the information were carried out using a Graphical User Interface [GUI], the first version of which was developed in the GUIDE environment of the MATLAB software. This tool allows for the automation of analysis, facilitates future methodological extensions and enables the analysis of wind resources in any region of the country and the world.

This approach seeks to improve the accuracy of wind resource characterisation in regions of medium altitude, where the influence of local atmospheric conditions on air density can significantly affect the technical viability of decentralised wind power generation projects [Murthy & Rahi, 2017; Patel, 2006; Holton, 2004].

The rest of the article is organised as follows: Section II provides a general description of the methodologies used to calculate average energy density [AED], extrapolate wind speed at different heights, and estimate air density; Section III presents the analysis of wind resources in the Mapimí Biosphere Reserve, with an emphasis on the impact of each approach to calculating air density on the estimation of AED. Finally, Section IV presents the conclusions.

II. Methodologies used for wind resource analysis in a region

Wind energy potential can be estimated using empirical or numerical methods. Empirical methods allow the average energy density [AED] to be calculated from wind speed records taken every 10 minutes for at least one year, which makes it possible to capture daily and seasonal variations in the resource. For a more accurate analysis, it is necessary to calculate air density considering actual atmospheric conditions, which involves the simultaneous use of temperature, atmospheric pressure and relative humidity data, recorded with the same frequency and duration.

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This information, complemented by wind direction analysis, allows for a more accurate determination of the wind potential of the area. Numerical methods, on the other hand, use probability density functions [PDFs], such as the Weibull distribution, to model wind behaviour and estimate its energy potential, using only the average annual wind speed in some methodologies [Meraz-Becerra & Carrillo-Martínez, 2023].

2.1. Wind Energy Density

The instantaneous power of the wind represents the energy that a turbine can extract at a given moment, based on the difference in air speed before and after passing through the rotor.

This magnitude is essential for determining the energy in the wind and optimising the design of wind turbines. The kinetic energy [Ec] contained in a mass of air m moving at speed V is calculated as [Wu, Lang, & Zargari, 2011]:

$$Ec = \frac{1}{2}\ m\,V^2 \qquad [1]$$

The power available in a free air stream is the flow of kinetic energy per unit time through the cross-sectional area of the rotor blade of the wind turbine [Patel, 2006]:

$$P = \frac{EC}{t} = \frac{1}{2}\frac{m}{t}\ v^2 = \frac{1}{2}Mv^2 = \frac{1}{2}\rho\ A\ v^3 \qquad [2]$$

where P is the instantaneous mechanical power of the moving wind [w], M is the mass flow rate [$\frac{Kg}{s}$], ρ is the density of air [$\frac{Kg}{m^3}$], $A = \pi r^2$ is the area swept by rotor blades [m^2], t is the time [s] and v is the air speed [$\frac{m}{s}$].

For general wind resource analyses, where a specific swept area has not yet been defined, it is common to calculate the instantaneous energy density [IED] of the wind, expressed in W/m², by means of the following equation:

$$DEI = \frac{1}{2}\rho\ v^3 \qquad [3]$$

2.1.1. Average Energy Density Obtained by Integrating the Instantaneous Energy Density Curve [DEM_IEI]

1. The instantaneous energy density in the wind [DEIi] is calculated from each of the 52560 wind speeds recorded by the EMA every 10 minutes for at least one year in the region of interest, as expressed in equation [4], where v_i is the i_{th} wind speed recorded in the region.

$$DEI_i = \frac{1}{2}\ \rho\ v_i^3 \qquad [4]$$

2. The annual instantaneous power density curve for the region of interest is generated and the instantaneous power curve is numerically integrated with respect to time to obtain the total annual wind energy density. [DET]:

$$DET = 10\left(\frac{1}{2}\ DEI_1 + \sum_{i=2}^{n-1} DEI_i + \frac{1}{2}\ DEI_n\right)n \qquad [5]$$

Where n is the total number of data and 10 is a factor used to derive the energy density in $[W * min]/m^2$, yThe wind speed recordings are usually made every 10 min by the EMAs.

3. Finally, the TED is divided by the corresponding factor to obtain the energy in the desired period. Equation 6 determines the annual energy density [AED] in Wh/m^2.

4.

$$DEA = DET\left[\frac{W*min}{m^2}\right]\left[\frac{1\ hora}{60\ min}\right] = \frac{DET}{60}\left[\frac{w*h}{m^2}\right] \qquad [6]$$

2.1.3. Average Energy Density Using Average Cubic Velocity [DEM_VRMC].

The monthly wind speed varies around ±30% to ±35% above the average wind speed at a typical location during the year [Patel, 2006]. Therefore, the wind speed used to determine the energy density in [3] should be [Pishgar-Komleh et al., 2014]:

$$V_{rmc} = \left(\frac{1}{n}\sum_{i=1}^{n} vi^3\right)^{\frac{1}{3}} \qquad [7]$$

Finally, the mean energy density per hour in the wind [DEMH] will be obtained in Wh/m^2 [Patel, 2006]:

$$DEMH = \frac{1}{2}\rho\ {V_{rmc}}^3 \qquad [8]$$

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2.2. Methods for vertical extrapolation of wind speed.

Accurate estimation of wind speed at different heights is crucial for assessing available wind resources, as anemometers installed at weather stations are usually located at lower heights than those at which wind turbines operate. For this reason, various methods have been proposed for vertical extrapolation, based on physical models or empirical adjustments. Three methodologies are used in this research: the Monin–Obukhov Method [MMO], Hellmann's Exponential Law [LEH] and the Logarithmic Wind Profile Law [PEL], widely recognised in scientific literature for their usefulness and adaptability to different environments [Bañuelos-Ruedas et al., 2010; Allouhi et al., 2017; Lázár et al., 2024].

2.2.1. Monin–Obukhov Method [MMO]

The MMO considers atmospheric stability conditions through the L parameter, known as the Monin–Obukhov length. It is particularly useful for vertical profiles in non-neutral conditions and with high-frequency records, but should not be applied to monthly or annual averages [Bañuelos-Ruedas et al., 2010].

The extrapolated velocity V[h] is obtained by:

$$V(h) = \frac{Vf}{K}\left[\ln\left(\frac{h}{Zo}\right) - \xi\left(\frac{h}{L}\right)\right] \quad [9]$$

Where $V(h)$ is the speed extrapolated to height , is the friction speed [equation 10], is the Von Karman constant, generally 0.4 [Bañuelos-Ruedas et al., 2010], is the roughness length of the surface, and $\xi[h/L]$ the stability correction function, which depends on the degree of thermal stratification of the atmosphere.

$$Vf = \frac{Vo}{K*\ln\left(\frac{h}{Zo}\right)} \quad [10]$$

For slightly unstable conditions, a common way to approximate $\xi[h/L]$ is:

$$\xi\left(\frac{h}{L}\right) = 2\ln\left(\frac{1+X}{2}\right) + \ln\left(\frac{1+X^2}{2}\right) - 2\tan^{-1}(X) + \frac{\pi}{2} \quad [11]$$

with:

$$X = \left(1 - 16 * \frac{h}{L}\right)^{\frac{1}{4}} \quad [12]$$

Due to the complexity of this function, for values close to 0 it is usually simplified as [Stull, 1988]:

$$\xi\left(\frac{h}{L}\right) = -2 * \frac{h}{L} \quad [13]$$

The Monin-Obukhov length can be calculated as follows:

$$L = -\frac{u_*^3 * \bar{T}}{K*g*\overline{w'\theta'}} \quad [14]$$

whereu_*^3 is the frictional velocity, T¯is the mean air temperature, g is the gravitational acceleration, and $\overline{w'\theta'}$ is the turbulent flow of sensible heat. Given that the L estimate requires specialised sensors that are not usually available at conventional weather stations, various studies have opted to assume neutral conditions in order to apply the logarithmic law directly.

This simplification allows wind speed to be extrapolated with acceptable errors up to 100 m in height, especially when extensive data series are available and there are no extreme atmospheric conditions [Breedt et al., 2018; Lange et al., 2001; Pelliccioni et al., 2021]. In this study, this assumption is methodologically appropriate due to the limitations of the measurement equipment used.

2.2.2. Hellmann's exponential law [HEL]

HEL is one of the most widely used formulas for extrapolating wind speed due to its simplicity [González et al., 2025]. The model is expressed as:

$$V[h] = Vh0\left(\frac{h}{h0}\right)^{\alpha} \quad [15]$$

Where $V[h]$ is the extrapolated velocity at altitude h, $Vh0$ is the velocity measured at height and is the coefficient of friction. For neutral conditions, α=1/7 is commonly adopted, which allows for acceptable extrapolation [Kamau et al., 2010]. However, there are tables with recommended values of α depending on the type of surface [Patel, 2006; Masters, 2013]. The higher the value of α, the more the wind speed will increase with height [González et al., 2025].

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Box 1

Table 1

Coefficient of friction of various terrains

Type of terrain	Coefficient of friction α
Lakes, oceans and smooth and/or hard terrain.	0.10
Grass at foot level on flat ground.	0.15
Tall crops, hedges and shrubs.	0.20
Wooded landscape with many trees.	0.25
Small town with trees and shrubs.	0.30
Urban area with tall buildings.	0.40

2.2.3 Logarithmic Wind Profile Law [LPL]

The LPL is a model based on the boundary layer theory of fluid mechanics, with strong aerodynamic support. Although it requires knowledge of the terrain roughness length Z0, it can provide more realistic estimates in flat and homogeneous terrain [Okorie, 2017]. Its formulation is:

$$V(h) = Vh0 \left[\frac{\ln\left(\frac{h}{Zo}\right)}{\ln\left(\frac{ho}{Zo}\right)}\right] \quad [16]$$

Where Zo is the roughness length coefficient in m and dependent on terrain type, spacing and roughness factor height [Bañuelos et al., 2010; Masters, 2013; Okorie, 2017]. Table 2 shows Zo values for regions with common characteristics. [Wieringa, 1992].

Box 2

Table 2

Length coefficient of various terrains.

Clase	Description	Zo
0	Water surface.	0.0002
0.5	Completely open land with a smooth surface [concrete runways of airports and cut grass].	0.0024
1	Open agricultural area without fences and hedges, and with widely scattered buildings. Only gently rounded hills.	0.03
1.5	Agricultural land with a few houses and hedges 8 m high at a distance of approximately 1250 m.	0.055
2	Agricultural land with a few houses and hedges 8 m high at a distance of approximately 500 m.	0.1
2.5	Agricultural land with many houses, bushes and plants, or 8 m high hedgerows at a distance of approximately 250 m.	0.2
3	Villages, small towns or agricultural land with many or high hedgerows, forests and very hilly terrain.	0.4
3.5	Large cities with tall buildings.	0.8
4	Very large urban centres with tall buildings and skyscrapers.	1.6

2.3. Air Density Estimation.

Air density is a fundamental parameter in wind resource assessment, as it directly influences the calculation of the energy contained in the wind. Its value depends on atmospheric conditions such as pressure, temperature and relative humidity.

There are three approaches used for its estimation, each with a different level of accuracy and complexity.

2.3.1. Standard value of air density.

Multiple studies adopt a standard value of 1,225 kg/m³ for air density, corresponding to ideal conditions: dry air [0 % relative humidity], atmospheric pressure of 101,325 kPa and temperature of 288.15 K [15 °C], at sea level [IEC, 2005; Burton et al., 2011]. This value facilitates comparisons between regions and allows preliminary estimates when detailed local atmospheric data are not available.

2.3.2. Formula based on the ideal gas equation for dry air.

To obtain an estimate of the density of air under actual local conditions, a simplified form of the equation of state of ideal gases applied to dry air is used:

$$\rho = \frac{p}{R*T} \quad [17]$$

Where ρ is the density of the air in kg/m^3, p es is the atmospheric pressure in Pa, T is the absolute temperature in K and R is the specific constant of dry air. $\left(287.05\frac{J}{kg\ K}\right)$. This formula allows quick and reliable approximations if reliable pressure and temperature data are available, ignoring the contribution of water vapour. [Stull, 1988; Holton & Hakim, 2013].

2.3.3. Equation proposed by CIPM-2007.

For conditions where high accuracy is sought, it is recommended to use the full equation of the International Committee of Weights and Measures [Picard et al., 2008]. This equation includes the contribution of air humidity through the mole fraction of water vapour and is expressed as:

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$$\rho = \frac{p*M_a}{Z*R*T}\left(1 - x_v\left(1 - \frac{M_v}{M_a}\right)\right) \quad [18]$$

Where M_a is the molar mass of dry air and M_v is the molar mass of water vapour. Z is the compressibility factor expressed as:

$$Z = 1 - \frac{p}{T} * [a_0 + a_1 t + a_2 t^2 + (b_0 + b_1 t)x_v + (c_0 + c_1 t)x_v^2] + \frac{p^2}{T^2}(d + e x_v^2) \quad [19]$$

x_v is the mole fraction of water vapour:

$$x_v = h_r * f(p,t) * \frac{p_{sv}(T)}{p} \quad [20]$$

In equation 20, h_r is the relative humidity, f is the improvement factor [equation 21] and $P_{sv}[T]$ is the vapour pressure at saturation [Equation 22].

$$f = \alpha + \beta p + \gamma t^2 \quad [21]$$

$$P_{sv}[T] = 1\ Pa * e^{\left(AT + BT + C + \frac{D}{T}\right)} \quad [22]$$

Table 3 summarizes the other parameters necessary for the implementation of equations 19, 20, 21 and 22. [Picard et al., 2008].

Box 3

Table 3

Coefficients for calculating air density.

Symbol	Value	Unit
Coefficients For Saturation Vapour Pressure [P_{sv}]		
A	0.000012378847	$1/K^2$
B	-0.019121316	$1/K$
C	33.93711047	
D	-6343.1645	K
Coefficients for the Improvement Factor [f]		
α	1.00062	
β	0.0000000314	$1/Pa$
γ	0.00000056	$1/K^2$
Compressibility Factor Coefficients [Z]		
a_0	0.00000158123	K/Pa
a_1	-0.000000029331	$1/Pa$
a_2	0.00000000011043	$1/[K*Pa]$
b_0	0.000005707	K/Pa
b_1	-0.00000002051	$1/Pa$
c_0	0.00019898	K/Pa
c_1	-0.000002376	$1/Pa$
d	0.0000000000183	K^2/Pa^2
e	-0.00000000765	K^2/Pa^2
Coefficients for Air Density [ρ]		
R	8.314472	$J/[mol*K]$
M_a	0.02896546	Kg/mol
M_v	0.01801528	Kg/mol

Picard et al., 2008; Peña & Becerra, 2010

The use of this formula is particularly relevant in regions with considerable variations in relative humidity and temperature.

III. Results

The study was carried out using data recorded every 10 minutes during the year 2024 by the EMA of the ejido La Flor in charge of CONAGUA located outside the Mapimí Biosphere Reserve [Latitude: 26.55, Longitude: -103.99].

Table 4 presents the annual average air density values calculated with three different approaches: the standard value [1.225 kg/m³], the simplified formula based on the ideal gas equation for dry air, and the full equation proposed by the CIPM-2007.

The results show that, under the specific atmospheric conditions of the region, the methods incorporating pressure, temperature and relative humidity provide considerably lower values of air density compared to the standard value.

Box 4

Table 4

Annual average air density calculated by each methodology.

Method	Value [kg/m^3]
Ecuación propuesta por el CIPM-2007	0.89369
Ecuación del gas ideal para aire seco	0.89861
Valor estándar [$T = 15°C$ y $P = 101325\ kPa$]	1.225

Tables 5, 6 and 7 show the mean energy density [DEM] at different heights, from 10 m to 200 m, using two calculation methods: instantaneous energy integration [DEM_IEI] and mean cubic velocity [DEM_VRMC]. In each case, the three wind speed extrapolation methodologies were applied: the Monin-Obukhov method [MMO], Hellmann's Exponential Law [HLE] and the Logarithmic Wind Profile [LEP].

The difference between the tables lies in the method used to calculate the air density: Table 5 uses the standard value, Table 6 uses the ideal gas equation for dry air, and Table 7 applies the full CIPM-2007 formula.

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Box 5

Table 5

DEM calculated from the standard value of air density.

H [m]	Average Hourly Energy Density $\left[\frac{Wh}{m^2}\right]$					
	DEM_IEI			DEM_VRMC		
	MMO	LEH	LPL	MMO	LEH	LPL
10	36.57	36.57	36.57	36.98	36.98	36.98
20	55.69	55.43	55.69	56.32	56.05	56.32
30	69.48	70.70	69.48	70.26	71.49	70.26
40	80.54	84.02	80.54	81.44	84.96	81.44
50	89.87	96.05	89.87	90.88	97.13	90.88
60	98.02	107.16	98.02	99.12	108.36	99.12
70	105.28	117.54	105.28	106.46	118.86	106.46
80	111.84	127.34	111.84	113.10	128.78	113.10
90	117.86	136.67	117.86	119.19	138.21	119.19
100	123.42	145.59	123.42	124.81	147.23	124.81
110	128.60	154.16	128.60	130.05	155.89	130.05
120	133.46	162.42	133.46	134.96	164.25	134.96
130	138.03	170.41	138.03	139.58	172.33	139.58
140	142.35	178.16	142.35	143.95	180.16	143.95
150	146.46	185.69	146.46	148.11	187.78	148.11
160	150.37	193.02	150.37	152.06	195.19	152.06
170	154.11	200.17	154.11	155.84	202.42	155.84
180	157.69	207.15	157.69	159.46	209.48	159.46
190	161.12	213.98	161.12	162.94	216.39	162.94
200	164.43	220.67	164.43	166.28	223.15	166.28

Box 6

Table 6

DEM calculated from ideal gas equation for dry air.

H [m]	Average Hourly Energy Density $\left[\frac{Wh}{m^2}\right]$					
	DEM_IEI			DEM_VRMC		
	MMO	LEH	LPL	MMO	LEH	LPL
10	28.27	28.27	28.27	27.13	27.13	27.13
20	43.06	42.86	43.06	41.31	41.12	41.31
30	53.72	54.66	53.72	51.54	52.44	51.54
40	62.27	64.96	62.27	59.74	62.32	59.74
50	69.49	74.26	69.49	66.67	71.25	66.67
60	75.78	82.85	75.78	72.71	79.49	72.71
70	81.39	90.88	81.39	78.10	87.19	78.10
80	86.47	98.46	86.47	82.97	94.47	82.97
90	91.13	105.67	91.13	87.43	101.38	87.43
100	95.43	112.56	95.43	91.56	108.00	91.56
110	99.43	119.19	99.43	95.40	114.36	95.40
120	103.18	125.57	103.18	99.00	120.48	99.00
130	106.72	131.75	106.72	102.39	126.41	102.39
140	110.06	137.74	110.06	105.60	132.16	105.60
150	113.23	143.56	113.23	108.65	137.75	108.65
160	116.26	149.23	116.26	111.55	143.18	111.55
170	119.15	154.76	119.15	114.32	148.49	114.32
180	121.92	160.16	121.92	116.98	153.67	116.98
190	124.57	165.44	124.57	119.53	158.74	119.53
200	127.13	170.61	127.13	121.98	163.70	121.98

Box 7

Table 7

DEM calculated from the equation proposed by the CIPM-2007

H [m]	Average Hourly Energy Density $\left[\frac{Wh}{m^2}\right]$					
	DEM_IEI			DEM_VRMC		
	MMO	LEH	LPL	MMO	LEH	LPL
10	28.13	28.13	28.13	28.13	26.98	26.98
20	42.83	42.63	42.83	42.83	40.89	41.09
30	53.44	54.37	53.44	53.44	52.16	51.26
40	61.94	64.62	61.94	61.94	61.98	59.42
50	69.12	73.87	69.12	69.12	70.86	66.30
60	75.39	82.41	75.39	75.39	79.06	72.31
70	80.97	90.40	80.97	80.97	86.72	77.67
80	86.02	97.94	86.02	86.02	93.95	82.51
90	90.65	105.11	90.65	90.65	100.83	86.95
100	94.93	111.97	94.93	94.93	107.41	91.06
110	98.91	118.56	98.91	98.91	113.73	94.88
120	102.64	124.92	102.64	102.64	119.82	98.46
130	106.16	131.06	106.16	106.16	125.72	101.83
140	109.48	137.02	109.48	109.48	131.44	105.02
150	112.64	142.81	112.64	112.64	136.99	108.05
160	115.65	148.45	115.65	115.65	142.40	110.94
170	118.52	153.95	118.52	118.52	147.68	113.69
180	121.28	159.32	121.28	121.28	152.83	116.34
190	123.92	164.57	123.92	123.92	157.87	118.87
200	126.46	169.72	126.46	126.46	162.80	121.31

The results show a significant reduction in DEM when using more realistic methods to calculate air density. For example, at a height of 10 metres, the DEM_IEI estimated with the standard density value [Table 5] reaches up to 36.55 Wh/m², while it decreases to 28.26 Wh/m² using the simplified formula [Table 6] and to 28.03 Wh/m² with the complete CIPM-2007 formula [Table 7].

This reduction pattern remains constant at all heights analysed, reflecting the direct impact of the density value on the estimation of available energy.

At 50 metres, the differences between the extrapolation methods begin to become more pronounced. LEH tends to slightly overestimate DEM compared to MMO and PEL, particularly when using more accurately calculated air density. At higher heights, such as 150 m and 200 m, the discrepancy between the methods is amplified, with LEH consistently showing the highest DEM values, while MMO and PEL offer more conservative results.

This behaviour is consistent with what has been reported in the scientific literature. For example, studies such as those by Allouhi et al. [2017] and Kamau et al. [2010] have pointed out that LEH, while useful for its simplicity, tends to overestimate wind speed and, therefore, available energy, especially in complex terrain or with atmospheric variability. On the other hand, the logarithmic profile method and the Monin–Obukhov method offer greater robustness, especially when detailed data on local conditions are available [Allouhi et al., 2017].

In terms of impact, the differences observed between using the standard value and the adjusted densities are equivalent to a reduction of up to 23.3% in the estimate of wind energy available at the study site. This implies that if the standard value is used without considering local altitude, pressure, and humidity, there is a risk of significantly overestimating wind resources, compromising the actual viability of generation projects in isolated areas such as the Mapimí Biosphere Reserve.

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Figure 01 shows the average annual wind speed extrapolated to different heights [10 to 200 m] using the three methodologies analysed. The horizontal axis represents height and the vertical axis represents wind speed in m/s. The red curve corresponds to Hellman's Exponential Law, while the blue curve represents the Logarithmic Wind Profile Law.The Monin–Obukhov Method curve is not visible because, assuming neutral conditions [L = 0] due to a lack of specialised data, its expression is reduced to the logarithmic law, generating identical values. It should be noted that the extrapolated wind speed does not depend on the air density value used.

Box 8

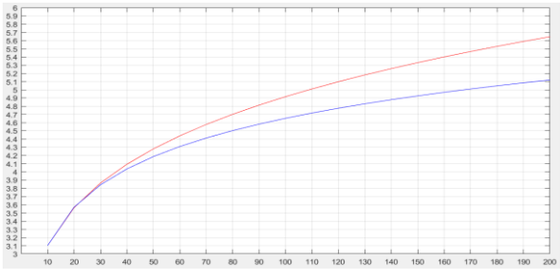

Figure 1

Average annual wind speed profile extrapolated by three methodologies.

In addition to the energy density analysis, a wind rose was generated for the year 2024 [Figure 2], which shows a predominance of flows from the southwest and northeast.

The red segments indicate class 3 winds [5-8 m/s] with less than 1000 occurrences per year per direction; the green ones represent class 2 [2-5 m/s], with higher frequency during the year, exceeding 4000 occurrences in the southwest direction; and the blue ones correspond to class 1 [0.5-2 m/s], reaching frequencies above 2000 in the southeast. It is worth mentioning that there were also occurrences of velocities exceeding 23 m/s, however, their frequency was not very representative and they are not clearly visualised in the graph.

3.1 Methodological inputs

All calculations were performed using a Graphical User Interface [GUI] developed in MATLAB GUIDE, which allows loading of historical meteorological data, selection of extrapolation methods and air density, and visualisation of both numerical and graphical results.

This tool represents an accessible solution for wind analysis in data-limited regions. The first version of this application is shown in Figure 3.

Box 9

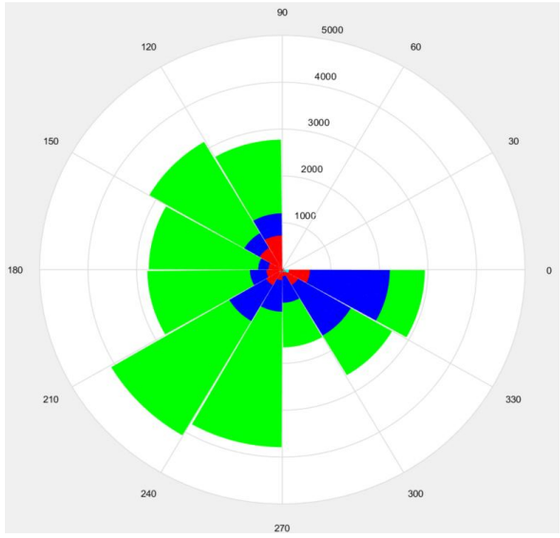

Figure 2

Polar plot of the wind direction, during the year 2024, in the analysed region.

Box 10

Figure 3

Graphical interface developed for wind resource estimation in any region.

Conclusion

This study demonstrates that the value used for air density has a significant impact on the estimation of the average energy density [AED] of wind, especially when applying exact methodologies based on the integration of the instantaneous power curve. Based on the results obtained, it can be observed that using the standard value of 1,225 kg/m³ tends to overestimate energy availability, compared to calculations made using the simplified formula based on the ideal gas equation and, even more precisely, the complete CIPM-2007 equation.

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The latter represents the most rigorous methodology, as it incorporates corrections derived from actual temperature, pressure and relative humidity conditions.

The values obtained show a reduction of approximately 23.3% in DEM when moving from the standard value to calculations using the complete density equation, which is particularly relevant in wind resource assessment projects for design or investment purposes.

The study also confirms that the three wind speed extrapolation methodologies [Monin–Obukhov, Hellmann's exponential law and Logarithmic Wind Profile] present consistent results, especially when neutral conditions [L = 0] are assumed, which reduces mathematical complexity without significantly affecting the accuracy of the model.

The implementation of a graphical interface in MATLAB to automate the calculations allowed the analysis process to be systematised and the information to be visualised clearly. The ability to extrapolate results to different heights, obtain energy densities both by integration and by average cubic velocity, and generate representative graphs such as the wind rose confirms the potential of this tool as a support in feasibility studies for wind projects in different regions.

Furthermore, based on the average energy density results obtained for heights of 10 to 50 metres, the Mapimí Biosphere Reserve can be considered a suitable region for the installation of low-power wind turbines for rural or autonomous applications. Various studies, such as that by Akhmatov [2007] and the recommendations of IRENA [2015], suggest that for this type of technology it is feasible to operate in regions with average speeds greater than 3 m/s or with a DEM equal to or greater than 50 W/m², values that were exceeded in multiple scenarios simulated in this work.

Studies such as those by Al-Nassar et al. [2005], Fagbenle et al. [2011], Mostafaeipour [2010], Mostafaeipour [2013] and Sánchez [2006] support the use of low-power wind turbines in semi-arid regions with profiles similar to the one analyzed here, provided that their design and height are adjusted to the actual wind behavior.

Finally, it is concluded that consideration of local atmospheric conditions and the appropriate selection of the air density model are key factors for an accurate estimation of wind resources.

In future work, it is recommended to validate these results with data from specialized EMAs or multi-height anemometric towers, as well as to extend the analysis to different regions of the country with emerging wind potential.

Declarations

Author contribution

Meraz-Becerra, Fernando: Responsible for the conceptualization of the study, complete development of the Graphical User Interface [GUI] in MATLAB, compilation, processing and analysis of meteorological data, preparation of figures and tables, drafting of the manuscript, and final editing of the article.

Juárez-Ortiz, Cinthia Andrea: Support in bibliographic research, data systematization, verification of intermediate calculations, and preliminary review of the manuscript format in accordance with the journal's editorial standards.

Martínez-Marroquín, Juan: Participation in the cross-validation of results obtained with the GUI, contribution to the preparation of comparative graphs, and support in the orthotypographical correction of the manuscript.

Availability of data and materials

The datasets generated and/or analyzed during this study, as well as the graphical user interface [GUI] developed in MATLAB for the analysis, are available upon reasonable request to the corresponding author.

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Abbreviations

CIPM	International Committee of Weights and Measures [Comité International des Poids et Mesures]
DEI	Instantaneous Energy Density
DEM	Average Energy Density
FDP	Probability Density Function
GUI	Graphical User Interface [Graphical User Interface]
K	Von Kármán constant [≈ 0.4]
L	Monin-Obukhov length
LEH	Hellmann's Exponential Law
MMO	Monin-Obukhov method
PEL	Logarithmic Aeolian Profile [LPL: Logarithmic Profile Law]
VRMC	Velocidad Relativa Media al Cubo [Velocidad cúbica media]
Zo	Longitud de rugosidad
ξ[h/L]	Función de corrección de estabilidad atmosférica

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Antecedents

Meraz Becerra, F., & Carrillo Martínez, J. M. [2024]. Estimación del recurso eólico en la Reserva de la Biósfera de Mapimí utilizando tres métodos para la aproximación de la velocidad del viento promedio a distintas alturas. *Revista Energías Renovables, 11*[54].

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Article

Aquaponic culture with application of biostimulants in floating root recirculation systems

Cultivo acuapónico con aplicación de bioestimulantes en sistemas de recirculación de raíz flotante

Chávez-Rangel Moisés * [a], Arellano-Rodríguez, Luis Javier [b], Neri-Luna, Cecilia [c] and Rodríguez William David [d]

[a] UdeG, University of Guadalajara [en] • OSH-2099-2025 • 0000-0002-8255-567X • 1068082
[b] UdeG, University of Guadalajara [en] • ORK-4592-2025 • 0000-0002-3188-0245 • 65995
[c] UdeG, University of Guadalajara [en] • X-2192-2018 • 0000-0002-8941-2305 • 72630
[d] UdeG, University of Guadalajara [en]] • NJT-1200-2025 • 0000-0002-2252-5540 554194

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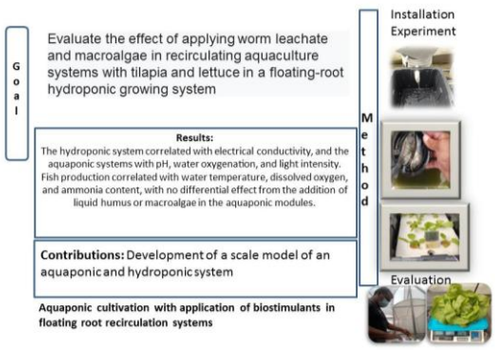
* ✉ [javier.arellano@academicos.udg.mx]

Abstract

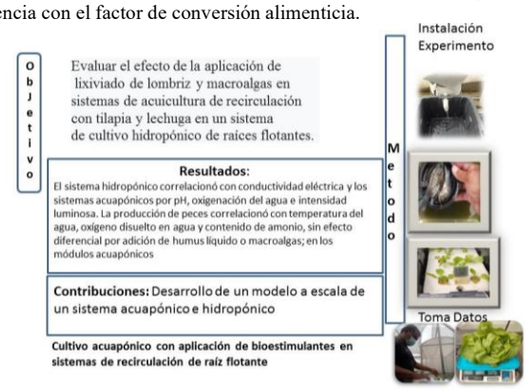
The objective was to evaluate the effect of the application of earthworm leachate and macroalgae [*Macrocystis pyrifera*] [L.] C. Agardh] in recirculating aquaculture systems with tilapia [*Oreochromis niloticus*] and lettuce [*Lactuca sativa* var. capitata] with floating root. An aquaponics system produces food by incorporating components [fish, plants, and microorganisms] into a recirculating system. Lettuce production was compared against a hydroponic system, where a higher weight of lettuce was obtained, followed by aquaponics with macroalgae. The hydroponic system correlated with electrical conductivity and aquaponics systems by pH, water oxygenation and light intensity. Fish production correlated with water temperature, dissolved oxygen in water and ammonium content, with no differential effect due to the addition of liquid humus or macroalgae; in the aquaponics modules; The greatest growth was in December-January where there was a higher absolute growth rate and weight gain without differentiation.



Acuaponía, Hidroponía, Bioestimulantes, Lixiviado de Lombriz, Macroalga

Resumen

El objetivo fue evaluar el efecto de la aplicación de lixiviado de lombriz y macroalgas [*Macrocystis pyrifera* [L.] C. Agardh] en sistemas de recirculación acuícola con tilapia [*Oreochromis niloticus*] y lechuga [*Lactuca sativa* var. Capitata] en sistema de cultivo hidropónico en raíz flotante. El sistema acuapónico produce alimentos mediante la incorporación de componentes [peces, plantas y microorganismos] en un sistema recirculante. La producción de lechuga se comparó contra un sistema hidropónico, donde se obtuvo mayor peso de lechuga, seguido del acuapónico con macroalgas. El sistema hidropónico correlacionó con conductividad eléctrica y los sistemas acuapónicos por pH, oxigenación del agua e intensidad luminosa. La producción de peces correlacionó con temperatura del agua, oxígeno disuelto en agua y contenido de amonio, sin efecto diferencial por adición de humus líquido o macroalgas; en los módulos acuapónicos; el mayor crecimiento fue en diciembre-enero donde hubo mayor tasa de cr7ecimiento absoluto y ganancia de peso sin diferencia con el factor de conversión alimenticia.



Aquaponics, Hydroponics, Biostimulants, Worm Leachate, Macroalgae

Area: Advocacy and attention to national problems

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Introduction

Currently, the agri-food sector faces the challenge of producing sustainable food without harming the environment and reducing the excessive use of agrochemicals by producers seeking higher yields and better crop quality [Gutiérrez et al., 2008]. Aquaponics is a technique that allows organic food to be produced and obtained, which is why it is considered an alternative that reduces the environmental pollution caused by conventional agricultural production, as well as avoiding nitrogen waste discharges into water bodies [Ramírez et al., 2008] from fish farming through a recirculation system [Rivas et al., 2021] that allows the reuse of nutrient-enriched water [Ramírez-Sánchez et al., 2016].

An aquaponics system allows food to be produced by incorporating two or more components into the same production system. The principle is based on water recirculation, harnessing the energy of the system, as well as the combination of fish, plants and microorganisms [heterotrophic bacteria, fungi and other higher organisms] that use organic carbon to break down solid waste [faeces and food scraps] [Colorado et al., 2021].

There are also nitrifying bacteria [Nitrosomonas spp. and Nitrobacter spp.] which, in the presence of oxygen, transform ammonium into nitrites and subsequently into nitrates, allowing these nitrogenous metabolites to be absorbed by plant roots and enabling the return of "clean" water to where the fish are located, functioning as a biological filter that maintains the proper functioning of the production system [Andrade et al., 2015]. The main advantage of producing food in aquaponic systems is the added value that can be given to food, because by not incorporating agrochemicals, the use of pesticides and fertilisers is eliminated, obtaining organic food and avoiding soil and water quality deterioration [Valdez-Martínez et al., 2023].

On the other hand, natural products such as biostimulants and biofertilisers have also begun to be sought and used to improve the process of food production and procurement [Espinosa-Antón et al., 2020] in an environmentally friendly and respectful manner, as well as to improve crop yield and/or quality [Florez-Jalixto et al., 2021].

Biostimulants are classified into: humic substances, products containing amino acids, and products containing hormones. The latter include seaweed extracts containing growth phytohormones [auxins, cytokinins and other derivatives], which are considered useful for plants [Kauffman et al., 2007; Kurepin, et al., 2014; du Jardin, 2015; Alcántara et al., 2019]. Based on the above, the objective was as follows:

Objective

To evaluate the effect of applying worm leachate and macroalgae [*Macrocystis pyrifera* [L.] C. Agardh] in recirculating aquaculture systems with tilapia [*Oreochromis niloticus*] and lettuce [*Lactuca sativa* var. Capitata] in a floating root hydroponic cultivation system.

Methodology

The study was conducted at the Bofish Aquaponics Farm [20°33' 22.7" N, 103°25' 48.3 W] in Santa Anita, Tlaquepaque, Jalisco, Mexico, at an altitude of 1,575 metres above sea level, in an area of 17.1 m² inside a reinforced zenithal greenhouse [15 m wide x 45 m long x 6 m high]. Two similar experiments were conducted: the first from October to November 2021 and the second from December 2021 to January 2022.

Organic lettuce seeds [*Lactuca sativa* var. capitata], butter type variety 'Nancy' [Isla®] and tilapia [*Oreochromis niloticus*] obtained from the 'BOFISH' aquaponics farm were used.

Obtaining seedlings and estimating the fish biomass ratio per plant, per module. One hundred and fifty seeds were sown in a 200-cell tray with BP [Berger®] peat substrate. After 33 days, seedlings with 5-6 true leaves [Maroto, 1983] were obtained for transplanting. The roots were washed and placed in polystyrene plates in the aquaponic-hydroponic modules. The ratio of fish [tilapia] biomass to planted plants was determined based on Racocy [2007] proposal for raft systems, of 60 to 100 g of fish food per square metre of cultivation area.

Considering the floating root area of each aquaponic module [0.2318 m^2] and the initial amount of food [60 g], this resulted in 13.9 g of food, equivalent to 4% of biomass, which at 100% biomass resulted in 347.5 g of biomass per module, divided between four fish of 86.9 g per module.

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Installation and management of the aquaponic and hydroponic modules. The aquaponic modules consisted of nine plastic boxes [102 L], where the tilapia were placed and a biological filter was incorporated into each of the modules, consisting of a 19 L plastic bucket, soft drink lids and anti-aphid mesh [reused materials] where the microorganisms and bacteria responsible for transforming the floating solid waste from the fish water into secondary metabolites useful for aquaponic cultivation were fixed [Figure 1].

The hydroponic modules for the lettuce under the floating root system were constructed in three boxes similar to those containing the fish, placed on top of them and covered with polystyrene plates 5 cm thick, 68 cm long x 42 cm wide, with six holes 20 cm apart for transplanting the plant material. For the recirculation of water with the nutrient solution, the aquaponic and hydroponic modules were interconnected, generating mechanical oxygenation by the fall of water [Figure 1].

*Treatments***.** The treatments and their acronyms are shown in Table 1.

The conventional control was the hydroponic system with nutrient solution [SHSN], which was prepared and applied at the beginning of each experiment [25 days], using per 75 L of water: Monopotassium phosphate [KH_2PO_4] 15 g, Magnesium sulphate [$MgSO_4$] 38 g, Potassium nitrate [KNO_3] 26 g, micronutrients 3.5 g, calcium nitrate [$Ca\,[NO_3]^2$] 53 g, based on Steiner's Universal Nutrient Solution [Juárez et al., 2006].

The aquaponic system without biostimulants [SASB] was used as the aquaponic control. Worm humus [in SAHL] and the macroalgae product [in SAM] Seaweed Dry ® [Macrocystis pyrifera] powder [Table 2] were the treatments to be evaluated within each experiment and the three respective replicates.

They had a capacity of 35 L, so the biostimulants were applied based on that volume of water.

Water quality variables in the aquaponic-hydroponic module and greenhouse environmental variables.

In each of the two experiments and replicates, variables related to water quality were recorded every three days: hydrogen potential [HANNA potentiometer mod. HI98127]; water temperature [°C, thermometer], dissolved oxygen [Smart sensor oximeter mod. AR406, mg L^{-1}] electrical conductivity [HANNA conductivity meter mod. HI98304, mS/cm], ammonium [YSI® Mod 9500 spectrophotometer, mg L^{-1}] and nitrites and nitrates [Fluval® tests, mg L^{-1}].

The following environmental parameters were recorded inside the greenhouse: minimum and maximum temperature, relative humidity [hygrometer with probe Mod. TA318, %] and light intensity [Smart sensor lux meter Mod. AS803, Lux].

Determination of lettuce yield. To evaluate the yield [g] of the lettuce crop in the aquaponic and floating root hydroponic modules, three lettuces were taken and weighed for each repetition and treatment at the time of cutting [fresh weight].

Determination of aquaponic crop yield. To evaluate the yield of tilapia expressed by the weight and length of each tilapia, two biometric measurements were taken for each repetition of the experiment: the first at the start and the second after 15 days to avoid stress on the fish due to handling. With each biometry, the amount of food to be given daily to the Tilapia during the 25 days of each experiment was recorded. The yield formulas [Table 3] for tilapia proposed by [Ramírez-Sánchez et al., 2016] were used.

Statistical analysis.

The possible combinations between the four treatments were evaluated in a completely randomized block design [CRBD] with three replicates each and two dates or experiments. Normality [Shapiro-Wilk] and homoscedasticity of variance [Bartlett's test] tests were performed.

An analysis of variance [ANOVA] and the adjusted Bonferroni method test [STD] were performed to evaluate the biostimulants in the recirculating aquaculture system [RAS] with the yield of lettuce cultivation and tilapia cultivation.

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A permutational analysis of variance [PERMANOVA] was performed to determine the response of the study variables on the fresh weight of lettuce and fish biometrics, in addition to canonical principal coordinate analysis [ACC] based on the Bray-Curtis distance matrix and 1,000 permutations [Anderson and Willis, 2003], to explore the relationships between the parameters studied and the yield of lettuce and tilapia crops through the standardization of total variance and the selection of the variables that concentrated the highest percentage of variability among the four treatments [Ramírez & Nienhuis, 2012]. The analyses were performed with the agricultural packages [De Mendiburu, 2023], ggplots2 [Wickham, 2016], MASS 7.3-58.2 [Ripley, 2022] and Vegan 2.2-1 [Oksanen et al., 2022], in R Studio 4.2.3 [R Core Team, 2023].

Results

In both experiments, the PERMANOVA analysis of lettuce weight identified significant differences [p = 0.017] in the electrical conductivity of the water, which influenced the weight of the lettuce. The rest of the variables estimated in the water [pH, water temperature and dissolved oxygen] did not show significant differences, nor did the environmental variables Temperature and Relative Humidity [T and RH].

This indicates that the weight of the lettuce in both experiments was similar and that EC was related to the treatments used: nutrient solution, aquaponic water and the application of macroalgae or worm humus. For Sambo et al., [2019], the characteristics to be controlled in the hydroponic nutrient solution include electrical conductivity [EC], pH and temperature, which affect the chemical balance of the nutrient solution and influence nutrient absorption.

The highest weight values [714.5 g] of lettuce showed a greater correlation with water temperature and electrical conductivity [Ta and EC] for treatments with nutrient solution [SHSN] and aquaponic treatment with macroalgae [SAM] [Figure 2]. The variability in lettuce weight in the aquaponic treatment without biostimulant [SASB] and the aquaponic treatment with worm humus [SAHL] was correlated with pH [6.3–7], oxygenation [2.9–3.9%], and light intensity [5082 to 11506 lux].

The highest electrical conductivity was found in the SHSN treatment, with values of 2.3 to 2.6 dS m^{-1}.

In this regard, García-Terrazas et al., [2022] agree that the highest fresh weights of lettuce were found in nutrient solution with an EC of 2.5 dS m^{-1}, although they attribute this good tolerance to salinity to the variety of lettuce used [Paris Island Cos romaine lettuce].

With regard to the treatments, significant differences were obtained in the ANOVA and Tukey's test at a 5% probability. The treatment with nutrient solution [T1:SHSN] yielded the highest lettuce weight [244 g], followed by the treatment with macroalgae [T4:SAM] [216 g], aquaponics [T2:SASB] [193 g], and finally the treatment with liquid humus [T3:SAHL] [176 g]. In the last three treatments, their average EC values were 0.9, 1.0 and 1.3 dS m^{-1}, respectively.

Bautista et al., [2021] reported that lettuce in a hydroponic system [HS] had a higher yield [1,847 kg/m^2] compared to the aquaponics system [AS with tilapia, 1,080 kg/m^2], which they attributed to the fact that the HS had the necessary nutrients from the start of cultivation due to the use of fertilizer.

In the present study, among the aquaponic systems, the one that received macroalgae [SAM] obtained the highest fresh weight [645.5 g], statistically similar to SHSN [714.5 g] with an average difference of 27.9 g, while the greatest difference was found with SASB [577.7 g], with 67.4 g less.

In relation to water temperature, [Ramírez-Sánchez et al., 2016] point out that between 21.1 and 23.3 °C there is optimal plant growth, at higher temperatures there is low growth and susceptibility to pathogens begins. In this regard, Urdiales-Ponce and Espínosa-Ortega [2018] pointed out that the optimal temperature for lettuce development is between 15 and 25 °C, with lettuce able to withstand maximum temperatures of 26 °C and minimum temperatures of 7 °C, confirming that temperature is a variable that affects plant development. In the hydroponic modules where the lettuce was grown, the water temperature was the same as that recorded in the aquaculture area due to the recirculation system.

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The overall yield [fresh weight] of the lettuce crop showed a normal distribution [p = 0.26] and homogeneity of variances [p = 0.58]. The ANOVA for the fresh weight of lettuce showed significant differences [p = 0.038] between the conventional SHSN control and the aquaponic treatments [SASB, SAHL, and SAM] [Figure 3].

During the first experiment, a higher fresh weight of lettuce was obtained than in the second, despite having the same average temperature, probably due to a greater amount of light compared to the second [9,999 and 7,225 lux, respectively]. Neff and Fankhauser [2000] point out that light, in addition to being an indispensable source of energy for plant photosynthesis, is also an important factor for plant growth, development and morphology, responding to light intensity [Fukuda et al., 2008].

Fuentes-Morales et al., [2022] obtained the highest fresh weight in lettuce under an intensity of 5700 lux; at 2100 lux, the highest plant height was observed, and it was the treatment with the highest energy consumption. On the other hand, with regard to the results on tilapia growth, in the PERMANOVA analysis [p = 0.001], only water temperature [p = 0.001] showed highly significant differences, while dissolved oxygen [p = 0.079] showed a tendency to show differences.

The principal coordinate graph [Figure 4] confirms that in both experiments, water temperature influences the length and weight of tilapia, in addition to dissolved oxygen and ammonium.

The weight and length of fish increased with higher dissolved oxygen content and/or lower water temperature [Table 2], such that in the second experiment [December-January], fish weighing 116 g and measuring 16.4 cm were obtained at an average temperature of 18.6 °C and 4% dissolved oxygen; higher values than in the first experiment [October-November], where fish weighing 89 g and measuring 11 cm were obtained at 23.8 °C and 3.7% oxygenation.

The ammonium concentration reached an average of 0.2 and 0.4 mg L^{-1} in each experiment, respectively.

According to Lama et al., [2025], an optimal temperature range between 18 and 30°C is recommended for aquaponic systems; according to Santoyo-Telles et. al., [2019], the appropriate water temperature for tilapia farming in aquaponic systems is 28 to 32°C. In this study, temperatures of 18° to 22° were recorded. Despite this, the tilapia did not show stress or symptoms of disease due to the low water temperatures, although there was an effect on performance [weight and length], because tilapia are ectothermic organisms [characterized by their dependence on external sources for heat], which generated metabolic differences related to the food consumed, reflected in performance [Ramírez-Sánchez et al., 2016].

According to the ANOVA for the weight and length of the tilapia, there were no statistically significant differences between the treatments [p = 0.9316] despite the normal distribution of the data and homogeneity of variances.

The feed conversion ratio obtained in the three aquaponic treatments [Figure 5] falls within the range reported by Sánchez-Sequeira [2006] as optimal [1.5-1.9], with no statistical difference between treatments of 1.87 to 1.9, except for SASB in the second experiment with 1.8. Comparatively, the FCA [feed conversion factor] values obtained were higher than those reported by Pérez et al., [2015] in a similar growth period of 25 days from 0.82 to 0.91 under a semi-intensive system and similar to those obtained by Zafra et al., [2019] with an average of 1.76 in a closed system.

For the absolute growth rate [AGR], there were also no differences between the aquaponic systems [without biostimulant, humus or algae] but there were differences between experiments, with higher values in the second experiment [December-January] at 0.4 g day $^{-1}$ than in the first experiment, which remained between 0.22 and 0.26, and in the second experiment between 0.6 and 0.63, lower than those obtained by Burgos et al., [2023] of 0.72 to 0.92 in red tilapia [*Oreochromis mossambicus*]. Similarly, there were no differences in weight gain between the aquaponic treatments except between experiments, with higher values in the second experiment of 15 to 15.9 compared to 5.25 to 6.6 in the first experiment; in the latter, similar to those obtained by Burgos et al., [2023] of 6.2 to 6.8 in red tilapia [*O. mossambicus*].

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Conclusions

The highest lettuce weight was obtained in the hydroponic system with nutrient solution, where EC had the greatest influence, while the second-best weight was obtained when macroalgae were applied. The aquaponic treatments were affected by the pH and oxygenation of the water, as well as the light intensity.

Fish production in aquaponic systems was influenced by water temperature, followed by dissolved oxygen in water and ammonium content, with no differential effect from the addition of liquid humus or macroalgae in the aquaponic modules.

The best growth was observed during the months of December-January [second experiment], where higher TCA and GP were observed with no difference in the Feed Conversion Ratio [FCR].

Declarations:

Conflict of interest:

The authors declare that they have no conflict of interest.

Authors' Contribution

Chavez-Rangel: Contributed to the idea of the Project, which is part of his master's thesis.

Arellano-Rodriguez: As thesis director Contributed to the documentary review, and monitoring of the work and additions modifications

Neri-Luna: Contributed to the introduction and the analysis of the results.

Rodriguez: Contributed to the statistical analysis and review of results

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Abbreviations

ACC	Canonical principal coordinate analysis
AGR	Absolute growth rate
ANOVA	analysis of variance
CRBD	Completely randomized block desing
EC	Electrical conductivity
HANNA	Potentiometer
pH	Hydrogen Potential
PERMANOVA	Permutational analysis of variance
R1, R2, R3	Repetitions
RH	Relative humidity
RAS	Recirculating aquaculture systems
SAHL	Aquaponic system plus liquid humus
SAM	Aquaponic system plus macroalgae
SASB	Aquaponic system without biostimulants
SHSN	Hydroponic system with nutrient solution
T	Temperature

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Article

Assessment of lung function using spirometry in brick factory workers: impact of air pollution in Tlaquepaque, Jalisco [2023-2024]

Evaluación de la función pulmonar mediante espirometría en trabajadores de ladrilleras: impacto de la contaminación del aire en Tlaquepaque, Jalisco [2023-2024]

Vargas-Chi, Melisa del Carmen [a], Figueroa-Montaño, Arturo * [b], Orozco-Medina, Martha Georgina [c] and Martínez-Abarca, Javier Omar [d]

[a] Universidad de Guadalajara • 0009-0002-7527-122X• 1238774.
[b] Universidad de Guadalajara • 0000-0001-7442-6301 • 76655.
[c] Universidad de Guadalajara • 0000-0002-2619-3408 • 25755
[d] Universidad de Guadalajara • 0000-0003-4592-7822• 948374

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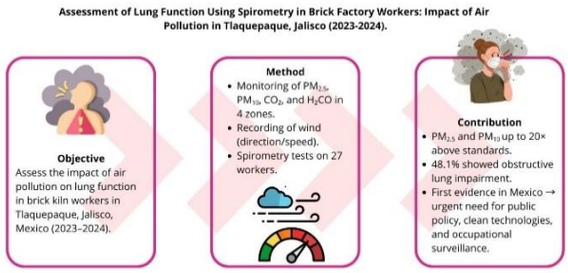
* ✉ [arturo.fmontano@academicos.udg.mx]

Abstract

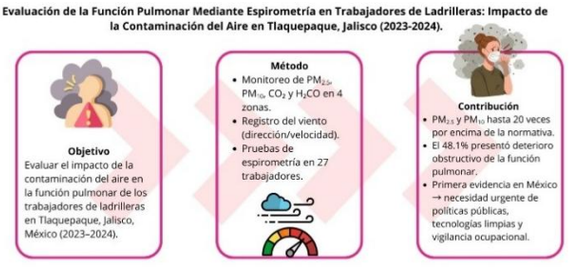
Air pollution poses a significant public health risk, particularly in artisanal brick production. This study assessed air quality and its impact on the pulmonary function of workers in Tlaquepaque, Jalisco, Mexico [2023–2024]. Measurements of $PM_{2.5}$, PM_{10}, CO_2, and formaldehyde [H_2CO] were conducted across four zones using portable monitoring devices, complemented by spirometry tests. $PM_{2.5}$ concentrations [up to 636 µg/m³] and PM_{10} [up to 902 µg/m³] exceeded regulatory limits, with higher severity in zones 2 and 4. CO_2 and H_2CO levels also surpassed reference thresholds, especially during and after rainfall. Weak winds limited the dispersion of pollutants. Spirometry results revealed that 48.1% of workers showed obstructive impairment, predominantly in the Suggestive of Obstruction category, with Mild, Moderate, and Severe obstruction each accounting for 11%. The findings underscore the urgent need for mitigation measures and public policies to safeguard occupational and environmental health.



Air pollution, Artisanal brick kilns, Pulmonary function

Resumen

La contaminación del aire representa un riesgo significativo para la salud pública, especialmente en la producción artesanal de ladrillos. Este estudio evaluó la calidad del aire y su impacto en la función pulmonar de trabajadores en Tlaquepaque, Jalisco, México [2023–2024]. Se midieron $PM_{2.5}$, PM_{10}, CO_2 y formaldehídos [H_2CO] en cuatro zonas utilizando equipos portátiles, complementados con espirometrías. Las concentraciones de $PM_{2.5}$ [hasta 636 µg/m³] y PM_{10} [hasta 902 µg/m³] superaron los límites normativos, con mayor severidad en las zonas 2 y 4. El CO_2 y H_2CO excedieron valores de referencia, sobre todo durante y después de lluvias. Vientos débiles limitaron la dispersión de contaminantes. El 48.1% de las espirometrías evidenció compromiso obstructivo, predominando la categoría Sugerente a Obstrucción y con Obstrucción Leve, Moderada y Grave distribuidas homogéneamente con 11% cada una. Los hallazgos evidencian la urgencia de medidas de mitigación y políticas públicas para proteger la salud laboral y ambiental.



Contaminación del aire, Producción artesanal de ladrillos, Función pulmonar.

Area: Advocacy and attention to national problems

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Introduction

Air pollution plays a crucial role in public health, especially in industrial areas where emissions of various pollutants are a significant concern [Rodríguez et al., 2020]. This phenomenon is a key feature of developing urban environments, where industrial activity and population density combine to create conditions that can be harmful to human health [Peña, 2023]. In the context of brick production, a predominant artisanal sector in Tlaquepaque, Jalisco, Mexico, exposure to air pollutants is a major concern due to the combustion processes involved in brick manufacturing [Gallegos et al, 2006].

Inhalation of fine particles, such as $PM_{2.5}$ and PM_{10}, as well as gases such as CO_2 and H_2CO, has been shown to have significant adverse effects on lung function, highlighting the need for studies assessing the respiratory health of exposed workers [Nicolaou, 2024; Raza et al 2022; Berumen et al 2021; Wei et al 2024].

Artisanal brick production in Tlaquepaque represents a vital economic sector that nevertheless carries considerable environmental and health risks. Brick kilns, typically fuelled by solid fuels such as wood and waste, are sources of pollutant emissions that contribute to the degradation of air quality [Berumen et al, 2021].

Recent studies have linked prolonged exposure to fine particles and harmful gases to a range of respiratory problems, including decreased lung function, increased incidence of chronic respiratory diseases, and exacerbation of pre-existing conditions such as asthma [Raza et al., 2021; Angarita 2023; Pannia 2023]. Previous research has documented that $PM_{2.5}$ and PM_{10} particles, due to their ability to penetrate deep into the lungs, are particularly harmful, causing inflammation and reducing respiratory capacity [Chen et al., 2022].

Recent evidence suggests that exposure to H_2CO, a volatile organic compound commonly emitted by combustion processes, can have acute and chronic toxic effects on the respiratory system and is referred to as a major urban risk factor for the development of some types of cancer [Khoshakhlagh et al, 2024; Olaguer, 2023].

It has previously been observed that CO_2, although less studied in the context of air pollution, can contribute to the burden of air pollutants and indirectly affect respiratory health [Taha et al, 2025; Salthammer, 2024].

The assessment of lung function through spirometry is a fundamental diagnostic tool that allows the quantification of the effects of exposure to these pollutants on the respiratory capacity of individuals [Rahman et al, 2019].

This approach is essential for understanding the magnitude of the impact of air pollution on the health of brick production workers and nearby residents.

Despite growing evidence of the adverse effects of air pollution, there are significant gaps in knowledge about how these specific pollutants affect occupationally exposed populations in artisanal settings. Most studies have focused on urban areas with more diverse sources of pollution, leaving a gap in understanding the effects on communities that depend on artisanal brick production.

This study addressed the need for a comprehensive assessment of air quality and respiratory health in Tlaquepaque, Jalisco, an area with a high concentration of brick kilns, where pollutant emissions are an environmental and public health concern.

The overall objective of this research was to investigate the relationship between air pollution and lung function in brick production workers in Tlaquepaque, Jalisco, Mexico, during the period 2023-2024. Systematic air quality monitoring was carried out, measuring concentrations of $PM_{2.5}$, PM_{10}, CO_2 and H_2CO, together with meteorological variables such as wind direction and speed, to assess the impact of these pollutants on the respiratory health of workers.

Spirometry tests carried out during a health campaign determined the prevalence of lung function disorders, providing empirical data on the effects of exposure to specific pollutants.

This study is pioneering in its focus on artisanal brick poduction and its impact on respiratory health, offering a unique perspective on the environmental and public health challenges in areas of artisanal brick production.

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By addressing gaps in the literature, this research provides a solid basis for the development of public health policies and mitigation strategies that protect the health of workers in Tlaquepaque and similar areas.

The combination of detailed environmental monitoring and respiratory health assessment provides a comprehensive view of the impact of air pollution in this specific context, highlighting the importance of effective interventions and mitigation strategies to reduce exposure to harmful pollutants. This holistic approach not only seeks to improve the quality of life of brick production workers, but also to set a precedent for future research in similar artisanal settings.

In the context of increasing industrialisation and urbanisation, it is imperative to understand how specific pollutants affect respiratory health in communities dependent on artisanal production. The lack of comprehensive studies in these areas has limited the ability of policymakers to implement effective measures that address environmental and public health concerns. By filling these gaps, this study provides empirical data that can guide policy formulation and the implementation of safer and more sustainable practices in brick production.

Furthermore, the research highlights the importance of integrating occupational health and environmental protection into regional economic development. By providing scientific evidence on the risks associated with exposure to pollutants in artisanal brick production, it underscores the need to adopt cleaner technologies and safer production practices that can reduce emissions and improve the health of workers and surrounding communities.

Methods

This study was conducted in the southern part of the municipality of Tlaquepaque, Jalisco, Mexico, a region known for its intense artisanal brick production activity.

The study areas were selected according to specific criteria that ensured the representativeness and relevance of the data obtained. Four study areas were defined, each of which included at least five brick kilns and were located in proximity to residential or educational areas [Figure 1].

Box 1

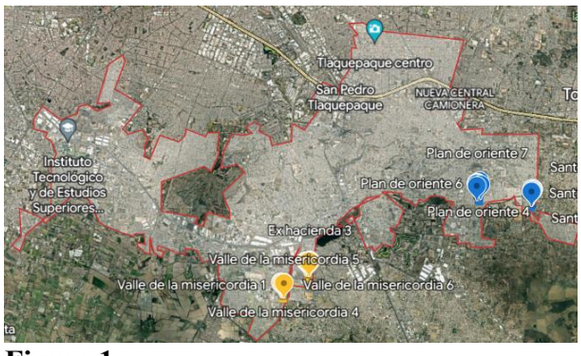

Figure 1

Location of the study areas, south of the Municipality of Tlaquepaque, Jalisco

A total of nine measurement points were established in each of the study areas: eight distributed around the perimeter of the study area and one in the centre, providing a representative value for each of the pollutants studied [Figure 2]. The choice of these points was based on a systematic design that considered the potential dispersion of pollutants from emission sources to surrounding areas.

Box 2

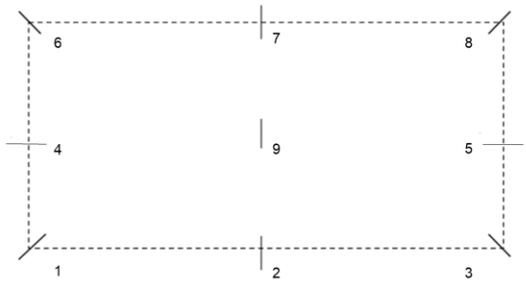

Figure 2

Distribution of measurement points in each study area

The study was conducted during the period from August 2023 to April 2024, covering three monitoring phases that corresponded to different climatic conditions: during rains [August-September], after the rains [November-December], and before rains [March-April].

This temporal design allowed for the evaluation of seasonal variations in pollutant concentrations and their relationship to prevailing weather conditions.

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For air quality monitoring, the Temptop M2000 portable device was used, designed to measure concentrations of $PM_{2.5}$ and PM_{10}, CO_2, and H_2CO. Meteorological variables, specifically wind direction and speed, were recorded using the Vantage Pro2 Duo 6162 portable weather station. This equipment provided accurate data on wind patterns, which is crucial for understanding the dispersion of atmospheric pollutants. At each of the established points, measurements were taken for 10 minutes, seeking stability and safety, both for air quality and wind, ensuring that the meteorological data could be correlated with the pollution data.

In October 2023, in order to assess the lung function of workers, a respiratory health campaign was carried out with the support of the Respiratory Therapy Unit of the Civil Hospital of the University of Guadalajara. For this purpose, a PC-Easy on PC-based intuitive solution spirometer was used to perform spirometry on volunteer workers. Participants were selected through convenience sampling, obtaining a sample of 27 adult participants, ensuring the inclusion of individuals of different ages, working conditions, and an almost equal distribution between women and men, 14 and 13 respectively, providing a comprehensive view of respiratory health in the study community.

Spirometry was performed following strict protocols to ensure the accuracy and reproducibility of the results. Individuals were instructed on the procedure and provided with a controlled environment for testing.

To categorise the results, the Respiratory Therapy Unit relied on the Global Initiative for Chronic Obstructive Lung Disease [Agustí et al, 2022] and the Spirometry Manual by Pérez et al., 2016, resulting in five categories of spirometry:

1] *Suggestive of obstruction*, indicating the presence/onset of a loss of lung function but without confirmed damage,

2] *Mild obstruction*, damage is already present but in its early stages,

3] *Moderate obstruction*, the damage is more pronounced, there is already a loss of lung function and medical follow-up is required,

4] *Severe obstruction*, the patient's lung function is already compromised and in many cases irreversible

5] *Normal*, airflow percentages are within normal ranges, but lung disease cannot be ruled out.

The data obtained were analysed to identify patterns of deterioration in lung function that could be related to exposure to air pollutants, especially those emitted by brick-making activity. This comprehensive methodological approach allowed not only for detailed characterisation of air quality and meteorological conditions, but also for direct assessment of the impact of these variables on the respiratory health of exposed individuals.

The combination of environmental and clinical data provides a solid basis for identifying risks and developing mitigation strategies that can be implemented in the future to protect the health of communities affected by air pollution in this region.

Results and discussion

The results are described with an emphasis on the distribution of pollutants and the comparison of the recorded pollution levels with national air quality standards for PM_{10} and $PM_{2.5}$ [NOM-025-SSA1-2021].

With regard to CO_2 and H_2CO, Mexico does not have specific regulations for their monitoring and control, so the description and comparative analysis of these is based on the information reported in the work of Xia et al, 2020 on regional transport and combustion efficiency, using CO_2 and CO as markers. With regard to H_2CO, the value of 0.010 ppm recommended by the Office of Environmental Health Risk Assessment [OERSA] and referred to in the work of Zhang et al., 2022, was taken as a reference. The distribution of the meteorological variables of wind speed and direction, which are key to the transport and dispersion of pollutants, is also described.

Table 1 summarises the results of the study, emphasising the pollutants studied, the standardised value or value referred to in the literature, and the range of concentrations above the specific values for each pollutant, for each of the study periods and areas.

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The standard values of 33 and 60 µg m^{-3} for $PM_{2.5}$ and PM_{10} particles correspond to the daily average value as the exposure limit that defines the threshold for the manifestation of health impacts. In particular, these levels have been adjusted based on the review and recommendations proposed by the World Health Organisation in September 2021, supported by scientific evidence collected and analysed since the last update of the air quality guidelines, which had been in force since 2005.

The new WHO global guidelines [WHO, 2021] on air quality provide clear evidence of the damage that air pollution inflicts on human health at concentrations even lower than previously assumed. The guidelines recommend new air quality levels to protect the health of populations by reducing levels of key air pollutants, some of which also contribute to climate change.

$PM_{2.5}$

The results of measurements taken at artisanal brick production sites recorded highly variable concentrations of $PM_{2.5}$, which was to be expected given the diversity of conditions present.

These include the weather, the size of the kilns, and the type and amount of fuel used for firing, as wood, sawdust, firewood and a wide variety of solid waste are used as fuels.

Overall, in the study, the range of concentrations that exceeded the standard value of 33 µg m^{-3} ranged from 40 to 636 µg m^{-3}. Regarding the distribution of these values by study period, it was observed that the period Before Rainfall [28%] and the period After Rainfall [25%] had a slightly higher distribution compared to the period During Rainfall [21%] [Table 1].

The occurrence of the highest average during the During Rainfall period [216 µg m^{-3}] could be related to the conditions of the magnitude and direction of the prevailing wind at the time of measurement, which may have directed the pollution plume towards the sensor, or perhaps coincided with the most active moment of combustion when the highest particle emissions are generated.

In each of these study periods, zones 2 and 4 stand out with the highest proportion of records above the limit established for health protection, with a percentage of occurrence of 56% and 67% of the total number of points sampled in each of the study zones [Table 1].

The findings of the study on fine particles pose significant health risks due to their ability to penetrate deep into the lungs, enter the bloodstream, and distribute to various organs. Their systemic effects are largely mediated by inflammation and oxidative stress, contributing to a wide range of diseases [Carazo et al, 2013; Balmes, 2015; Carmo et al, 2013]

Box 3

Table 1

Air pollutants and comparison with standard values based on Mexican Official Air Quality Standards

Pollutant/standard or reference value	*Range of concentrations > to standard or reference value*	*Average and percentage of violations per period*	*Percentage of violations by area*
*$PM_{2.5}$/33 µg m^{-3} **[NOM-025-SSA1-2021]***	*40-636 µg m^{-3}*	*Before the rains [179 / 28%]* *During rains [216 / 21%]* *After the rains [171 / 25%]*	*Zone 2 [56%]* *Zone 2 [56%]* *Zone 4 [67%]*
*PM_{10}/60 µg m^{-3} **[NOM-025-SSA1-2021]***	*66-901.6 µg m^{-3}*	*Before the rains [301 / 28%]* *During rains [306 / 21%]* *After the rains [314/ 26%]*	*Zone 2 [56%]* *Zone 1 [33%]* *Zone 4 [67%]*
*CO_2/550 ppm **[Xia et al, 2022]***	*556-834 ppm*	*Before the rains [678/ 8%]* *During rains [605/ 3%]* *After the rains [649/ 14%]*	*Zone 2 [22.22%]* *Zone 1 [11.11%]* *Zone 4 [44.44%]*
*FORMALDEHIDOS H_2CO/0.010 ppm **[Zhang et al, 2022]***	*0.010-0.102 ppm [91%]*	*Before the rains [0.025 / 81%]* *During rains [0.041 / 91%]* *After the rains [0.029 / 100%]*	*Zone 1 and zone 2 [100%]* *Zone 3 and Zone 4 [100%]* *In the 4 study areas 100% of values exceeded the reference value.*

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PARTICLES PM_{10}

Concentrations of this pollutant that exceeded the standard value of 60 µg m^{-3} ranged from 66 to 902 µg m^{-3}. Similar to the distribution of fine particles, the highest proportion of values above the standard were recorded in the pre-rainfall period [28%] and post-rainfall period [26%]. For the period during rainfall, the number of records above the reference value reached 21% [Table 1]. By study area, the distribution of critical conditions was recorded most frequently in areas 2 and 4, where pollution levels for this pollutant exceeded the reference value in 56% and 67% of cases during the pre-rainfall and post-rainfall periods, respectively [Table 1].

The coarsest particles, measuring 10 µm, are efficiently trapped by the body's respiratory defences, including the nasal passages and the mucociliary clearance system. This limits their penetration into the deepest parts of the lungs and reduces their potential to cause systemic health effects. However, they can still contribute to respiratory irritation and exacerbate conditions such as asthma. Their overall impact on health is considered less significant compared to smaller particles. Continuous and long-term exposure to airborne particles has been linked to respiratory diseases, cardiovascular diseases and premature death [Belkin, 2024; Adah, 2023; Raju, 2020].

CO_2

CO_2 is a colourless, odourless gas that is a natural component of the Earth's atmosphere. Although essential for processes such as photosynthesis, CO_2 is considered an air pollutant when its levels in the atmosphere increase significantly due to human activities, mainly the burning of fossil fuels, deforestation, and certain industrial processes.

The main anthropogenic sources of CO_2 include the combustion of coal, oil, and natural gas, as well as cement production and deforestation. Unlike other air pollutants such as carbon monoxide or sulphur dioxide, CO_2 is not toxic at typical outdoor air concentrations.

However, its role as a pollutant focuses more on its indirect effects through climate change and its impact on indoor air quality.

In enclosed spaces, high levels of CO_2 can indicate inadequate ventilation, which can lead to the accumulation of other pollutants. Exposure to high levels of indoor CO_2 [above 1000 ppm] can cause symptoms such as headaches, dizziness, fatigue, and difficulty concentrating [Chakraborty et al, 2014; Medgyesi et al, 2017].

Xia et al, 2022, in their work on regional transport and combustion efficiency, describe the worst pollution events as those with concentrations above 550 ppm. As there are no specific regulations for this pollutant in Mexico, the description of results is based on this reference. Thus, the range of concentrations above the reference value ranged from 556 to 834 ppm. Table 1 shows that the highest number of concentrations above this value was 14% in the period after rainfall, followed by the period before rainfall with 8%. Similarly, for this pollutant, zones 2 and 4 most frequently exceeded the number of records above the value referred to by Xia et al, 2022.

H_2CO

Formaldehyde is a volatile organic compound that plays an important role in atmospheric chemistry. It is a common air pollutant of natural and anthropogenic origin, and its presence in the atmosphere can have implications for air quality and human health [Dubey et al, 2021]. Due to the wide variety of sources emitting this pollutant, its presence was recorded in more than 80% of the study periods, in a range of concentrations from 0.010 to 0.102 ppm, exceeding the value of 0.10 ppm recommended by OERSA [Zhang et al., 2022].

For each of the study periods, the average concentrations were 0.025, 0.041, and 0.029 ppm, respectively. However, the period after rainfall stands out because in 100% of the study areas, the records were higher than the value recommended by OERSA [Table 1].

This is because, in addition to the emissions generated by the brickworks, the areas studied during all periods were highly urbanised areas, which had other fixed and mobile sources that emitted or contributed to the presence of this pollutant. Likewise, in the low humidity season, this phenomenon is accentuated due to the dry conditions of the atmosphere.

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Wind distribution

Studies on the urban climate of the Guadalajara Metropolitan Area [ZMG] that refer to the annual surface wind regime in the region identify a higher occurrence of warm, humid winds from the east, which are more intense and prevalent in March, June, October, and December, when the city reaches its maximum ventilation.

On the other hand, winds from the west are of medium intensity [average speed of 2 to 3 m s^{-1}], so in January and July they tend to affect the city's ventilation more frequently due to their presence, and together with periods of calm, which on average reach periods of up to eleven hours, significantly affect the city's ventilation as they are responsible for the accumulation of pollutants in the atmosphere of the ZMG. [Jáuregui, 1992].

Box 4

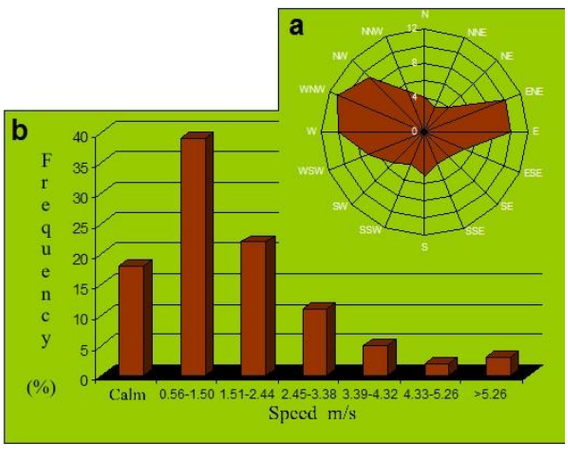

Figure 3

Wind rose of the most frequent wind directions [a] and magnitudes [b] recorded by the wind pattern in the ZMG [Figueroa, 2005].

Figure 3 shows a clear predominance of westerly [W] and easterly [E] winds and the highest frequency magnitudes between 0.56-1.50 m s-1. Also important are the wind magnitudes classified as calm [<0.56 m s-1] and light breeze [0.56-2.44 m s-1], which cumulatively account for 61% of all the records analysed in the graph.

This confirms the high potential for accumulation of pollutants due to lack of ventilation in the ZMG.

Box 5

Table 2

Wind distribution during monitoring

Direction	SPEED m/s							Total
	0.0-0.5	0.5-1.0	1.0-1.5	1.5-2.0	2.0-2.5	2.5-3.0	3.0-3.5	
N	2	5	4	1		1	1	14
NE	1	4	1	1				7
E	5	6	8	8	4			31
SE	4	7	2	1	1			15
S	4	5	2					11
SW	1	2	3					6
W	4	6	2	3				15
NW		4	2	1	1			8
TOTAL	21	39	24	15	6	1	1	107

Table 2 shows the distribution of winds recorded during the study. In line with the studies by Jáuregui, 1992, and Figueroa, 2005, the wind pattern recorded a predominant direction of E and W winds with frequencies of 31 and 15 of the total. The most frequent wind speeds ranged from 0.0 to 1.5 m/s, which, based on the Beaufort wind scale [Fortín, 2002], these winds are classified as calm [<0.56 m/s] and light [0.6-1.6 m/s], highlighting the favourable conditions for the accumulation of pollutants in the study areas, as 78% of the winds were calm to light.

Statistically significant differences in pollutants

The results of the hypothesis tests to assess statistically significant differences in $PM_{2.5}$ and PM_{10} in relation to the different study periods [before rain, during rain, after rain] were not statistically significant [P>0.05]. Similarly, the hypothesis tests for CO_2 did not yield statistically significant results for either the periods or the study areas [P>0.05].

With regard to the hypothesis tests to assess statistically significant differences between the different zones of study, the results were statistically significant, with Zone 2 standing out with the highest average value for both pollutants, P<0.05 [Figures 4a and 4b].

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Box 6

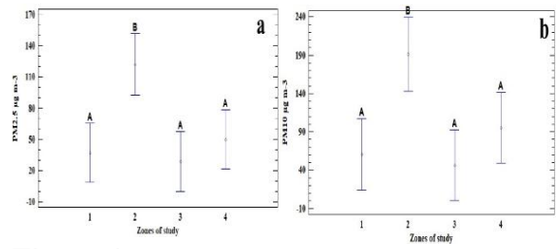

Figure 4

Medians for the concentration of PM2.5 particles [a] and PM_{10} [b]

Table 3 shows the results of the contrast of means between the different zondes of study, and emphasises the difference of the average concentration recorded in zone 2 with the rest of the study zones. In particular, the table distinguishes two homogeneous groups, group A with zones 3, 1, and 4; and group B consisting exclusively of zone 2, where the average value exceeds the average concentration for both pollutants by two to three orders of magnitude.

Box 7

Table 3

Homogeneous groups for study zones based on statistically least significant differences [LSD 95%].

$PM_{2.5}$ µg m^{-3}			PM_{10} µg m^{-3}		
Zone	**Media**	**Homogeneous Groups**	**Zone**	**Media**	**Homogeneous Groups**
3	28.5	A	**3**	46.3	A
1	36.9	A	**1**	60.6	A
4	49.9	A	**4**	95.3	A
2	122.3	B	**2**	191.1	B

Concerning H_2CO, hypothesis tests were highly significant for both periods and zones of study [P=0.0007]. The results distinguish the period during rainfall with the highest average concentration of 0.038 ppm [Figure 5a]. These levels could be justified due to the high solubility of formaldehyde in water, so that higher humidity or intermittency in water may facilitate its dissolution and dispersion in the air.

Box 8

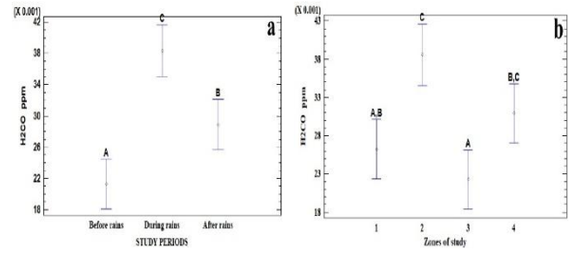

Figure 5

H_2CO concentration averages for periods [a] and study areas [b].

In relation to the zones, the highest average was recorded in zone 2 [Figure 5b], although the difference observed with zone 4 is not statistically significant, so they form the same homogeneous group C [Table 4]. Other homogeneous groups that the table also distinguishes by their overlap between the range of concentration values, are groups A made up of zones 3 and 1 with means of 0.022 ppm and 0.026 ppm, as well as zones 1 and 4 in group B with means of 0.026 ppm and 0.031 ppm respectively.

Box 9

Table 4

Homogeneous groups for periods and zones of study based on least significant statistical differences. [LSD 95%].

H_2CO ppm			H_2CO ppm		
Period	**Media**	**Homogeneous Groups**	**Zone**	**Media**	**Homogeneous Groups**
Before the rains	0.021	A	**3**	0.022	A
After the rains	0.029	B	**1**	0.026	AB
During rains	0.038	C	**4**	0.031	BC
			2	0.039	C

Lung function analysis

Based on the results obtained through spirometry tests voluntarily administered to artisanal brick production workers during the respiratory health campaign, a worrying pattern of lung function deterioration was identified. Of the 27 participants evaluated, 51.9% had results consistent with normal lung function, while 48.1% showed some degree of obstructive impairment in their respiratory capacity [Table 5]. This distribution shows a high burden of respiratory morbidity, as almost half of the workers show functional impairment consistent with obstructive lung disease, probably of occupational origin.

Box 10

Table 5

Pulmonary function status of study participants

Sex	**Suggestive of obstruction**	**Mild obstruction**	**Moderate obstruction**	**Severe obstruction**	Normal	**Row total**
Woman	1	2	2	2	7	14
	3.7%	7.4%	7.4%	7.4%	25.9%	51.9%
Man	3	1	1	1	7	13
	11.1%	3.7%	3.7%	3.7%	25.9%	48.2%
Column total	4	3	3	3	14	27
	14.8%	11.1%	11.1%	11.1%	51.9%	100.00 %

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Of the 48.1% of cases with some degree of obstructive impairment in their respiratory capacity, 14.8% of participants were classified in the suggestive to obstructive category, corresponding to evidence of a loss of lung function, but without confirmed damage. However, the categories of mild, moderate, and severe obstruction were evenly distributed at 11.1%.

These categories are related to the presence of initial damage to compromised and, in many cases, irreversible lung function [Table 5].

The presence of obstructive alterations in almost half of the workers evaluated highlights an alarming pattern. These findings are consistent with chronic exposure to atmospheric pollutants typical of the artisanal brick production process, such as fine particles capable of penetrating deep into the respiratory tract, generating chronic inflammation and bronchial remodelling, as well as toxic gases and irritating chemical compounds that can induce bronchoconstriction and exacerbate respiratory diseases [Moreira et al, 2018; Montoya et al, 2014, Nicolaou et al, 2023; Patel et al 2023].

When comparing the spirometry results in relation to the gender of the participants, similar percentages of impairment were observed [Table 5]. However, women show a higher concentration of cases with moderate and severe obstruction [4 of the 6 most severe cases], which could suggest greater biological vulnerability or differential exposure, possibly associated with the type or duration of work tasks, since women, in addition to participating in the production process, are also involved in food preparation, where they mainly use firewood for cooking.

The higher prevalence of findings suggestive of obstruction in men could indicate earlier stages of lung deterioration, which represents an opportunity for early detection and intervention. These findings reinforce the hypothesis that constant exposure to pollutants such as PM_{10}, $PM_{2.5}$, CO_2 and H_2CO can progressively compromise the respiratory health of workers. Consequently, it is imperative to establish preventive strategies, periodic monitoring of lung function, improvements in ventilation conditions, and mandatory use of personal protective equipment in these industries.

From a clinical point of view, the alterations detected by spirometry are indicative of a respiratory obstructive pattern, characteristic of diseases such as chronic obstructive pulmonary disease, chronic bronchitis, or pulmonary emphysema. The distribution of degrees of obstruction among participants shows that lung damage is not only common but, in some cases, has already reached advanced stages.

Finally, this profile of respiratory abnormalities is consistent with what is described in the literature on the occupational effects of working in industries exposed to incomplete combustion and constant release of atmospheric pollutants [Balmes, 2015]. The lack of protective measures and adequate ventilation in the workplace considerably increases the risk of developing chronic respiratory diseases.

Thus, the data analysed highlight the urgent need for health and regulatory interventions to mitigate the respiratory risk associated with this traditional productive activity in Tlaquepaque.

Conclusions

This study identified that $PM_{2.5}$ and PM_{10} particle concentrations in the southern part of the municipality of Tlaquepaque, Jalisco, significantly exceeded the regulatory values established for health protection, particularly in zones 2 and 4. $PM_{2.5}$ concentrations ranged from 40 to 636 µg m^{-3}, with the periods before and after rainfall showing a higher proportion of records above the regulatory limit.

Similarly, PM_{10} concentrations reached critical levels, ranging from 66 to 902 µg m^{-3}, with adverse conditions prevailing during the periods before and after rainfall. These findings suggest that emissions from artisanal brick production pose a significant risk to air quality and, therefore, to the health of nearby communities.

The present study reveals that CO_2 and H_2CO concentrations also exceeded the reference and recommended values, respectively. In the absence of specific regulations in Mexico, CO_2 concentrations were evaluated based on the value referred to by Xia et al., 2022, showing that zones 2 and 4 frequently recorded higher levels, especially during the period before rainfall.

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On the other hand, H_2CO was detected in more than 80% of the study periods, with concentrations exceeding the value recommended by OERSA, with the post-rainfall period being the most critical. These results are important because they provide information on the presence of pollutants that not only affect air quality but also have direct implications for public health.

The wind distribution, predominantly from the east and west and classified mainly as weak winds, suggests a dispersion pattern that could influence the concentration and transport of pollutants. Low wind speeds could contribute to the accumulation of particles and gases in the study areas, exacerbating local pollution conditions.

Clinically, the finding of obstructive alterations in 48.1% of workers evaluated by spirometry confirms the presence of significant lung function impairment, consistent with chronic conditions such as chronic obstructive pulmonary disease. The similarity in prevalence between sexes, together with the greater severity observed in women, raises new lines of research on biological differences and occupational exposure in this sector.

This work contributes to the field of environmental health by integrating environmental measurements, statistical analysis, and clinical evaluation within the same methodological framework, generating local evidence that strengthens the understanding of the respiratory risks associated with artisanal brick production. The results support the urgent need for public policies that regulate emissions, promote clean technologies, strengthen environmental monitoring, and ensure occupational respiratory surveillance programmes.

Further work is needed to deepen the understanding of pollutant emission and dispersion mechanisms in this region. Future research should focus on the detailed characterisation of emission sources and the development of predictive models that can anticipate episodes of high pollution. In addition, further research is needed to examine the long-term effects of exposure to these levels of pollution on the respiratory health of brick factory workers.

Further research should be conducted to explore mitigation strategies that include cleaner production technologies and environmental regulation policies that specifically address the problem of air pollution in artisanal brick production areas. These efforts would contribute significantly to improving air quality and protecting public health in Tlaquepaque and similar regions.

Declarations

Conflict of interest.

The authors declare that they have no conflict of interest. They have no known competing financial interests or personal relationships that could influence what is reported in this article.

Author contribution.

Vargas-Chi, Melisa del Carmen: Conceptualisation, methodology, validation, formal analysis, research, writing of the original draft.

Figueroa-Montaño, Arturo: Conceptualisation, methodology, statistical software, validation and formal analysis, data curation, writing, review and editing, supervision.

Orozco-Medina, Martha Georgina: Conceptualisation, methodology, research, resources, supervision, project management, funding acquisition.

Martinez-Abarca, Javier Omar: Data curation, editing software, validation, and formal analysis. Availability of data and materials.

Availability of data and materials

The original data presented in this study are available upon request to the corresponding author.

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Abbreviations

OERSA Office of Environmental Risk Assment
WHO World Health Organization
ZMG Guadalajara Metropolitan Area

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Article

Complex thinking to transcomplexity: artificial intelligence teachers perceptions in neurodidactic activities design with educational neuroengineering

Del pensamiento complejo a la transcomplejidad: Percepción docente sobre la inteligencia Artificial en el diseño de actividades neurodidácticas con neuroingeniería educativa

Valladares-Ríos, Luis [a], Duarte-Reaño, Jhonny [b] and Lino-Gamiño, Juan Alfredo [c]

[a] ISENCO • 0000-0002-9151-1441
[b] Universidad de Colima • 0009-0004-8733-6611
[c] Universidad de Colima • 3059393• 0000-0002-7022-5438 • 26894

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* ✉ [jlino@ucol.mx]

Abstract

Transdisciplinary education converges neurodidactic processes with neuroengineering and Artificial Intelligence, allowing the neurocognitive, neuroethical, and sociocultural student analysis characteristics; to link educational models from a holistic, integrative, and multidisciplinary perspective. This research explores, among teachers, the prospect of using neurodidactics to develop activities with educational neuroengineering through Artificial Intelligence, from a complex perspective toward transcomplex projects. Proposing the multi-method approach to transcomplex projects; the methodology combines a non-experimental, exploratory, descriptive, and cross-sectional quantitative approach with a qualitative approach using focus groups, administering a self-directed online questionnaire to 14 CISET students. The higher the academic level, the more information and openness to the design of activities with artificial intelligence and neurodidactics, facilitating the transition toward more interdisciplinary and neuroethical educational models. Although 85.7% are unaware of the usefulness of neuroengineering for the design of neuroeducational activities.

Neuroengineering, Neuroeducation, Artificial Intelligence, Complexity, Transcomplexity.

Resumen

La educación transdisciplinaria converge procesos neurodidácticos con la neuroingeniería y la Inteligencia Artificial; permitiendo analizar las características neurocognitivas, neuroéticas y socioculturales de cada estudiante para vincular los modelos educativos desde una visión holística, integradora y multidisciplinaria. Está investigación explora, en docentes, la prospectiva de emplear la neurodidáctica para el desarrollo de actividades con la neuroingeniería educativa a través de la Inteligencia Artificial desde una mirada compleja hacia proyectos transcomplejos. Proponiendo el multimétodo de proyectos transcomplejos; la metodología combina un enfoque cuantitativo no experimental, exploratorio, descriptivo y transversal, junto con un enfoque cualitativo mediante grupos focales, aplicando un cuestionario autodirigido en línea a 14 estudiantes del CISET. A mayor grado académico; más información y apertura al diseño de actividades con inteligencia artificial y neurodidáctica; facilitando la transición hacia modelos educativos más interdisciplinarios y neuroéticos. Aunque el 85.7 % desconocen la utilidad de la neuroingeniería para el diseño de actividades neurodidácticas.

Neuroingeniería, Neuroeducación, Inteligencia artificial, Complejidad, Transcomplejidad

Area: Development of strategic leading-edge technologies and open innovation for social transformation.

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Introduction

In today's world, we must accept that we are immersed in a physical, emotional and immersive reality governed by chaos and order as part of disorder. Non-linearity, uncertainty and indeterminacy, among other things, are significant markers of everyday life, directly impacting individuals in their daily tasks. This new reality poses a great challenge for scientists and researchers who are accustomed to working with certainties and truths, predictability and stability.

Therefore, one of the few certainties we have today is the constancy of change accompanied by an increasingly accelerated pace, which brings with it uncertain times, rendering obsolete the techniques and tools that until recently worked to address reality. There is no doubt that we are facing a new era, governed by the paradigm of complexity and migrating towards transcomplexity.

Consequently, when observing the present, one can appreciate the great complexity of the times and events; for this reason, it is necessary to migrate from simplistic thinking to complex and transcomplex thinking. Understanding that in order to address this reality, the generation of knowledge must be based on novel forms, which must be complemented as we move forward, where each phenomenon dictates how it should be approached disciplinarily, in those phenomena that can be addressed from a uni-, multi-, inter- or transdisciplinary perspective.

The approach to complexity is not limited to the use of disciplinary forms, but also involves generating an epistemic approach specific to that paradigm. This is how transcomplexity is understood, which is based on complex thinking and transdisciplinarity. From this perspective, there is a need to promote neuroscience, which allows us to study the neurophysiological behaviour of humans in order to understand the brain as an ally in addressing this new reality in education.

Neuroscience promotes advances in the worldview of the functioning of thought and basic and higher functions to promote the adaptability of living beings in this dimensional and complex space.

From the development and use of neuroscience strategies and techniques, it is beginning to be used from various perspectives or thoughts in different areas of life.

Among these studies is one focused on the area of education, as one of the fundamental pillars of development in a society. Bringing neuroscience to the service of individual learning from the perspective and support of neurodidactics, neurolearning, and educational neuroengineering in the development of neurodifferentiated activities per student.

Studies on brain function have led to technological advances that neuroscience seeks to replicate in order to reflect on the neurophysiological bases of human brain behaviour in biological and adaptive learning processes for survival. Understanding this interaction between biological processes and their effects on student behaviour and learning is strengthened by the use of technological tools such as artificial intelligence, which allow the creation of activities enriched with immersive environments and accompanied by information provided in an ethical manner, facilitating the construction of neurocognitive experiences to connect previous experiences with new knowledge.

These immersive environments, created as part of digital simulators, will assist humans in many fields of learning, including engineering, medicine, architecture, and ergonomics, among others. The aim is to train and reduce occupational costs and risks in order to enhance skills and strengthen neurocerebral fields that allow technology to be humanised in the field of education without neglecting emotional intelligence and the importance of the essence of human beings in a transcomplex world.

From complex thinking to transcomplexity.

According to Morin [1999, p. 36], there is a loop between science, technology and society; therefore, the technology produced by science transforms societies and, conversely, technologised society transforms science itself.

It can be said that the structure of the function of objects designed in nature and the function shapes the structure of those objects existing in space and time.

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This complex thinking provides us with a reflection on the adaptability of the brain using a computational analogy.

Over time, the anthroposocial aspect has its origin and roots in a biophysical component, where the mechanisms of certain mental processes store information in certain structures of the brain that can be compared to the functioning of a computer that needs information to store and process it in order to execute an action. Martínez, A. & Fornaguera J. [1998] highlight that the brain is a complex organ that is still being studied and that computers are being developed by humans. Both perform processes of evaluating external information with the criterion of learned value that produces movement, action, emotion, and language expression. This allows for free and reflective decision-making. The need for a science with consciousness is important to identify the validity of the local and the unique; by identifying mental errors, intellectual errors, and intellectual errors, Morin [1999, p. 5] explains that in each neuro-cerebral system, they connect the organism with other systems of ideas and interpretation of each assimilated reality. This perception is mediated by the assimilation and sensation of electrical stimuli in dendrites in the axons of neurons specific to each function.

When interpreting theories, beliefs, or intellectual paradigms, they must be analysed under the large amount of information stored in three sub-organs of the brain that allow for the creation of a conscious cortical system, a subconscious limbic system, and even the control of reflexes to perform survival functions such as breathing, heart rate, and essential movements. These allow the individual to react to their interconnected environment under a criterion of action. This set of events, actions, interactions, determinations, retractions, randomness and decisions weave a paradox of the one and the multiple that seems to be in disorder, but is not; the brain presents it in an interwoven manner, as explained by Morin [1990, p. 32] in complex thinking about phenomena, rejecting disorder in order to classify, distinguish and hierarchise facts from the binary thinking of the subject of the object, soul of the body, spirit of matter, quality of quantity, real of the virtual, feeling of reason, and of existence or essence. Each individual values this disjunction of perception of a parallel world subjected to the observation and interpretation of events.

This interconnected terrestrial world, which for Morin [1983: 418] in Lira [2004] refers to the fact that every system is constituted as a complex unit that involves diversity and multiplicity, even antagonism. Where disorder can be understood within the tetragram of order, disorder, interaction, and organisation. Within an open system such as fractals, which show a pattern of disorder at first glance.

But when applying the Fibonacci formula, the organisation of these fractals is found to be conditioned by factors that affect that structure based on its function. When disorder increases, order and organisation grow. To give an example: when measuring brain activity within an explanatory mechanism of neurological processes, there is an increase in action potentials, stimulus response and inhibition of certain areas that can be observed when measuring the brain with electroencephalograms or magnetic resonance imaging during academic activities.

Miklos & Tello [co-author], [1991] take foresight to a style of planning in line with current circumstances where the attitude of the mind is directed towards the problems of the future. However, foresight does not go from the present to the future, it comes from the future to the present, building means and alternatives to construct that desirable future [p. 42]. This is where paradigms help us to understand reality. Just as the human brain does to arrive at critical thinking about the solution to a problem to be solved, integrating various areas of the brain and activating various neural networks that connect to provide a holistic and integrative response from biological, affective, cognitive, sociocultural, institutional and/or political processes that can be studied under transcomplexity. A simple way of understanding what transcomplexity consists of is offered by Arce, R. [2019], who presents it as a new science that goes beyond complexity, conceived as a philosophy and episteme oriented towards the investigation of multiple realities, thus bringing together the strands of complex thinking and transdisciplinarity [p. 60].

Villegas [2006] in Perdomo, W., Salazar, S., Pérez, R., Rodríguez, J., Ruiz, B. and Villegas, C. [2017] defines transcomplexity as a bio-affective-cognitive, but also sociocultural, institutional and political process of producing complex knowledge.

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The recognition of the existence of a plurality of neglected approaches, such as the everyday, the imaginary, the poetic, among others [p.15], thus evidencing its integrative spirit.

Villegas [op. cit.] argues that transcomplexity is a construct that originates in the link between complexity and transdisciplinarity, taking three of the latter's characteristics, namely rigour, openness and tolerance towards other points of view, seen as a commitment to reconcile differences [p. 16].

This transcomplex interaction of different perspectives of analysis allows neuroscience, educational neuroengineering, and artificial intelligence to be integrated under an educational situation to attend to, understand, and diagnose; to design, implement, and evaluate the processes of educational achievement. This allows for the enhancement of the neurophysiological, emotional, behavioural, cognitive and sociocultural impact of this construction of activities from a transdisciplinary approach to improve students' quality of life and harmonise their learning from the human aspect to the adaptability of their processes in language acquisition, fine and gross motor skills, and even higher mental processes with the support of different professionals, methods, techniques and instruments to learn to live and coexist.

Martínez, M. [2009] defines transdisciplinarity as an intellectual and academic movement that seeks to go beyond or through [trans] unidisciplinarity, multidisciplinarity and even interdisciplinarity, thereby demonstrating its sole intention to overcome the fragmentation and compartmentalisation of knowledge reflected in particular disciplines. [p.27]

For Villegas [2010], a multidisciplinary approach to current phenomena is insufficient because this methodology seeks to study the phenomenon from various disciplines. Each discipline works separately, conducting an analysis from its own perspective. For this reason, interdisciplinarity was born, which integrates the visions of the disciplines, making this integration reach the epistemological and methodological levels.

Transdisciplinarity, on the other hand, is characterised by its integrative spirit, but is more comprehensive when addressing reality, thus broadening the vision and enabling it to take on complexity as an organised form.

Thus, research from the point of view of transdisciplinarity cannot be conceived as an end, but as a constant process of knowledge production, where one learns, unlearns, and relearns. In this way, it is recognised that the object learned has several facets that lead to different ways of thinking and reflecting on it in order to grasp its complex essence. In addition to transforming the practice of research into a process where there is learning, relearning, unlearning and complexification; or, in other words, transdisciplinary research must lead to the complexification of the object to be learned:

This reaffirms the position of complex thinking in which research is to investigate and follow the complex trail of something.

In this perspective on complexity, research must be transdisciplinary, developing the researcher's creativity by using all their thinking skills to immerse themselves in the background of reality, of which they are an active participant, in order to generate knowledge from there, making use of their previous and current experiences.

These experiences could be learned through observation, analysis, or implementation in order to construct their thought patterns and develop the skills and aptitudes acquired through formal and non-formal education in the teaching-learning processes at school in an informal way by interacting in society.

Neuroengineering in neuroeducation

Education in the 21st century requires us to look at open systems of knowledge generation with a complex, systemic, dialogical, and reflective view of the events of reality in each educational subject, from students, teachers, family members, support staff, educational authorities, and the knowledge society.

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To address the blind spots and illusions of knowledge in education, Gómez [2018] suggests that the classroom should be creative in multiple directions with emerging objects of study, overcoming linearity by interacting skilfully, with a democratising, dialectical and creative education capable of creating scaffolding to face the uncertainties of reality [p.97].

Neuroengineering, as defined by Panuccio et al. [2018], is the discipline that develops instruments that enable dialogue with a neural system, using computational tools and communication techniques that allow the electrical activity of the nervous system to be measured, analysed and manipulated. The aim is to identify patterns, algorithms and communication codes to resolve brain injuries, treat brain disorders and promote neural plasticity to enhance neural connections in order to make everyday activities more efficient. [p.1]

For Ahsan et al. [2024], neuroengineering is a rapidly expanding interdisciplinary field that connects neuroscience, biomedical engineering, and electrical engineering. It focuses on diagnosing and understanding brain function to create solutions for neurological disorders or enhance brain areas. The aim is to increase brain capacity, restore lost functions and decipher the complexities of brain function or alteration in the body. [p.1]

For Morín in Delgado [2017], it is necessary to humanise technology through soft digital skills that allow for reflection, self-regulation and self-knowledge of individuals in order to strengthen ecological awareness, autonomy, creativity and environmental ethics to build a participatory and democratic citizenship in an interconnected knowledge society. Where each subject's way of life is related to a system of acculturation that allows them to learn and tacitly and pre-reflexively apprehend the ways of living and managing their socially possible life [p. 20].

The act of learning pedagogically, as Alberto et al. [2024] comment, requires us to seek in subjects the principle of self-regulation and maturation. Identifying sociocultural meanings to generate products about current, comprehensive and open situations and contexts.

Therefore, teachers must consider the humanistic axis, recognising others from their philosophical, sociocultural, historical, political, ethical and aesthetic axis of the school environment.

They must also consider the scientific axis to develop the construction of knowledge through epistemology, discipline, research, critical thinking, and praxis. Without losing sight of the pedagogical aspect of what is communicated, listened to, and discussed in order to address the dimensions of pedagogical and curricular knowledge [p. 491].

Education, according to Potter [as cited in Gómez, 2018], must be transdisciplinary, global, interdisciplinary, ecological, critical and situated. It must allow for the appreciation of scientific and technological advances for humanity, with the aim of acquiring inclusive, equitable, fair, democratic and humanistic knowledge.

From moral reflection on the problems of nature that are experienced every day when interacting in society to understand and transform it with a holistic and systemic perspective.

Therefore, neuroeducation supported by artificial intelligence requires a comprehensive understanding of the subject in the educational process, considering their mind, perception, sensation, emotion, knowledge, physicality, context and intelligence in order to achieve more effective, ethical and symbiotic educational models with themselves and other living beings [p.92].

The need to reinvent an educational process with an interdisciplinary, transdisciplinary and systemic vision based on complex, ethical and humanistic thinking; should encourage reflection as a process of knowing what is known and co-constructing in dialogue what we are and what we have to recognise in the uncertainty that there are not only linear paths, but multiple trajectories to allow interaction with the diversity of knowledge, attitudes, feelings, perspectives, sensations and conceptions of reality in each subject.

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Neuroeducation proposes a perception from a complex system that studies and explains the assimilation of knowledge in brain structures that allow for the differentiated explanation of each individual to weave that network of information and generate experiential learning with knowledge, doing science with practice using techniques linked to feelings, sensations and emotions; to connect with various neurons from different cortical areas and brain systems that link emotions with previous information and new information; and thus be able to process a structured thought, processed and linked by the experience of information from an inductive or deductive analysis in the tetragram of complexity.

For Montoya-Restrepo I.A. and Montoya-Restrepo L.A. [2023], neuroscience is the study of the nervous system from its structure, development and functioning. From this, it can be deduced that neuroscience generates valuable information about neural mechanisms and their responses when the individual is subjected to stimuli such as emotion, reward, memory, motivation and decision-making processes, among others. These reactions help us understand individuals' affective and social behaviours [p. 29].

Neuroeducation shows us the importance of the link between emotions, context, and physicality with cognition. to create biopsychological technological tools that address the uniqueness and individuality of students' emotions, learning rhythms, and history based on differentiated teaching; supported by the use of artificial intelligence as a mediator for the protection of human dignity, promoting social justice and avoiding focusing on the subject as an object of study.

Rather, as a complex living being who interacts systemically in their individuality towards the collective.

Now, according to Fernández Pérez [2009], considering the Proteus effect to be a stereotype of users of these environments in neuroeducation, Heraclitus mentions in his enantological thinking that there are categories of opposition and relationship between events.

Allowing the experienced reality to be composed, decomposed and recomposed depending on the neurodidactic activities applied in the classes, the subjects interpret the conditions for interacting within or outside an immersive environment, real or fictional, novel or classic, orderly or disorderly, being or becoming, and easy or difficult. Creating neural networks to activate prior knowledge linked to an emotion or anchored characteristic [p.159].

Pineda et al. [2012], understanding of the mind has been studied over time, where theory of mind [ToM] is a cognitive ability directed towards predicting the behaviour of others in relation to social skills.

This mental representation, elaborated in the brain of each subject, is classified in external behavioural expression through gestures, with the visible physical world and hypothetical models alternative to that reality. Therefore, the use of digital, virtual and immersive environments to recreate a conscious virtual mental state in a living organism can create confusion between the real and the unreal, presenting a state of the Proteus effect. [p.181]

In the study by Yim et al. [2024] on the Proteus effect on pain management by users of virtual environments, they recorded that the perception of the stereotype influenced the subject's perception when performing activities with the viewers. Therefore, the thought generated from a computer to be visualised in a headset in virtual reality generates a complex thought that the user's brain will have to interpret as something real without physical matter in a digital, virtual, and immersive environment within a computational cyberspace. [p.10]

Artificial intelligence in neurodidactics.

Fredes [2025], in conjunction with neuroeducation, artificial intelligence is used to generate ideas, provide emotional support, organise routines, manage topics of interest, perform specific searches and solve problems for improved and personalised learning. Thus, the subject emerges from their self-concept of their cultural and social reality and begins to use digital, virtual, or immersive tools that allow them to self-regulate, organise, and self-discover under the awareness of themselves as a reasonable autonomous being immersed in a complex and interconnected system.

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According to García et al. [2024, p. 7], the use of generative artificial intelligence in education has made it possible to provide answers based on the evaluation of large data corpora supplied to train its information management system within a given probability to the question asked without reasoning, although coherent at times, it is not always correct.

Therefore, users must critically assess the veracity of the response provided by generative artificial intelligence. It is here that its use in education promotes critical thinking in the construction of knowledge as digital tools that can be used as augmented intelligence to analyse the large amount of data that provides information in a short time for reflection and analysis. Likewise, for experimentation with the use of chatbots for collaborative work in the design of a prototype or simulation of projects in digital, virtual or immersive environments, before developing the physical model.

In the research on the perception and use of chatbots among online postgraduate students: An exploratory study by Segovia-García [2023] reports that students use the Generative Pre-trained Transformer [ChatGPT] for its usefulness, ease of use, support, complement to study, easy search, source verification and knowledge generation.

Therefore, being able to interact with Artificial Intelligence allows for dialogue between participants and the construction of tools or prototypes based on a hypothesis or idea. Thus, the use of chatbots can facilitate the search for large amounts of information in different digital bibliographic resources from different subject areas to plan activities and provide effective and efficient feedback with human quality from neurodidactics.

Neurodidactics is a branch of pedagogy based on neuroscience, envisioning an innovative path in the field of education [Paniagua, 2013]. It is here that cognitive sciences and neuroscience converge with education, with the aim of designing didactic and methodological strategies in line with the current complex times.

Brain development and learning are closely related, and thus every meaningful learning experience for the individual leads to new neural connections accompanied by chemical secretions in the brain, which leads us to imagine that learning is a way of modifying the brain and keeping it active. Thus, educators can be thought of as modifiers of the brain through the teaching of novel and interesting content that will have an impact on the structure of the brain, with its chemical composition and electrical activity, thereby improving synapses or neural communication.

Ramírez et al. [2025] comment that the use of technological tools to develop hard and soft digital skills, such as augmented, generative, and investigative artificial intelligence assistants, encourages teachers to use methodologies related to complex thinking. The aim is to transform systemic, scientific, critical, and innovative thinking, as well as digital transformation for literacy in artificial intelligence.

This should adopt a dynamic model of thinking assistant that reinforces the need to design strategies for the executive functions of each subject, as well as to encourage critical reasoning, perception, attention, memory, emotion, thinking, learning, and motivation in material, digitised, virtual, and immersive environments.

Understanding this interaction of biological processes and their effects on student behaviour and learning is strengthened by the use of technological tools such as artificial intelligence, which allow the creation of activities enriched with immersive environments and accompanied by information provided in an ethical manner, facilitating the construction of neurocognitive experiences to connect previous experiences with new knowledge. These immersive environments, created as part of digital simulators, will assist individuals in many fields of learning, including engineering, medicine, architecture, and ergonomics, among others. The aim is to train and reduce occupational costs and risks in order to enhance skills and strengthen neurocerebral fields that allow technology to be humanised in the field of education without neglecting emotional intelligence and the importance of the essence of human beings in a transcomplex world.

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With the support of neuroscience, teachers are supported in the construction of activities based on neuroeducation. Actions in the classroom will always generate neurotransmitters that will either promote or hinder learning. Thus, if the educator promotes cortisol and adrenaline, they will cause a feeling of stress in the student that will in no way benefit learning. On the other hand, if they generate serotonin and dopamine, they will be gaining a great ally in fixing or generating knowledge through the student's openness and involvement in the teaching process.

Neurotherapy can be assisted with portable devices that improve people's learning conditions, as suggested by Ghannam Rami et al. [2020, p. 212397] through the use of microelectronics, artificial intelligence, neuroscience, and integrated circuits with portable devices such as immersive reality glasses, smart watches and bracelets, contact lenses, haptic gloves, hearing devices, footwear, and clothing, with the use of digital assistants such as chatbots that allow data analysis to suggest actions to enhance learning.

The use of electroencephalographic biofeedback analysis, known as neurofeedback, citing Carrobles, J. A. [2016], has made it possible to monitor biological activity from a system, brain region or process using electronic instruments that record brain activity signals in certain regions linked to clinical symptoms or problems in order to establish learning techniques to learn how to voluntarily control the functioning of that system.

This constitutes a field developed by learning psychology to use electronic devices to decode brain activity, supported by principles and techniques of operant conditioning learning from neurotherapy.

Using these devices provides access to information about the subject, so it is essential to consider ethical, legal and social issues regarding the use of data by support and monitoring devices, with techniques that promote the free and ecological development of the individual in question. According to Moritz et al. [2016], the capacity for self-determination must be considered and the ability to adapt to a dignified and healthy way of life must be enhanced by being able to choose based on preferences and values [p. 1362].

Neuroethics in neuroeducation and neuroengineering

Neuroethics has become a key issue due to advances in neuroscience and neurotechnology have advanced the medical treatment of certain neurological disorders or conditions in patients to improve their quality of life; the study of the brain has provided personal information about their brain processes in relation to their mental states and behaviour [Ienca, M., & Andorno, R., 2021, p. 144], so this information must be protected to ensure user privacy.

Neuroethics, according to Sánchez Vilanova, M. [2020], considers it a new branch of bioethics that debates the moral, social, and legal issues that accompany advances in neuroscience in order to protect the safety and autonomy of patients and avoid distorted interpretations that may arise during studies without affecting users or scientific advances that will allow other patients to be treated. [p. 194]

Considering the principle of agency in which each individual is the owner of their own thoughts and feelings, it becomes a priority to consider, according to [Floridi and Tadeo 2016 cited in Monasterio Astobiza, A. et al. 2019], the ethics of brain-machine interfaces for data management, algorithmic ethics and the ethics of practices; where the acquisition, storage and processing of data must follow privacy and patient authorisation standards. As well as data support by artificial intelligence, chatbots, machines and robots that use and interpret this data with the recording of neural activity; so that they protect the integrity of the user and the privacy of their information. Furthermore, ethical practices must be considered when developing information privacy codes for future research, supporting human rights, dignity and freedom with ecological responsibility. [p.4].

Methodology

The multi-method of transcomplex projects is proposed; according to Arango [2013] in "Investigación intervención y enfoque multimétodo en Ciencias Humanas y educación matemática" [2017], transcomplex research is suggested as a new paradigmatic worldview that requires a comprehensive, systemic and multivariate position.

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Synergy is achieved by using the methodology combined with a non-experimental, exploratory, descriptive and cross-sectional quantitative approach, together with a qualitative approach using focus groups, applying a self-directed online questionnaire to 14 teacher students from the Tecomán A.C. Centre for Social and Educational Research [CISET].

The research question posed is: What is the perception of postgraduate teacher students regarding neuroeducation and educational neuroengineering enhanced by artificial intelligence? Can they facilitate the transition from complex thinking to transcomplex thinking in education?

In the non-experimental, exploratory, descriptive, and cross-sectional quantitative approach, the following hypotheses are established:

H1: Postgraduate students have a high perception of the potential of artificial intelligence combined with educational neuroengineering as facilitators of the transition from complexity to transcomplexity in teaching.

H2: There is a significant relationship between the integration of multimethode with artificial intelligence and the perception of the ability to solve complex problems in postgraduate students.

H3: Most postgraduate students have an adequate level of awareness of the neuroethical challenges associated with the use of educational neuroengineering and the Proteus effect.

With the following operational variables for the study: 1.- Prospective perception of neurodidactics with AI and neuroengineering.

2.- Level of awareness of neuroethical challenges and the Proteus effect.

With the operational definition of: - Degree of assessment, expectations and attitudes of CISET teacher training students regarding the applicability of educational neuroengineering and AI in advanced educational processes.

- Degree of knowledge, recognition and concern of students about the ethical and neuroethical dilemmas related to the implementation of educational neuroengineering.

In the qualitative approach, the focus group is used to address the research questions:

1.- What is the perspective of postgraduate students on the use of Artificial Intelligence with educational neuroengineering as facilitators of the transition of teaching from complexity to transcomplexity? 2.- What is the perception of postgraduate student teachers regarding the integration of the use of multi-methods with artificial intelligence for solving complex problems? 3.- What is the level of awareness of postgraduate students regarding the neuroethical challenges linked to the use of educational neuroengineering with artificial intelligence and its relationship with the Proteus effect in education?

Proposing the multi-method of transcomplex projects, the general objective is constructed as follows: To explore the prospects of neurodidactics with the development of educational neuroengineering using artificial intelligence from complexity to transcomplexity. With specific objectives to achieve this knowledge in: 1.- Measuring the prospective perception of neurodidactics among postgraduate students on the use of artificial intelligence combined with educational neuroengineering in their professional practice as a facilitator for the transition of teaching from complexity to transcomplexity. 2.- Evaluate the quantitative perception of postgraduate students in integrating educational neuroengineering to solve complex problems from a multi-method and multiple perspectives approach using adaptive Artificial Intelligence. 3.- Identify the prevalence of awareness of the neuro-ethical challenges associated with the application of educational neuroengineering in relation to the Proteus effect in educational contexts.

Using a non-probabilistic sample of 14 postgraduate students at CISET with bachelor's, master's and doctoral degrees.

A mixed online questionnaire was conducted using Google Forms with closed and open questions.

It began with questions on three aspects: Aspect I. 1.- How old are you? I.2.- What is your gender? I.3.- What is your profession? I.4.- What is your highest level of education?

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Aspect II.- Closed questions with: yes/no. II.1.- Are you familiar with the contributions of neuroscience to education? II.2.- Are you familiar with the contributions of artificial intelligence to education? II.3.- Are you familiar with the contributions of educational neuroengineering to society? Aspect III.

Mixed questions with a Likert scale from 1 to 5. 1]. Strongly disagree. 2]. Disagree. 3]. Neither agree nor disagree. 4]. Agree. 5]. Strongly agree.

III.1.- I believe that educational neuroengineering, combined with artificial intelligence, can significantly improve teaching and learning processes. III.1.1.- Why? III.2.- I believe that the integration of artificial intelligence with neurodidactics and educational neuroengineering facilitates the transition to more complex and flexible educational models.

III.2.1.- Why? III.3.- Do I see myself using neuroscience with educational neuroengineering to solve complex problems from a multi-method and multi-perspective approach to solutions with the use of Artificial Intelligence? III.3.1.- Why?

III.4.- I believe it is essential to identify the neuroethical challenges associated with the application of educational neuroengineering in relation to the Proteus effect in educational contexts. III.4.1.- Why?

For the analysis of the results for the quantitative approach, the SPSS programme is used with descriptive statistics, frequencies and percentages for the graphical representation of relevant findings. Likewise, for the qualitative approach, the categories established a priori in the specific objectives will be used with the Atlas.ti 9 programme. Creating a network of words and code schemes.

Results

Seven of the participants who are familiar with the contributions of neuroscience are classroom teachers and one is an engineer. Of these, one has a postdoctorate, two have doctorates, one has a master's degree and three have bachelor's degrees. Those who are unfamiliar with the subject have master's degrees and bachelor's degrees.

Three are teachers, one is a preschool director, two are lawyers and one is an engineer. 85.7% of participants are familiar with the contributions of generative artificial intelligence in education.

Only two participants, equivalent to 14.3%, are unfamiliar with it; they are a preschool director and a telesecondary school teacher. The prospective perception of neurodidactics among CISET postgraduate students on the use of artificial intelligence combined with educational neuroengineering in their professional practice as a facilitator for the transition of teaching from complexity to transcomplexity tells us about the benefits and disadvantages of this use.

Among the benefits, they establish that the use of these technological and mental processes in subjects promotes attention, favours teaching and learning, and develops technological tools for meaningful and playful learning.

By generating attention in students, cognitive and metacognitive processes are initiated that allow students to become interested in the subject, understand the processes for acquiring knowledge from their brain activity, and generate knowledge linked to their neural connections and brain structures through neuroplasticity and brain adaptability. This allows them to learn in a differentiated, divergent, meaningful, and emotionally connected way that is linked to their learning style and brain maturity. Complexity shows us that everything is interrelated, and the way in which activities that stimulate or enhance certain areas of the brain are developed will yield results that enrich understanding, critical thinking, and the use of multiple intelligences to reinvent activities that modernise teaching styles in a creative, humanistic, and down-to-earth way.

When evaluating the quantitative perception of postgraduate students, educational neuroengineering is integrated to solve complex problems from multiple methods and perspectives using adaptive artificial intelligence. 85.7% of participants are unaware of the contributions of educational neuroengineering to society, while only 14.3% are aware of it and have a postdoctoral academic level with teaching functions and are biochemical engineers.

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The use of neuroscience with educational neuroengineering through a multi-method approach allows teachers with an open and flexible mind to understand students' learning patterns and models from an integrative and holistic perspective in order to establish different functional methods depending on the situation to be resolved. This line of thinking allows us to learn how to apply different useful, innovative, and even personalised tools to get to know people and be able to serve them by providing them with excellent service with dignity and humanism.

The need to solve complex problems from a holistic understanding refers to integrating different points of view in order to design, apply, and evaluate strategies that allow goals and objectives to be achieved from a humanistic worldview. They refer to the importance of being open to using multiple methods to integrate biological, neural, cognitive, emotional, behavioural, cultural and social processes, among others, for intercultural learning for the development of transdisciplinary projects.

These will be supported by the use of neurodidactics for the development of neuroeducational activities based on the analysis of each individual with the support of tools used by neuroengineering to understand the metacognitive and emotional processes involved in knowledge acquisition in students with the help of artificial intelligence and technological assistants such as chatbots.

Identify the prevalence of awareness of the neuroethical challenges associated with the application of educational neuroengineering in relation to the Proteus effect in educational contexts. It is observed that 78.6% of teachers, engineers, lawyers and directors agree on identifying neuroethical challenges, 14.3% neither agree nor disagree because they are unfamiliar with the subject, and 7.1% disagree, although their response is contrary, arguing that the future is changing.

Participants consider it essential to understand the ethical use of digital citizenship behaviours on platforms, applications, and networks, as well as to take values into account when resolving any personal or educational issues, behaviours, relationships, and contexts.

Conclusions

It is important to mention that generations are changing, from Generation X, born between 1965 and 1979, aged between 46 and 60, to millennials born between 1980 and 1994, aged between 31 and 45, and generation Z or centennials born between 1995 and 2009, aged between 16 and 30, who currently make up the working population; to generation alpha, born between 2010 and 2024, aged 15 and under, who are currently studying.

In addition, in 2025, the beta generation will emerge, which will be in contact with the Internet of Things [IoT]. It is therefore important to address ethics in society in order to take care of the information collected by technological devices and the information shared by computer networks, whether via the internet or other means.

Hence the importance of valuing each student's personality in order to protect their personal data and make good use of their studies, analyses and sensitive information to enhance their cognitive abilities, skills and emotions based on their study records provided by neuroengineering to design, apply and evaluate the impact of the activities carried out by the student during the sessions.

From a usability perspective based on human dignity and freedom of thought, this avoids the Proteus effect, a classic conditioning where a dissociation occurs between the real physical world and the immersive digital virtual world.

All of this leads to strengthening the proper use of technology with topics such as cybersecurity, values, and digital citizenship to understand its usability and promote data protection strategies and safe internet browsing. Addressing neuroethical challenges in data security and confidentiality, as well as the storage of data in locations protected against malicious computer systems.

Declarations

Conflict of interest

The authors declare that there is no conflict of interest, either financial or personal, that could influence the results of the research.

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Author contribution

Valladares-Ríos, Luis: Project idea, design and writing, design, application and qualitative analysis.

Duarte-Reaño, Jhonny; project idea, design and writing, design, application and quantitative analysis.

Availability of data and materials

The research information is available.

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Abbreviations

AI Artificial Intelligence.

GPT Generative Pre-trained Transformer

Annexes

Box 1

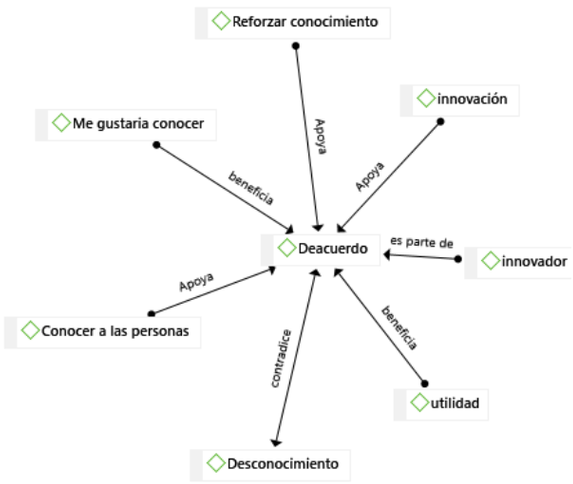

Figure 1

Code diagram on the visualisation of using neuroscience with educational neuroengineering to solve complex problems from multi-method and multi-perspective solutions using Artificial Intelligence.

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Article

Expanded polystyrene and whey goat as substrates for *Tenebrio molitor* breeding

Poliestireno expandido y suero de leche de cabra como sustratos para la cría de Tenebrio molitor

Alonso-Segura, Diana [a], Hernández-Ballejo, Pedro D. [b], González-Huerta, Jenifer [c] and Mandujano-González, Virginia * [d]

[a] Universidad Tecnológica de Corregidora, Academia de Ingeniería en Biotecnología 0000-0003-2297-3850• 44457.
[b] Universidad Tecnológica de Corregidora, Academia de Ingeniería en Biotecnología 0009-0004-6734-389X • 2171638
[c] Universidad Tecnológica de Corregidora, Academia de Ingeniería en Biotecnología 0009-0009-7712-5446 • 2171850
[d] Universidad Tecnológica de Corregidora, Academia de Ingeniería en Biotecnología 0009-0006-2938-314X • 334980.

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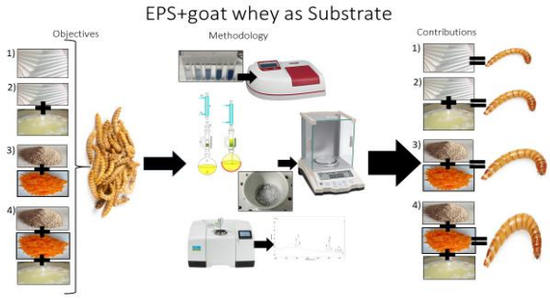
* ✉ virginia.mandujano@utcorregidora.edu.mx

Abstract

The objective of this work was to evaluate expanded polystyrene [EPS] and goat whey as substrates for rearing *Tenebrio molitor* larvae, analyzing microplastic residues in their flour and feces, and assessing nutritional impacts. Larvaes were raised in darkness at 20 ± 1 °C for 28 days across four treatments [substrates]: wheat bran/carrot [control], EPS, EPS with whey, and bran/carrot with whey. Flours obtained were analysed: protein, fat, and ash content using Lowry, Soxhlet, and gravimetric methods, respectively. FTIR spectrometry were used to determined microplastics residues in feces and in flour. Results showed microplastics in feces, indicating incomplete EPS degradation by the larvae. The bran/carrot/whey treatment yielded the highest larval weight [77.5 ±4.94 g] and a flour yield of 32-33%w/w, while EPS groups had the lower fat content 2.65-5.24%w/w and higher ash content 8.55-10.07%w/w.



Insects, Microplastics, whey

Resumen

El objetivo fue evaluar poliestireno expandido [PEE] y suero de leche de cabra como sustratos en la crianza de larvas de *Tenebrio molitor*. Las larvas fueron criadas en obscuridad a 20 ± 1 °C durante 28 días con 4 tratamientos: salvado de trigo/zanahoria [control], PEE, PEE con suero y salvado de trigo/zanahoria con suero. A las harinas obtenidas de las larvas se les determinó: proteína, grasa y cenizas por los métodos de Lowry, Soxhlet y gravimetría respectivamente. Por espectrometría de FTIR se determinó la presencia de microplásticos en las heces, indicando una degradación incompleta del PEE. El uso de salvado de trigo/zanahoria con suero resultó con el mayor rendimiento de la larva y de la harina [77.5±4.94 g y un 32-33%w/w respectivamente], mientras que con el consumo de PEE se obtuvo menor grasa 2.65-5.24%w/w y un mayor contenido de cenizas 8.55-10.07%w/w.

Insectos, Micro-plásticos, Suero de leche

Area: Dissemination of and universal access to science

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Introduction

This work explores the use of *Tenebrio molitor*, commonly known as mealworm, a coleopteran insect from the Tenebrionidae family, as a promising organism for addressing global challenges in sustainability and food security. Its four-stage life cycle makes it a versatile candidate for biotechnological applications due to its high nutritional value [van Huis et al., 2013], and ability to consume synthetic materials like expanded polystyrene [EPS] [Yang et al., 2015].

These larvaes offers a sustainable protein source with a lower environmental footprint compared to traditional livestock, requiring less water and emitting fewer greenhouse gases [Oonincx et al., 2015].

On the other hand, here it is explored the potential of goat whey, a nutrient-rich dairy byproduct with proteins, lactose, and minerals, to enhance larval growth and its nutritional content. Combining EPS and goat whey as substrates, includes waste management and valorizing agro-industrial byproducts, emphasizing the evaluation of microplastic residues in *T. molitor* flour and feces.

Tenebrio Molitor

It is a coleopteran insect from the Tenebrionidae family, has a life cycle of 280–600 days, comprising egg, larva, pupa, and adult stages. Eggs, laid by females [250–1000], hatch in 10–12 days at 18–20°C into white larvae that turn yellowish, growing to 2.5–3.75 cm and 160 mg. The larval stage lasts 3–4 months, followed by a 7–20 day pupal phase and a 2–3 month adult stage [Medrano, 2019]. As a nutritional source, *T.molitor* larvaes are rich in protein [approximately 20%w/w dry basis] and can be fed with cereals, vegetables, or synthetic materials like polystyrene, requiring hydration from moist substrates to enhance fertility and growth. They convert low-quality residues into high-quality protein and fat efficiently [Ramos, 2021].

The nutritional composition includes 52.8 ± 4.2%w/w crude protein, 36.1 ± 4.1%w/w fat, and 3.1 ± 0.9%w/w ash in dry larvae, making *T. molitor* a sustainable alternative protein source for human and animal consumption with a low environmental footprint [Ramos, 2021].

Plastics like polystyrene and agroindustrial byproducts pollution.

Expanded Polystyrene, EPS, a petroleum-derived plastic, composed of 98% air and 2% polystyrene, valued for its lightweight, thermal insulation, and moisture resistance properties [Muthukumar, 2024], is widely used in packaging, construction, and food industries [e.g., disposable cups and containers]. The global EPS market is projected to grow at 4.8% until 2028, yet recycling rates remain low in Latin America with Brazil at 24%, Mexico at 15%, and lower in Guatemala, Honduras, and Panama, exacerbating environmental issues. EPS's environmental challenges are related to soil contamination, chemical risks from styrene [a potential carcinogen] and toxic additives like hexabromocyclododecane [HBCD], persistence due to non-biodegradability, and impacts on soil organisms, contributing to microplastic formation [Chae & An, 2018; Lithner et al., 2011].

On the other hand goat whey, a nutrient-rich dairy byproduct from cheese production, is rich in proteins, lactose, and minerals; however the improper disposal of it, poses environmental challenges, but proper management can transform it into a valuable resource, unlike persistent EPS [Borba et al., 2022].

In this sense the use of tow residues from different sources are evaluated as an alternative to breed *Tenebrio molitor*, also a potential source of protein.

Box 1

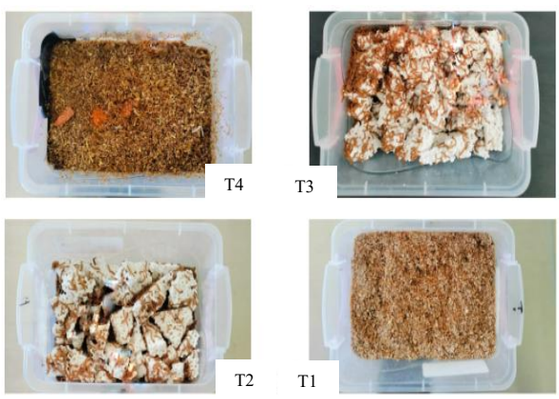

Figure 1

Treatments for *T.molitor* breed, corresponding to Table 1 description

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Box 2

Table 1

Substrate portion given every 7 days

Treatment	Bran+Carrot [g]	EPS [g]	Whey [mL]
T1 [Control]	30+10	-	-
T2	-	3	-
T3	-	3	10
T4	30+10	-	10

Methodology

Substrates and *T. molitor* larvaes

Larvaes were reared in darkness at 20 ± 1 °C for 21–28 days. Four treatments were designed, each treatment, substrate, had a duplicate, in containers with lids for controlled conditions, as shown in Figure 1. Substrates were provided based on formulations outlined in Table 1. Larval weight, EPS consumption, and pupation rates were recorded weekly.

After 24 h of fasting larvaes were sacrificed by freezing at -50 °C [MDF-C8V1, SANYO, Moriguchi] for 72h, then dried at 60 °C for 2 h in an oven [GTF7460, Gourmia, CDMX], and ground into flour in a food processor [WF2212114, Waring, Connecticut]. Flour yields were measured for each treatment and stored in a cool, dry place until their corresponding analysis.

Protein, fat and ashes determination.

For protein determination a Bradford Protein Assay Kit were used [Bio-Rad Laboratories, Inc. 2023] and a calibration curve was made [shown in Annexes 1] with bovine serum albumin as standar [EuroNutec, Qro., Mexico], as well as the samples preparation to be read in a Microplatereader at 595nm [Allsheng, AMR-100, Hangzhou], all by triplicate. Fat and ashes determination was made according to FAO [2011], using 2.5g of each sample, by triplicate for each treatment, using the equations:

$$\%\text{raw fat} = [[m3-m2]/m1]*100$$

Where m1= sample weight
m2= flask weight
m3= flask and fat residue weight

$$\%\text{ashes} = [m2-m0/m1-m0]*100$$

Where m0 = empty melting pot
m1= melting pot with sample
m2 = melting pot and calcinated sample

FTIR analysis

Flour of the *T.molitor* larvaes of each treatment, 1mg, was mixed with 1mg of KBr in an Agatha mortar, then press until getting a tablet and scanned with a FTIR [Bruker Vertex 70v, Germany], from 400 - 4000cm^{-1}.

Results

Larvaes weight of each treatment were determined weekly as reported in Table 2, and was observed that treatment 4 [T4] increased larval weight by circa 8g more than the control, on the contrary, treatments 2 and 3 based on EPS did not promote weight gain in the larvaes, which concides with the work of Bonilla-Amaya [2023]; even when whey and EPS where consumed by the larvae the weight gain was almost 30g less than with the control.

Box 3

Table 2

Larvae´s weight recorded every 7 days

Day	T1 [g]		T2 [g]		T3 [g]		T4 [g]	
0	30	30	30	30	30	30	30	30
7	45	47	37	39	40	35	64	64
14	70	71	36	47	41	35	78	83
21	71	71	35	44	41	36	94	89
28	71	69	36	47	42	36	81	74
Average Final weight	70 ±1.41		41.5±7.77		39±4.24		77.5 ±4.94	

The nutritional content of the treatments can be reflected in the transformation from larvae to pupa, so the lesser the nutrients the less transformation, when EPS only was consumed there were cero pupas, and with the richer in nutrients diet [T4] there were an average of 5.5±0.7 pupas. In this sense the flour yields were related with the larvae weight gain, being T2 and T3 the smaller yields on dry basis [24.35%w/w and 28.04%w/w, respectively] compared to the diets where no EPS was used [T1 and T4, 32.14%w/w and 33.12%w/w respectively].

Protein, fat and ashes determination.

The proximal analysis of the flours obtained with each treatment are shown in Table 3, where can be observed that the use of EPS as substrate reduces almost 50%w/w the protein content of the larvaes, so this reduction impact in the potential of the *T.molitor* to be use as an alternative protein source as proposed elsewhere [Ramos, 2021; Ramos-Elorduy et al., 2002].

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Box 4

Table 3

Proximate analysis of *T.molitor* larvae flours

Treatment	Protein [%w/w]	Ashes [%w/w]	Fat [%w/w]
T1 [Control]	62.54±0.31	4.42±0.13	21.02±1.6
T2	33.65±0.56	8.55±0.16	2.65±3.79
T3	35.85±2.33	10.07±0.88	5.24±1.98
T4	63.77±0.22	4.26±0.14	26.41±0.6

Is worth notice that ashes content represents the minerals with in the sample, generally the ash corresponds to less than 5% of the dry matter of the foode, when the percentage is higher it suggests contamination with some inorganic component [Márquez, 2014]. Based on this, the high percentage of ash obtained with diets T2 and T3 indicates contamination by remnants of the same EPS.

FTIR analysis of *T.molitor* larvae and feces

Figure 2 shows the spectra from the analysis of three flours, blue line corresponds to the flour made with larvaes fed with T3, green line corresponds to the flour from larvae fed with T1 and the red line corresponds to the flour from larvae fed only with EPS, T2.

Box 5

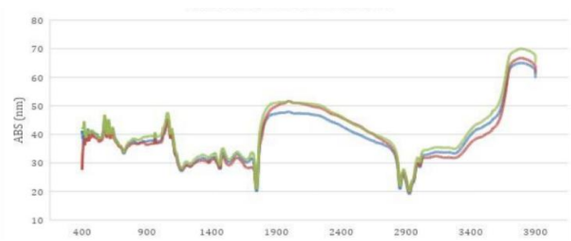

Figure 2

FTIR spectra of T.molitor larvae flour obtained with: T1-green line, T2-red line, T3-blue line

Some of the peaks observed in the spectra, found in all the flours analyzed, are at 1226, 1350, 1433, 1496, and 1619 cm^{-1}. According to Pérez-García [2021], these peaks correspond to proteins, since the region from 1100 to 1700 cm^{-1} is characteristic of peptide bonds. Furthermore, the region from 1680 to 1800 cm^{-1} is associated to fats, so a peak at 1723 cm^{-1} is observed in figure 2; this confirms the presence of proteins and fats and the absence of microplastics in *T. molitor* larvae flours. On the other hand, figure 3 shows the spectra obtained with the feces of mealworms fed with the control diet T1, which corresponds to the red line, and from the feces of larvaes fed with EPS, T2, which corresponds to blue line.

Box 6

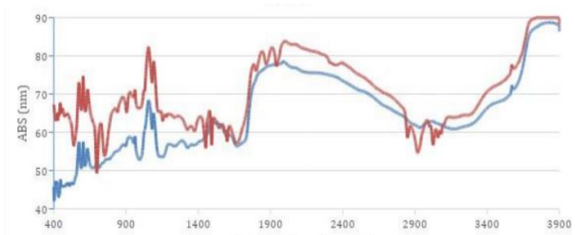

Figure 3

FTIR spectra of *T.molitor* larvae feces obtained with: T1-red line and T2- blue line

According to Torres-López [2016], some characteristic peaks of expanded polystyrene are found in the 2700 to 3150 cm^{-1} region, which is due to the C-H bond strain of the aromatic ring present in the EPS. In the spectra obtained in this work, peaks were observed in the feces of larvae fed with EPS [T2, red line] within this region, specifically at wavelengths of 2881, 2995, and 3052 cm^{-1}, which could indicate the presence of EPS residues. On the other hand, blue line corresponding to the feces from the control diet does not show these peaks. Another interesting region is between 1670 and 2000 cm^{-1}, characteristic peaks of aromatic rings. In Figure 3, we observe that the feces of larvae fed only with EPS have peaks at 1793, 1854, and 1922 cm^{-1}, in contrast to the feces of larvaes fed with the control diet, T1. The regions between 1451 and 1492 cm^{-1} represent the vibration of the C-C bond of aromatic compounds, and in Figure 3, a peak at 1483 cm^{-1} is observed in the feces of larvaes fed with the control diet.

Based on these results, it can be concluded that *T. molitor* larvae do not completely degrade EPS.

According to Álvarez-Estepa and Botache-Laguna [2020], 50% of the consumed substrate is transformed into CO_2, and the other 50% is excreted by the larvae in the form of biomass and even small biodegraded plastic fragments.

Conclusions

The absence of microplastics in the flours made with larvaes fed with EPS was confirmed. Furthermore, the presence of EPS residues in the larval feces was determined. This means that although the larvae consume the EPS, it does not remain within their organisms and is excreted in their feces.

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This also confirms that *Tenebrio molitor* does not completely degrade the polymer.

On the other hand, the results obtained from the nutritional content of the flours confirmed the low viability of producing/consuming flour from larvaes fed with EPS due to its low nutritional content. However, the flour from larvae fed with an EPS diet supplemented with whey showed high percentages of protein, fat, and ash, due to the fact that whey provides some of the necessary nutrients for their growth.

It is concluded that flour made from larvae fed with carrot/wheat bran and whey, T4 had a higher yield compared to the initial and final biomas. The larvae from this treatment showed greater weight gain during the 28 days of rearing, in addition to greater pupal development, which could reduce rearing time and optimize flour production.

It is proposed to continue the research in this field and work with the EPS-degrading microorganisms present in *Tenebrio molitor* microbiome, isolate them, and better understand their mechanism of action.

Annexes

Protein determination.

Box 7

Table 3

Dilutions made for calibration curve

Test Tube #	Estándar Vol [uL]	Standar	Water [uL]	[Protein] [uL/mL]
1	20	Stock 2 mg/mL	0	2000
2	30	Stock 2 mg/mL	10	1500
3	20	Stock 2 mg/mL	20	1000
4	20	Tube 2	20	750
5	20	Tube 3	20	500
6	20	Tube 5	20	250
7	20	Tube 6	20	125
8	-	-	20	0

Box 8

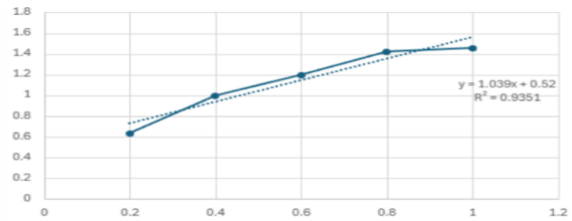

Figure 4

Calibration curve for protein determination

Declarations

Conflict of interest

The authors declare no interest conflict. Authors have no known competing financial interests or personal relationships that could have appeared to influence the article reported herein.

Author contribution

Alonso-Segura, Diana and Mandujano-González, Virginia: Contributed to the project idea and research methods.

González-Huerta, Jenifer and *Hernández-Vallejo, Pedro D.*: contributed with the experimental work and technical analysis.

Availability of data and materials

Data obtained in this research was experimentally based and its discussion was according work published elsewhere and are available free on-line.

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Abbreviations

EPS	Expanded Polyestirene.
PEE	Poliestireno expandido.

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Article

Implementation of a Reforestation Restorative Plan in Jilotzingo, Estado de Mexico

Implementación de un Plan Restaurativo de Reforestación en Jilotzingo, Estado De México

Rangel-Ruiz Karelia Liliana [a] *, Granados Olvera Jorge Alberto [b], Cureño-Jiménez, José Domingo Agustín [c] and Calvillo-Beltrán, Sofía Valentina [d]

[a] Universidad Politécnica de Cuautitlán Izcalli • GLQ-8704-2022 • 0000-0003-1805-0447 • 225798
[b] Universidad Politécnica de Cuautitlán Izcalli • S-5756-2018 • 0000-0003-0546-5328 • 946998
[c] Subdirección de Desarrollo Regional de la Secretaria del Bienestar del Gobierno del Estado de México• OBO-5365-2025 0009-0004-9095-834X •2163659
[d] Universidad Politécnica de Cuautitlán Izcalli • KYU-8461-2024• 0009-0003-8078-7172 • 2052292

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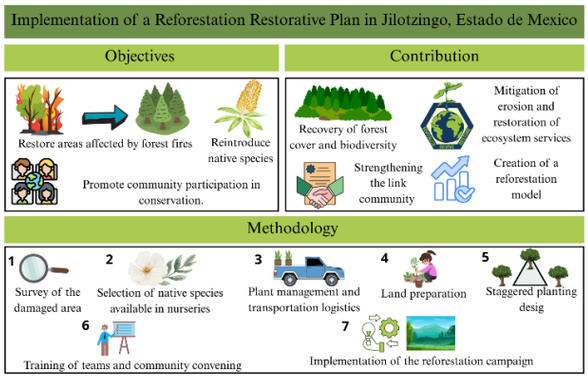
* ✉ [karelialiliana.rr@upci.edu.mx]

Abstract

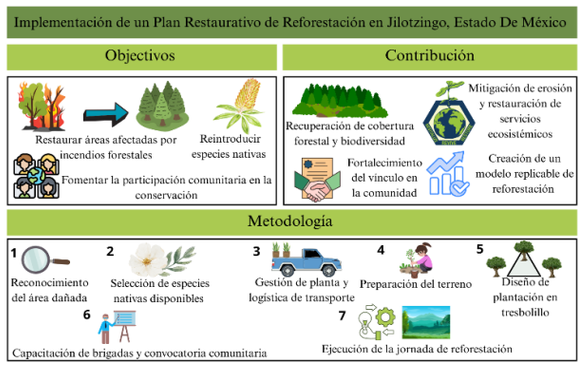
In the past few months, the forest area of the municipality of Jilotzingo, State of Mexico, was struck by a series of fires that affected a significant part of its forests, these provide important ecosystem services for the inhabitants of the CDMX and its conurbated area. Therefore, the need to implement a reforestation plan that would help restore the affected area was evident. The objective of this work was to implement a restorative reforestation plan in this key area. The area to be reforested, and the tree species were selected keeping in mind various criteria. The design was based on what was previously reported in the literature according to the type of land. During reforestation, 14,000 organisms of the species *Abies religiosa* and *Pinus spp.* were planted in approximately 10 ha. It is necessary to follow up on the reforested area to guarantee the survival of most trees.



Forestal fire, Jilotzingo, Reforestation

Resumen

En los pasados meses, el área forestal del municipio de Jilotzingo, Estado de México, se vio azotado por una serie de incendios que afectaron una parte importante de sus bosques, los cuáles aportan importantes servicios ecosistémicos para los habitantes de la CDMX y su área conurbada por lo cual, se hizo evidente la necesidad de implementar un plan de reforestación que ayudara a restaurar la zona afectada. El objetivo del presente trabajo fue implementar un plan restaurativo de reforestación en esta importante zona. El área por reforestar, y las especies arbóreas fueron seleccionadas tomando en cuenta diversos criterios. El diseño se basó en lo reportado previamente en la literatura de acuerdo con el tipo de terreno. Durante la reforestación se plantaron 14, 000 organismos de las especies *Abies religiosa* y *Pinus spp.* abarcando una extensión aproximada de 10 ha. Es necesario realizar el seguimiento de la zona reforestada para garantizar la supervivencia de la mayor cantidad de árboles



Incendios forestales, Jilotzingo, Reforestación

Area: Dissemination of and universal access to science

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Introduction

The municipality of Jilotzingo is situated in the central region of the Estado de Mexico, within the geographic coordinates 19º24'59" to 19º33'26" N latitude and 99º19'56" to 99º28'25" W longitude. The prevailing climate throughout the year is classified as temperate sub-humid. The mean annual temperature is approximately 13.7°C, with recorded minimum and maximum values of 5.6°C and 29.5°C, respectively.

Forestry production constitutes a significant economic activity that utilizes the region's natural resources, given that approximately 80% of the municipal territory is covered by forest.

The dominant arboreal species within these ecosystems include oaks [*Quercus spp*.], firs [*Abies spp.*], and pines [*Pinus spp.*]. These forested areas play a critical role in maintaining the ecological equilibrium of the northern sector of Mexico City and its surrounding metropolitan zone [Jilotzingo, 2024].

In March 2024, the municipality experienced a severe series of wildfires that devastated approximately 760 ha. Among the most affected localities were San Luis Ayucan, Santa María Mazatla, and Rancho Blanco [Facebook Gobierno Municipal de Jilotzingo, 2024]. Figure 1 presents a map of the territory corresponding to Jilotzingo; the dotted lines indicate the municipal boundaries. Yellow markers denote areas where low-intensity fires occurred, while red markers indicate sites impacted by high-intensity wildfires [Dirección de Catastro de Jilotzingo, 2024].

Reforestation can be defined as the process through which tree planting is carried out [Mohan y cols., 2021; Fesenmeyer y cols., 2025] encompassing the stages of planning, implementation, monitoring, and supervision.

There are two primary types of reforestations: urban and rural.

Urban reforestation refers to tree planting within city environments and may serve various purposes, such as providing shade, noise mitigation, aesthetic enhancement, among others.

Rural reforestation is conducted on forested or potentially forested lands that previously supported tree cover, which has been lost due to factors such as illegal logging, wildfires, or soil erosion.

Rural reforestation is further classified into four categories based on its intended purpose: conservation, protection and restoration, agroforestry, and productive reforestation [Comisión Nacional Forestal, 2010].

Box 1

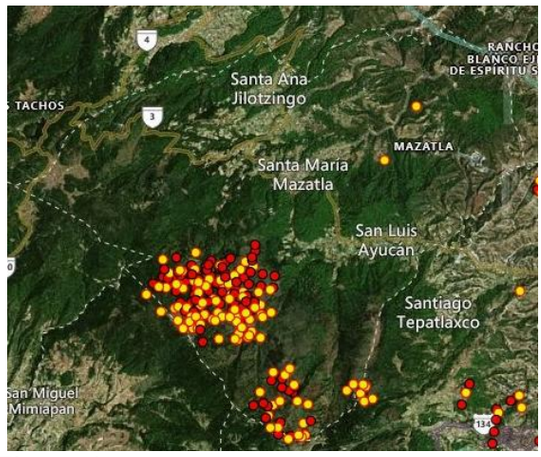

Figure 1
Fire Alerts in the Municipality of Jilotzingo during March 2024.
Source: Dirección de Catastro de Jilotzingo, 2024

To carry out restoration-oriented reforestation, it is necessary to employ a range of tools that enable the reestablishment of the forest's original structural composition and species diversity, as well as to avoid actions that reduce plant germination rates and survival [Drake et al., 2015]. This is why several strategies can be applied to achieve ecosystem restoration, among which the most common are Rehabilitation, Replacement, and Revegetation.

The Rehabilitation strategy aims to reintroduce certain plant and animal species that previously inhabited the site, with the objective of halting ongoing environmental degradation processes [Vanegas López, 2016]. The purpose of this article is to propose the implementation of a restoration plan for the reforestation of an area affected by wildfire in the municipality of Jilotzingo, Estado de Mexico.

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Methodology

Selection of the Area for Reforestation and Tree Species

Periodic field visits were conducted in the areas affected by wildfires in the municipality of Jilotzingo to assess the extent of damage and to identify the types of flora present in the surrounding zones. This evaluation enabled the selection of appropriate plant species for the reforestation process.

The reforestation area was selected based on the following criteria:

- Degree of damage caused by the fires
- Type of native vegetation
- Terrain characteristics
- Surface area
- Accessibility via existing roads
- Willingness of nearby residents to participate in the project [Bustamante y cols., 2022]

Regarding the selection of tree species, the following criteria were considered:

- Characteristics of the selected reforestation area
- Preexistence of species in the affected zone
- Availability of seedlings [Di Sacco y colaboradores, 2021]

Land Preparation and Seedling Acquisition

Once the reforestation area was selected, manual weeding of invasive vegetation was carried out, along with manual soil preparation.

The clearing was performed using tools such as hoes, pickaxes, and shovels, with the aim of preventing competition for resources [water, light, nutrients] between the seedlings and surrounding weeds. Additionally, soil preparation included digging planting holes for transplanting the seedlings [Secretaría de Medio Ambiente y Recursos Naturales, 2018]. To obtain the seedlings, donations were sought from institutions or nurseries located near the reforestation zones, with support requested from the municipal government of Jilotzingo.

Reforestation Design

Based on the terrain conditions, a Tresbolillo planting design was proposed, as the slope of the land ranges between 20°C and 80°C.

In this configuration, plants are arranged in equilateral triangles. Given that tree density must not exceed 1,100 plants per hectare [Bustamante y cols., 2022; Preece y cols, 2023], the spacing between plants was set between 3.2 and 3.4 meters, both between rows and individual plants. Planting holes were excavated with dimensions of 40 x 40 x 40 cm [length, width, and depth].

During excavation, the first 20 cm of topsoil were placed on one side, and the deeper 20 cm on the other. This allowed for differentiation of soil layers. Once reforestation has been carried out, periodic visits will be conducted to assess the survival and growth of the planted trees.

Results

Selection of the Reforestation Area and Tree Species

During the series of field assessments conducted to evaluate the damage in the burned areas, it was observed that the understory was the most severely affected.

Although the canopy cover also sustained damage, it did not appear to be significantly compromised [see Figure 2].

Box 2

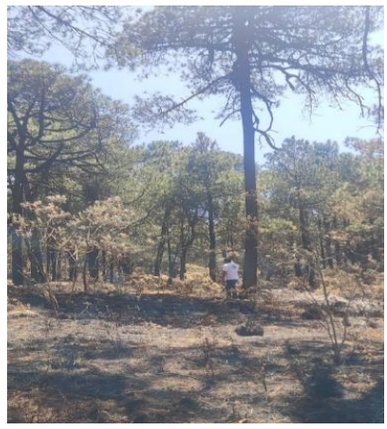

Figure 2

Understory vegetation in Peñuelas

Source: Author's own.

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In the surveyed areas, the soil appeared to have been preserved due to the presence of a thick layer of pine litter, which acted as a protective cover [see Figure 2].

Therefore, no reduction in nutrient availability was expected. A decrease in tree density was also observed [see Figure 3].

Box 3

Figure 3

Tree Density Sample in the Peñuelas Region.
Source: Author's own.

Finally, based on the established criteria, the most suitable site for reforestation was determined to be the locality of Villa Alpina [see Figure 4], located at coordinates: -99.380329 and 19.437050. Figure 4 shows the location of Villa Alpina; the yellow markers indicate areas affected by low-intensity fires, while the red markers represent sites where high-intensity forest fires occurred [Dirección de Catastro de Jilotzingo, 2024].

Regarding the species proposed for reforestation, since the majority of the forest is composed of *Abies religiosa* [Oyamel], *Pinus patula* [Mexican yellow pine / Ocote colorado], and *Pinus hartwegii* [Ocote blanco or high-altitude pine] [Rosaliano Evaristo y cols., 2022; Tellez y cols., 2020], these species were selected for reforestation.

Box 4

Figure 4

Location of Villa Alpina.
Source: *Dirección de Catastro de Jilotzingo, 2024*

Land Preparation and Seedling Acquisition

Site clearing was conducted one week prior to the reforestation. Weeding was performed carefully to avoid excessive removal of ground cover. Additionally, 14,000 seedlings of *Abies religiosa* and *Pinus* spp. were donated by PROBOSQUE, State of Mexico. The seedlings were transported with support from the Municipality of Jilotzingo [Figure 5].

Box 5

Figure 5

Transport of Seedlings for Reforestation.
Source: *Facebook page of the Municipal Government of Jilotzingo, 2024*

3.3 Reforestation

One month prior to the reforestation, a training process was conducted during which brigade leaders were selected [see Figure 6].

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Box 6

Figure 6

Training of brigade leaders.

Source: Facebook Municipal Government of Jilotzingo, 2024.

The municipal government of Jilotzingo issued a public call inviting local businesses and residents to participate in the reforestation effort.

The campaign, titled "Reforestación Jilotzingona," was promoted through social media platforms [see Figure 7].

Box 7

Figure 7

Banner of the "Reforestación Jilotzingona" Campaign.

Source: Facebook Municipal Government of Jilotzingo, 2024.

Finally, on August 11 at 7:30 a.m., the reforestation began with the participation of approximately 1,000 people, who planted 14,000 trees following the recommended protocols [see Figure 8].

Box 8

Figure 8

Participants in the Reforestation of Jilotzingo's Forests.

Source: Facebook Municipal Government of Jilotzingo, 2024.

Conclusions

One of the most critical aspects of any reforestation effort is post-implementation monitoring. Some authors measure the level of success as the degree to survival of trees at different points in time [Herborhn y cols., 2023].

For this reason, the design proposed in this study includes tracking tree growth to assess survival rates, evaluating the health status of individual trees and the forest, and detecting potential pests that could threaten tree survival [Mexal y cols., 2008]. Likewise, identifying the presence of illegal loggers or early signs of forest fires is essential to achieving the long-term goals of this initiative [Carrión-Vera, 2025; Souza Mosqueda, 2025].

Based on the above, it is concluded that the objective of this study was achieved. However, considering that the forests of Jilotzingo are part of the "Bosque de Agua," one of the main hydrological basins of Mexico City and its metropolitan area [López-García and Navarro-Cerrillo, 2021], it is crucial that projects like this be carried out on a continuous basis. Damage to these ecosystems could lead to the loss of vital ecosystem services necessary for the survival of residents in these densely populated regions of the country.

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Therefore, it is imperative that more initiatives of this nature be implemented.

Declarations

Conflict of interest

The authors declare no interest conflict. They have no known competing financial interests or personal relationships that could have appeared to influence the article reported in this article.

Author contribution

Rangel-Ruiz, Karelia Liliana: Design and implementation of training and Reforestation
Granados-Olvera, Jorge Alberto:

Implementation of training and Reforestation
Cureño-Jiménez, José Domingo Agustín:

Logistics of training and Reforestation
Calvillo-Beltrán, Sofía Valentina:

Implementation of training

SECIHTI area: Advocacy and attention to national problems

Availability of data and materials

Data are available

Funding

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Abbreviations

am: antes meridiano
C°: Centigrade grades
CDMX: Mexico City
cm: centimeters
ha: hectareas

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Article

Synthesis of Chitosan from Shrimp Exoskeleton an its Characterization by SEM and DRX

Síntesis de quitosano a partir del exoesqueleto del camarón y su caracterización mediante SEM y DRX

Fuentes-Romero, María Teresa [a], Bermúdez, Jesús Nicolás [b], Medina-Mendoza, Manuel [c] and Maldonado-Mondragón, Erick Antonio [d]

[a] Universidad Tecnológica Fidel Velázquez • LBI-6364-2024 • 0009-0002-6981-3045 • 160544
[b] Universidad Tecnológica Fidel Velázquez • KZT-8961-2024 • 0000-0001-8104-4096 • 705130
[c] Universidad Tecnológica Fidel Velázquez • KZU-6258-2024 • 0000-0003-0912-0124 • 714543
[d] Universidad Tecnológica Fidel Velázquez • KZT-9152-2024 • 0009-0005-6674-5296 • 1105931

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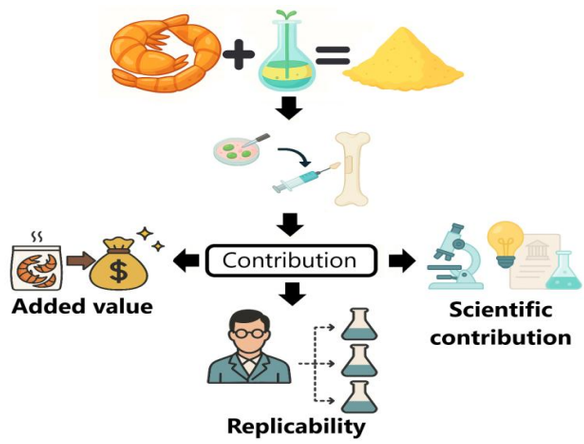
*✉[erick.maccio@gmail.com]

Abstract

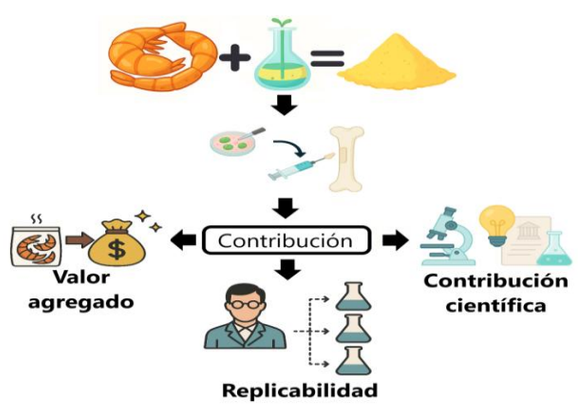
Currently, sustainable alternatives are being explored for the development of biomaterials from organic waste. In this study, **chitosan [CTS]** was extracted from shrimp shells through a process of demineralization, deproteinization, and deacetylation. The resulting material was **characterized** using SEM and DRX techniques, confirming its structure and purity. To assess its **biocompatibility**, **in vitro** assays were performed using **C6 glial cells** [from rat], applying the **Alamar Blue** method to evaluate **cell viability**. The results showed good cell tolerance to chitosan, indicating its potential as a biomaterial for **tissue engineering** applications. This approach represents an eco-friendly, feasible, and low-cost alternative for the development of functional materials derived from marine waste.



Biomaterial, Chitosan, Tissue

Resumen

Actualmente, se buscan alternativas sostenibles para desarrollar biomateriales a partir de desechos orgánicos. En esta investigación, se obtuvo **quitosano [CTS]** a partir de cáscaras de camarón mediante un proceso de desmineralización, desproteinización y desacetilación. El material obtenido fue **caracterizado** mediante técnicas como SEM y DRX, confirmando su estructura y pureza. Para evaluar su **biocompatibilidad**, se realizaron ensayos **in vitro** utilizando células **C6** [gliales de rata], aplicando el método **Alamar Blue** para determinar la **viabilidad celular**. Los resultados mostraron una buena tolerancia de las células al quitosano, lo que indica su potencial como biomaterial para aplicaciones en **ingeniería de tejidos**. Este enfoque representa una alternativa ecológica, viable y de bajo costo para el desarrollo de materiales funcionales a partir de residuos marinos.



Quitosano, Biocompatibilidad y tejidos

Area: Dissemination of and universal access to science

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Introduction

In recent decades, the increasing volume of agro-industrial and marine waste has motivated an active search for sustainable solutions that allow their reuse as raw materials for the development of new functional materials. In this context, shrimp shells, rich in chitin, represent an economical and abundant source for the production of chitosan [CTS], a versatile biopolymer with biomedical, pharmaceutical, and environmental applications [Zhou et al., 2023].

Chitosan is a linear polysaccharide obtained by the partial deacetylation of chitin, a natural polymer mainly found in the exoskeletons of crustaceans. The transformation process of chitin into chitosan is based on three successive stages"demineralization, deproteinization, and alkaline deacetylation"in which inorganic salts, proteins, and acetyl groups are removed, respectively, generating a material with high chemical and biological functionality [Ahmed et al., 2025]. This conversion may also include a final chemical precipitation step, where the pH is adjusted to induce controlled insolubilization of the chitosan and facilitate its recovery [Olafadehan et al., 2021].

Various factors such as temperature, reagent concentration, reaction time, and the type of solvent used during the extraction process can significantly affect the characteristics of the obtained chitosan. Recent studies have reported products with a low content of impurities, such as ash [0.72%] and residual proteins [less than 0.13%], evidencing the efficiency of modern purification processes [Shahidi &Abuzaytoun, 2005].

Interest in chitosan has grown significantly due to its biodegradability, biocompatibility, antimicrobial activity, and its ability to form films, gels, micro- and nanoparticles, among other functional structures [Chen et al., 2021]. These properties make it an ideal candidate for applications in tissue engineering, where the need for biodegradable and bioactive scaffolds is essential to promote cell regeneration. In addition, its cationic nature allows the formation of electrostatic bonds with cell surfaces and materials, which further expands its functionality [Elias et al., 2022].

From a structural characterization perspective, techniques such as Scanning Electron Microscopy [SEM], which allows observing the surface morphology of the material, and X-Ray Diffraction [XRD], which provides information about its degree of crystallinity and internal structure, are commonly used [Zhong et al., 2023; Nouj et al., 2021].

Regarding its biocompatibility, chitosan has been shown to be well tolerated by various cell lines, such as fibroblasts, tumor cells, and neuronal cells. In this study, cell viability was evaluated using the C6 cell line [rat glioma], widely used as a neurotoxicity model. For this evaluation, the colorimetric Alamar Blue assay was employed, which quantifies cellular metabolic activity through the conversion of resazurin to resorufin, without generating significant cytotoxicity [Thermo Fisher Scientific]

Recent studies support the use of chitosan as a safe and efficient material in biomedical applications. For example, it has been reported that chitosan extracted from shrimp shells exhibits high compatibility with HEK 293 cells and induces a minimal inflammatory response. Furthermore, its behavior in solution and strong adsorption capacity for dyes and bioactive compounds underscore its potential not only in regenerative medicine but also in water treatment and other environmental applications. [Çelikçi et al., 2020; Mathew et al., 2020].

Specifically, it was observed that chitosan nanoparticles with silibinin [SCNP] were internalized by C6 cells and induced apoptosis by increasing Bax and caspase-3 expression, without affecting H9C2 cells [Takke et al., 2021]. In addition, chitosan-eugenol nanoparticles induce apoptosis and inhibit metastatic signals in C6 cells, confirming their antitumor potential [Li et al., 2020].

In summary, the valorization of marine waste, such as shrimp shells, for the production of chitosan represents a strategy that combines environmental sustainability with technological innovation. This biopolymer, obtained through efficient chemical methods, can be characterized and validated using structural tools and cellular assays, thereby contributing to the development of new functional biomaterials with a wide range of applications.

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The main objective of this research work was to synthesize and characterize chitosan from shrimp shells through a chemical precipitation process, using analysis techniques such as scanning electron microscopy [SEM], X-ray diffraction [XRD], and the Alamar Blue cell viability assay.

This proposal aims to add value to an agro-industrial waste by revaluing a marine residue through its transformation into a functional biomaterial with potential biomedical applications.

Through the study of C6 cells, the aim is not only to validate the biocompatibility of the obtained chitosan but also to explore its ability to support the viability, proliferation, and possible differentiation of neural-origin cells, which is key for future applications in nervous system tissue engineering, glioma therapies, or neurologically oriented drug delivery platforms.

Evaluating the interaction between chitosan and this cell line will allow identifying whether the biomaterial possesses the necessary properties to be considered a bioactive, safe, and efficient scaffold capable of functionally integrating into complex cellular systems.

2. Materials and Methods

Shrimp exoskeletons were obtained from local markets in Nicolás Romero, State of Mexico, representing an abundant and inexpensive raw material rich in chitin.

Throughout the entire procedure, analytical-grade reagents and freshly prepared solutions were employed to maintain experimental consistency and to minimize possible interferences during the structural and biological characterization of the resulting chitosan.

Using high-purity reagents—particularly hydrochloric acid and sodium hydroxide at controlled concentrations—has been recognized in previous studies as a critical factor for producing chitosan of high quality, with a uniform surface morphology and an elevated degree of deacetylation [Hisham et al., 2021].

Box 1

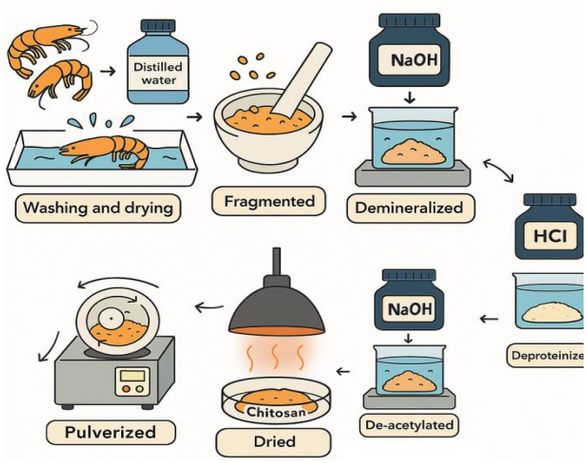

Figure 1
Methodology for obtaining chitosan

2.1. Chitosan synthesis

The process of obtaining chitin was carried out through chemical precipitation applied to shrimp exoskeletons collected from local markets in the municipality of Nicolás Romero, State of Mexico. Initially, the residues attached to the shells were removed by continuous jet washing, eliminating any superficial organic matter [Xie et al., 2021].

Subsequently, the exoskeletons were dried under an infrared lamp for one hour, simplifying water removal and facilitating the subsequent grinding process. The dried exoskeletons were subjected to primary grinding using a ceramic mortar and then pulverized in a planetary ball mill model BIC0400-0.4, in order to reduce particle size and improve the material's handling properties [Tuhua et al., 2020].

Box 2

Figure 2
Planetary mill model BIC0400-0.4

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This procedure follows efficient protocols described in the literature, which state that "shrimp shell waste is one of the main sources used for chitin preparation, with a content of approximately 15 to 40% of the dry weight of the shrimp shell" [Nanoxhitosan study, 2024]. Therefore, it is possible to obtain chitosan powder using a planetary mill. Likewise, more recent research has pointed out that particle size directly influences key characteristics of chitosan. For example, it has been found that "prolonged milling significantly reduces the molar mass of chitosan," which is a desirable property for certain applications, demonstrating that the milling operation is decisive for the final structure of the polymer [Helton et al., 2017].

Once the shrimp exoskeleton powder was pulverized, three fundamental phases were carried out to obtain chitosan: demineralization, deproteinization, and deacetylation. The first demineralization step was performed by immersing the material in a 0.6N HCl solution for 2 hours at room temperature, with the aim of eliminating calcium carbonate. This procedure coincides with that reported by Marina et al. [2022], who state that "shrimp shell waste is one of the main sources used for chitin preparation, and demineralization is essential for removing the mineral content present in the biological matrix."

Subsequently, the sample was subjected to deproteinization using a 1% NaOH solution for 24 hours under constant stirring, a process that facilitates the breaking of peptide bonds. Gao et al. [2024] note that alkaline treatment is the most effective method for removing proteins from chitinous materials without altering the integrity of the polymer.

Finally, the deacetylation of chitin was carried out to transform it into chitosan. Initially, a pretreatment with 3% acetic acid was applied, followed by filtration, and then the material was introduced into a 50% NaOH solution for 2 hours at 100°C. This protocol aims to achieve a high degree of deacetylation, a condition that is essential to improve the solubility and reactivity of chitosan. According to a study published in *Polymers* [2023], "NaOH concentrations of 40 to 50% are commonly used to induce deep deacetylation under heterogeneous conditions while maintaining the fibrous structure of chitin."

Box 3

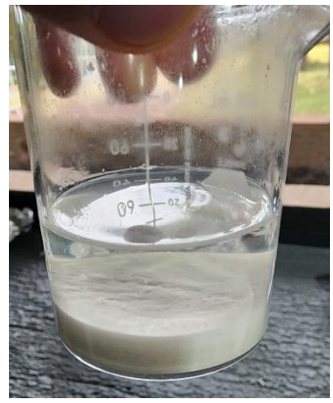

Figure 3
Chitosan production

Regarding the cell viability tests, rat glioma cells [C6 cell line] were used, which were incubated for 24 hours with chitosan at concentrations of 100 and 200 mg/mL, performing three independent replicates.

Subsequently, the Alamar Blue reagent was added, and fluorescence was measured to quantify cell viability. The data were analyzed using one-way ANOVA followed by Dunnett's post hoc test, with the aim of comparing each treatment against the control. This statistical approach is commonly used in cytotoxicity studies, including the evaluation of chitosan nanoparticle formulations on C6 cells, where the correlation between cellular metabolism and viability after 24 hours of exposure is observed.

Box 4

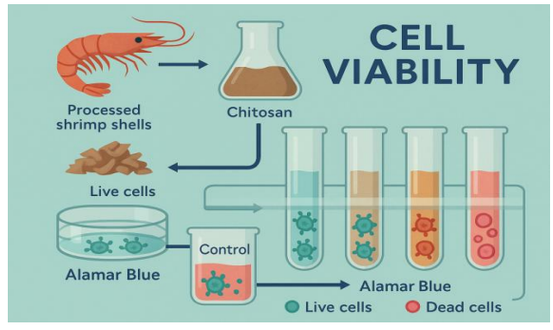

Figure 4
Cell viability test in C6 cells

3. Results

3.1 Morphological Analysis

The agglomerate obtained from shrimp shells was processed using a planetary ball mill, with the purpose of reducing the particle size to an ultrafine range.

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Box 5

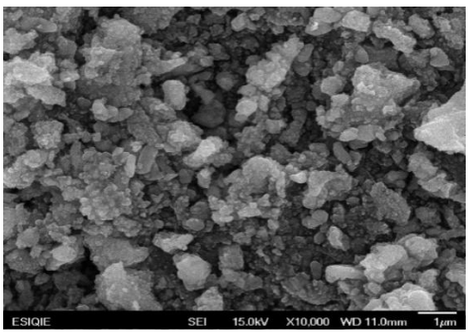

Figure 5

Chitosan scanning by scanning electron microscopy [SEM]

Subsequently, the samples were analyzed using electron scanning, and the morphology and particle size were evaluated with the Digital Micrograph software from Gatan Microscopy.

Box 6

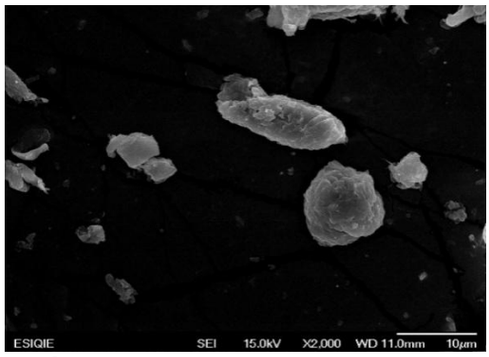

Figure 6

Scanning of a chitosan particle using

As a result of the milling process, a particle size distribution between 1.0 and 1.5 µm was obtained, with an average size of 1.3393 µm and a standard deviation of 0.5 µm.

Box 7

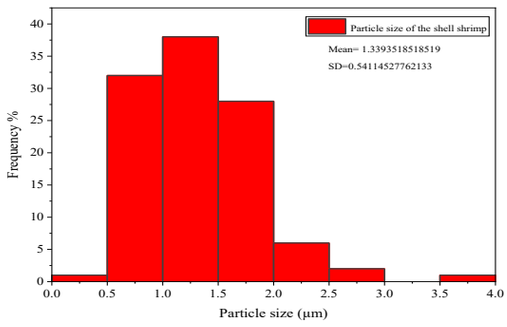

Figure 6

Determination of chitosan particle size after milling with a planetary mill

This behavior is consistent with previous studies where the use of planetary mills has made it possible to achieve fine micrometric sizes. For example, Alves et al. [2018] reported intermediate sizes [D50] around 1.3 microns when milling chitosan in a ball mill.

The planetary mill operates through centrifugal forces combined with repetitive impacts between the balls and the material inside the container. These impacts cause fractures and exfoliation of the polymeric structure, reducing the size and potentially decreasing the molar mass [depolymerization] without significantly altering the degree of deacetylation.

Box 8

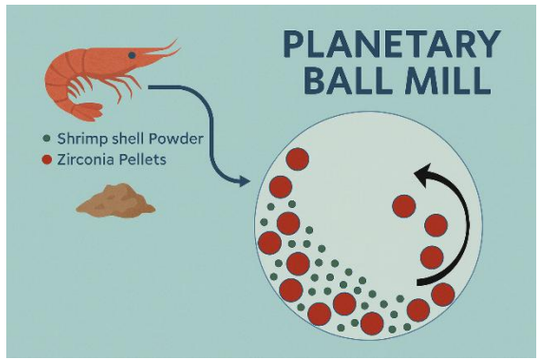

Figure 8

Internal operation of a planetary mill

3.2. Characterization by XRD

The results of the X-ray diffraction [XRD] analysis for the chitin samples extracted from shrimp shells are presented in the following figure [Figure 9].

In the spectrum, two high-intensity peaks were observed at 0° and 19.45° [2θ], as well as secondary peaks of lower intensity at 22.95°, 26.01°, 32.90°, and 39.22°. These diffraction patterns are characteristic of the crystalline structure of α-chitin, which is widely reported in the literature as the main allotrope of chitin present in crustacean exoskeletons.

The peaks of highest intensity around 9°–10° and 19°–20° correspond to the crystallographic planes [020] and [110], respectively, which reflect the orthorhombic conformation typical of α-chitin [Liu et al., 2012; Ravindra et al., 2014].

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Meanwhile, the minor signals observed at 22.95°, 26.01°, 32.90°, and 39.22° have also been reported in previous studies and are attributed to less intense reflections characteristic of the laminar organization of partially ordered chitin [Younes &Rinaudo, 2015].

Box 9

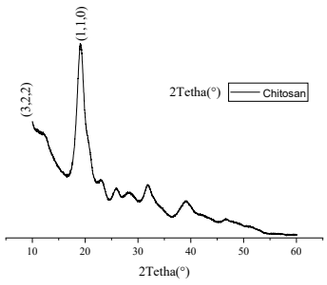

Figure 9

X-ray diffraction spectroscopy of chitosan

Although slight variations in peak position and intensity may occur due to factors such as the degree of deacetylation, moisture content, or the presence of residual impurities, the results obtained show a high degree of similarity with the patterns reported by other authors for chitins extracted from crustaceans, which validates the efficiency of the extraction process used.

3.3. Viability with C6 Cells

In the interpretation of cell viability performed with the C6 cell line using the Alamar Blue assay, treatments with chitosan were applied on different plates [1–4]. According to the statistical analyses performed [ANOVA and Dunnett's multiple comparison test], slight differences were observed compared to the control group.

The concentrations analyzed were 100 mg/mL and 200 mg/mL. As shown in the following figure, the concentration of 100 mg/mL resulted in a slight increase in cell viability, reaching approximately 101% compared to the control group, which suggests a possible metabolic stimulation without evidence of cytotoxicity. On the other hand, the concentration of 200 mg/mL showed a slight decrease in viability, recording a value close to 99% compared to the control.

Although both variations are subtle, these results indicate that chitosan, at the evaluated concentrations, does not exhibit marked cytotoxic effects, and its impact on cell viability may depend on the dose.

Box 10

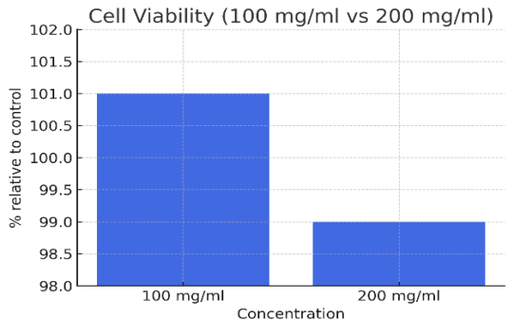

Figure 10

Cell viability of chitosan compared to the control in C6 cells

Box 11

Table 1

Cytotoxicity analysis of chitosan at doses of 100mg/ml and 200mg/ml in plates with C6 cells

Compound	Concentration	Plate 1	Plate 2	Plate 3	Plate 4
Chitosan	T1 100 mg/mL	–	–	Yes [+]	–
Chitosan	T1 200 mg/mL	–	–	Yes [+]	–

4. Discussion

During this research study, the synthesis of chitosan was carried out using the chemical precipitation method, employing biological residues from shrimp exoskeletons as raw material, with the aim of adding value to this type of marine waste.

The process focused on the extraction of chitin and its subsequent deacetylation to obtain chitosan, a biopolymer with well-known bioactive properties and potential applications in the biomedical and pharmaceutical fields.

For the characterization of the synthesized materials, three main analytical techniques were employed: scanning electron microscopy [SEM], X-ray diffraction [DRX], and the Alamar Blue cell viability assay, which

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allowed the evaluation of the biological behavior of chitosan in contact with C6 cells.

The DRX analysis enabled the identification of the crystalline structure of the extracted material. In the diffraction pattern, two high-intensity peaks were observed at 2θ angles of 10° and 19.45°, accompanied by lower-intensity peaks at 22.95°, 26.01°, 32.90°, and 39.22°.

According to the consulted literature, also reported by Kaya et al. [2014] and Alma et al. [2019], the main peaks in the range of 9° to 19° correspond to the crystallographic planes [020] and [110], which are characteristic of the orthorhombic crystal lattice that defines α-chitin. These results confirm that the structure of the biopolymer was preserved after the extraction and modification process.

On the other hand, the morphological analysis using SEM allowed the observation of the shape and size of the chitosan particles, which were previously optimized by milling in a planetary ball mill.

This mechanical size reduction technique proved to be effective, as it allowed the production of particles with sizes ranging between 1.0 and 1.5 µm, with an average size of 1.3393 µm. This reduction in particle size is relevant, as it improves the surface reactivity of chitosan and its performance in applications involving cellular interaction, encapsulation, or controlled release.

Finally, the results obtained from the cell viability assay using Alamar Blue, applied to C6 cell lines treated with different chitosan concentrations, indicate that this biopolymer does not exert significant cytotoxic effects under the evaluated conditions.

The slight increase in viability observed at 100 mg/mL [≈101 %] could be related to a mild stimulation of cellular metabolic activity, which has been previously reported in studies where moderate chitosan concentrations promote cellular proliferation or activation processes. In contrast, the reduction observed at 200 mg/mL [≈99 %] suggests that, at higher concentrations, chitosan could begin to generate an inhibitory cellular response, although not enough to be considered cytotoxic.

The absence of statistically significant differences compared to the control group indicates that, within the studied concentration range, chitosan exhibits a biocompatible profile in the C6 cell line. This finding is consistent with reports in the literature, which recognize that cellular response to chitosan may vary depending on its concentration, molecular weight, degree of deacetylation, and physical form [soluble or particulate].

In conclusion, the chitosan synthesized from shrimp exoskeletons shows safe behavior in terms of cell viability in C6 cells at concentrations of up to 200 mg/mL. These results support its potential as a biocompatible material for biomedical applications, although additional studies will be necessary to evaluate its performance in other cell types, as well as its influence on processes such as proliferation, differentiation, or apoptosis.

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To the team that collaborated in the development of this research project.

Declarations

Conflict of Interest

The authors declare that they have no conflicts of interest. They have no known competing financial interests or personal relationships that could appear to influence the work reported in this article.

Authors' Contribution

*Fuentes-Romero, María Teresa***:** Contributed to the project idea, research method, and technique. Supported the design and concept of this research. Helped guide the study through previous investigations carried out by her.

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*Bermúdez, Jesús Nicolás***:** Contributed to the research design, type of study, methodology, and the writing and editing of the article, as well as providing suggestions for the synthesis of this research.

*Medina-Mendoza, Manuel***:** Contributed to data analysis for the characterization of the equipment used, such as X-ray diffraction spectroscopy analysis, scanning electron microscopy, energy-dispersive spectroscopy, and article revisions related to technical processes.

*Maldonado-Mondragón, Erick Antonio***:** Contributed to experimental design, optimization of the synthesis process, and prior research for the production of hydroxyapatite, as well as in writing the article.

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Antecedents

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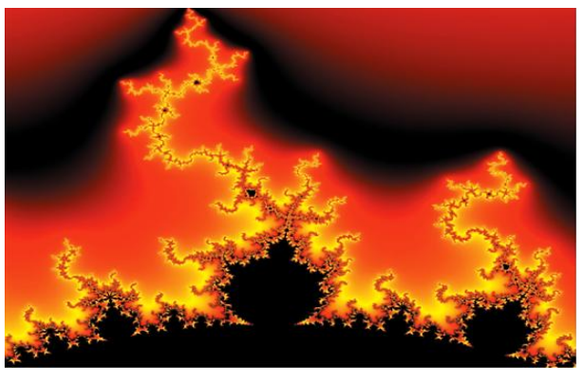

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