Handbook T-XXV

CIERMMI Women in Science

Applie Social Innovation

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Handbooks

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ECORFAN CIERMMI Women in Science
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Volumen XXV

The Handbook will offer volumes of selected contributions from researchers who contribute to the scientific dissemination activity of the Colegio de Ingenieros en Energías Renovables de Querétaro A.C in areas of Innovation social. In addition to having a full evaluation, in the hands of the coordinators of the Colegio de Ingenieros en Energías Renovables de Querétaro A.C, quality and punctuality in its chapters, each individual contribution was refereed to international standards [V|LEX, RESEARCH GATE, MENDELEY, GOOGLE SCHOLAR and REDIB], the Handbook thus proposes to the academic community, recent reports on new developments in the most interesting and promising areas of Innovation social.

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Preface

The volume Applied Social Innovation brings together works focused on the advancement of innovative solutions that respond to social challenges in various areas of science and technology. Each paper stands out for its contribution in addressing real problems that affect everyday life, health and sustainable development in Mexico and the world.

The first chapter examines the human right to water in Mexico, analyzing how the water crisis challenges the capacity of government and communities to guarantee adequate access to water, considering critical legislative and environmental aspects. The second chapter explores the interaction of enzymes and electric fields, seeking future applications in biotechnology through an in-depth understanding of their biophysical effects. BTEX inhalation risk analysis in urban areas, as presented in the third chapter, assesses the health risks of communities exposed to contaminants from service stations.

Other studies analyze public health issues, such as the research in the fourth chapter on the relationship between ghrelin polymorphism and overweight and obesity in Mexican youth, which raises new possibilities to address this health crisis. In the fifth chapter, the development of a mobile application for sex education in adolescents shows the potential of digital technologies to improve the prevention of sexually transmitted diseases and contraceptive practices. The sixth chapter focuses on the experience of nurses with electronic systems in the early detection of cervical cancer, highlighting the relevance of technology in improving clinical records.

The Handbook closes with a discussion of biotechnology in the chapter on in vitro fertilization in small animals, a technique that represents a breakthrough in animal breeding and could be widely applied in livestock production.

The research offers a comprehensive perspective on social innovation, showing how scientific knowledge can transform challenges into opportunities for development and welfare for society.

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Content

Content and scope of the human right to water. The water crisis in Mexico

Contenido y alcance del derecho humano al agua. La crisis hídrica en México

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Key Handbooks

The research has a dogmatic approach, however it makes contributions to the science of law regarding the water crisis, the rights involved and the existing problems in this regard. The aspects to understand to apply to the generation of universal knowledge?. Scales and content of the human right to water, essential in the global crisis we are experiencing, are established, as well as an analysis of what is causing the problem. The main conclusions of the research. The humanright to water focuses on the possibility of accessing this vital liquid so that it is accessible, available, in sufficient quantity for human subsistence and personal hygiene requirements and the home where one resides. It is now unquestionable that it is an independent human right despite its close connection with other rights such as health, food, housing and the right to a healthy environment.

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Abstract

The water crisis in Mexico is worsening day by day and the solutions seem distant in the face of the government's constant efforts to alleviate the effects of its shortage on the population, particularly in the most pressing needs of the vital liquid in its domestic use, its accessibility , insufficiency, lack of infrastructure and poor quality; as well as the causes that give rise to the emergency situation such as climate change, waste and contamination of aquifers. In the face of this problem, the full exercise of the human right to water seems unattainable. The present work examines the content and scope of the right, considering its scarce legislative regulation and what are the impacts that the aforementioned crisis causes in its exercise. This is a dogmatic investigation, which is based on the hypothesis of the current impossibility of guaranteeing the human right to water in Mexico. Its objective focuses on highlighting the problems that afflict the law that is the subject of the study in the face of the problems that the water situation in Mexico reveals. The methods used in the research are historical, legal epistemology, comparative and legal hermeneutics. The main techniques are the review of literature, legislation and jurisprudence that allow us to verify the hypothesis.

Content and scope of the human right to water. The water crisis in Mexico		
Goals	**Methodology**	**Contribution**
General: Examine the human right to water and its impact on the water crisis	Literature review	Establishes the characteristics of the human right to water and determines its safeguarding and exercise
General: Analyze the content of the human right to water Establish the possible limitations to the right	Literature review	Establishes what is the protected content of the human right to water Determines whether the right can be limited in its exercise by the state and the scope of its obligation in its protection.
Examine existing legislation	Information analysis	Determines the ineffectiveness of existing legislation to safeguard the right
Review applicable jurisprudence	Information analysis	Establishes the precariousness of existing jurisprudence

Water crisis, Human rights, Mexico

Resumen

La crisis hídrica en México se agrava día a día y las soluciones parecen lejanas de cara a los constantes esfuerzos del gobierno para paliar los efectos de su escasez en la población, particularmente en las necesidades más apremiantes del vital líquido en su uso doméstico, su accesibilidad, insuficiencia falta de infraestructura y mala calidad; así como las causas que originan la situación de emergencia tales como el cambio climático, el desperdicio y la contaminación de los mantos acuíferos. De cara a esta problemática el pleno ejercicio del derecho humano al agua parece inalcanzable. El presente trabajo examina el contenido y alcances del derecho en comento su escasa regulación legislativa y cuáles son los impactos que en el ejercicio del mismo causa la mencionada crisis. Se trata de una investigación de carácter dogmático, que parte de la hipótesis de la imposibilidad actual de garantir el derecho humano al agua en México. Su objetivo se centra en evidenciar los problemas que aquejan al derecho materia del estudio ante los problemas que la situación hídrica de México patentiza. Los métodos empleados en la investigación son el histórico, epistemología jurídica, comparativo y la hermenéutica jurídica. Las principales técnicas son la revisión de literatura, legislación y jurisprudencia que nos permiten comprobar la hipótesis.

Crisis hídrica, Derechos humanos, México

Contenido y alcances del derecho humano al agua. La crisis hìdrica en Mèxico		
Objetivos	**Metodología**	**Contribución**
General: Examinar el derecho humano al agua y su afectación por la crisis hídrica	Método analítico aplicado con la técnica de revisión de lietratura	Establece las características del derecho humano al agua y determinar su salvaguarda y ejercicio
Generales: Analizar el contenido del derecho humano al agua Establecder las posibles acotaciones al derecho	Método analítico aplicado con la técnica de revisión de lietratura	Establece cuál es el contenido protegido del derecho humano al agua Determina si el derecho puede ser limitado en su ejercicio por el estado y los alcances de la obligación de éste en su tutela
Examinar la legislación existente	Hermenéutica jurídica aplicada con la técnica de análisis de leyes	Determina la ineficacia de la legislación existente para salvaguardar el derecho
Revisar la jurisprudencia aplicable	Epistemología jurídica	Establece la precariedad de la jurisprudencia existete

Introduction

Given the unstoppable climate change that affects the entire world, there are multiple problems that arise from them and that affect the exercise of some essential rights for human survival. This work and research focuses on one of those rights that undeniably correspond. To those without which human beings cannot exist, we speak of the so-called human right to water, whose scope and responsibility for protection will be examined with the aim of establishing with crystal clarity what the former right protects and who is responsible. and to what degree, of its safeguarding.

In principle we must establish that the right in question has not been expressly enshrined in international instruments, however international regulations establish guidelines for states to guarantee access to drinking water for their nationals within the guidelines of international instruments. in which accessory mention is made of the right, for example the International Convention on the Rights of Persons with Disabilities or the Universal Declaration of the Rights of the Child, which in their articles make reference to this right in a random manner when referring to their right to health (UN, s/f) The UN recognizes it as an independent right as of 2014 and the Manual was prepared in that same year, *Realization of Human Rights to Water and Sanitation*, by the first Special Rapporteur on the Human Rights to Water and Sanitation, Catarina de Albuquerque. (UN, 2014)

Likewise, it is necessary to emphasize that On July 28, 2010, through Resolution 64/292, the United Nations General Assembly expressly recognized the human right to water and sanitation, establishing the need for clean and potable water. , as well as sanitation for the realization of all human rights (UN, 2010)

As we will examine in subsequent paragraphs, the right is made up of a series of conditions that, rather than defining it, establish its content and limits, the latter not necessarily limited by the law, since there are extralegal factors that prevent access to water in many communities.

It is also debated whether it is an individual, social or development right, given the diverse conditions that affect its exercise, such as absence of rainfall, difficult access, insufficient budget, among others that will clearly affect the feasibility of the project. exercise of law.

Turning to the doctrine we find concepts that define it as “the most notable innovation in water management in modern history, to the extent that it seeks to return the individual to the center of the administration of the resource” (Mc Graw, 2011) that in our opinion Judgment does not even give us a glimpse of what the law is or contains; Salmón (2012:246) tells us that considering it as a human right constitutes “the legal reaction to ensure that every human being has access to quality water and in sufficient quantities.” It is complex to find a true definition of the right as such, but we could say that it is a human right inherent to human subsistence that safeguards the right of people to access the vital liquid called water for their personal and domestic consumption in a healthy, equitable and egalitarian way. Among the essential characteristics of access to water, Acosta and Martínez (2010:19) distinguish four, namely, that it is a human right, it is a national asset that is considered strategic and for public use, it is the heritage of society and finally, but of vital importance, water is a fundamental component of nature itself that has its own rights to exist and maintain its life cycles.

I. Legislative and jurisprudential framework of the human right to water

The legislative framework of law, regardless of what has already been mentioned regarding international instruments in the previous section, is part of its constitutional consecration since it expresses that our fundamental charter in its numeral 4, sixth paragraph, which to the letter determines, Article 4.- [...] Every person has the right to access, disposal and sanitation of water for personal and domestic consumption in a sufficient, healthy, acceptable and affordable manner. The State will guarantee this right and the law will define the bases, supports and modalities for the equitable and sustainable access and use of water resources, establishing the participation of the Federation, the federal entities and the municipalities, as well as the participation of citizens to the achievement of said purposes. (Congress of the Union, 1917)

The content of article 27 of the Constitution, which confers on the nation the original ownership of national waters and allows its concession by it to individuals, is also essential; Article 27. [...]

The waters of the territorial seas are property of the Nation to the extent and terms established by International Law; inland marine waters; those of the lagoons and estuaries that communicate permanently or intermittently with the sea; those of naturally formed inland lakes that are directly linked to constant currents; those of the rivers and their direct or indirect tributaries, from the point of the channel where the first permanent, intermittent or torrential waters begin, until their mouth into the sea, lakes, lagoons or estuaries of national property; those of constant or intermittent currents and their direct or indirect tributaries, when the channel of those, in all their extension or in part of them, serves as a limit to the national territory or to two federative entities, or when it passes from one federative entity to another or cross the dividing line of the Republic; that of lakes, lagoons or estuaries whose basins, areas or banks are crossed by dividing lines of two or more entities or between the Republic and a neighboring country, or when the limit of the banks serves as a boundary between two federative entities or the Republic with a neighboring country; those from springs that arise on beaches, maritime areas, channels, basins or shores of lakes, lagoons or estuaries of national property, and those extracted from mines; and the channels, beds or banks of the lakes and inland streams to the extent established by law. The subsoil waters can be freely illuminated by artificial works and appropriated by the owner of the land, but when the public interest requires it or other uses are affected [...] (Congress of the Union, 1917)

Article 115 of the Constitution establishes that municipalities must provide public services of drinking water, drainage, sewage, treatment and disposal of their wastewater. (Congress of the Union, 1917) The construction of the law under study in Mexico starts from this constitutional framework, emanating from it the ordinary laws that regulate and limit it, highlighting in this regard the National Water Law, the Federal Law of Rights and its respective regulations; the General Law of ecological balance and environmental protection,

The jurisprudence in this regard is quite precarious, since the majority of existing theses and jurisprudence resolve on issues related to the Law of Rights regarding national waters and we rarely find that with respect to the law in question it has been said that,

The human right to water recognized in article 4, paragraph six, of the Constitution is based on the recognition that access to healthy, safe, sufficient and affordable water for personal and domestic uses is an essential requirement for the realization of other human rights. . In any circumstance, the requirements of availability, quality and accessibility (physical, economic, non-discrimination and access to information) outlined in General Comment No. 15 of the ESCR Committee must be observed, as well as the obligations of respect, protection and State compliance. State authorities have the obligation to maintain a supply of water necessary for people to satisfy their personal and domestic uses. (SCJN, 2016) As noted, the court focuses on characteristics and content but does not establish a concept where we can find the legal nature of the right,

To the above we can add a couple of criteria that refer to human rights in relation to other rights, thus we find the jurisprudence that under the heading ECOLOGICAL BALANCE AND ENVIRONMENTAL PROTECTION. DISTRIBUTION OF POWERS THAT MUST BE ATTENDED FOR THE CONTROL OF WATER POLLUTION, which essentially establishes that,

In accordance with article 5, section XI, of the General Law of Ecological Balance and Environmental Protection, the powers of the Federation are, among others, the protection and preservation of national waters; For its part, article 4. of the National Water Law specifies that the authority and administration in matters of national waters and their inherent public goods corresponds to the Federal Executive, who will exercise it directly or through the National Water Commission; while, in accordance with articles 7o., 8o. and 9th. of the first-mentioned law, it corresponds to the States and Mexico City, among other powers: (I) the preservation and, where appropriate, the restoration of water; (II) the control of pollution of waters under state jurisdiction, as well as the national waters assigned to them; and (III) the application of legal provisions regarding the prevention and control of pollution of water discharged into the drainage and sewage systems of population centers, as well as the national waters assigned to them.

According to the above, it can be concluded that in the matter related to the prevention, preservation, protection and sanitation of water, the powers of the authorities are designed in accordance with a "territorial" scope, in which the federal public administration must ensure the ecological balance and the protection of natural resources of federal jurisdiction, that is, of the "national waters" referred to in the fifth paragraph of article 27 of the Political Constitution of the United Mexican States, while it will correspond to the States and Mexico City, the prevention and control of pollution of waters under state jurisdiction, that is, specifically, local authorities are responsible for controlling wastewater discharges into drainage and sewage systems; the surveillance of the corresponding official Mexican standards regarding the waters that are within its jurisdiction, as well as requiring those who generate discharges to said systems and do not comply with them, to install treatment systems.

Likewise the one under the heading RESPONSIBLE AUTHORITY FOR PURPOSES OF THE AMPARO TRIAL. THE MUNICIPAL WATER AND SANITATION SYSTEM OF SAN PEDRO DE LAS COLONIAS, COAHUILA DE ZARAGOZA HAS THIS CHARACTER, WHEN THE LACK OF DRINKING WATER SUPPLY IS COMPLAINED, which determines the quality of the responsible authority, which in the relevant case alleges,

Facts: A District Judge dismissed the indirect protection trial filed by the plaintiffs against the lack of supply of drinking water in conditions of quantity, quality and frequency, considering that the cause of inadmissibility provided for in article 61, section XXIII, was updated. in relation to the various 1st, section I and 5th, section II, of the Amparo Law, under the argument that the Municipal Water and Sanitation System of San Pedro de las Colonias, Coahuila de Zaragoza, does not have the character of responsible authority.

Legal criterion: This Collegiate Circuit Court determines that the Municipal Water and Sanitation System of San Pedro de las Colonias, Coahuila de Zaragoza, has the character of responsible authority for the purposes of the amparo trial when the lack of supply of drinking water is claimed, in conditions of quantity, quality and frequency.

Justification: The above, because article 4, sixth paragraph, of the Political Constitution of the United Mexican States (added by decree published in the Official Gazette of the Federation on February 8, 2012), provides as a fundamental right of individuals, that The State must guarantee the right to access, disposal and sanitation of water for personal and domestic consumption in a sufficient, healthy, acceptable and affordable manner, and that the law will establish the bases, supports and modalities for access and use of that resource. natural.

However, in the aforementioned State, the acts carried out in connection with the provision of water service for personal and domestic consumption, related to the collection and suspension of supply, are governed by articles 1, first paragraph, 2, 4, 11, 13, 21, sections II, XIII and XVIII and 86 of the Water Law for the Municipalities of the State of Coahuila de Zaragoza, which regulate the activity and set the limits of the systems municipal water and sanitation, so it is clear that said acts are vested with public power and, therefore, are considered issued on a level of supra to subordination, since they depend on what is established in this regard by the General Constitution and the own law of the matter and not what could be indicated in the adhesion contract as if it were an act of commerce, since the human right of access to water that was established in the Magna Carta is guaranteed for all people by providing of the public drinking water service, so it does not have its origin in the contractual will.

Without the jurisprudence thesis P./J. 92/2001, of the Plenary of the Supreme Court of Justice of the Nation, published in the Judicial Weekly of the Federation and its Gazette, Ninth Period, Volume XIV, July 2001, page 693, with digital registration number: 189353, of item: "DRINKING WATER. WHEN THE STATE PROVIDES THE SERVICE THROUGH ADMINISTRATIVE ADHESION CONTRACTS, THE RELATIONSHIP LEGAL BETWEEN THE PARTIES DOES NOT CORRESPOND TO THE SUPRA SUBORDINATION THAT EXISTS BETWEEN AN AUTHORITY AND A GOVERNED, BUT TO A RELATIONSHIP OF VOLUNTARY COORDINATION BETWEEN THE SERVICE PROVIDER AND THE INDIVIDUAL.", since this arose before the aforementioned constitutional addition. and taking as a basis that the water supply only depended on the agreement of wills expressed in the administrative adhesion contract, without considering that the provision of this service obeys a human right that the State must guarantee to individuals.

It is evident in light of the content of the inserted theses that regarding the exercise of law and its safeguarding, the court's pronouncements are very few and that is why not much has been paid to its consolidation through jurisprudence, although it has It has allowed us to clarify some procedural aspects such as legitimation, which will be analyzed in the subsequent section.

II. The exercise and safeguard of the right and its legitimation

Holder of the human right to water

If we look at the content that the right is given through UN Recommendation No. 15 as that which every person has. Observation No. 15 also defines the right to water as “the right of each person to have sufficient, healthy, acceptable, physically accessible and affordable for personal and domestic use” (UN, 2002) we would have to conclude that it is an individual right, however there are doctrinal discussions about this connotation of the right, since it is alleged which is an essential part of the right to a healthy environment and that gives it the characteristic of a social right.

We consider that the right allows individual exercise, but also has aspects of a social nature that allow collective actions in its defense.

The legitimate interest and procedural legitimacy for the exercise and safeguard of the human right to water has certain particularities that, as we stated in the preceding paragraph, generate the possibility of an individual exercise of the right, which does not generate any controversy, but can also be protected by the collective actions provided for in the Mexican federal constitution, the exercise of which is controversial and it is difficult for groups of citizens to prove their legitimate interest in said exercise, a dilemma recognized by the SCJN by establishing that,

Collective actions provided for by Article 17 of the Constitution and regulated in the Federal Code of Civil Procedures can be brought in matters of environmental protection. The right to a healthy environment is essential to live with dignity and, furthermore, is a precondition for achieving the enjoyment of other fundamental rights, such as the right to water, also protected by article 4. constitutional. In this context, it is amply justified to adopt the laxest possible view to appreciate the cause of asking for collective action linked to the rights to a healthy environment and water. (Rabasa Salinas and Medina Amaya, 2021:71)

A) Authorities responsible for safeguarding the right

In principle and in accordance with the aforementioned constitutional regulation, the federal government is responsible for everything that pertains to administration, control, protection of rights and everything that pertains to water in the country, with the exceptions that the law itself establishes, there being a body which par excellence manages everything related to water in Mexico and is the National Water Commission (CONAGUA) which in the terms of the National Water Law, in its third article section XII is, Deconcentrated Administrative Body of the Secretariat of Environment and Natural Resources, with Public Law functions in matters of management of national waters and their inherent public goods, with technical, executive, administrative, budgetary and management autonomy, to achieve its object, the performance of its functions and the issuance of the acts of authority that, in accordance with this Law, correspond to both it and the authority bodies to which it refers;

Regarding the responsibilities of the state as such, the Committee on Economic, Social and Cultural Rights establishes basic obligations in relation to the right to water, of a non-derogable nature,

a) Guarantee access to the minimum essential quantity of water, which is sufficient and suitable for personal and domestic use and to prevent diseases;
b) Ensure the right of access to water and water facilities and services on a non-discriminatory basis, especially with regard to vulnerable or marginalized groups;

c) Guarantee physical access to water facilities or services that provide a sufficient and regular supply of safe water; that they have a sufficient number of water outlets to avoid prohibitive waiting times; and that they are a reasonable distance from the home;

d) Ensure that personal safety is not threatened when people have to go to obtain water;

e) Ensure an equitable distribution of all available water facilities and services;

f) Adopt and implement a national water strategy and action plan for the entire population; The strategy and action plan must be developed and periodically reviewed based on a participatory and transparent process;

g) Monitor the degree of realization, or non-realization of the right to water (Bautista Justo, s/f:3-4)

What is related to judicial protection is exercised in actions of an administrative nature and protection, with administrative actions being regulated by the Federal Law of the contentious administrative procedure, although it is true that the law in Mexico is just being developed and there is little construction of the It exists despite the enormous problems that afflict the country regarding it.

Consequently, the exercise of the right corresponds to the person, who can exercise it individually or collectively, but it's safeguarding is the responsibility of the state in all its areas, in such a way that to do so it must refrain from directly or indirectly hindering its enjoyment; prevent interference from third parties and, finally, ensure that the legislation, administrative mechanisms and budgetary items are suitable to make the right fully effective, in summary it is about "respecting, protecting and making effective the human right to water, that is, on the one hand, that no one can be deprived of access to water and, on the other, proactive actions by the State are required and necessary to ensure that people gain access to it when they do not have it." (Milán Rodríguez, 2021:774)

III. Its correlation with other human rights

Most rights present interrelationships among themselves, therefore, it is not surprising that the human right to water presents them. In this section we will examine the rights with which it has a close relationship, to the extent that it has been considered as part of some of them.

A) Right to health 

There is undoubtedly a close relationship between the right under study and the right to health, since in principle human survival depends on water, avoiding diseases that reach such levels of severity that they can cause death depends on the quality of the water, as well The World Health Organization (WHO) has highlighted this by establishing that

Contaminated water and poor sanitation contribute to the transmission of diseases such as cholera, other diarrheal diseases, dysentery, hepatitis A, typhoid fever and polio. If there are no water and sanitation services, or if they are insufficient or inappropriately managed, the population is exposed to health risks that, in reality, can be prevented. This prevention could be achieved especially in health facilities without water, sanitation and hygiene services, where both patients and professionals are at greater risk of contracting infections and diseases. (OMS, 2022)

Consequently, the close relationship between both rights is incontestable and the health of contemporary states definitely depends on the realization of the human right to drinking water.

B) Right to a healthy environment 

The interaction between this human right and that of water is unquestionable, to the extent that the second has been considered a necessary presupposition to achieve the first, thus Nava Escudero (2021:694) tells us in this regard that

Specifying or limiting the content of what the well-being or quality of, as well as what the natural and built environments encompass, is the task of those who influence the development of the respective regulations. In this work, it must be taken into account that there are legal rights related (although independent) to the environment, which means that the right to the environment is linked to other rights (constitutionally recognized or not), such as the right to health, the right to sustainable development, the right to water, the right to the atmosphere, the right to cultural heritage, etc.

And later the author adds in a quote that "from the legal point of view, the right to the environment is simultaneously an individual and collective right, which generates duties of action and abstention of the State. It is a right that complements, but that at the same time is complemented by other rights."(Nava Zamora, 2021:696). Consequently, we cannot speak of a healthy environment when citizens cannot access drinking water and rely on contaminated water to meet their personal, hygiene and domestic needs.

C) Right to food

With an express constitutional consecration, this right is contained in the fourth article of the fundamental charter, together with the right to water, health and housing, so it seems that the constitutional legislature considers them so closely interconnected that he regulates them in the same constitutional precept, this following the reform of said article in 2011 and 2012.

The UN Committee on Socio-Economic, Social and Cultural Rights tells us that "The right to adequate food is realized when every man, woman and child, whether alone or in common with others, has physical and economic access, at all times. , to adequate food or means to obtain it."(UN, 2002:3); It also makes the pertinent clarifications so as not to confuse the right with a right to be fed, on the contrary the right to food is translated as "the right to feed oneself in conditions of dignity. People are expected to meet their own needs with their own efforts and using their own resources. A person must live in conditions that allow him or her to either produce food or buy it" (UN, 2002:5)

Like the right to water, it demands accessibility, sufficiency. availability and absence of contamination, although in the latter case the forms of contamination may coincide as in the case of pesticides or differ as in the case of feeding hormones to consumer meat products.

D) Right to housing

In this right, its relationship with that of water is based on the content of the latter in the sense of being able to access the water necessary for domestic consumption, therefore to be able to realize it, we first require a home, this correlation Although it may seem idle, it is not in light of the conditions of poverty in which millions of people live, which prevents them from having decent housing.

In this regard, the Committee on Socioeconomic, Social and Cultural Rights, in recommendation No. 4 (1991) has said that "[t]he right to adequate housing is considered as the right of all people to live in security, peace and dignity somewhere", and adds that housing ownership can take various forms such as "rental, cooperative and property, among others. However, all people must enjoy a certain degree of security of this tenure that guarantees legal protection against eviction, harassment or other threats. (UN, 1991)

According to UN data (2010: 1), The number of people who do not have adequate housing easily exceeds 1 billion. Millions of people around the world live in conditions that are dangerous to life or health, crowded in slums and makeshift settlements, or in other conditions that do not respect their human rights and dignity. It would seem unnecessary to clarify that by not having decent housing the difficulty in accessing the human right to water becomes evident and perhaps we can say that both rights are so closely linked that one cannot be accessed without the other.

E) Right to self-determination of peoples

Finally, it is related to the right to self-determination of peoples because, as stated by Valdés de Hoyos and Uribe Arzate (2016:6), indigenous peoples and communities were able to use their natural resources in this way, in particular which concerns their aquifers and other forms of obtaining water essential for their subsistence, as determined by the International Covenant on Civil and Political Rights in article six, first paragraph.

There are many conflicts that arise in this sense in relation to the use of waters that are part of the heritage of these communities, and it is in this matter where we find greater construction of the right by the SCJN.

IV. Water crisis in Mexico 

In this context that we have been weaving regarding the human right to water, we can now examine the situation currently experienced in Mexico, which, like most countries in the world, faces an unprecedented water crisis. In a universe of 164 countries, Mexico is at number 24 among those with the greatest water stress (Tinoco Morales, 2024), an unfavorable place in a country with a population of 126,705,138 people who demand sufficient, healthy and accessible water. , in addition to the requirements of the vital liquid for aspects such as agriculture, industry, commerce, recreation, in a country of great extension (1,964,375 km2) with needs that cannot be met with the available water resources.

The problems that led to the present crisis are very different in nature. In principle, it is evident that climate change is modifying the planet's rainfall conditions and generating unprecedented heat waves. In this sense, Zavala Hidalgo and Romero Centeno (2024:4- 5) they emphasize that

Differential warming of the planet causes transformations in the dominant patterns of winds and ocean currents, as well as in the spatial and temporal distribution of precipitation. This motivates changes in the climate of different regions and is reflected in the modification of regional temperature: its variability increases extreme phenomena such as heat waves and droughts. Likewise, it alters meteorological phenomena such as the jet stream or tropical cyclones; For example, research indicates that, on average, tropical cyclones will have stronger winds and the precipitation associated with them will increase in intensity.

It is incontestable then the impact that climate change is generating and, derived from it, the worsening of the heat has generated additional problems in Mexico that are further aggravating the water problem that it currently suffers from. The absence of rainfall in most states is evidently one of the factors that most pressingly affects water scarcity, in the month of March 2024 alone the North American Drought Monitor among the points that we consider to have the greatest impact established that,
[...] 16 of the country's 32 states ranked March 2024 among their 5 warmest Marches. The national average temperature in March 2024 was 20.7 °C, 0.6 °C above the normal for the month of March, and was ranked as the sixth warmest March since 1953. The combination of a warm and dry month favored the increase of areas with drought, mainly in Chihuahua, Sinaloa, Durango, Tamaulipas and the center of the country.

As of March 31, 2024, extreme to exceptional drought (D3 to D4) covers 26.03% of the national territory, with the main drought sources in the northwest and center of the country; 31.47% remain in moderate to severe drought (D1 to D2) and only 25.57% of the country is drought-free. As of April 1, 2024, storage in the country's 210 main dams stands at 45%, according to reports from the National Water Commission. This figure represents a decrease of 16.83% compared to the same date in 2023. Of the 210 dams, 122 have storage of less than 50% of their capacity, therefore, in most regions of Mexico the average storage is below average.[...] For April 2024, the CONAGUA Drought Monitor (2024) determined that 64.6% of the national territory had some degree of drought, within which, 11% reported unprecedented droughts. From these worrying data it is clear that the problem is increasing and it seems that there is no turning back as far as the water scarcity derived from the meager rainfall, which once allowed the resource to be replenished and for it to be considered *to infinity*, unfortunately that has changed and modern states consider it as a finite resource given the prevailing climatic conditions.

Thirdly, we must analyze water waste as an essential factor of the crisis, starting from what the state itself causes due to its neglect in the maintenance of the networks, since "[a]around the **40% of drinking water in Mexico is lost due to leaks**[1] in the distribution network and in 2023 alone, almost 27 thousand reports of ruptures in pipes were reported in Mexico City due to wear and compliance with their useful life" (Tinoco Morales,2024, s/p) It is evident that said waste is in no way scarce and that it affects the distribution of drinking water and its effective performance.

[1] Bold in the original

Waste in agriculture is the one that has the greatest impact on the distribution of water in Mexico since this sector currently uses 67.8% of the country's water (Lara and Li Ng, 2024: s/p), however, in accordance with data from the various state agencies such as CONAGUA; INEGI, among the most notable, waste in agriculture is very high, there is even talk that in 2024 agricultural consumption will be 77% of the country's total water resources and this enormous percentage 65% is lost due to leaks in agricultural irrigation, the use of inadequate irrigation systems and lack of modernization in the field. (S/A, 2024)

The industry also has high rates of waste, despite the fact that despite the problem, the installation of industrial lines, generally of foreign capital, that demand large volumes of water, continues to be authorized. This is what Lemus (2019:55) tells us that of 61,693 companies of these types, censused by the Mexican federal government in 2019, 84% require a very high use of water resources that did not allow them to settle in their countries of origin.

Then to all of the above we have to add the corruption that allows regulations to be violated and continue affecting Mexico's precious water resources despite the existence of this never-before-seen crisis.

Regarding domestic use, it is evident that we cannot yet speak of total coverage to guarantee the human right to water, however it is recognized that the percentages have been increasing, but inequalities persist, which are sadly an important part. of the problem in the exercise of the right, since in households with the highest poverty rate the coverage reaches from 81.9% to 88.9% while in those with the highest income the coverage rate reaches from 97.6% to a 98.6%; It is also worth clarifying that in the majority of the national territory the supply is rationed and is provided with increasingly restricted schedules. (Villarreal Páez *et al* , 2024:4)

According to Guillén (2024:s/p), 47.7% of homes in Mexico do not have a constant supply of water, but there are differences between federal entities, while in states such as Colima where the supply is constant in 78% of the houses or Baja California, with 75%, we find other states such as Guerrero where it is 25% or Oaxaca 30%. Added to the above is the fact that only 20.9% of households receive water that can be drunk without consequences for health.

From what has been analyzed we can infer that the problem is multifaceted and requires various measures from the state based on more efficient public policies on the matter in each sector according to the water it receives and the efficiency with which it is used.

Concluding reflections 

The human right to water focuses on the possibility of accessing this vital liquid so that it is accessible, available, in sufficient quantity for human subsistence and personal hygiene requirements and the home where one resides.

It is now unquestionable that it is an independent human right despite its close connection with other rights such as health, food, housing and the right to a healthy environment.

The state in the case of Mexico, according to the constitutional order, is responsible for safeguarding the past right, however currently it is not only about will or legal regulation, it is also necessary to take advantage of the country's water resources in an efficient and effective manner to be able to avoid a national catastrophe.

Agriculture and industry must modify their practices and modernize their infrastructure to achieve a greater degree of use of water and stop wasting so many resources that the country needs in other areas, particularly now that domestic use is sought to be privileged.

At the same time, citizens must be more responsible in their homes, avoid wasting the vital liquid and make its domestic use more efficient, also taking care of proper use of it in personal hygiene, for this it is necessary to educate citizens from childhood to Make them aware of the seriousness of the problem and the simple practices with which each person can save thousands of liters with responsible use of water.

The current situation is unsustainable and the problems tend to worsen with the poverty and inequality in which millions of Mexicans still live. Water pollution and access difficulties are already generating social conflicts that will tend to worsen as long as solutions are not developed. suitable for the problem that, although it is one of scarcity, is to a greater extent one of inadequate distribution of existing resources.

Declarations

Conflict of interest

The author declares that she has no conflict of interest. He has no financial interests or personal relationships that could have influenced this book.

Authors' contribution

Cobos-Campos, Amalia Patricia: The author carried out the work in its entirety

Availability of data and materials

Printed and electronic literature was available, as well as the consultation pages of the SCJN and other public order bodies.

The materials used were computer equipment, internet, personal and institutional libraries. The data collected is accessible and available for consultation by the general public, the texts from the personal library are accessible to the author. The investigation found no obstacles in accessing the required information.

Abbreviations and acronyms

CONAGUA(Comisiòn Nacional del Agua
S/A (Sin autor)
OMS (Organizaciòn Mundial de la Salud)
SCJN (Suprema Corte de Justicia de la Naciòn)
UN (United Nations)

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Bridging the world of enzymes with electric fields

Integrando el mundo de las enzimas con los campos eléctricos

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Area: Biology, Chemistry and Life Sciences
Field: Life sciences
Discipline: Biochemistry
Subdiscipline: Catalysis technology

Key Handbooks

Examines how electric fields influence enzyme catalysis and optimize biotechnological applications using advanced electrochemical methodologies. Describes electrochemical techniques such as protein film voltammetry to study enzymatic behavior under the influence of electric fields. Explore how electric fields improve the functionality of enzymes in industrial and medical applications, optimizing their stability and activity. This research significantly contributes to science and technology by introducing electric fields (EF) as a novel parameter in bioprocesses. It demonstrates how EFs influence enzyme catalysis, affecting the orientation and behavior of charged particles in biological systems, and disrupting biomolecular conformations to induce physiological changes. The study integrates advanced biotechnological techniques, showcasing the potential of EFs alongside traditional environmental factors like temperature, pH, and nutrient concentration. Furthermore, the research employs advanced electrochemical methodologies, such as nano-impact electrochemistry and protein film voltammetry, providing detailed insights into electron transfer and enzymatic mechanisms. Computational simulations further elucidate the effects of EFs on enzyme structure and function, particularly in redox reactions. These advancements enhance the industrial and medical applications of enzymes by optimizing processes and improving functionality under various conditions. By establishing EFs as an independent and reproducible variable, the study opens new avenues for future microbial and enzymatic research. Overall, this research offers groundbreaking knowledge in electrostimulation, setting the stage for innovative applications in biotechnology. Gain insights into enzymes from a thermodynamic perspective, linking electric field concepts and applications, and ultimately grasp their catalytic response to this stimulus. Electric fields significantly influence enzymatic catalysis through voltammetry and computational simulations, advancing biotechnological applications and providing a tool to modify catalysis.

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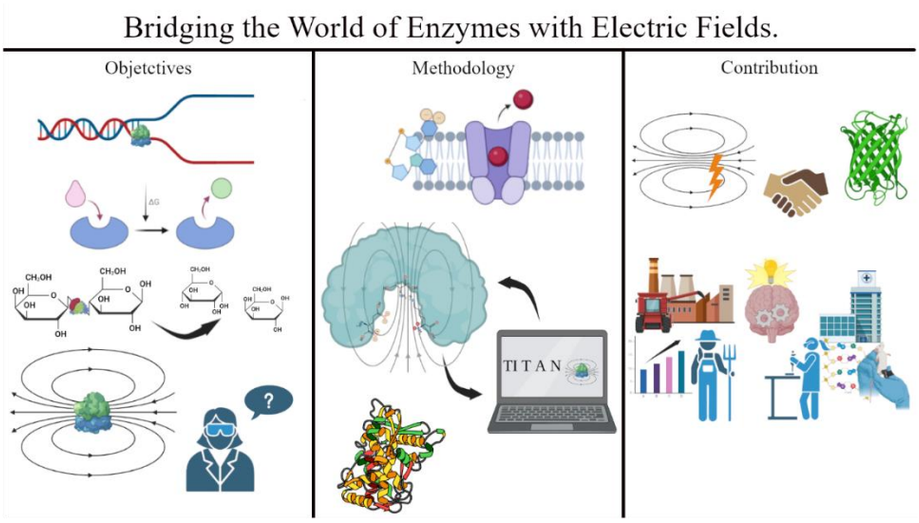
Abstract

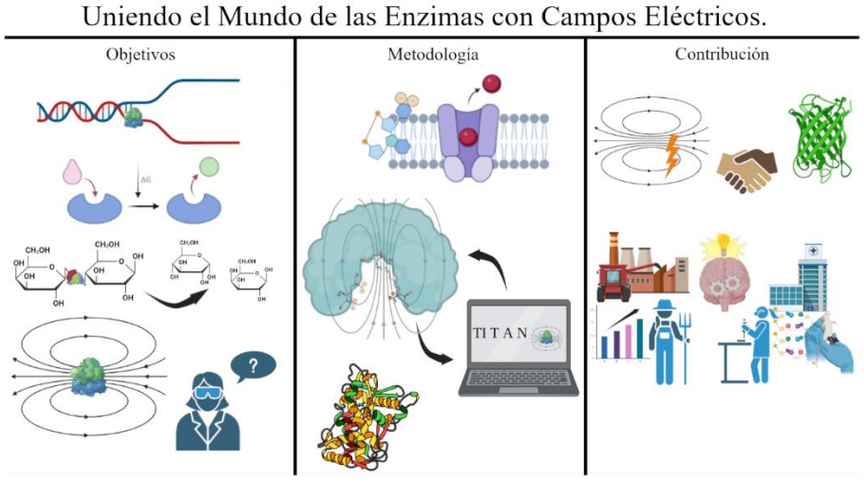
Enzymes are essential proteins involved in metabolism, gene expression, cell division, and immune responses. They play a significant role in industry due to their efficient catalysis of chemical reactions. The diversity of enzyme actions is attributed to their varying substrate specificities and reaction types. Recently, researchers have studied electric fields as a biophysical factor that can stimulate or inhibit biological or catalytic responses, although the mechanisms remain unclear. Understanding the role of enzyme amino acid structures and electric fields offers new insights into catalysis. It is crucial to establish a foundational understanding to comprehend these phenomena. By reviewing and relating fundamental concepts, we can broaden our interpretation and study of enzyme technology, leading to future research and potential applications.



Electrostatic, Catalysis, Biophysical

Abstract

Las enzimas son proteínas esenciales en procesos vitales como el metabolismo, la expresión génica, la división celular y las reacciones del sistema inmunológico. Son importantes en la industria por su eficiencia en catalizar reacciones químicas. La diversidad de acciones y aplicaciones de las enzimas se debe a sus diferentes especificidades de sustrato y reacción. En años recientes, investigadores han estudiado los campos eléctricos como factores biofísicos capaces de estimular o inhibir respuestas biológicas o catalíticas, aunque sus mecanismos no están completamente resueltos. Esto requiere comprender el papel de la estructura de los aminoácidos en las enzimas y los campos eléctricos, ofreciendo nuevos fundamentos sobre la versatilidad de la catálisis. Es esencial establecer un conocimiento básico que permita comprender estos fenómenos, revisando y relacionando conceptos fundamentales, para ampliar el panorama y lograr una mejor interpretación y estudio de la tecnología enzimática, con aplicaciones potenciales en futuras investigaciones.



Electrostática, Catálisis, Biofísica

Introduction

Enzymes play a crucial role in all stages of metabolism and cellular biochemical reactions. The planet's biodiversity has underscored the importance of certain enzymes, particularly those of microbial origin, which are extensively used in commercial-scale industrial applications (Nigram, 2013). Since the 20th century, there have been significant advances in studies related to the isolation, characterization, large-scale production, and bioindustrial applications of enzymes. Moreover, their manipulation has been made possible through techniques such as protein engineering, biochemical, metagenomic, and molecular techniques, enhancing the quality and performance of enzymes, thereby expanding their industrial applications (Chirumamilla *et al*., 2001; Nigram, 2013).

Consequently, the rapid development of the enzyme industry, as we know it today, has been particularly remarkable over the past four decades, thanks to significant progress in modern biotechnology (Kirk *et al.*, 2002). This ongoing advancement allows for the full exploitation of enzymes' potential in various industrial applications.

Enzymatic catalysis has expanded its application field to processes in the pharmaceutical, food, and beverage industries. However, to achieve optimal biocatalytic processes in the energy sector, such as the production of biofuels and natural gas conversion, further improvements in the stability and functionality of biocatalysts are required (Chapman *et al*., 2018).

The uniqueness of enzyme biological activity lies in the precision of their action, requiring minimal energy to catalyze a specific reaction (Ali *et al*., 2023). Although enzymatic design and manipulation are often approached from the perspectives of pH, substrate concentration, and temperature - crucial factors for catalysis - the exploration of other factors, such as electric fields (EF), has begun.

EF are considered biophysical factors capable of stimulating or inhibiting a biological response by exerting work to move a unit charge from one point to another in a given space. Due to the electrical properties and physicochemical nature of the material exposed to the field, it experiences forces of attraction and repulsion (Pataro *et al*., 2011). Consequently, these fields are essential for the reactivity and selectivity of enzymatic active sites, highlighting electrostatic interactions and their role in enzyme organization. Moreover, when applied appropriately, they can modify direct chemical interactions, reactivity, and catalysis by manipulating activation energies, which depend on molecule orientation (Fried & Boxer, 2017; Hanoian *et al*., 2015; Liu *et al*., 2014; Wu *et al*., 2020).

Enzymatic manipulation from the perspective of EF is based on protein composition, whose amino acid residues have a balance of positive and negative charges. The application of EF can affect these residues, causing reorientations and changes in catalysis or alterations in active sites. However, the application of EF presents challenges related to the lack of standardized measurement techniques for the phenomenon, which hinders the reproducibility of results, a consequence of the complex composition of biological systems (Colello & Alexander, 2003; Lewczuk *et al*., 2014).

Methodology

The Catalytic Power of Enzymes: A Thermodynamic and Structural Approach.

An enzyme is defined as a protein that acts as a biological catalyst, accelerating chemical reactions in living organisms. However, this definition can be ambiguous, making it relevant to link it with thermodynamic terms. In this context, a chemical reaction should be considered in terms of Gibbs free energy (ΔG), which represents the energy available to perform work in a system (Warshel *et al*., 2006; Lui, 2017; Aledo & Medina, 2019).

In biochemistry, chemical reactions vary according to the value of ΔG: if it is negative, the reaction is exergonic, releasing energy; if positive, it is endergonic, requiring energy. The uniqueness of enzymes lies in that they do not alter the ΔG of a reaction, but rather reduce the activation energy, that is, the energy barrier that substrates must overcome to reach the transition state and become products (equation 1) (Warshel *et al*., 2006; Lui, 2017; Aledo & Medina, 2019).

$$Enzyme - Substrate \leftrightarrow Produt \quad [1]$$

In this way, enzymes optimize reactions by reducing the activation energy, allowing them to occur at much higher speeds than a chemical or physical catalyst. In other words, enzymes do not alter the thermodynamic direction of a reaction, but they enable and accelerate the process by making the transition of states from substrates to products more accessible (Grahame *et al*., 2015).

However, the extraordinary power of enzymes is attributed to their chemical composition, that is, the sequence of amino acids, arrangement of atoms, chemical groups, as well as weak interactions like hydrogen bonds, ionic forces, and Van der Waals forces, among others (Roskoski, 2014).

All these components are distributed in such a way that they form a specific three-dimensional structure, capable of carrying out its catalytic function by efficiently interacting with the substrates. This task occurs in the active site, a specific region where catalysis takes place. The conformation of this site is crucial for the specificity of the substrate and the enzyme, as it determines the molecules that can bind and interact during catalysis (Roskoski, 2014).

The conformation of an enzyme can change in response to various stimuli such as changes in pH, temperature, presence of cofactors, and substrate concentration (Grahame *et al*., 2015). Recently, the use of EF has been explored as an additional variable capable of inducing changes in the enzyme structure, as well as the factors mentioned above. Understanding the underlying principles of this emerging variable is vital for advancing the study of enzymes and the effects derived from their exposure to this factor.

In this context, the relationship between EF and enzymes is not a novel topic. Evidence has been accumulating that links the catalytic ability of enzymes with electrostatic interactions (Stark, 1913; Warshel, 1981; Fried *et al*. 2014). This contrasts with Pauling's hypothesis (Pauling, 1946), which attributes such catalytic power to a reduction in the activation barrier, resulting from changes in conformational strain during the progression of the reaction. Furthermore, it has been observed that enzymes possess an intrinsic EF within their three-dimensional structure, and when interacting with an external field of different intensity, they modify their behavior, which is precisely what will be explored.

Results

Exploring the Interactions Between Electric Fields and Biology: From Fundamental Principles to Applications in Biological Systems.

An EF is defined as a force field generated by electric charges. The response in the EF varies depending on the nature of the material (its polarization capacity) and the inherent or acquired surface charges (Kandelousi, 2018). Additionally, it can be conceived as a vector field where forces of attraction and repulsion are exerted (Pataro *et al*., 2011). This fundamental concept dates back to Benjamin Franklin's discovery, who observed that like charges repel each other while opposite charges attract (Home, 1972). Coulomb's Law (Equation 2) (Ida, 2000) is a quantitative relationship between two-point charges and the space surrounding them, describing the force they generate, which is also fundamental in the study of electrostatic phenomena at different levels. Thus, the EF is grounded in fundamental principles of electrostatics, enabling the understanding and prediction of electrical interactions between charges.

$$F = K \frac{q_1 q_2}{r^2} \quad [2]$$

Where
q_1 *y* q_2 = magnitude of charges in coulomb (C).
r^2= distance separating the charges in meters (m).
F= force of attraction or repulsion in Newtons (N).

The force of attraction or repulsion between molecules is intrinsically linked to the distance between the charged particles, being inversely proportional to the distance separating them (Ida, 2000; Zhou & Pang, 2018). This phenomenon leads to two types of behavior: conductors and insulators.

Biological systems, characterized by their versatility, can exhibit both properties, classifying as semiconductors, as they contain charges capable of moving systems (Colello & Alexander, 2003).

In biological materials, the distribution of charges tends to be homogeneous; however, in exceptional cases, they may concentrate in specific regions within a molecule, creating a charge separation or polarization (Colello & Alexander, 2003).

The application of an EF to a molecule entails significant changes in its charge distribution, which can result in the breaking or formation of bonds that affect or favor the generation of products. A detailed understanding of this process could be achieved by characterizing the fundamental electronic state of the species involved in the reaction (Vaissier-Welborn, 2024).

In this regard, it connects with the electrostatic property of biological systems, such as enzymes. This characteristic refers to the interaction between charge densities and their distributions, which can be permanent or induced, encompassing permanent or induced dipoles, as well as solvation effects, hydrogen bonds, and hydrophobic forces, analyzed from an electrostatic perspective. In this context, distributions tend to distort to adapt to a new environment, achieving once again an electrostatic preorganization. Thus, the effect of the enzymatic environment on the reaction's evolution is considered as an electrostatic potential associated with an EF. This EF will dictate the temporal evolution of the system's charge distributions, suggesting to induce an interaction energy in a substrate proportional to its dipole, in accordance with the physics convention that indicates the orientation of dipoles towards positive charges (Vaissier-Welborn, 2024).

As an example, in aqueous environments, like biological ones, ions (Na^+, Ca_2^+, K^+, and Cl^-) interact with the EF. We also find this in proteins, composed of amino acids with ionizable groups, polar, and side chains. In a typical biological environment with pH 7.4, eight amino acids act as hydrophobic and nonpolar, adopting dipole characteristics. Under similar pH conditions, seven amino acids are hydrophilic and polar, capable of acquiring a charge or behaving ionically. Two amino acids have a negative charge and three, a positive one. In summary, the net charge and polarity of a protein depend on its distribution, which is intrinsically related to its sequence and, in turn, to the pH (Ida, 2000; Zhou & Pang, 2018). Other biological molecules, such as nucleic acids, vitamins, lipids, and cofactors, which possess a net charge, are also influenced by electric fields (Colello & Alexander, 2003; Ren *et al*., 2012; Zhou & Pang, 2018; Vaissier-Welborn, 2024).

Under physiological conditions, proteins, enzymes, and peptides generally adopt their native conformation, thereby favoring their biological functions. However, exogenous factors can disturb this balance, triggering a transition to denatured states. Research based on molecular dynamics simulations has revealed that agents like EF can induce conformational changes in enzymes, even affecting their denaturation and stability (Budi *et al*., 2005; English *et al*., 2009). Biologically, it is not possible to find an EF in its traditional definition and representation, but rather in a relatively more abstract form where electrochemical phenomena manifest as transmembrane potentials derived from ionic exchange. It is well-known that any system with mobile charges, when exposed to an EF, generates an electric potential. This potential is the result of electron flow in metallic conductors or ions in electrolytes. The point where this exchange occurs is known as the electrode (Colello & Alexander, 2003) (see Figure 1).

Box

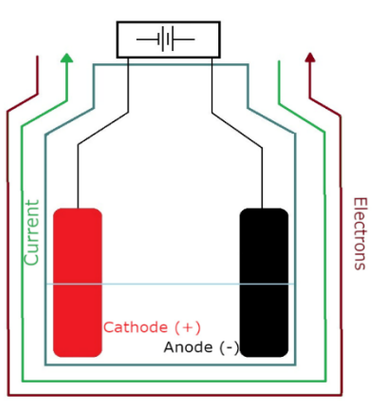


Figure 1

General Schematic of an Electrobio-reactor

Source: Own elaboration

In Figure 1, the direction of ion movement in relation to the current and the EF is observed. It is crucial to highlight that the current flow is oriented in a positive direction, a significant clarification since metals carry a negative charge, whereas electrolytic solutions contain both positive and negative charges. When an accumulation of electrons occurs in the metallic conductor at the left electrode, a negative charge is generated on the surface, favoring the attraction of positive ions (cations), which is why it is called the cathode. Conversely, at the anode, the attraction of anions is due to the lack of electrons, creating a positive charge.

This process implies that anions donate an electron to the anode, while cations head towards the cathode, leading to an exchange of charges. However, this exchange is not so simple because many ions do not easily give up their electrons, attributable to their electronegativity (Colello & Alexander, 2003).

Moreover, an ion barrier known as the 'electrical double layer' or insulating barrier is formed.

This barrier originates from a coating of water that covers the electrode in an aqueous environment, due to the polar nature of water and the excess charge on the electrode surface, requiring more energy to complete the exchange of molecules (Colello & Alexander, 2003; Gonella *et al*., 2021).

This situation, termed overpotential of energy, is essential for overcoming the electrochemical reaction at the electrode-aqueous medium interface (Eberhard, 1986). At this point, two types of reactions can be distinguished to accomplish the exchange: the first occurs when the electrode acts as an inert electron, i.e., chemically inactive, and the second occurs if the electrode acts as a donor/acceptor of ions, i.e., chemically active (Colello & Alexander, 2003).

This is where redox reactions become significant, as they facilitate charge transfer through the reduction and oxidation of ions at the cathode and anode, respectively. Additionally, water decomposition mechanisms can occur at any electrode, a phenomenon known as electrolysis (Colello & Alexander, 2003).

For instance, in a covalent solution like water, which contains few ions, a current can be generated by adding a diluted acid or a salt like NaCl, which provides enough ions to conduct the current. However, when using H_2SO_4, which contains H^+ ions and $(SO_4)^-$, the hydrogen ions are attracted to the cathode, where they collect electrons, forming a gas molecule. At the anode, the negative sulfate ions are attracted, undergoing neutralization and reacting with water to produce more H_2SO_4 and release O_2 (Chang *et al*., 2020).

It is crucial to emphasize that the presence of electrolysis during the application of EFs is not always desirable, as it can generate toxic byproducts for a biological system (Jasper *et al*., 2017). One way to prevent this phenomenon is to create a barrier that inhibits the diffusion of byproducts, allowing the flow of ionic current (Colello & Alexander, 2003). Understanding the nature of the electrodes to be used, as well as the composition of the fluids involved, is one of the alternatives for achieving this.

Another critical aspect is the manner in which the EFs are applied, as variations in magnitude and direction can have a substantial effect on the rate of enzymatic reactions. The application of EFs above 0.1 V can alter the energies of molecular orbitals, modifying the activation barriers of reactions and the kinetic parameters associated with catalysis. Working with lower field intensities allows the molecules to remain intact but oriented and polarized according to their dipole moment and polarization (Shaik *et al*., 2018).

Applications of Electric Fields in Enzymes

In the field of enzymes, research since the 1970s has laid the groundwork for a more quantitative than qualitative application of EF (Stark, 1913; Warshel, 1981; Warshel *et al*., 2006; Roskoski, 2014; Grahame *et al*., 2015). Phenomena triggered by exposure to these fields affect the spectroscopy of molecules (Stark effect), promote electron transfer and redox reactions. They also induce polarized spin conductivity, alterations in molecular geometry, isomerization of molecules, and even spin-crossover transitions in Fe (II) complexes, acting as molecular switches (Léonard *et al*., 2021).

In 1997, Armstrong and colleagues developed a voltammetric technique called Protein Film Voltammetry (PFV), primarily aimed at studying the behavior of complex metalloproteins. This technique involves the adsorption of the protein onto an electrode to which an electric potential is applied, and the flow of electrons between the electrode and the active site is monitored, measured as current consumption (essentially similar to cyclic voltammetry). Thanks to this technique, important thermodynamic and kinetic information has been obtained on various proteins (cytochrome P450, catalases, cytochrome c nitrite reductase, hemoglobin, etc.), enabling a greater understanding of their mechanisms of action (Armstrong *et al*., 1997; Davis *et al*., 2021).

In recent decades, researchers have actively explored the use of EF in various applications, spanning fields as diverse as health and biology, such as in cancer treatment and cell sorting, as well as in engineering and technological applications, where the aim is to improve heat transfer, study colloidal hydrodynamics, and address stability issues (Kandelousi, 2018).

Thanks to technological advances and the development of tools that allow for more complex and specific techniques, the field of electrochemistry has become actively involved in the study of protein models (Davis *et al.*, 2021). Among them, nano-impact electrochemistry (NIE) stands out, through which more in-depth study of mechanisms such as electron transfer has been achieved (Han *et al*., 2016; Davis *et al.*, 2021). Since electron transfer is a crucial process in biochemical reactions, special emphasis has been placed on its study. Perhaps one of the most relevant in the biological field is the relationship between conductance capacity and secondary structure (Zhang *et al*., 2015; Yu *et al*., 2019; Davis *et al*., 2021).

In addition to the electroporation of cell membranes, the electrostimulation of cellular metabolism through electric or electromagnetic fields has become a valuable tool in non-invasive processes to stimulate living organisms. These processes include organism proliferation, enzymatic reactions, biopolymer synthesis, morphological changes, membrane transport phenomena (Berg, 1993; Berg, 1995), and bone tissue regeneration (Tsong, 1990).

It is important to note that these effects are conditioned by factors such as exposure time, intensity of the applied current, distance between the electrodes, among others (Pataro *et al*., 2011). Most studies have focused on chemical catalysis and computational simulations, largely overlooking enzymatic aspects. Among the pioneer researchers, Fried *et al.* (2014), have explored the Stark vibrational effect, which involves the spectral shift of atoms and molecules in the presence of an EF.

This approach allows measuring the EF experienced by the substrate when it binds to the active site, thus supporting the importance of electrostatic contribution in enzymatic catalysis (Boxer *et al.*, 1987). Furthermore, using Stark spectroscopic vibration, Fried *et al.* (2014) identified that the active site of the enzyme ketosteroid isomerase (KSI) presents an EF that stabilizes the C=O bond dipole, orienting it towards the transition state, correlating with an improvement in the catalytic rate.

Computational studies of the enzyme cytochrome P450, present in the bacterium *Jeotgalicoccus sp*. ATCC 8456, have used a software called "elecTric fIeld generaTion And maNipulation (TITAN)". This software aids in generating uniform and non-uniform EF and quantifying local EF present in proteins and other biomolecules. This approach has allowed exploring the effects and evolution of EF in biological systems. In particular, the study focused on the hydrogen transfer reaction of the P450 enzyme, and its modifications induced by the application of an externally oriented EF. The main impact was observed on the Fe-O bond (Stuyver *et al.*, 2020). In a simulation by Lai *et al.* (2010), it was demonstrated that an external EF has the capability to control the activation of the enzyme's catalytic activity, its O_2 consumption, its resting state geometry, and the spin state arrangement, thus highlighting the relevance of electric fields in enzymatic processes (Nam et al., 2024).

While some researchers have applied EF as a means of enzymatic stimulation, the mechanism is not yet fully elucidated. It is primarily attributed to structural changes, but it has also been studied that transmembrane enzyme, such as ATPase, can harness the free energy from the EF to modify their catalysis, although this depends more on the characteristics of the EF than on the enzyme (Westerhoff *et al.*, 1986). This leads to the so-called 'electrogenic enzymes,' of which ATP synthase is probably the most representative.

In this enzymatic complex, the exchange of Na^+ and K^+ ions has been well defined for several decades (Hernández *et al.*, 1983); however, based on this premise, the electrochemical implications in protein and cellular systems were studied more deeply, leading to the description of another factor known as 'electrochemical homeostasis' or 'ionic homeostasis,' which in turn led to the description of mechanisms of ionic exchange regulated by electromotive forces, where transmembrane proteins are of primary importance (Mehta *et al.*, 2008).

It should be noted that the opposite effect can occur, namely, the denaturation of enzymes, resulting from the association or dissociation of functional protein residues, inducing charge separation (induced dipole) (Poojary *et al.*, 2017; Zhou &Pang, 2018). Although the process of enzymatic inactivation is better characterized compared to stimulation, it is presumed that this phenomenon may be due to the additional formation of active sites or their modification, reducing the activation energy of the reaction. This may be related to the induction of a favorable orientation of the substrate and the enzyme. Therefore, it is crucial to continue research in this area to understand and establish the real contribution of EF (Zhou &Pang, 2018).

While enzymes have been classified according to their biochemical function and all share essential thermodynamic parameters (optimal pH and temperature, activation energy, pKa, redox state, etc.); oxidoreductases, especially those involved in respiratory processes, stand out among others due to the electrochemical potential, which adds as an additional factor (Elliot *et al.*, 2002; Léonard *et al.*, 2021).

Due to this electrochemical nature, the active sites of these enzymes can be presented in three defined states as oxidized (O), intermediate (I), or reduced (R), where each state then has different properties and therefore can lead to different metabolic pathways (Elliot *et al.*, 2002); where the response will depend on the existence of additional electron transport centers (e.g., heme groups or Fe-S) (Craig & Marszalek, 2002).

In 1984, Serpesu & Tsong (1984) used a solution of blood cells to study the effect of imposing an EF on the activity of (Na,K)ATPase; the results obtained from this work showed that protein modifications resulted from changes in the flow of Na^+, K^+, and Rb^+ ions as well as the addition of compounds that showed to inhibit or favor ionic exchange under the study conditions.

Conclusions

EF are a phenomenon commonly associated exclusively with physical aspects and, due to their traditional definition and representation, are seldom linked to biological processes; however, it has been established that EF, and therefore electric potential, are parameters implicitly involved in enzymatic processes.

Nevertheless, the manipulation of enzymes through EFs still presents several challenges due to the rigorous conditions necessary for their study; this is why many works within this field employ bacterial models in specific processes where the results are associated with enzymatic processes. However, this has not been a limitation for studying in depth the effect and relationship that EFs have with enzymes. While concrete observations of the implications at the structural and/or conformational level have not yet been achieved, contemporary tools and methods allow for a more precise approach. Combined with decades of research, it is possible to conclusively assert that electric fields are a physicochemical factor of utmost importance for enzymatic processes and a useful tool in optimizing these processes, which are extremely attractive for various industries.

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 in this chapter.

Author contribution

Alonso- Vargas, Monserrat: Conceptualization, methodology, investigation, writing—original draft preparation.

García- Esquivel, Yarely: Methodology, investigation, writing—review and editing, supervision.

Cadena- Ramírez, Arturo: Conceptualization, resources, data curation, writing—review and editing, visualization, supervision, project administration, funding acquisition.

Availability of data and materials

No extra data are available.

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Abbreviations

EF	Electric Fields
TITAN	Electric Field Generation and Manipulation
ΔG	Gibbs Free Energy
I	Intermediate
KSI	Ketosteroid Isomerase
NIE	Nano-impact Electrochemistry
O	Oxidized
PFV	Protein Film Voltammetry
R	Reduced

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Determination of the risk level from BTEX inhalation at a gas station in Ciudad del Carmen, Campeche

Determinación del nivel de riesgo por inhalación de BTEX en una estación de gasolina en Ciudad del Carmen, Campeche

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Field: Atmospheric Sciences
Discipline: Atmospheric Chemistry
Subdiscipline: Other

Key Handbooks

The main contribution of this work is to know the current levels of BTEX in ambient air near a gas station and to determine the possible health risk associated to this exposure. Results obtained in this study deserve to be disseminated not only at a local level but also at regional and global level. Results obtained can be compared with those reported in other regions of the world. This will allow a diagnosis of the level of risk that this type of gas station in Mexico represent within a global context. The cancer risk index for benzene exposure exceeded the reference value proposed by the US EPA (LTCR $1x10^{-6}$), suggesting a significant risk, and being even higher for the child population. The overall potential for no carcinogenic effects due to BTEX exposure was determined as an HQ risk quotient. In all cases, a value of HQ<1 was obtained, indicating that the population is not exposed to a significant risk of contracting diseases other than cancer (respiratory and cardiovascular diseases) due to the daily exposure to the BTEX levels found in the study area. It is necessary to carry out more studies around the city including other climatic seasons and a greater number of sampling sites (service stations) to obtain more precise conclusions regarding the risk associated to this type of emissions.

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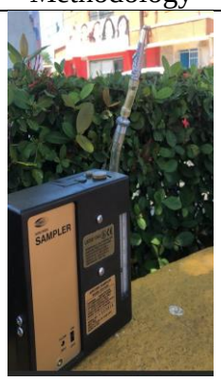
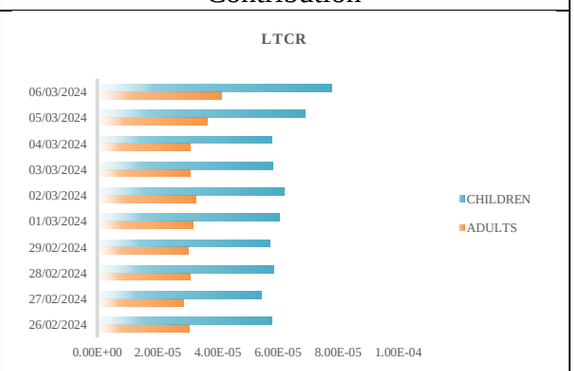
Abstract

Health risks associated with inhalation of BTEX were determined near a service station in Ciudad del Carmen, Campeche. BTEX levels and meteorological parameters were measured in the ambient air of an urban site during the dry season. Samples were analyzed by GC with FID detection. The BTEX showed high concentrations during sampling B1. The X/E and B/T ratios indicate that the emissions were recent, of local origin and from vehicle and service station emissions. The cancer risk index for benzene exposure exceeded the guideline value proposed by the US EPA, suggesting significant risk. The overall potential for non-carcinogenic effects was determined as a hazard ratio (HQ). The value of HQ<1 indicates that the population is not exposed to a significant risk of contracting diseases other than cancer.

Determination of the risk level from BTEX inhalation at a gas station in Ciudad del Carmen, Campeche

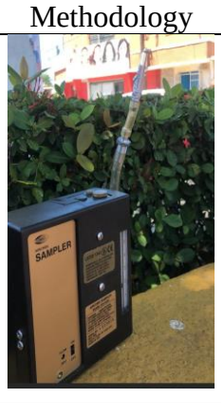
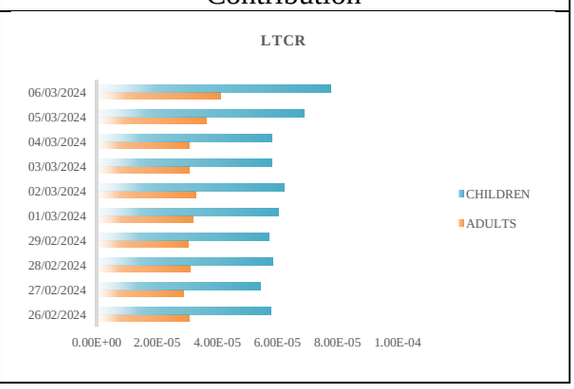
Objectives	Methodology	Contribution
		

BTEX, Health risk, Gas station

Resumen

Se determinaron riesgos para la salud asociados con la inhalación de BTEX cerca de una estación de servicio en Ciudad del Carmen, Campeche. Se midieron niveles de BTEX y parámetros meteorológicos en el aire ambiente de un sitio urbano durante la estación seca. Las muestras se analizaron por CG con detección FID. Los BTEX mostraron altas concentraciones durante el muestreo B1. Las relaciones X/E y B/T indican que las emisiones fueron recientes, de origen local y proveniente de emisiones de vehículos y de la estación de servicio. El índice de riesgo de cáncer por exposición al benceno excedió el valor de referencia propuesto por EPA de EE. UU. lo que sugiere riesgo significativo. El potencial general de efectos no cancerígenos fue determinado como índice de riesgo (HQ). El valor de HQ<1, indica que la población no está expuesta a un riesgo significativo de contraer enfermedades distintas al cáncer.

Determinación del nivel de riesgo por inhalación de btex en una estación de gasolina en Ciudad del Carmen, Campeche

Objectives	Methodology	Contribution
		

BTEX, Riesgo a la salud, Estación de gas

Introduction

The World Health Organization (WHO) reports that 99% of the global population breathes air that exceeds the recommended limits for health protection due to high levels of contaminants. This exposure is particularly high in low- and middle-income countries, including Mexico (WHO, 2023). The WHO's global burden of disease report identifies exposure to air pollutants as the main environmental risk leading to premature mortality. In 2019, there were 48,331 deaths attributable to air pollution in Mexico (IHME, 2019; ONU, 2021; SEMARNAT, 2024), making air pollution the primary environmental risk to health, causing around 7 million premature deaths annually.

A recent World Bank publication revealed that air pollution cost the world approximately $8.1 trillion in 2019, equivalent to 6.1% of global GDP. Over 95% of air pollution-related deaths occur in developing countries, with the economic burden of premature mortality and morbidity equivalent to 5-14% of GDP in these nations. Furthermore, the impact of air pollution on health surpasses that of other well-known risk factors like chronic diseases such as high blood pressure, diabetes, tobacco use, obesity, high cholesterol, and malnutrition (State of Global Air, 2019).

Volatile organic compounds (VOCs) are a group of chemical compounds emitted from highly volatile liquids at room temperature and from industrial processes (FAO & UNEP, 2022). These compounds and their reaction products pose an unacceptable risk to public and occupational health and biological and physical environments (Bloemen & Burn, 1993). Studies worldwide have reported that BTEX (benzene, toluene, ethylbenzene, and the three isomers of xylene) are potentially dangerous for the environment and human health (Campos-Candel, Llobat-Estelles & Mauri-Aucejo, 2007). While these compounds can be released from natural sources like forest fires, the majority are emitted by human-related activities such as oil and natural gas extraction and combustion, petrochemical activities, and various industrial processes. Due to their volatile nature, humans are primarily exposed to VOCs through inhalation. Both chronic and acute exposure can have serious health consequences, including neurological diseases, cancer, and teratogenic effects (ATSDR, 2004; Ma et al, 2024; Chaiklieng et al, 2024; Baghani et al, 2024; Hosseinpour et al, 2024). The World Health Organization (WHO) estimates that exposure to 1 mg/m³ of benzene puts 4 in 1 million people at risk of developing leukemia during their lifetime (PAHO & WHO, 2012).

Background

The presence of BTEX compounds in the atmosphere is concerning because there are no established maximum limits for these pollutants. As a result, there is a lack of continuous or systematic measurements of these contaminants in Mexico. To address this, we need to establish a theoretical framework by reviewing the available regulatory standards and examining results from other national and international studies. Criterion pollutants have well-established limits and extensive information on their sources and health impacts. In contrast, non-criterion pollutants, like BTEX compounds, are not as well studied and lack sufficient information for regulatory frameworks. Therefore, it is necessary to conduct a health risk assessment to determine if the levels of BTEX in a specific area are acceptable or pose a risk to public health and well-being (Comisión Federal para la Protección contra Riesgos Sanitarios, 2017).

Regulations for non-criterion pollutants

The current regulations in our country only address criterion pollutants. However, no Mexican regulation sets the Maximum Permissible Limit (MPL) for BTEX pollutants released into the air. There are official Mexican standards that focus on recognizing, evaluating, and controlling chemical agents in the workplace and for occupationally exposed personnel (POE). Since no standard indicates maximum exposure values, international organisms such as the World Health Organization (WHO), the National Institute for Occupational Safety and Health (NIOSH), and some foreign government agencies are frequently consulted. WHO (2005) developed the Global Air Quality Guidelines (GCA) for the entire world. These guidelines were created to minimize the health effects of air pollution and contain recommendations for indicative levels of air quality and targets for the six major air pollutants.

Table 1 shows the reference values established by NIOSH for the concentrations of non-criterion contaminants, specifically BTEX compounds.

Box 1

Table 1

Maximum Permissible Limits for exposition to BTEX

Pollutant	Maximum Permissible Limits	Source
Benzene	1 ppm, 3.25 mg/m^3 , 24 h	NIOSH, 1976
Toluene	100 ppm, 375 mg/m^3, 10 h	NIOSH, 1973
Ethylbenzene	100 ppm, 435 mg/m^3, 10 h	OSHA, 2021
O-Xylene	100 ppm, 435 mg/m^3, 10 h	NIOSH, 2014
m-Xylene	100 ppm, 435 mg/m^3, 10 h	NIOSH, 2014
p-Xylene	100 ppm, 435 mg/m^3, 10 h	NIOSH, 2014

Source: Own elaboration

National Background

In previous studies, García et al. (2014) conducted research on the emission of BTEX at gas stations in Ensenada, Baja California, Mexico. The selected sites were in areas with high population density and vehicular traffic, and a total of 37 service stations were studied. The study measured the annual BTEX concentrations (tons per year) in Ensenada, Baja California, Mexico in 2014, and the results are summarized in Table 2.

Box 2

Table 2

Annual concentrations of BTEX (Tons per year) Ensenada, Baja California, Mexico (2014)

Year	Benzene	Toluene	Ethylbenzene	mp-Xylene	o-Xylene
2010	18.17	54.99	0.078	3.35	1.40
2011	17.70	53.57	0.075	3.26	1.36
2012	14.40	43.58	0.061	2.65	1.11

García et al. (2014) concluded that human exposure to volatile organic compounds, such as benzene, toluene, and xylene (BTEX), has various effects on the short and long-term health of men, women, and children. These compounds can interact with the genome and epigenome during cell division in somatic and germ cells. The presence of gas stations in urban areas leads to BTEX emissions, which are associated with a higher risk to the population's health (García et al. 2014). The study used a Geographic Information System (GIS) methodology, employing ARC/INFO for digitization and ArcView for visualization and attribute creation. The resulting maps indicated a higher presence of BTEX in areas closest to the service stations. García et al. (2014) suggested that the high BTEX emissions at Ensenada gas stations may be due to the absence of a vapor removal and recovery system in the gasoline delivery pumps at the service stations, rather than in the fuel transfer from the pipes to the storage tanks.

On the other hand, Cerón et al, (2018) conducted a study in the city of Tijuana, Baja California, Mexico, to characterize and determine the sources of aromatic hydrocarbons (BTEX). Samples were collected over four sampling periods, resulting in a total of 24 samples during January 2017 (See Table 3).

Box 3

Table 3

Mean Concentrations of BTEX (µg/m^3) in Tijuana, Baja California, Mexico (January 2017)

Site	Benzene	Toluene	Ethylbenzene	p-Xylene
Tijuana, Baja California México (µg/m^3)	32.40	13.28	7.02	17.16

The levels of BTEX compounds at the study site followed a daily pattern, with the highest concentrations occurring during the morning and afternoon sampling periods. Benzene was found to be the most abundant aromatic compound, followed by p-Xylene, toluene, and ethylbenzene. Analysis showed that vehicular traffic was the main source of benzene, toluene, and p-xylene during the sampling period, and that toluene and p-xylene could contribute to tropospheric ozone. Meteorological analysis revealed that BTEX levels were influenced by local and fresh emissions, particularly from vehicle traffic (Cerón et al., 2018).

Cerón et al. (2020) assessed the health risk associated with BTEX levels in the ambient air at the CICEG monitoring station, in an industrial area with the highest population density in the state of Guanajuato, Mexico. BTEX samples were collected three times a day for a week during both summer and autumn, resulting in a total of 48 samples (refer to Table 4).

Box 4

Table 4

Mean Concentration of BTEX (µg/m^3) in Leon, Guanajuato (2020)

Season	Benzene	Toluene	Ethylbenzene	Xylene
Summer and Autumn (µg/m^3)	1.73	11.85	11.86	3.31
Summer (µg/m^3)	2.633	15.78	15.28	3.46

The Mann-Whitney test showed significant differences in BTEX concentration between summer and autumn. The test also revealed significant differences in wind speed, direction, and temperature between the two seasons, suggesting that air masses from different directions could contribute to BTEX levels, influenced more by local and regional sources such as industrial emissions and vehicular traffic rather than by photochemical activity. BTEX levels were higher in summer due to higher wind speeds. The estimated cancer risk (LTCR values) for adults and children ranged from 5.26×10^{-6} to 4.33×10^{-5}, exceeding the limit values set by the US EPA (1×10^{-6}) and the World Health Organization (WHO) (1×10^{-5}) (Cerón, et al., 2020).

In another study by Estéves et al. (2015) in Orizaba, Veracruz, BTEX levels in ambient air and the carcinogenic risk levels of benzene in an urban site were determined during the autumn season, with a total of 36 samples collected (See Table 5).

Box 5

Table 5

Mean Concenration of BTEX (µg/m^3) in Orizaba, Veracruz (2015)

Site	Benzene	Toluene	Ethylbenzene	Xylene
Orizaba, Veracruz, Mexico (µg/m^3)	74.51	5.33	2.26	3.35

The authors found that BTEX levels were significantly affected by winds from the south and south-southeast, primarily due to emissions from the Veracruz-Mexico highway, indicating that these compounds mainly came from vehicular sources. The PCA analysis revealed that Benzene was strongly influenced by vehicle emissions. The results for Benzene exceeded the maximum permissible limits set in Europe and the United States. The average daily exposure at the study site was 22.83 x10^{-3} mg/kg per day.

The lifetime benzene cancer risk (LTCR) was 68 x 10^{-5}, surpassing the acceptable LTCR value of 1 x 10^{-6} for adults, as per the US EPA. The authors emphasized that the potential for cancer risk due to environmental benzene exposure through inhalation should be a concern for health authorities in the region of Orizaba, Veracruz, Mexico. In terms of non-cancer risk, HQ values ranged from 2.483 to 3.038, exceeding the limit set by the US EPA, which suggests that contaminants may pose a risk of producing cardiovascular and respiratory effects, among others, if the HQ value exceeds 1.

Justification

The rapid population growth, expansion of urban areas, and increased utilization of natural resources, energy, and transportation lead to the release of pollutants into the air of many cities worldwide (Lan & Minh, 2013). Air pollution disrupts ecosystems and results in economic and social costs, as well as significant health risks to humans at local and regional levels globally. In 2019, according to the World Health Organization (WHO), outdoor air pollution, in both urban and rural areas was estimated to cause 4.2 million premature deaths annually worldwide. This mortality is attributed to exposure to fine particles, which contribute to cardiovascular and respiratory diseases, as well as various types of cancer (WHO, 2022).

In 2019, the World Health Organization (WHO) estimated that outdoor air pollution contributed to 37% of premature deaths from ischemic heart disease and stroke, 18% from chronic obstructive pulmonary disease, 23% from acute lower respiratory tract infections, and 11% from respiratory tract cancer. This highlights the importance of monitoring ambient air quality. Volatile organic compounds (VOCs) are some of the most dangerous air pollutants (Bloemen & Burn, 1993; FAO & UNEP, 2022). Evaluating VOCs is crucial in understanding atmospheric pollution due to their high volatility, fat solubility, toxicity, and flammability. They are highly reactive and contribute to ozone formation and, consequently, to climate change (Finlayson & Pitts, 1993). Benzene, toluene, ethylbenzene, and o-, p-, and m-xylene (BTEX) are among the VOCs emitted into the atmosphere from both anthropogenic and biogenic sources and can also be formed photochemically (FAO & UNEP, 2022). Studies have shown that in densely populated and industrialized cities, BTEX can significantly contribute to the total VOCs in the atmosphere (Yalcin et al., 2020).

The monoaromatic hydrocarbons benzene, toluene, ethylbenzene, and xylenes can cause various health issues such as asthma, dizziness, fatigue, and irritation of the eyes, nose, and throat. Nausea and other non-specific symptoms have also been linked to BTEX exposure (US EPA 1987, 1991). Humans are exposed to BTEX through the respiratory system or skin contact (Li et al., 2014). In developing countries like ours, it's common to employ attendants to pump fuel at service stations. These attendants come into direct contact with vehicles using multi-octane unleaded fuels, lead substitute gasoline, and diesel, putting them at risk of inhaling volatile organic compounds (VOCs) released by these fuels. The released contaminants include benzene, toluene, ethylbenzene, and xylenes (BTEX), which are highly toxic. As a result, service station personnel experience long-term exposure, leading to adverse health effects and potential environmental impact. As a result, this study aims to analyze the daily variation of BTEX levels in the ambient air of a service station (gas station) during the dry season using active samplers.

Methodology

Study Site

The study site is located in the State of Campeche, in the southeastern region of Mexico. It shares borders with the State of Yucatan to the North, the state of Quintana Roo and Belize to the East, the Republic of Guatemala to the South, and the state of Tabasco and the Gulf of Mexico to the West. The state covers an area of 57,507 km^2 and has a total population of 928,363 inhabitants, which represents 0.7% of the country's total population (INEGI, 2020). The predominant climate in the state is warm subhumid in approximately 92% of its territory, while 7.75% has a warm humid climate, mainly in the Eastern and Northern parts. A small percentage of 0.05% has a semi-dry climate. The average annual temperature ranges from 26 to 27°C, with the highest temperature frequently exceeding 30°C and the minimum at 18°C. Rainfall is abundant, varying between 1200 and 2000 mm annually, except in the Northern region with a semi-dry climate, where it is around 800 mm per year (INEGI, 2020).

Ciudad del Carmen is the main city in the municipality of Carmen, in the state of Campeche. It is situated in the Southwestern part of the Yucatan peninsula, on the Western side of the Island of Carmen, between the Gulf of Mexico and the Terminos Lagoon. The primary economic activity in the city is the production and extraction of oil. Ciudad del Carmen is also recognized as the leading port in the country for transporting personnel and materials to the oil platforms of the Campeche Sound. Currently, it has 20 service stations supplying gasoline and diesel, benefiting the population, and over 100 companies operating in the area, which, in turn, have environmental effects due to the consumption of these fuels.

In this study, we will assess the levels of BTEX in the ambient air of an area affected by urban and oil activities in the municipality of Carmen, Campeche. The sampling site was chosen at a service station (gas station) that is frequently visited by motorists and other means of transportation. The sampling site's location is presented in Figure 1.

Box 6

Figure 1

Location of the sampling site

Sampling

Active sampling of ambient air was conducted using the "Determination of aromatic hydrocarbons (benzene, toluene, ethylbenzene, p-xylene, 1, 2, 4-trimethylbenzene) in air - Activated carbon adsorption method/Gas chromatography" method, which is approved by the National Institute of Safety and Hygiene at Work (INSHT) (MTA/MA-030/A92) (INSHT, 1995). A total of 30 samples of ambient air were gathered in glass tubes containing activated carbon brand 226-01 Anasorb CSC from SKC. These tubes are 7 cm in length, with an external diameter of 6 mm and an internal diameter of 4 mm. Each tube contains two sections of activated carbon separated by a 2 mm portion of polyurethane foam (see Figure 2). It is important to note that the tubes should have well-fitting polyethylene caps to prevent leaks and contamination during the transport and storage of the samples.

Box 7

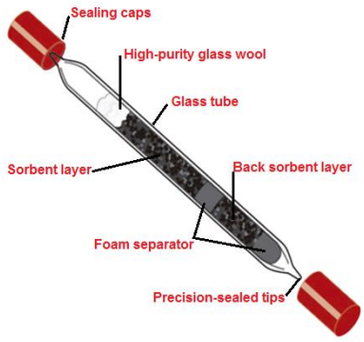


Figure 2

Characteristics of the active sampling tubes

Ambient air was drawn through a GAST-type vacuum pump at a controlled flow rate of 200 ml/min through horizontally placed tubes. The ends of both sections were broken, and section 2 (containing 50 mg of activated carbon) was securely positioned on a rubber or plastic tube holder of suitable length and diameter. The tube was covered with aluminum foil to prevent photochemical reactions and interference when reading the samples. The process of obtaining ambient air samples was conducted during three time periods based on diurnal variation: morning B1 (7:00 a.m. to 8:00 a.m.), midday B2 (12:00 p.m. to 1:00 p.m.), and afternoon B3 (5:00 p.m. to 6:00 p.m.), each lasting 1 hour. A total of 30 samples were collected over 10 sampling days in the 2024 dry season. After each sampling, the tubes were covered, labeled, and stored in special bags to prevent direct sunlight exposure and potential photochemical reactions. Subsequently, they were refrigerated to preserve them for no more than 20 days until analysis at the Environmental Protection laboratory of the Autonomous University of Carmen (UNACAR).

Chemical Analysis

The chemical analysis was conducted at the Environmental Protection Laboratory of the Autonomous University of Carmen following the method established by the National Institute of Safety and Hygiene of Spain MTA/MA-030/A92 (INSHT, 1995). Calibration curves were prepared using reagent-grade solutions of 99.98% purity from the Sigma-Aldrich brand. After this, chemical desorption was carried out, by using 2 ml amber vials with lids and septa. These vials were labeled and subjected to pre-treatment by rinsing with distilled water and then dried. A small amount of HPLC-grade carbon disulfide was added as an adsorbent reagent, allowing it to dry to avoid contamination of the samples with impurities. After pre-treatment, desorption was carried out by carefully breaking the end of section 1 of the tube to remove the glass wool using a metal clamp. The material was then emptied into the vial, and 2 ml of CS_2 was added. The vial was closed, and the steps were repeated for section 2 and for each sample. After closing the vials, they were shaken vigorously for 5 minutes and refrigerated for a maximum of 24 hours before analysis by gas chromatography.

Statistical Analysis

Statistical analysis on the BTEX concentration data and meteorological data was conducted by using the Excel statistical package XLSTAT version 2019. Several statistical tools were using, including:

1. Pearson correlation to identify relationships between BTEX, criterion pollutants, and meteorological variables.
2. Principal component analysis (PCA) to explain the variance and discover the structure of the data set. PCA results help identify whether a contaminant is secondary or primary, or to identify the specific source of the contaminants.
3. Friedman non-parametric tests ($\alpha = 0.05$) to evaluate the differences between the sampling periods of the concentrations of atmospheric pollutants measured. This helps determine whether the data studied come from the same population and if there is significant diurnal variation.
4. Box plot to display the descriptive statistics of the results obtained, including the concentrations, means, maximum, and average of each of the aromatic hydrocarbons. This allows for easy identification of the contaminant with the highest average concentrations and any diurnal pattern or trend during sampling.

Meteorological Analysis

Wind analysis was performed to determine the places where the BTEX emissions probably came from. Windrose program and Google Earth were used to create wind roses and to identify potential emission sources based on wind direction over ten sampling days. Then, Excel was used to estimate the average wind direction during morning, afternoon, and night for the sampling period. Additionally, the HYSPLIT trajectory model from the National Oceanic and Atmospheric Administration (NOAA) was applied to estimate air mass trajectories and to identify regional influences. Finally, Radar Chart were obtained to visually represent the possible origin of BTEX concentrations (NOAA, 2024).

Health Risk Analysis

The carcinogenic potential of benzene is well known (Moolla et al., 2015). The European Union recommends an annual limit of 5 µg m^{-3} for benzene in ambient air, while the USEPA establishes a value of 4.0 ppbV for this contaminant (US EPA, 2012). To determine the daily exposure (E), the cancer risk potential (LTCR), and the non-cancer risk potential (HQ), we used the methodology proposed by Zhang et al. (2012). The daily exposure (mg/kg per day) of an individual by inhalation can be calculated as follows:

$$E = \frac{C * Ira * Da}{Bwa} \quad (1)$$

Where:
C (mg/m^3), is the mean concentration of benzene
Ira, is the inhalation rate for an adult person (0.83 m^3/hr) (US EPA, 1998)
Da, is the exposure duration for an adult (24 hr/día)
Bwa, is the mean weight for an adult -- 65 kg -- (US EPA, 1998)

In addition, the lifetime cancer risk (LTCR) is calculated as follows:

$$LTCR = E * SF \quad (2)$$

Where:
E, is the daily exposure for a person by inhalation
SF, is the slope factor for benzene toxic inhalation risk when considering the linear carcinogenic effect due to exposure. The proposed SF value for benzene (0.029 kg/mg per day) was based on US EPA 2012. Finally, the non carcinogenic risk of BTEX was estimated as a risk quotient (HQ):

$$HQ = \frac{C}{Rfc} \quad (3)$$

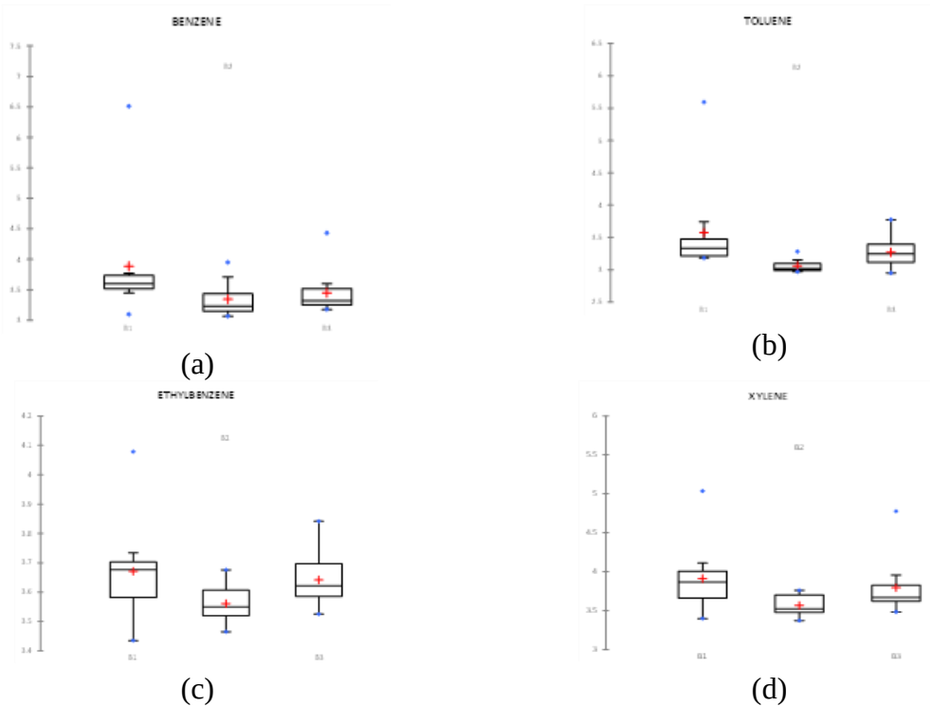
Where:
C, is the daily mean concentration (µg/m^3)
Rfc, is the reference concentration of inhalation, proposed by U.S. EPA for benzene (0.03 µg/m^3), toluene (5 µg/ m^3), ethylbenzene (1 µg/m^3) and xylene (0.1 µg/m^3) (USEPA, 2012).

Results

Descriptive Statistic

In the case of Benzene, the average concentration in schedule B1 was 3.88 µg/m^3, with maximum and minimum values of 6.51 and 3.10 µg/m^3, respectively, and a standard deviation of 0.96 µg/m^3. For schedule B2, the average concentration was 3.34 µg/m^3, with maximum and minimum values of 3.95 and 3.07 µg/m^3, respectively, and a standard deviation of 0.29 µg/m^3. Lastly, for schedule B3, the average concentration was 3.45 µg/m^3, with maximum and minimum values of 4.43 and 3.18 µg/m^3, respectively, and a standard deviation of 0.37 µg/m^3. Figure 3 (a) illustrates the descriptive statistics for benzene concentrations during the 3 sampling times at the study site. It is evident that benzene concentrations were significantly higher during sampling period B1, which corresponds to the time from 07:00 to 08:00 hrs.

Box 8



(a) (b) (c) (d)

Figure 3
Descriptive Statistic for (a) Benzene, (b) Toluene, (c) Ethylbenzene and (d) Xylene

To assess the significance of these differences, a hypothesis test was conducted. Normality testing of the benzene concentration data obtained at three different sampling times indicated that they follow a normal distribution. Therefore, Levene's inferential statistical test was used to determine the significance of the observed differences in benzene concentration at different sampling times.

The null hypothesis Ho states that the variances are identical, while the alternative hypothesis suggests that at least one of the variances is different from the other. In this case, the calculated p-value exceeded the significance level alpha=0.05. Therefore, the null hypothesis should be accepted, and the alternative hypothesis rejected. In other words, it can be inferred that no significant differences were found between benzene concentrations at different sampling times.

For Toluene, the average concentration in schedule B1 was 3.57 µg/m^3, with maximum and minimum values of 5.59 and 3.18 respectively, and a standard deviation of 0.73 µg/m^3. In schedule B2, the average concentration was 3.06 µg/m^3, with maximum and minimum values of 3.28 and 2.97 respectively, and a standard deviation of 0.10 µg/m^3. Lastly, for schedule B3, the average concentration was 3.27 µg/m^3, with maximum and minimum values of 3.78 and 2.95 respectively, and a standard deviation of 0.24 µg/m^3.

Figure 3 (b) shows the descriptive statistics for toluene concentrations at the study site during the 3 sampling times. Similar to benzene, the concentrations of toluene were notably higher during sampling period B1, which corresponds to the time from 07:00 to 08:00 hrs.

In order to determine if the differences in the concentration of toluene at different sampling times are statistically significant, a hypothesis test was conducted. The normality test of the concentration data for toluene during the 3 sampling times indicated that they do not follow a normal distribution. Therefore, the Friedman non-parametric test was used to determine the significance of the observed differences in the toluene concentration at different sampling times.

Based on the established null hypothesis Ho (the samples come from the same population) and the alternative hypothesis (the samples do not come from the same population), in this case, the calculated p-value was less than the significance level alpha=0.05. As a result, the alternative hypothesis should be accepted, and the null hypothesis rejected. Therefore, it can be concluded that the differences found between toluene concentrations at the different sampling times were significant.

For Ethylbenzene in schedule B1, the average concentration was 3.67 µg/m3, with maximum and minimum values of 4.08 and 3.44 µg/m3, respectively, and a standard deviation of 0.17 µg/m3. For schedule B2, the average concentration was 3.56 µg/m3, with maximum and minimum values of 3.68 and 3.47 µg/m3, respectively, and a standard deviation of 0.06 µg/m3. Finally, for schedule B3, the average concentration was 3.64 µg/m3, with maximum and minimum values of 3.84 and 3.52 µg/m3, respectively, and a standard deviation of 0.10 µg/m3.

The descriptive statistics in Figure 3 (c) show the concentrations of ethylbenzene during the 3 sampling times at the study site. Similar to benzene and toluene, the concentrations of ethylbenzene were notably higher during sampling period B1, which is from 07:00 to 08:00 hrs. To determine if these differences are statistically significant, a hypothesis test was conducted. The concentration data for ethylbenzene during the 3 sampling times were found to follow a normal distribution, and Levene's inferential statistical test was used to ascertain if the differences in ethylbenzene concentration at different sampling times are significant.

In this study, we set the null hypothesis Ho: the variances are identical, and the alternative hypothesis Ha: At least one of the variances is different from the other. After analyzing the data, the calculated p-value was greater than the significance level alpha=0.05. Therefore, we should accept the null hypothesis and reject the alternative hypothesis. This means that no significant differences were found between the concentrations of ethylbenzene at the different sampling times.

For Xylene in schedule B1, the average concentration was 3.91 µg/m^3, with maximum and minimum values of 5.03 and 3.40, respectively, and a standard deviation of 0.46 µg/m^3. In schedule B2, the average concentration was 3.57 µg/m^3, with maximum and minimum values of 3.76 and 3.37, respectively, and a standard deviation of 0.14 µg/m^3. Lastly, in schedule B3, the average concentration was 3.79 µg/m^3, with maximum and minimum values of 4.78 and 3.48, respectively, and a standard deviation of 0.37 µg/m^3. Figure 3 (d) provides the descriptive statistics for xylene concentrations during the 3 sampling times at the study site. Similar to benzene, toluene, and ethylbenzene, the concentrations of xylene were significantly higher during the sampling period B1, which corresponds to the time from 07:00 to 08:00 hr.

In order to determine if the differences in xylene concentration at different sampling times are statistically significant, a hypothesis test was conducted. The normality test of the concentration data for xylene during the 3 sampling times showed that they follow a normal distribution. Subsequently, Levene's inferential statistical test was applied to assess the significance of the observed differences in xylene concentration at different sampling times.

In this study, the null hypothesis (Ho) was that the variances are identical, while the alternative hypothesis (Ha) suggested that at least one of the variances was different from the other. After analyzing the data, it was found that the calculated p-value was greater than the significance level (alpha=0.05). Therefore, we accept the null hypothesis and reject the alternative hypothesis. In other words, we can conclude that there were no significant differences found between the xylene concentrations at the different sampling times.

Meteorological Analysis

From Figure 4, it can be observed that the prevailing wind direction was from SE.

Box 9

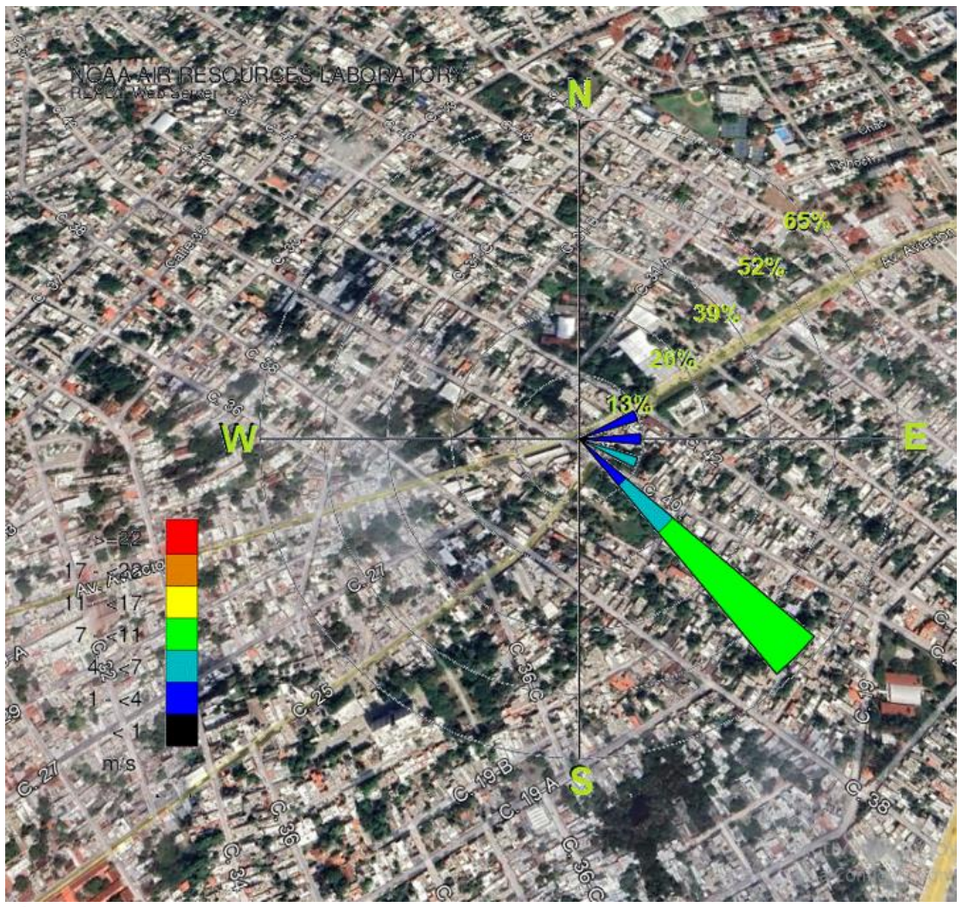


Figure 4

Prevailing wind direction in the sampling site

In Figure 5, the wind roses are plotted against the concentration for each of the BTEX compounds. Figures 5 (a-d) indicate that the concentrations of all BTEX compounds were higher when the wind blew from the southeast to the northwest.

Box 10

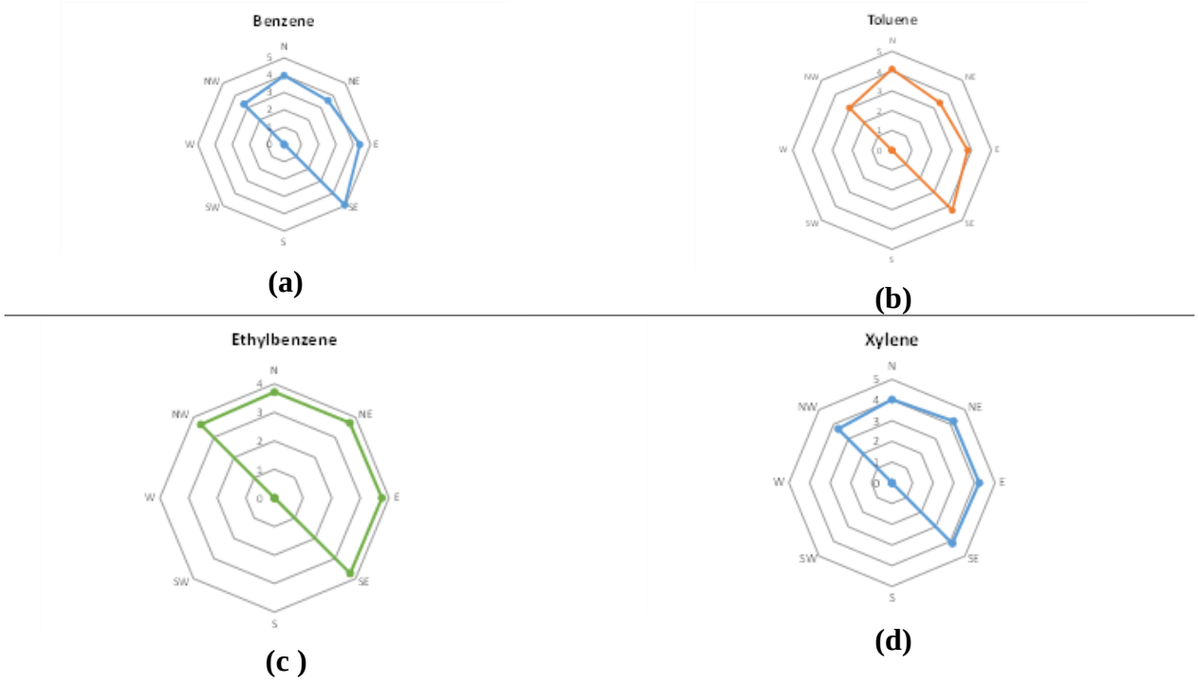


Figure 5

Wind roses vs concentration in the sampling site for (a) benzene, (b) toluene, (c) ethylbenzene and (d) xylene

Bivariate Analysis and Principal Components Analysis

To identify significant correlations between the BTEX compounds and the meteorological variables, we conducted a bivariate analysis using Pearson correlation (see Table 6).

From Table 6, we observed the following significant correlations: toluene-benzene (0.972), benzene-ethylbenzene (0.825), toluene-ethylbenzene (0.934), benzene-xylene (0.868), toluene-xylene (0.960), and xylene-ethylbenzene (0.997). It's important to note that the correlation between BTEX compounds in ambient air can vary based on factors such as emission sources, atmospheric conditions, and geographic location. These volatile organic compounds (VOCs) are commonly found in the air due to industrial, automotive, and other human-made sources.

BTEX compounds can be released into the air during processes such as incomplete combustion of fossil fuels, vehicle emissions, and various industrial activities. The correlation between these compounds may differ depending on the emission source. For instance, in urban areas with heavy traffic, the correlation is likely to be higher due to vehicle emissions. However, in industrial areas where toluene-containing solvents are used, the correlation may be different. It's crucial to note that although BTEX compounds may be correlated in certain situations, they may also have independent emission sources and different atmospheric behaviors. Additionally, each compound has different effects on human health.

Correlations between temperature-pressure (0.454), humidity-benzene (0.789), humidity-toluene (0.910), humidity-ethylbenzene (0.998), and humidity-xylene (0.990) were observed. Studies have shown that humidity can influence the volatility and transfer rate of BTEX compounds between air and other environmental matrices, such as soil and water. Furthermore, humidity can impact the atmospheric oxidation processes of these compounds, which can affect their concentrations in the air. However, the relationship between atmospheric humidity and BTEX concentration may not be direct and can be influenced by other factors such as temperature, human activity, local emission sources, and meteorological conditions. Therefore, while there may be a significant correlation in certain contexts or conditions, it's important to consider all these factors before generalizing this relationship. The ambient temperature had significant inverse correlations with benzene (-0.982), toluene (-0.999), ethylbenzene (-0.917), and xylene (-0.946). This means that as the temperature increases, the concentration of volatile organic compounds (VOCs) such as BTEX (benzene, toluene, ethylbenzene, and xylenes) in the ambient air decreases. This inverse relationship is due to several factors:

1. *Volatility:* BTEX compounds are highly volatile, so they evaporate more easily at higher temperatures. This leads to a greater release of BTEX from natural and human-made sources into the air.
2. *Atmospheric Stability:* Weather conditions, including temperature, can affect atmospheric stability. Higher temperatures can lead to thermal inversions, trapping contaminants near the ground and increasing their concentrations.
3. *Chemical Reactions:* Higher temperatures can trigger chemical reactions in the atmosphere that either produce or degrade BTEX, thus influencing their concentrations in the air.

It's important to consider that the relationship between ambient temperature and BTEX concentration can be influenced by various factors, such as season, geographic location, local emission sources, and specific meteorological conditions. While we generally expect an inverse correlation between temperature and BTEX concentration in ambient air, this relationship can vary in different contexts.

Additionally, there were correlations found between pressure and benzene (-0.277), pressure and toluene (-0.493), pressure and ethylbenzene (-0.772), pressure and xylene (-0.717), humidity and temperature (-0.891), and humidity and pressure (-0.809). The correlation between atmospheric pressure and the concentration of BTEX compounds in ambient air may be inverse in certain cases, but it's important to note that this relationship can be more complex due to the influence of multiple factors. For example, atmospheric stability, influenced by atmospheric pressure, can also affect the concentration of BTEX in the air. Under high-pressure conditions, thermal inversions are more likely to form, trapping contaminants near the ground and increasing their concentrations. The inverse correlation between pressure and humidity indicates that high-pressure systems are associated with low humidity, while low pressures are accompanied by high humidity, which is a characteristic phenomenon of the atmosphere.

Box 11

Table 6

Pearson correlation matrix between BTEX concentrations and meteorological variables in the sampling site

Variables	Benzene	Toluene	Ethylbenzene	Xylene	Temperature	Pressure (Hpa)	Humidity
Benzene	1	0.972	0.825	0.868	-0.982	-0.277	0.789
Toluene	0.972	1	0.934	0.960	-0.999	-0.493	0.910
Ethylbenzene	0.825	0.934	1	0.997	-0.917	-0.772	0.998
Xylene	0.868	0.960	0.997	1	-0.946	-0.717	0.990
Temperature	-0.982	-0.999	-0.917	-0.946	1	0.454	-0.891
Pressure (Hpa)	-0.277	-0.493	-0.772	-0.717	0.454	1	-0.809
Humidity	0.789	0.910	0.998	0.990	-0.891	-0.809	1

Principal component analysis (PCA) on the BTEX concentration data collected at the sampling site was carried out. The analysis revealed that 2 factors were sufficient to explain the variability of the data. Factor F1 contributed the greatest proportion (86.77%) to the total observed variability. In Factor 1, benzene, toluene, ethylbenzene, xylene, temperature, and humidity were grouped together, while atmospheric pressure was grouped in Factor 2 (Table 7).

Box 12

Table 7

Results of the Principal Components Analysis (PCA) for the sampling site

	F1	**F2**
Own value	6.074	0.926
Variability (%)	86.774	13.226
% accumulated	86.774	100.000

Figure 6 displays the biplot of the PCA analysis. There is noticeable clustering between BTEX compounds and humidity, as well as a significant inverse correlation between BTEX compounds and temperature. Additionally, there is a significant inverse correlation between atmospheric pressure and xylene, ethylbenzene, and humidity.

Box 13

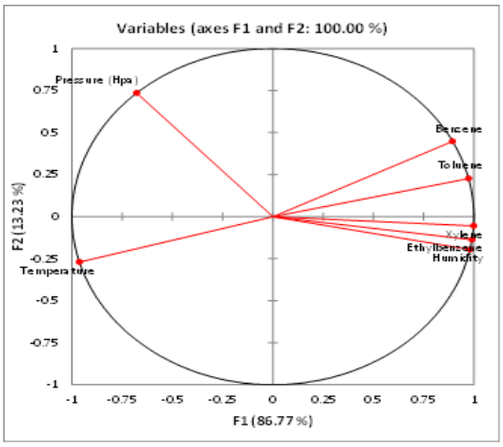


Figure 6

Biplot graph of PCA applied to BTEX concentrations and meteorological variables in the sampling site

Mapping

The BTEX concentrations were determined using the Kriging method to create a new set of estimated data. These estimates were then used to create concentration isolines in Surfer. The isolines were georeferenced to produce maps showing the spatial distribution of each BTEX compound.

Box 14

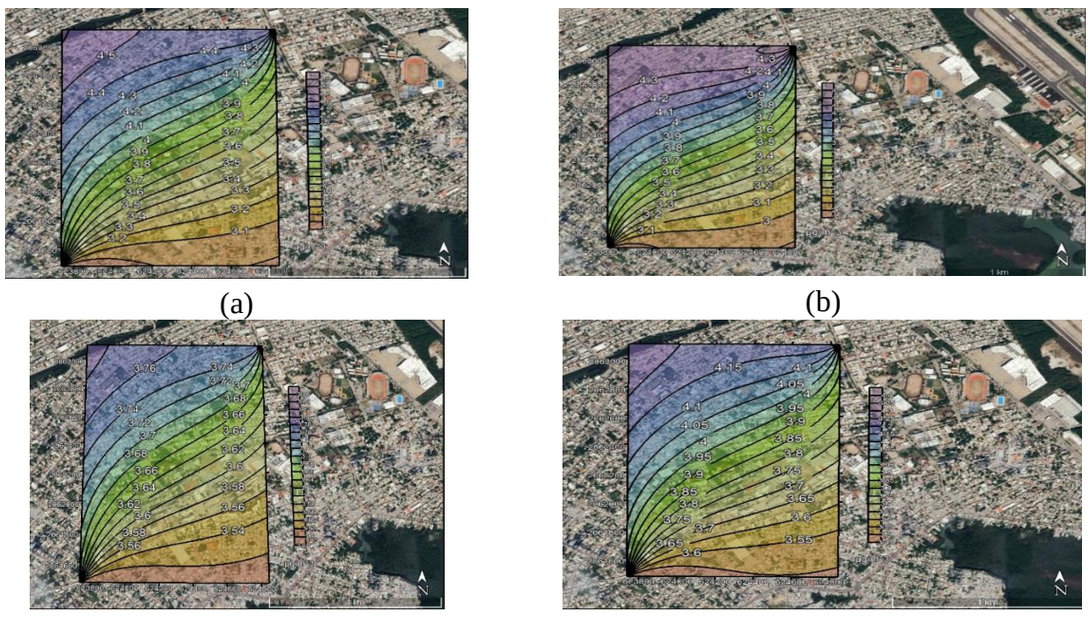


Figure 7

Map of isolines for (a) Benzene, (b) Toluene, (c) Ethylbenzene and (d) Xylene concentrations in the study area

The spatial distribution of benzene is depicted in Figure 7 (a), indicating higher concentrations towards the northwest of the sampling area, specifically near the fishing port, North Beach, and a section of the city center. This area also encompasses the Atasta sour gas recompression station and the offshore platform region. Similarly, Figure 7 (b) displays the spatial distribution of Toluene, revealing a similar pattern.

Figure 7 (c) illustrates the spatial distribution of ethylbenzene, indicating that ethylbenzene concentrations were higher toward the northwest of the sampling area, specifically near the North Beach, Fishing Port, and City Center area. Conversely, Figure 7 (d) demonstrates the spatial distribution of xylene, displaying a similar spatial pattern to the other BTEX compounds.

B/T and X/E ratios

The B/T and X/E ratios can help determine the likely origin of BTEX at a specific site. A toluene/benzene ratio (B/T) of less than 2-3 indicates a high influence of vehicular emissions, suggesting that BTEX comes from motor vehicle emissions, as both toluene and benzene are present in gasoline (Elbir et al., 2007). On the other hand, in the case of the xylene/ethylbenzene (X/E) ratio, this ratio is used to estimate the age of air masses and to infer whether the emissions are local and come from fresh emissions, or if they come from aged air masses, that is, of regional nature and with a certain history of photochemical processing. This way, values below 4.4 in this ratio indicate that the air masses are recent and carry fresh emissions, so their origin can be considered local (Keymeulen et al., 2001).

The B/T ratios for the 3 sampling times (B1, B2, and B3) from Table 8 were equal to or greater than 1, indicating a high influence of vehicular emissions at the sampling sites. This suggests that BTEX emissions likely originated from vehicular sources, possibly even from the same service station where the sampling was conducted.

Box 15

Table 8

Results of the Principal Components Analysis (PCA) for the sampling site

B/T		
B1	**B2**	**B3**
1.0162	1.0417	1.0209
0.9499	1.0250	1.0218
1.0765	1.0576	1.0383
1.0895	1.0510	1.0230
1.1019	1.0677	0.9286
1.1065	1.1298	1.0239
1.0953	1.0758	1.1047
1.1298	1.0669	1.0639
1.0823	1.2516	1.1733
1.1644	1.1543	1.1448
1.0812	**1.0921**	**1.0543**

B/T ratios obtained in the sampling site

In Table 9, it is evident that the X/E ratios for the 3 sampling times (B1, B2, and B3) were close to 1. This suggests that these are recent air masses carrying fresh emissions, indicating that the BTEX compounds measured can be considered to have a local origin.

Box 16

Table 9

X/E ratios obtained in the sampling site Results of the Principal Components Analysis (PCA) for the sampling site

X/E		
B1	**B2**	**B3**
0.9894	0.9732	0.9934
0.9869	0.9827	1.0101
1.0152	1.0142	1.0340
1.0762	0.9863	0.9882
1.1099	0.9887	1.0462
1.0736	1.0395	1.0050
1.0595	1.0132	1.2433
1.0401	0.9864	1.0041
1.0411	1.0289	1.0662
1.2343	0.9988	1.0114
1.0626	**1.0012**	**1.0402**

B/T ratios obtained in the sampling site

The B/T ratios are used to determine the relative abundance between vehicular and non-vehicular sources. In this case, the B/T ratios for the sampling site were less than 3, ranging from 0.998 to 1.168 with an average value of 1.076. This indicates that BTEX emissions come from vehicular sources, as this range has been reported in various urban areas worldwide (see Figure 8).

Box 17

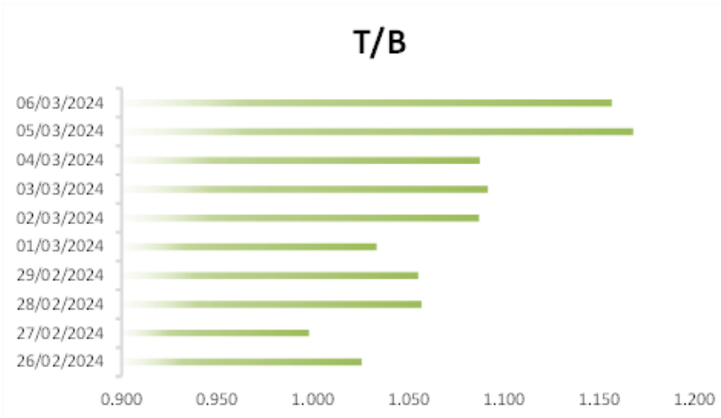


Figure 8

B/T ratios in the sampling site

The X/E ratios are used as an indicator of the photochemical age of the air masses at the sampling site. This is because xylene is more reactive compared to ethylbenzene, leading to photochemical reactions. In this study, the X/E ratios indicate that the air masses are fresh (see Figure 9).

Box 18

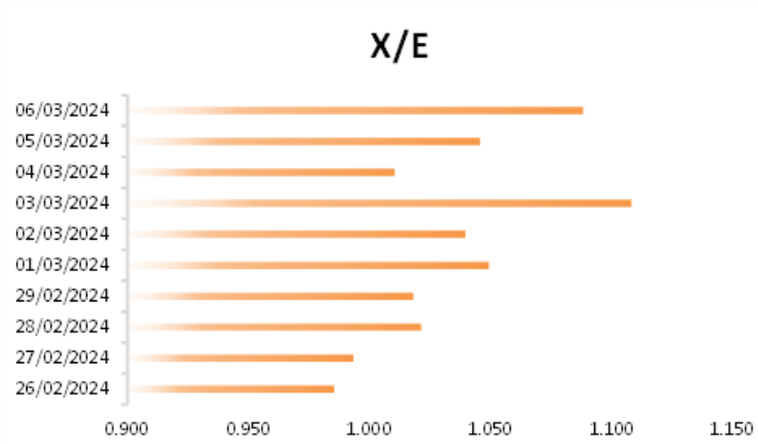


Figure 9

X/E ratios in the sampling site

Therefore, from B/T and X/E ratios, it can be concluded that BTEX levels found in the sampling site had a local origin, specifically in fresh emissions from vehicular sources at NW of the sampling sites, probably, in the surroundings of the fishing port, the downtown of the city, and North Beach.

Health Risk Assessing

The cancer risk index due to exposure to benzene at the sampling site was calculated for both, adult and child populations (Figure 10 a). Lifetime cancer risk (LTCR) values for the sampling site were from $2.88x10^{-5}$ to $4.12x10^{-5}$, with a mean value of $3.25x10^{-5}$ for adults; and $5.45x10^{-5}$ to $7.8x10^{-5}$ with a mean value of $6.15x10^{-5}$ for children. The averages in both cases can be seen to exceed the reference value proposed by the US EPA (LTCR $1x10^{-6}$); being the risk significantly higher for the child population.

Box 19

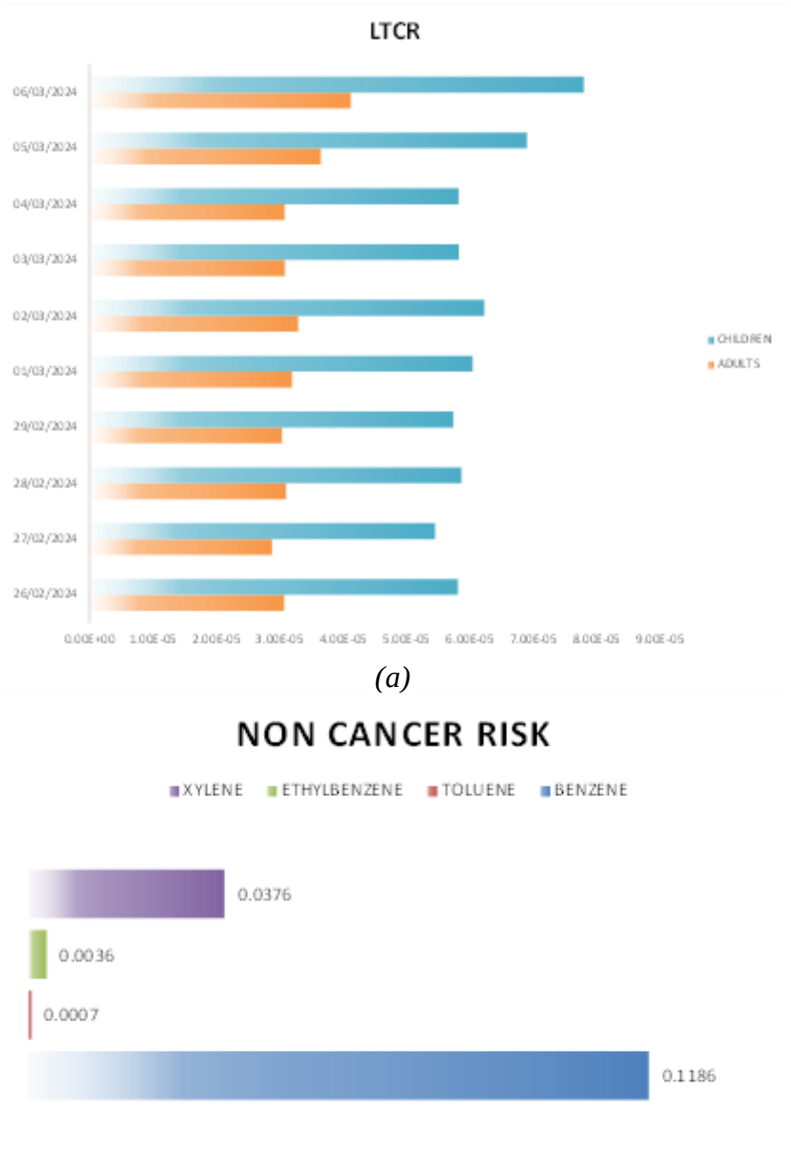


Figure 10

(a) Cancer risk (LTCR) due to benzene exposure, (b) Non-cancer risk quotient (HQ) due to exposure to BTEX

Non-carcinogenic risk quotients were estimated for the BTEX compounds. The overall potential for non-carcinogenic effects due to exposure to more than one chemical was determined as an HQ risk quotient, where a value of HQ <1 indicates that the population is not exposed to a significant risk of contracting diseases other than cancer (respiratory and cardiovascular diseases) as a consequence of daily exposure to this type of compounds. From Figure 10 (b) the HQ values were in all cases less than unity, which suggests that the population near the study area does not present a risk of suffering respiratory and cardiovascular diseases.

Comparison with other works

Table 10 compares the results obtained in the present study with those reported by other authors in other regions of the world. In the case of Benzene, it can be observed that the levels obtained in the study site were considerably lower than those reported by Baimatova et al. (2016) in Kazakhstan; Chaiklieng (2021) in Thailand, Cerón et al. (2018) in Tijuana, Estéves et al. (2015) in Orizaba, and by Cerón et al. (2017) in Mérida. However, they were higher than those found by Mainka and Kozielska (2016) in a kindergarten in Poland, by Tecer et al. (2018) in Turkey, by Moolla et al. (2015) in South Africa, and by Cerón et al. (2020) in Leon.

The Toluene levels found were lower than those reported in other studies and considerably lower than those reported by Kerchich and Kerbachi (2012) in Algeria, and by Chaiklieng (2021) in Thailand. On the other hand, the levels of Ethylbenzene obtained were comparable to those found by Molla et al. (2015) in South Africa and lower than those reported by other authors. Finally, in the case of xylene, the concentrations found were lower than those reported by other authors, and significantly lower than those found by Chaiklieng (2021) in Thailand and by Cerón et al. (2018) in Ensenada.

Box 20

Table 10

Comparison with results obtained in other regions of the world

Location	B	T	E	X	Reference
indergarten in Gliwice, oland (µg/m^3)	1.24	0.78	0.22	0.46	Mainka and Kozielska (2016)
Almaty, Kazajistán (µg/m^3)	53	57	11	14	Baimatova et al. (2016)
Algiers, Alger (µg/m^3)	16.7	40.5	6.8	10.74	Kerchich and Kerbachi (2012)
Yalova, Turkey (µg/m^3)	2.6	11	1.32	3.8	Tecer et al. (2018)
Johannesburg, South Africa (µg/m^3)	1.41	3.22	0.67	4.1	Moolla et al. (2015)
Khon Kaen, Thailand (µg/m^3)	33.1	142.7	14.4	41.3	Chaiklieng (2021)
Ensenada, Baja California (µg/m^3)	16.8	50.7	0.1	3.1	García et al. (2014)
Tijuana, Baja California, (µg/m^3)	32.4	13.28	7.02	17.16	Cerón et al. (2018)
Leon, Guanajuato (µg/m^3)	1.73	11.85	11.86	3.31	Cerón et al. (2020)
Orizaba, Veracruz (µg/m^3)	74.51	5.33	2.26	3.35	Estéves et al. (2015)
Merida, Yucatan (µg/m^3)	40.91	6.87	6.23	13.87	Cerón, et al. (2017)
Merida, Yucatan (µg/m^3)	32.86	3.29	4.48	8.29	Cerón, et al. (2017)
This study (µg/m^3)	3.56	3.30	3.62	3.76	Cerón et al. (2024)

Conclusions

Temporal variability in BTEX levels was evaluated at a site adjacent to a gas station in Ciudad del Carmen, Campeche, during the dry season of 2024. The concentrations of all BTEX were highest during the B1 sampling period, which corresponds to the time from 07:00 to 08:00 hrs; However, when applying hypothesis tests, it was found that these differences were only significant for toluene. The BTEX concentrations in the present study were generally lower than those reported by other authors in other world regions.

From plots of wind vs concentration, it was assessed the influence of surface meteorology on the measured BTEX levels, regarding this, it was found that the trend was the same for all BTEX compounds, with the highest concentrations when the wind had a SE component, that is, when the wind blew from the SE to the NW.

Applying geo-statistical tools, the spatial variability of BTEX compounds in the study area was evaluated, finding in all cases that the levels of BTEX tend to be higher towards the NW of the sampling area, that is, towards the zone of North Beach, the downtown of the city and the Fishing Port. This area includes avenues and roads that connect the East edge of the island with the West edge; so, at certain times of the day, these roads stay congested and have high vehicle traffic.

In this regard, to determine whether vehicular sources had a significant impact on the emission of BTEX compounds, the B/T and X/E ratios were estimated. The B/T ratios for the 3 sampling times (B1, B2, and B3) were close to 1, which can be considered characteristic of sites with a high influence of vehicle emissions. Therefore, we conclude that BTEX emissions had their origin in vehicular emissions and, also from the service station where the sampling was done.

On the other hand, since the X/E ratios are used as an indicator of the photochemical age of the air masses at the sampling site, in this study, all cases, in a range of 0.998 to 1.168 with an average value of 1.076, which indicates that the emissions were recent and of local origin, indicating that they are fresh air masses. The sour gas recompression station and the offshore platform area are also located in the NW direction; However, according to what was found from the B/T and X/E ratios, it is concluded that the influence of regional sources was not significant.

Finally, the cancer risk index for benzene exposure exceeded the reference value proposed by the US EPA (LTCR $1x10^{-6}$), suggesting a significant risk, and being even higher for the child population. The overall potential for no carcinogenic effects due to BTEX exposure was determined as an HQ risk quotient. In all cases, a value of HQ<1 was obtained, indicating that the population is not exposed to a significant risk of contracting diseases other than cancer (respiratory and cardiovascular diseases) due to the daily exposure to the BTEX levels found in the study area. However, it is necessary to carry out more studies around the city including other climatic seasons and a greater number of sampling sites (service stations) to obtain more precise conclusions regarding the risk associated to this type of emissions.

Annexes

Tables and adequate sources.

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 in this chapter.

Author contribution

Cerón-Bretón, Rosa María: Contributed to the project idea, research method, technique, financial support, result analysis and paper redaction.

Cerón-Bretón, Julia Griselda: Contributed to the project idea, research method, technique, financial support, result analysis and paper redaction.

Pérez-Vera, Joselyn Itzell: Contributed to the sampling and chemical analysis.
Reyna del Carmen Lara Severino: Contributed to research method and technique, statistical analysis.

Availability of data and materials

The Data is available if required.

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Abbreviations

ATSDR	Agency for Toxic Substances and Disease Registry
UNACAR	Autonomous University of Carmen
BTEX	Benzene, Toluene, Ethylbenzene, and the three isomers of Xylene
FAO	Food and Agriculture Organization of the United Nations
UNEP	UN Environment Programme
GIS	Geographic Information System
GCA	Global Air Quality Guidelines
INEGI	National Institute of Statistic and Geography
LTCR	Lifetime cancer risk
MPL	Maximum Permissible Limit
NIOSH	National Institute for Occupational Safety and Health
INSHT	National Institute of Safety and Hygiene at Work)
NOAA	National Oceanic and Atmospheric Administration

HQ	Non-cancer risk potential
POE	Occupationally exposed personnel
PAHO	Pan American Health Organization
PCA	Principal component analysis
LTCR	The lifetime benzene cancer risk
US EPA	EPA de EE. UU.)
VOCs	Volatile organic compounds
WHO	World Health Organization

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Association of the Arg51Gln Polymorphism of the Ghrelin Gene and Serum Ghrelin levels with overweight and obesity in young individuals

Asociación del polimorfismo Arg51Gln del gen de grelina y de los niveles séricos de grelina con sobrepeso y obesidad en población joven

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Key Handbooks

The research contributes to understanding the genetic (Arg51Gln polymorphism) and biochemical (serum ghrelin levels) factors influencing obesity and overweight, particularly in a young population from Western Mexico. The research offers insights into the monomorphic nature of the Arg51Gln polymorphism in the Western Mexican population, which is crucial for understanding genetic influences on obesity in specific demographics. Role of Ghrelin: Understanding ghrelin's role in regulating appetite and energy balance and its association with body weight and fat distribution. Genetic Variations: Comprehending how specific genetic polymorphisms (e.g., Arg51Gln) affect ghrelin secretion and activity, and how these variations influence obesity. Population-Specific Genetic Expressions: Recognizing that genetic factors influencing obesity may vary significantly across different populations, which necessitates studying diverse demographic groups. Hormonal and Metabolic Interactions: Investigating how hormonal levels (like ghrelin) interact with metabolic processes and contribute to conditions like overweight and obesity.

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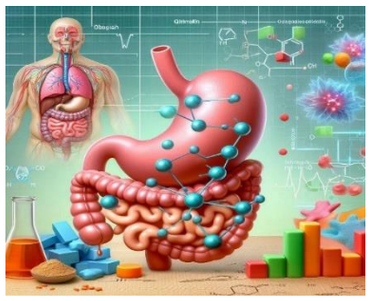
Abstract

Obesity and overweight are growing public health concerns, especially among young people. Ghrelin, an appetite-regulating hormone, and the Arg51Gln polymorphism in the ghrelin gene have been associated with obesity. Methodology: Serum ghrelin levels and the Arg51Gln polymorphism were studied in 289 university students from western Mexico. Anthropometric measurements and blood samples were taken to analyze ghrelin levels and genotype the polymorphism. Results: The polymorphic allele (T) was not found in the participants; all were homozygous for the wild-type allele (C/C). Ghrelin levels were higher in the obesity group and lower in the overweight group. A significant association was found between ghrelin levels and overweight, but not with obesity. Conclusions: Serum ghrelin levels are associated with overweight in young individuals, but the Arg51Gln polymorphism is not present in this population. These findings may inform prevention and treatment strategies for obesity**.**

Association of the Arg51Gln Polymorphism of the Ghrelin Gene and Serum Ghrelin Levels with Overweight and Obesity in young individuals		
Objectives	**Methodology**	**Contribution**
		
Describe the association of ghrelin and the Arg51Gln polymorphism with overweight and obesity in young populations	*Selection of young subjects with overweight and obesity *Determination of Ghrelin by sandwich ELISA *Characterization of the polymorphism through real-time PCR	Joint analysis of obesity with ghrelin Evidence of the possible existence of the monomorphic region for this coding zone in the Mexican population from the western region

These images were created with AI Dall-E

Obesity, Ghrelin, Polymorphism

Resumen

La obesidad y el sobrepeso son crecientes preocupaciones de salud pública, especialmente entre los jóvenes. La grelina, una hormona reguladora del apetito, y el polimorfismo Arg51Gln se han asociado con la obesidad. Metodología: Se estudiaron los niveles séricos de ghrelina y el polimorfismo Arg51Gln en 289 jóvenes del occidente de México. Se tomaron medidas antropométricas y muestras de sangre para analizar los niveles de grelina y realizar la genotipificación del polimorfismo. Resultados: No se encontró el alelo polimórfico, todos fueron homocigotos para el alelo de tipo salvaje. Los niveles de ghrelina fueron más altos en el grupo con obesidad y más bajos en el grupo con sobrepeso. Se encontró una asociación significativa entre los niveles de ghrelina y el sobrepeso, pero no con la obesidad. Conclusiones: Los niveles séricos de ghrelina están asociados con el sobrepeso en jóvenes. Estos hallazgos pueden informar estrategias de prevención y tratamiento de la obesidad.

Asociación del polimorfismo Arg51Gln del gen de grelina y de los niveles séricos de grelina con sobrepeso y obesidad en población joven		
Objetivos	**Metodología**	**Contribución**
		
Describir la asociación de grelina y el polimorfismo Arg51Gln con sobrepeso y obesidad en población joven	*Selección de sujetos jóvenes son sobrepeso y obesidad *Determinación de Grelina por ELISA tipo sándwich *Caracterización del polimorfismo a través de PCR en tiempo real	Análisis de forma conjunta de obesidad con grelina Evidencia de la posible existencia de la región monomórfica para esta zona codificante en población mexicana del occidente

Estas imágenes fueron creadas con AI Dall-E

Obesidad, Grelina, Polimorfismo

Introduction

Obesity and overweight are growing public health concerns worldwide, particularly among young adults, including university students. Understanding the genetic and biochemical factors that contribute to these conditions is crucial for developing effective prevention and treatment strategies. Ghrelin, a peptide hormone primarily produced in the stomach, plays a significant role in regulating appetite and energy balance. Variations in the ghrelin gene, particularly the Arg51Gln polymorphism, have been implicated in influencing ghrelin levels and potentially contributing to obesity.

The Arg51Gln polymorphism results from a single nucleotide change that replaces arginine (Arg) with glutamine (Gln) at position 51 in the ghrelin peptide. This genetic variation has been associated with altered ghrelin secretion and activity, which may influence body weight and fat distribution. Additionally, serum ghrelin levels, which regulate hunger and energy intake, may differ among individuals with varying body mass indices (BMI).

This study aims to describe the association of serum ghrelin levels and the Arg51Gln polymorphism of the ghrelin gene with overweight and obesity in young individuals from Western Mexico. By investigating these associations, we seek to contribute to the understanding of the genetic and biochemical factors underlying obesity in this population.

We hypothesize that there is an association between serum ghrelin levels and/or the Arg51Gln polymorphism of the ghrelin gene with overweight and obesity in university students from Guadalajara. This hypothesis is based on previous studies that have shown a link between ghrelin gene polymorphisms, ghrelin levels, and body weight regulation. Our research will provide further insights into the potential genetic and hormonal mechanisms influencing obesity in young adults, which may inform targeted interventions and therapeutic approaches.

In this chapter, we present the results of our research and explore the topics of obesity and overweight, satiety and intake control, ghrelin and related diseases, genetic variants of the ghrelin gene, the Arg51Gln polymorphism, and metabolic diseases in young populations.

Development

Overweight and Obesity

Recent statistics indicate that overweight and obesity continue to rise globally, with more than 2 billion people worldwide having excess weight, accounting for approximately 30% of the global population (Caballero, 2019; WHO, 2024). In Latin America, according to the latest joint report presented in the Panorama of Food and Nutrition Security in Latin America and the Caribbean, about 16% of the population is obese, and slightly over 43% are overweight, resulting in a combined prevalence of nearly 60%. The impact is more significant among women and shows an upward trend in adolescents and young adults (OPS, 2024). Meanwhile, in Mexico, the 2022 National Health and Nutrition Survey reported a combined prevalence of overweight and obesity of 75.2%. Notably, the prevalence of obesity increased by 21.4% from 2006 to 2022 (INSP, 2023).

Obesity is a complex and multifactorial condition affecting millions of people worldwide and significantly contributing to the burden of morbidity. It is associated with various pathologies, including cardiovascular diseases, type 2 diabetes, hypertension, and certain types of cancer (Erion et al., 2017). Its etiology involves genetic and environmental factors, as well as lifestyle factors such as dietary habits, physical inactivity, and the nature of food consumed (INSP, 2024). Excess weight is attributed to an imbalance between caloric intake and energy expenditure, leading to chronic excessive accumulation of adipose tissue (Erion et al., 2017).Energy metabolism and appetite regulation are influenced by a complex network of hormonal and neuroendocrine signals (Lustig et al., 2022). Multiple hypothalamic regions send and receive signals from the insula, orbitofrontal cortex, nucleus accumbens, and the dopaminergic reward system, as well as chemical signals, including peptides and gastrointestinal hormones, to regulate eating behavior (Van Loenen et al., 2020). Among these signals, ghrelin, known as the "hunger hormone," plays a crucial role due to its orexigenic effect.

Ghrelin

Ghrelin is a peptide hormone composed of 28 amino acids requiring an essential enzymatic modification (O-acyltransferase) to enable its bioactivity and ability to activate one of the two ghrelin receptors. Two different ghrelin receptors have been identified: GHS-R1a and GHS-R1b (Van Loenen et al., 2020). This hormone is primarily produced in the stomach but can also be found in small concentrations in the hypothalamus. It promotes food intake and regulates energy homeostasis by acting on the hypothalamus to increase appetite and stimulate the release of growth hormone (Ibrahim et al., 2023).

The activation of GHS-R1a by ghrelin induces multiple cells signaling pathway cascades, which can, in turn, regulate a wide range of metabolic processes such as gluconeogenesis and fat deposition (Van Loenen et al., 2020). Interestingly, ghrelin levels are shown to be reduced in the context of obesity and a high-fat, high-sugar diet. Ghrelin acts on all body systems, with well-known effects involving the regulation of the gut-brain axis, including appetite, food intake, energy, and glucose metabolism, which are key factors leading to obesity (Ibrahim et al., 2023).

Some studies indicate that ghrelin is a potent stimulator of growth hormone (GH) secretion, which leads to insulin suppression by glucose action. Ghrelin synthesized in the islets of Langerhans restricts insulin release (Dezaki, 2015). It has also been linked to Metabolic Syndrome (MS). The OPERA study showed a negative correlation between ghrelin levels and the number of MS components. MS itself decreased ghrelin levels as the number of MS components increased. Recently, it has been found that as the number of MS parameters increases, there is a reduction in circulating acylated ghrelin, while desacylated ghrelin increases. However, it remains unclear whether this association is causal and its behavior in apparently healthy individuals (Mora et al., 2015).

Ghrelin gene

The coding of the ghrelin gene and its genomic structure has allowed for detailed scrutiny of the gene. This has increased interest in the potential role of the ghrelin gene in regulating risk in various pathologies, including obesity and lipid metabolism disorders, as well as inflammatory and metabolic diseases such as metabolic syndrome, insulin resistance, and diabetes mellitus.

Ghrelin is encoded by the GHRL gene, and genetic variations in this gene can influence serum ghrelin levels and, consequently, body weight regulation (Becer & Ergoren, 2021). The best-known SNPs are Arg51Gln, located in the region that regulates the generation of the active mature hormone, and Leu72Met and Gln90Leu, located elsewhere in the gene (Mora et al., 2015).

Arg51Gln polymorphism (rs34911341)

Arg51Gln is located in exon 2 of the ghrelin gene. This polymorphism involves an amino acid change from arginine (Arg) to glutamine (Gln) at position 51, which can alter ghrelin's functionality and its interaction with the receptor (Pöykko, 2003). The Arg51Gln polymorphism (rs34911341) has been studied in various populations due to its potential impact on susceptibility to obesity and overweight (Becer & Ergoren, 2021).

Previous studies have explored the relationship between the Arg51Gln polymorphism and serum ghrelin levels, as well as its association with anthropometric parameters in various cohorts. However, the results have been inconsistent, and the influence of this polymorphism in specific populations, such as young individuals, is not yet fully understood. Given the importance of the young adult population in preventing long-term obesity and its comorbidities, it is crucial to better understand the genetic and hormonal factors contributing to overweight and obesity at this life stage.

This study aims to investigate the association of the Arg51Gln polymorphism of the ghrelin gene and serum ghrelin levels with overweight and obesity in a young population. Identifying these associations could provide a deeper understanding of the mechanisms underlying body weight regulation and offer new perspectives for preventive and therapeutic strategies in the fight against obesity.

Methodology

Before the beginning of the study, we shared the necessary information with students who met the inclusion criteria. Inclusion criteria were: 18 – 25 years, fasting 8 – 10 hours, BMI ≥ 19 Kg/m^2, not related with other participant of the study, sign inform consent and participated volunteer. Those who decided to participate were received in the Biochemistry Laboratory between 8:00 and 10:00 in the morning. Upon arrival, those meeting the inclusion criteria filled out a clinical history form. We took anthropometric measurements (weight, height) for BMI calculations. After that, a blood sample was taken in two different tubes for analysis. One tube was used to quantify serum ghrelin, and the other was used for DNA extraction to characterize the Arg51Gln polymorphism.

Body max index calculations.

For weight, we used a TANITA scale 300A with participants barefoot, standing, and recorded the weight in kilograms (kg). Height was measured with a stadiometer, with participants standing with heels together, arms at their sides, and their backs against a flat surface, recorded in meters. This data was used to calculate BMI, which is calculated by dividing the weight in kilograms by the height squared in meters. We used this calculation to divide the population into three groups: those with normal weight (19.0 kg/m^2 – 24.5 kg/m^2), overweight (25.0 kg/m^2 – 29.9 kg/m^2), and obesity (> 30.0 kg/m^2) according to WHO standards

Ghrelin serum levels assay

One of the blood samples was centrifuged to obtain serum, which was separated into aliquots of 500 µL in Eppendorf microtubes and frozen at -20°C for analysis once all volunteers were recruited. The stability of the samples is 6 months at frozen temperatures, avoiding freeze-thaw cycles. Once we completed the sample size, analysis was performed by immunoassay using the Magpix Luminex with the Bio-Plex 200 System Assay Human Diabetes kit, according to the manufacturer's specifications.

DNA Extraction

DNA from leukocytes was extracted using the modified Miller salt precipitation method. The obtained DNA was reconstituted with 0.3 mL of Tris-EDTA (T.E.) buffer and stored at -76°C (Miller, 1998)

DNA Quantification

The obtained DNA was analyzed by spectrophotometry to quantify concentration and purity. After that, it was homogenized with sterile water and separated into aliquots with a final concentration of 100 ng/µL to be used in the characterization of the Arg51Gln polymorphism.

DNA Analysis

DNA analysis was performed using the real-time PCR technique with a TaqMan® allelic discrimination probe. The characteristics used are described in the Table 1:

Table 1.

Box 1

Table 1

DNA Analysis conditions

General Conditions	Conditions		Program (50 cycles)			
		Final Volume	Stage	Temperature	Time	Cycles
Polymorphism: rs398123011	2x Master Mix	2.5 uL	Pre incubate	95 ° C	10 min	1
Gene: GHRL Polymorphism: C/T	40x Tub	0.125 uL	Amplification	92° C	15 seg	50
VIC: C (Wild type)	PCR Water	1.875 uL	Extension	60° C	90 seg	1
FAM: T (Polymorphic)	DNA [50 ng/uL)	0.5 uL	Cooling	37 °C	30 seg	1

Statistical Analysis

In this study, descriptive statistics were performed, reporting percentages for qualitative variables, mean and standard deviation for quantitative variables, or median and interquartile range according to distribution. Allelic and genotype frequencies were determined by direct counting of the observed genotypes. The Hardy-Weinberg equilibrium test was performed to verify the study population. The association between variables was assessed using the Chi-square test to determine the Odds Ratio. The relationship between ghrelin serum levels and the dependent variable was analyzed using an ANOVA test. To analyze intervening variables, logistic regression was used. A p-value ≤ 0.05 was considered significant. All data were analyzed using Excel and the statistical package Statgraphics Centurion 19.

Results

Two hundred eighty-nine volunteers were recruited: 94 men and 126 women. They were divided into three groups according to BMI: 97 were of normal weight, 54 were overweight, and 69 were obese. When the polymorphism was characterized, no subject presented the polymorphic allele (T). All volunteers were homozygous for the wild-type allele (C/C).

Study variables were analyzed according to BMI in the three previously mentioned groups. Table (2) shows descriptive data of the population. Ghrelin serum levels were within the normal range (300 – 900 pg/mL). Statistical differences were observed between the normal weight group and the obesity group, with higher levels in the obesity group. It was also observed that the overweight group had a tendency towards lower levels of ghrelin, although this difference was not statistically significant.

We also observed differences in SBP/DBP, triglycerides, and HDL between the overweight and obesity groups compared to the normal weight group, the data is shown in table 2. This could be mainly due to the pattern of adipose tissue accumulation, which is more pronounced in individuals with higher BMI.

Box 2

Table 2

Descriptive data according to BMI

	Normal weight	Overweight	Obesity	p-value
	n=97	n= 54	n= 69	
Ghrelin (pg/mL)	618.5±438.3	481.1±376.5	780.4±444.9*	0.0001
Age (years)	20.3±1.4	21.3±2.1*	21.2±1.7*	0.0001
BMI (Kg/m^2)	21.4±1.9	27.5±1.5*	33.8±4.3*	0.0001
SBP (mm Hg)	109.7±9.8	117.6±10.1	121.6±12.2*	0.0000
DBP (mm Hg)	72.7±8.2	74.6±11.6	79.7±10.3*	0.0001
Triglycerides (mg/dL)	80.8±35.6	116.7±63.3*	140.8±87.5*	0.0001
HDL (mg/dl)	52.3±10.7	41.2±9.9	37.6±10.8*	0.0001

Media ± SD; ANOVA test; post hoc LSD. *Difference respective normal weight group. p<0.05.

Ghrelin serum levels divided into two groups, men and women, are shown in Figure 3. No statistical differences were observed between them.

Box 3

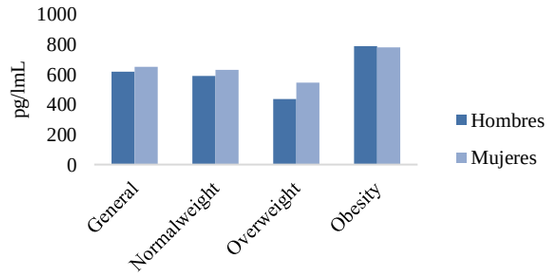


Figure 3

Ghrelin values in men and women

A Chi-square test was conducted to identify if there was an association between ghrelin levels and overweight or obesity, and an association with overweight was found, with an OR of 4.185 and a p-value < 0.0001 (CI 2.105-8.318). The results of all associations are shown in the table 3.

It can be observed that there is an association between ghrelin and overweight when compared to the reference group with normal weight, and even when contrasted with the rest of the volunteers who are not overweight. The OR value is greater than 2 and the results are statistically significant.

Box 4

Table 3

Association of ghrelin with overweight and obesity Descriptive data according to BMI

	Low ghrelin	Normal ghrelin
	Overweight vs Without OW	
OW	23	31
W/OW	25	141
OR	**4.255 (2.105-8.318) p < 0.0001***	
	Overweight/Obesity vs Normal weight	
OW/OB	32	91
NW	16	81
OR	1.780 (0.9102-3.482) p > 0.05	
	Obesity vs Sin Obesity	
OB	9	60
W/O	39	112
OR	0.4786 (0.2233-1.026) p > 0.0547	
	Overweight vs Normal weight	
OW	23	31
NW	16	81
OR	**3.756 (1.755-8.036) p<0.0001**	

*Frequency. OR: Odds ratio (Confident interval). *Significant p<0.05. NW: Normal weight, Ow: Overweight, Ob: Obesity.*

Finally, we conducted a logistic regression to evaluate the role of the intervening variables, shown at table 4. The variable sex was not included in the equation, indicating that it does not influence the ghrelin results. The adjusted OR value for overweight is 4.946 (CI 2.043-11.975) with a p-value < 0.0001.

Box 5

Table 4

Logistic regression to evaluate sex and BMI effect on ghrelin lower levels

Equation variables	B	Wald	Sig.	Exp (B)	CI (95%)
BMI		16.893	0.000		
BMI (1)	.275	.374	.541	1.317	0.545-3.182
BMI (2)	1.599	12.557	0.000	4.946	2.043-11.975
Constant	-1.897	28.167	0.000	0.150	

In addition, complementary analyses of the Arg51Gln genotyping were conducted in a population from western Mexico. A total of 432 individuals from Jalisco (149), Colima (192), and Nayarit (92) were studied. Among these individuals, no polymorphic subjects for Arg51Gln were found, suggesting that this polymorphism might be monomorphic in our population.

Discussion

We observed normal ghrelin levels in the population. When stratified by BMI, lower ghrelin levels were observed in overweight volunteers. Previous studies, such as that by Llamas-Covarrubias et al. (2015), have reported low ghrelin levels in individuals with obesity, but we did not find this in our study. The normal serum ghrelin levels observed in the obese group in our study can be explained in two ways: higher levels of the desacylated form in an attempt to control the energy imbalance, which has been mainly reported in young people; in contrast, reports of low ghrelin levels related to obesity have focused on adults over 40 years old (Akimoto-Takano et al., 2005; Becer & Ergoren 2021).

Another possible reason could be that we are observing an adaptive state where hyperghrelinemia is present, leading to resistance to the positive effects of the hormone due to alterations in receptor binding, receptor expression, or inadequate hormone transport. This could explain why postprandial ghrelin levels do not decrease in obese individuals (Cui et al., 2017). A limitation of this study is that we only measured serum hormone levels and did not measure each isoform or receptor expression. However, our strength lies in our age group, as previous studies have focused on adults who already have more significant metabolic alterations.

We did not find differences in ghrelin levels between men and women. Some studies, such as the one reported by Soriano-Guillén et al. (2016), have suggested that this hormone could exhibit sexual dimorphism. These differences can mainly be explained by variations described in adolescent groups or postmenopausal women. It has been observed that estrogen levels strongly relate to ghrelin synthesis, potentially causing differences not present in our study group.

Regarding the association of ghrelin with overweight, this has been previously described by other authors like Mora et al. (2015). However, they also describe an association of the hormone with obesity.

We only found an association in the overweight group, with an odds ratio greater than 2, indicating that low hormone levels could be considered a risk factor for developing overweight. We believe the lack of association with obesity could be due to an adaptive state by the body. Other studies have shown that once the overweight stage is surpassed, individuals reaching obesity can exhibit metabolic control, leading to what is known as metabolically healthy obesity (MHO). These individuals may have biochemical alterations, such as in lipid profiles, but can still control hormone and cytokine synthesis and secretion, as is the case with ghrelin.

Finding this situation can be advantageous, as we might be dealing with obese individuals who can still return to a healthy metabolic state without severe alterations like insulin resistance. Analyzing other hormones, such as insulin and leptin, could improve our understanding of the findings in this study.

Finally, since no Arg51Gln polymorphism was found in our volunteers, we decided to search in a larger population group with similar sociodemographic characteristics, and no polymorphic subjects were found. This indicates that while we have observed variations in ghrelin levels, they are not due to the presence of a genetic variation, at least not the one described here. Therefore, we might be looking at a population monomorphic for this coding region, opening the door to new questions on this topic.

Conclusions

- The study suggests the possibility of adaptive mechanisms in obese individuals, where normal ghrelin levels might indicate a state of metabolic control, known as metabolically healthy obesity (MHO).
- There were no significant differences in ghrelin levels between men and women within the studied age group.
- The population of western Mexico is monomorphic for the Arg51Gln SNP.
- The lowest ghrelin levels were found in the overweight group, showing statistically significant differences compared to the other groups.
- Low ghrelin levels are associated with overweight but not with obesity.

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 in this chapter.

Author contribution

Uvalle-Navarro, Rosario Lizette: Contributed to conception idea, writing the document, research method and technique.

González-Sandova, Claudia Elena: Contributed to conception idea, writing the document and research method.

Díaz-Burke, Yolanda: Contributed to conception idea and research method.

Mederos-Torres, Claudia Verónica: Contributed to conception idea, writing the document, research method and technique.

Availability of data and materials

Further data is available from the corresponding author on reasonable request.

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Abbreviations

ANOVA	Analysis of Variance
Arg	Arginine
BMI	Body Mass Index
CI	Confidence Interval
DBP	Diastolic Blood Pressure
GH	Growth Hormone
GHRL	Ghrelin gene
GHS-R1a	Growth Hormone Secretagogue Receptor type 1a
GHS-R1b	Growth Hormone Secretagogue Receptor type 1b
Gln	Glutamine
HDL	High-Density Lipoprotein
INSP	National Institute of Public Health
LSD	Least Significant Difference
MHO	Metabolically Healthy Obesity
MS	Metabolic Syndrome
OB	Obesity
OPS	Pan American Health Organization
OR	Odds Ratio
OW	Overweight
PCR	Polymerase Chain Reaction
SNP	Single Nucleotide Polymorphism
SBP	Systolic Blood Pressure
SD	Standard Deviation
W/O	Without Obesity
W/OW	Without Overweight
WHO	World Health Organization

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Development and testing of a mobile application prototype for counseling on the correct use of contraceptive methods, prevention of HPV and other sexually transmitted infections aimed at adolescents

Desarrollo y prueba de un prototipo de aplicación móvil para la consejería sobre el uso correcto de métodos anticonceptivos, prevención del VPH y otras infecciones de transmisión sexual dirigida a adolescentes

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Key Handbooks

The use of mobile applications through information and communication technologies (ICT) has contributed to the transition of a new paradigm based on technology that can be used to enable young people to obtain knowledge and safe information, so that they can become aware of the importance of having healthy sexual practices and behaviors, based on solid knowledge regarding their sexual life, allowing them to develop self-care and prevention measures as a means of protection. In this way, mobile devices can be used as an alternative to improve coverage and access to health services, with emphasis on the first level of care and preventive services. Currently, a gradual increase in the incorporation of risky sexual practices has been identified, since barriers still persist in access to sexual and reproductive health (SRH) services for adolescents, mainly access to contraceptive methods with the consequent lack of confidentiality. This is why the media, including interactive media such as the Internet through cell phones, represent a sector with significant potential to provide information and to influence values and norms that strengthen adolescent health. 3. Outline the main conclusions of the research An application for cell phones with Android operating system, freely distributed, aimed at promoting content related to sexual health, was obtained and made available to adolescents. The implementation of the educational intervention was effective, as it provided participants with experience in improving knowledge of sexually transmitted infections (STIs), and contraceptive methods. Its main focus is to respond to the information and knowledge needs provided through interactive and playful reading.

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Resume

Objective: To develop and test a prototype mobile application. **Methods:** For the development and evaluation of the prototype, 7 levels were applied according to the TRLS methodology (NASA technological maturity levels). The study was conducted from October 2021 to June 2023. 22 students from a public high school in San Luis Potosí, Mexico, participated. **Results:** In the global measurement, a mean of 54.48 was obtained in the pre-intervention and the post-intervention 67.67 (t=-9.12 p= <.001). In the indicator of sexually transmitted infections, a mean of 19.14 before the intervention and after 22.38 (t=-4.269 p= <.001). In risk sexual behaviors before the intervention 14.61 and after 22.38 (t=-6.00 p= <.001). In the secure sources of information, 4.95 before and 4.85 after the intervention (t= -54,3 p= .325). In contraceptive methods before the intervention 15.76 and after 18.04. (t=20.7 p=<.001). **Conclusion:** the educational intervention through digital technologies was effective.

Development and testing of a mobile application prototype for counseling on the correct use of contraceptive methods, prevention of HPV and other sexually transmitted infections aimed at adolescents				
Objetive		Methodology		Contribution
To develop and test a prototype mobile application to provide counseling to adolescents on the correct use of contraceptive methods and the prevention of HPV and other sexually transmitted infections.		For the development and evaluation of the prototype, 7 levels were applied according to the TRLS methodology (NASA technological maturity levels). The study was conducted from October 2021 to June 2023. 22 students from a public high school in San Luis Potosí, Mexico.		The participants' experience using the mobile app was outstanding. The indicators in which the most important increases in knowledge were identified were risk sexual behaviors, prevention of HPV and other sexually transmitted infections, contraceptive methods, and safe means of information. The educational intervention through digital technologies was effective.

Mobile application, prototype, sexually transmitted infections

Resumen

Objetivo: Desarrollar y probar un prototipo de aplicación móvil. Métodos: Para el desarrollo y evaluación del prototipo se aplicaron 7 niveles según la metodología TRLS (niveles de madurez tecnológica de la NASA). El estudio se realizó de octubre de 2021 a junio de 2023. Participaron 22 estudiantes de una escuela secundaria pública de San Luis Potosí, México. Resultados: En la medición global se obtuvo una media de 54,48 en la pre-intervención y en la pos-intervención 67,67 (t=-9,12 p= <.001). En el indicador de infecciones de transmisión sexual, una media de 19,14 antes de la intervención y después de 22,38 (t=-4.69 p= <.001). En conductas sexuales de riesgo antes de la intervención 14,61 y después 22,38 (t=-6 p= <.001). En fuentes de información segura, 4,95 antes y 4,85 después de la intervención (t= -54.3, p= .325). En métodos anticonceptivos antes de la intervención 15.76 y después 18.04 (t=20.7 p= <.001). Conclusión: la intervención educativa a través de tecnologías digitales fue efectiva.

Desarrollo y prueba de un prototipo de aplicación móvil para la consejería sobre el uso correcto de métodos anticonceptivos, prevención del VPH y otras infecciones de transmisión sexual dirigida a adolescentes				
Objetivo		Metodología		Contribución
Desarrollar y probar un prototipo de aplicación móvil para la consejería sobre el uso correcto de métodos anticonceptivos, prevención del VPH y otras infecciones de transmisión sexual dirigida a adolescentes.		Para el desarrollo y evaluación del prototipo se aplicaron 7 niveles según la metodología TRLS (niveles de madurez tecnológica de la NASA). El estudio se realizó de octubre de 2021 a junio de 2023. Participaron 22 estudiantes de una escuela secundaria pública de San Luis Potosí, México.		La experiencia de los participantes al utilizar la aplicación móvil fue buena. Los indicadores en los que se identificaron incremento de los conocimientos fueron las conductas sexuales de riesgo, prevención del VPH y otras infecciones de transmisión sexual, métodos anticonceptivos y medios de información seguros. La intervención educativa a través de tecnologías digitales fue efectiva.

Aplicación móvil, prototipo, infecciones de transmisión sexual.

Introduction

The onset of reproductive life in adolescence is part of a maturation process leading to puberty; there is a strong relationship between maturation rates and the age of onset of sexual activity. Considerable numbers of young people in this age range suffer the consequences of inadequate and insufficient information on reproductive health issues, resulting in large numbers of women becoming pregnant and having complications in childbirth or abortion. In general, adolescents are also at higher risk of contracting sexually transmitted infections, including HIV/AIDS (Encuesta Nacional de Salud y Nutrición ENSANUT 2018).

Important biological, psychological and social changes occur during early adolescence. These changes are related to significant events in the lives of individuals, such as the onset of sexual life, and the beginning of the reproductive trajectory, the conditions in which decisions are made about their sexuality, the elements and services available to them, as well as the development opportunities available, have an important effect on their quality of life (Güemes-Hidalgo Et al 2017).

In terms of sexual and reproductive health (SRH), the main risks for this population are unplanned, involuntary and unprotected sexual debut, exposure to unplanned, unwanted or unsafe pregnancies, or exposure to a sexually transmitted infection, thus becoming a serious public health problem. Globally, a large number of adolescents are sexually active before the age of 20, and the vast majority (around 60 per cent) do not use any protection against pregnancy or against the risk of acquiring an STI or becoming infected with HIV. In Mexico, young people begin their sexual lives between the ages of fifteen and nineteen, on average.

The vast majority of them (97 per cent) know at least one method of contraception; however, more than half of them did not use any at the time of their first sexual intercourse. Data from the Mexican Ministry of Health show that the greatest unmet demand for contraceptive methods corresponds to adolescents. In terms of information related to STIs, nine out of ten young Mexicans say they are aware of any of the prevention methods. The Mexican Institute of Youth makes a more detailed analysis since, in the case of those who live in urban areas, their level of knowledge to prevent STIs is 10 percentage points higher than that of young people living in rural areas. When analysing pregnancy among young women, it is found that 12.7% of young women between 15 and 19 years of age have been pregnant (UNFPA 2024).

In Mexico, Sexually Transmitted Diseases are increasing in the shadow of the HIV/AIDS pandemic. According to the Epidemiological Bulletin of the Ministry of Health, as of week 33 of 2021 (covering 15-21 August), 7,930 new cases of HIV (Human Immunodeficiency Virus) infection, 5,810 cases of syphilis and 4,250 cases of herpes had been registered. In addition, the states with the greatest increase in HIV infections were the State of Mexico, with 811; Veracruz, with 699; Jalisco, 589; Quintana Roo, 515; Puebla, 454; Nuevo León, 440; and Baja California, with 435 cases. This corresponds to 49.72% of all new cases in the country (Political Expansion 2022).

STIs are predominantly spread through sexual contact, including vaginal, anal and oral sex. Some STIs can also be transmitted from mother to child during pregnancy, childbirth and breastfeeding. STIs have profound effects on sexual and reproductive health worldwide. The World Health Organization (WHO) reports that an estimated 16 million 15-19 year olds and approximately 1 million girls under the age of 15 become pregnant, mostly in low- and middle-income countries; complications during pregnancy and childbirth are the second leading cause of death among adolescent girls aged 15-19 globally (World Health Organization 2022).

WHO estimates that, in 2020, there were about 374 million new infections of one of these four STIs: chlamydia (129 million), blennorrhoea (82 million), syphilis (7.1 million) and trichomoniasis (156 million). The number of people with genital HSV (herpes) infection was estimated to be more than 490 million in 2016, and more than 300 million women are infected with human papillomavirus (HPV), the leading cause of cervical cancer. Some 296 million people suffer from chronic hepatitis B (World Health Organization 2021). In this sense, and in terms of the incorporation of information and communication technologies (ICT) in education, the possibility of using mobile applications to obtain knowledge has been evaluated, as their usefulness is widespread among the population, so the creation of a technological tool could be an excellent resource to promote the prevention of STIs and encourage healthy sexual practices (Fandos-Garrido M 2003).

ICTs promote more interactive and participatory learning, favouring work in the classroom. It opens up the possibility of learning at a distance and in different contexts. It makes it possible for young people to maintain a pace that is more personalised to their needs. Society has become aware of the importance of science and its influence on issues such as health, resources and means of communication, conditions that improve the quality of human life. The growing influence of technology, its contribution to the transformation of our conceptions and ways of life, makes it necessary to consider the introduction of scientific and technological education as a key element of the general culture of future citizens, preparing them to understand the world in which they live and to make the necessary decisions during their sexual life (Fandos-Garrido M 2003).

A large percentage of adolescents seek health information online on their mobile devices, which means that mobile applications are useful for promoting and improving good practices. New IT solutions are continually being created in response to health problems. In relation to sexual and reproductive health, several computer applications have been created, their focus is to respond to the information and knowledge needs of the adolescent population, which is provided through interactive reading and constitutes, in turn, a means of access to sexual health services, allowing self-assessment of knowledge and information related to sexual health and characteristics specific to adolescents.

According to the World Health Organization (WHO), health developments mainly include applications (apps) aimed directly or indirectly at maintaining or improving people's healthy behaviours, quality of life and well-being. The abbreviation for mobile health is ‘mHealth’, a term used to refer to the practice of medicine and public health supported by mobile devices (University of Salamanca. Useful mobile applications for everyday life and health 2024).

According to the report ‘The Mobile Health Global Market Report 2013-2017: The Commercialization of mHealth apps’, 70 % of apps are intended for patients and 30 % are apps for professional use. Among all health apps, disease follow-up monitoring will be the main development of mHealth apps for patients: in general, the apps with the greatest future impact will be those that enable information gathering, diagnosis and treatment, as well as those dedicated to prevention. The greatest impact for the patient is expected to be related to counselling and follow-up after the initial visit to the doctor. In addition, it is estimated that the use of mobile applications (apps) could improve the efficiency of patient care and minimise up to 30% of the time spent accessing and analysing information, with an economic saving of 15% of healthcare utilisation costs through remote monitoring via mobile apps (Alonso-Arévalo Et al 2017).

In this way, service providers should also explore other less traditional and more innovative ways of guiding, contributing to and promoting health that raise users' awareness. It is also essential to mention the importance of health personnel as agents of change at the administrative level, when evaluating, designing or restructuring health programmes based on new ways of promoting health education. This helps to empower adolescents by enabling them to become active agents in maintaining their health.

The purpose of this study is to test and evaluate the use of a mobile application based on digital counselling aimed at adolescents through safe, easily accessible, private sources of information with all the data they need, so that they have a space that allows them to obtain reliable information, so that in the future they can be responsible in the exercise of their sexuality.

Firstly, the methodology for the design of the app is presented through the different levels of technological maturity according to NASA; secondly, the procedures for carrying out the first tests of the app at the prototype level are presented through an educational intervention based on constructivism; and at the end of the chapter, the results of the test of this technological development are presented in terms of the increase in knowledge in four basic indicators such as: the correct use of contraceptive methods, prevention of HPV and other sexually transmitted infections, use of safe information media and prevention of risky sexual behaviour.

Methodology

A pre-experimental and prospective pilot study was conducted; implemented as an educational intervention in the period from October 2021 to June 2023 in San Luis Potosí, Mexico. Twenty-two students from a public high school selected by non-probabilistic quota sampling participated.

A prototype of a mobile app was developed according to the TRLS methodology (NASA Technology Maturity Levels) (Hobbs et al), which represents a consensual way of measuring the degree of maturity of a technology, ranging from its initial idea to its commercialisation in the market or, in this case, to the stage in which the prototype is obtained to demonstrate the system in a relevant environment (Level 7). Figure No. 1.

Box 1

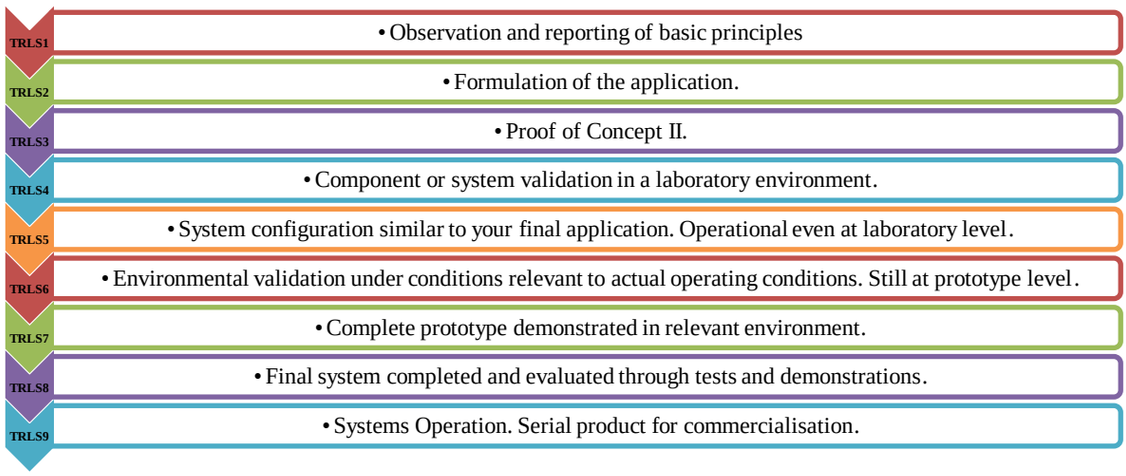

Figure 1

NASA's Technology Maturity Levels

Source: [Information taken from the Ministry of Economy. Technological Innovation Fund of CONAHCYT. Technological maturity stages, according to NASA's "Technology Readiness Level" methodology. Prepared by CONAHCYT's Technology Commercialisation Directorate, February 2015. Original source: Brian Dunbar. Technology Readiness Level [online]. U.S.A: National Aeronautics and Space Administration (NASA)].

Prior to the development of the app, an exhaustive systematic review was carried out, as well as the selection and validation of the contents. Three on-site tests were carried out before testing the prototype in a relevant environment (concept validation, critical and experimental analysis and internal pilot demonstration), the educational materials were designed based on Ausebel's theory of meaningful learning which, according to Matienzo R (2019), is the process in which new knowledge or information is related to the cognitive structure of the learner, in a non-arbitrary and substantive or non-literal way.

The app was presented and tested in a face-to-face manner in conjunction with other activities and implemented in the real environment through an educational intervention that took place in three phases: I).-Pre-intervention or Baseline Measurement, where a first test called "Afrodit-One" was applied, developed by Gutiérrez-Enríquez in 2021, which evaluated four indicators such as knowledge about the prevention of HPV and other sexually transmitted infections (STIs), the correct use of contraceptive methods (MAC), sexual risk behaviours (CSR) and safe means of information (MIS). II) - Intervention or Training, in which they went through the app, read the sections, watched videos, interacted with games and chatted. A series of lectures were given with information about the app and workshops were implemented. III) Post-intervention or final measurement in which the knowledge test and a survey on the experience of using the app were applied again. For data analysis, the parametric t-Student test was applied for paired samples. Informed consent was obtained from the parents of the students who participated in the study. The project was approved by the Ethics Committee of the Faculty of Nursing and Nutrition with registration number CEIFE-2022-417.

Results

In the global evaluation of all knowledge, a mean of 54.48 was obtained pre-intervention and 67.67 post-intervention, with a statistically significant difference (t=-9.12 p=<.001). The indicator with the greatest increase in knowledge was that of risky sexual behaviour, before the intervention the mean was 14.61 and after 22.38 (t=-6.0 p=<.001), followed by the indicator of sexually transmitted infections, before the intervention the mean was 19.14 and after 22.38 (t=-4.69 p=<.001), in contraceptive methods the mean before the intervention was 15.76 and after 18.04 (t=20.7 p=<.001). The indicator in which the least knowledge was obtained was in sources of safe information with a mean of 4.95 before and 4.85 after the intervention (t= -54.3, p=.325, p=.001). (Table 1).

Box 2

Table 1

Statistical scores and t-Student test obtained by students according to subject area

n=22

Knowledge About:	Statistics	Baseline measurement	Measurement final	Difference of Means	Value of t	*p*
Sexually transmitted infections	Media	19.14	22.38	- 3.24	-4.69	<.001
	DE*	1.74	2.55	- 0.81		
	Minimum	15	17	- 2		
	Maximum	22	27	- 5		
Contraceptive methods	Media	15.76	18.04	- 2.28	20.7	<.001
	DE*	2.54	1.11	1.43		
	Minimum	12	15	- 3		
	Maximum	20	20	0		
Risky sexual behaviour	Media	14.61	22.38	- 7.69	-6.00	<.001
	DE*	3.5	2.5	0.7		
	Minimum	12	19	- 7		
	Maximum	19	24	- 5		
Secure sources of information	Media	4.95	4.85	0.1	-54.3	.325
	DE*	.327	.288	0.03		
	Minimum	4	4	0		
	Maximum	5	5	0		
Overall scores	Media	54.48	67.67	-13.19	-9.12	<.001
	DE*	4.00	4.11	- 0.11		
	Minimum	48	58	-10		
	Maximum	62	73	-11		

(*) Standard Deviation

Source: Afrodit-One instrument to assess knowledge of sexual and reproductive education among adolescents.

Students in the first grade of secondary school had the highest post-intervention mean (67.6) compared to students in the second and third grades, who scored 65.0 and 67.0 respectively (Table 2).

Box 3

Table 2

Secondary school students' pre- and post-intervention scores by grade level

n=22

	Pre-intervention	Post-intervención	
Grade level	Media	Media	Difference in averages
First	54.16	67.66	13.5
Second	53.11	65.00	11.8
Third	56.83	67.00	10.1

Source: Afrodit-One tool to assess knowledge of sexual and reproductive health education among adolescents.

Most students' perception of the accessibility, structure, design and content of the mobile application was good (85.3%), while 14.7% found it fair and none found it excellent or poor.

Box 4

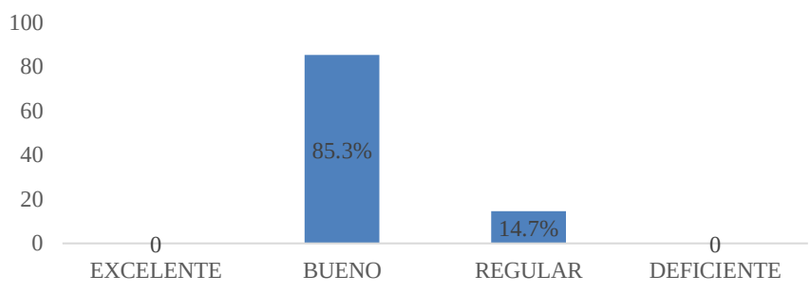

Figure 2

Overall assessment of mobile application usage among secondary school adolescents

Source: Afrodit-One instrument to assess the user experience with the mobile application through user satisfaction.

The source of support for information on sexuality and reproduction that students turn to most often is their mother (57.1%), followed by social networks and the Internet (26.5%), followed by family members (14.2%) and health personnel (2%). Table 3.

Box 5

Table 3

Sources of information on adolescent sexuality and reproduction issues

n=22

Source of support	Frecuency	Porcentage
Mother	12	57.1
Family members	3	14.2
Health personnel	1	2.9
Internet and social networks	5	26.5
Total	21	100

Source: Afrodit-One instrument to assess knowledge of sexual and reproductive education in adolescents

Discussion

WHO (2020) in the draft global strategy on digital health 2020-2025, notes that the 2030 Agenda for Sustainable Development emphasises that the expansion of information and communication technologies and global interconnectedness offer great potential for accelerating human progress, bridging the digital divide and developing knowledge societies.

According to León-Castañeda and Christian Díaz de. (2019), the evolution of mobile communication technologies such as smartphones and wearable devices (*wearables*, such as bracelets, watches or other accessories) enables the monitoring of activities (physical exercise) or health conditions for individuals and the monitoring of these conditions and lifestyles by healthcare professionals towards decision making. These devices are increasingly applied in various fields and make up the so-called mobile health (m-Health or m-Health).

From a public health perspective, these devices represent opportunities to intervene with the general population in health promotion or to collect information related to habits, behaviours or lifestyles of the population, which constitutes an opportunity to issue possible health risk alerts that can generate timely interventions for epidemiological surveillance systems.

On the other hand, the Mexican Official Standard (NOM 039-SSA2-2014) for the prevention and control of sexually transmitted infections establishes the criteria to be implemented in terms of prevention and control of sexually transmitted infections throughout the national territory, hence some of its recommendations are aimed at prevention through the maintenance of information provided to the population regarding the routes and mechanisms of transmission, forms of prevention and information services, detection and comprehensive care related to STIs, in addition to inviting the population to avoid risky sexual behaviour. In terms of education, it indicates that educational programmes should be developed that integrate the topics of STIs and their prevention so that people adopt preventive behaviours and healthy lifestyles in favour of the prevention and control of STIs in the country.

Studies such as Mederos Villalón et al (2019) and Palacios Gálvez et al (2020) provide a first approximation between health and technology in adolescents and to what extent electronic devices, games, social networks or mobile applications can play a relevant role in promoting healthy lifestyles. Another study carried out in Spain by Carrion et al in 2016, where mobile phones were used to measure the perception of adolescents and their parents regarding the promotion of healthy habits, shows that both adolescents and adults consider that technology can contribute to improving lifestyle habits and improving their self-esteem. The above has a point of coincidence with the present study, since it was identified that the sources of support to which adolescents turn to ask questions regarding sexuality issues, in the first place, is their mother and their second source of support is found in the use of social networks.

In this study, a significant increase was observed in knowledge about the prevention of sexually transmitted infections, risky sexual behaviour, contraceptive methods, as well as safe means or sources of information, showing significant learning, which coincides with a study on significant learning and its role in the social and cognitive development of adolescents, by Moriera (2019) in which it is pointed out that, according to this type of learning, new knowledge is incorporated in a substantive way into the student's previous cognitive structure, this is achieved when the student relates the new knowledge to previously acquired knowledge; But it is also necessary to interest the student in learning what he/she is being shown, thus bringing together the motivations of the teacher and the student to efficiently and effectively carry out the teaching-learning process. ICTs are therefore good tools for learning, as students can develop other skills through new ways of transmitting, processing and using information.

Most of the students were satisfied and had a good experience with the use of the mobile application, which was tested in a relevant environment and achieved a level 7 (NASA Technology Readiness Level), making it ready to progress to levels 8 (Final system completed and evaluated through testing and demonstration) and 9 (System Operation with a serial product for commercialisation).

Conclusions

The prototype of a freely distributed Android mobile application for promoting sexual health-related content available to adolescents was successfully tested. The implementation of the educational intervention was effective, as it provided participants with experience in improving knowledge of sexually transmitted infections and contraceptive methods. Its main focus is on responding to the information and knowledge needs provided through interactive and playful reading. It is necessary to encourage the creation of tools and content that address the promotion of sexual and reproductive health among the general population.

Declarations

Conflict of interest

The authors declare that there were no conflicts of interest in the execution of this work.

Authors' contributions

Gutiérrez-Enríquez, Sandra O: original project idea, design of the mobile app, project management, supervision of methods and techniques, and correction of the manuscript.

Rivera-Gómez, Jessica M: drafting of the manuscript, execution of the project, corrections to the manuscript.

Terán-Figueroa, Yolanda: correction of the manuscript and supervision of style:

Acuña-Aradillas, Jorge Martín: collaboration in the design, implementation and control of the mobile app.

Availability of data and materials

All data and bases are available.

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Background

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Nursing experiences before and after the use of an electronic clinical records system for the early detection of cervical cancer

Experiencias de enfermería antes y después del uso de un sistema electrónico de registros clínicos para la detección oportuna de cáncer cervicouterino

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The electronic system <SISCAP-DOCACU> used by the nurses is a technological development for process innovation in nursing that is original and innovative, capable of issuing and sending the results related to screening for the timely detection of cervical cancer electronically and in a reduced time directly to the health centers, to be delivered to the users of the program so that they can have a timely diagnosis. In addition, the electronic system allows easy access, data confidentiality and has a great competitive advantage with ecological benefits by migrating from manual to electronic format. Knowing the experiences of the health personnel helps to improve the acceptance of new ways of working and innovation in the processes. The application of new technologies are strategies that have been promulgated by national and international organizations as lines of action within their health policies to improve priority health programs and thus increase the quality of life of the population, so it is important to promote the continuous updating of health personnel and increase knowledge and skills with the use of technology. The electronic system is functional to perform cervical screening clinical records with legibility, accuracy and completeness. The nursing staff developed skills and competencies in making electronic health records. The nursing staff shared their experiences and expressed their acceptance of the electronic system because they verified its benefits, among which the following stand out: user-friendly system, easy access and adaptability, time optimization during the cytology collection process, reduction in the time it takes to deliver the results to the users, improved communication and interconnection with the pathology department, as well as legibility, accuracy and completeness of the data.

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Abstract

Objective: To analyze the experiences of nurses before and after the use of an electronic system for cervical screening records of the Cervical Cancer Timely Detection program, called "SISCAP-DOCACU". **Methodology**: A Qualitative study was conducted in a public health institution in San Luis Potosí, S.L.P., Mexico. 8 nurses from three health centers participated. Data collection was carried out through a systematization of experiences. The Taguette program was used for the analysis of the information. **Results:** Before the implementation of the electronic system, limitations were identified in manual recordings. After applying it, the nursing staff was very satisfied with the training and use of it. **Conclusions**: The analysis of the nurses' experience allowed us to know the self-perception of their practice, recognize the limitations and broaden their perspective on the importance of process innovation through health technology.

Nursing experiences before and after the use of an electronic clinical records system for the early detection of cervical cancer				
Objetive		Methodology		Contribution
To analyze the experiences of nurses before and after the use of an electronic system for cervical screening records of the Cervical Cancer Timely Detection program, called "SISCAP-DOCACU"		A qualitative study was conducted in a public health institution in San Luis Potosí, S.L.P., Mexico. 8 nurses from three health centers participated. Data collection was carried out through a systematization of experiences. The Taguette program was used for the analysis of the information		The analysis of the nurses' experience allowed us to know the self-perception of their practice, recognize the limitations and broaden their perspective on the importance of process innovation through health technology.

Screening cervical cancer; Innovation, Nursing perception

Resumen

Objetivo: analizar las experiencias de las enfermeras antes y después del uso de un sistema electrónico para los registros clínicos del tamizaje cervical del programa de Detección Oportuna de Cáncer Cervicouterino, llamado <SISCAP-DOCACU>. **Metodología:** estudio cualitativo realizado en una institución de salud pública de San Luis Potosí, S.L.P., México. Participaron 8 enfermeras de tres centros de salud. La recolección de datos se realizó a través de una sistematización de experiencias. Para el análisis de la información se utilizó el programa Taguette. **Resultados:** antes de la implementación del sistema electrónico se identificaron limitantes en los registros manuales. Tras aplicarlo el personal de enfermería se mostró muy satisfecho con el entrenamiento y el uso del mismo. **Conclusiones:** el análisis de la experiencia de las enfermeras permitió conocer la autopercepción de su práctica, reconocer las limitaciones y ampliar su perspectiva sobre la importancia de la innovación de los procesos mediante la tecnología en salud.

Experiencias de enfermería antes y después del uso de un sistema electrónico de registros clínicos para la detección oportuna de cáncer cervicouterino				
Objetivo		Metodología		Contribución
Analizar las experiencias de las enfermeras antes y después del uso de un sistema electrónico para los registros clínicos del tamizaje cervical del programa de Detección Oportuna de Cáncer Cervicouterino, llamado <SISCAP-DOCACU>.		Estudio cualitativo realizado en una institución de salud pública de San Luis Potosí, S.L.P., México. Participaron 8 enfermeras de tres centros de salud. La recolección de datos se realizó a través de una sistematización de experiencias. Para el análisis de la información se utilizó el programa Taguette.		El análisis de la experiencia de las enfermeras permitió conocer la autopercepción de su práctica, reconocer las limitaciones y ampliar su perspectiva sobre la importancia de la innovación de los procesos mediante la tecnología en salud.

Detección de cáncer de cuello uterino, Innovación, Percepción de enfermería

Introduction

According to the World Health Organisation (WHO), cancer is considered the second leading cause of death in the world by 2021, causing 10 million deaths each year. One in six deaths worldwide is due to cervical cancer. Oncogenic infections include human papillomavirus (HPV) infections, which cause 30% of cancer cases in low- and middle-income countries *(World Health Organization, 2021)*.

The incidence of cervical cancer has increased to 18.1 million new cases by 2020. An estimated 569,847 new cases are estimated annually in developing countries and its incidence is highest in East Africa and West Asia. In Latin America, it is the second most common neoplasm in women, with approximately 72 000 cases per year (24.3 cases per 100 000 women) *(Government of Mexico, Secretaría de Salud, Mexico, 2019)*.

In Mexico, in 2020, this disease caused 4185 deaths and the death rate was 11 per 100 000 women, with an average age of 53 years. Late treatment reduces life expectancy to five years on average in 95 % of cases; however, it is curable if detected early. This situation is contrary to what happens in the United States and Europe, where up to 75 % of the cases are diagnosed in early clinical stages, in Mexico, according to the National Institute of Cancerology, locally advanced stages are the most prevalent. Currently, global efforts to prevent cervical cancer have been based on cytological screening, quality, coverage and follow-up screening; if these aspects are properly addressed, the incidence of cervical cancer can be reduced by up to 80 % *(Government of Mexico, Ministry of Health, Mexico, 2022)*.

The World Health Organization and the Pan American Health Organization emphasise the importance of the development and availability of new technological tools as a public health strategy to promote and strengthen cervical cancer prevention and control, as well as to implement changes in programmes to achieve a positive impact for women *(Pan American Health Organization, 2021)*. Thus, one of the lines of research for continuous improvement is Health Information Systems.

The Mexican Health Services have a national database called ‘Women's Cancer Information System’, which integrates information to guide programme actions, as established by the Official Mexican Standard (NOM 014) for the prevention, diagnosis, treatment, control and epidemiological surveillance of cervical cancer *(Official Journal of the Federation, 2007)*.

This system is available to the Statistics Department, the Cytology Analysis Laboratory and the Colposcopy Department. Access to this system is not available to health personnel who take samples in clinics or screening areas and there is no interconnection with the place where the samples are analysed. This limits systematised care, with adequate organisation of the clinical data of the clients, ensuring the legibility, accuracy and completeness of the information collected, which is also related to more effective patient tracking and tracing, as well as a reduction in waiting times and delivery of results to the clients. On the other hand, the screening programme has a large number of forms for manual registration, which makes it difficult to process the information *(Vidales-Cerda M. 2017)*.

Based on the above, in 2014, the ‘Electronic system for the registration of cervical cytology and cytopathology reports (SISCAP-DOCACU)’ was created (*Gutiérrez-Enríquez SO et alt., 2022)*, which consists of a prototype of a functional operating system, this system allows migration from manual to electronic systems, promoting that all service providers involved in the sampling, registration and interpretation of cervical cytology have an accessible and useful information system for accessing information in a faster, more accurate, readable, complete and reliable way. To achieve this goal, it is necessary to know the opinions of the staff who will implement the changes, as the success of innovation projects depends to a large extent on the acceptance of health workers, as well as the training provided to them.

The aim of this study was to analyse the experiences of nurses before and after the use of an electronic clinical record system for the timely detection of cervical cancer (DOCACU).

Methodology

Qualitative study conducted in the period from 01 December 2022 to 22 April 2023. Eight nurses from the Women's Care Module of four health units belonging to four municipalities in the interior of the State of San Luis Potosí, Mexico, participated. The study was carried out in three stages: Phase 1 (initial), phase 2 (training) and phase 3 (final). To collect the data, the systematisation of experiences was used (*Jara O, 2012)* which aims to recover the experiences of the participating health personnel, through an exercise of analysis and reflection, based on the self-perception of their knowledge and skills in the process of recording the clinical data of the users of the Cervical Cancer Early Detection programme. A total of 14 hours were invested. First phase 2 hours, second phase 10 hours and third phase 2 hours. A total of two interviews were conducted, one in the initial phase and the other in the final phase after using the electronic system.

In the initial or pre-phase, interviews were conducted with a nurse manager and the operational nurses in the health units, who are responsible for taking cervical cytology smears. Six trigger questions were asked about the types of records used in their practice, the limitations they observe in terms of accuracy, readability and completeness, and their perspective on innovations to improve recording systems. These interviews were recorded and lasted 15 minutes per participant. The conversation was conducted both face-to-face and virtually. The information was collected by video-call recording with prior authorisation of the participating staff.

The training phase was carried out based on professional competencies according to the International Labour Organisation *(International Labour Organisation, 2020)*. Five basic activities were carried out: 1) awareness-raising on the use of digital systems, 2) importance of the legal framework and current regulations on Health Information Systems, 3) demonstrations on the use of the system in person and through video-tutorials, 4) practice with the system without users, and 5) final practice of the records with users. The training was carried out in a hybrid modality (face-to-face and on-line).

At the end of the training programme activities, the use of the SISCAP-DOCACU electronic system began, which was implemented for 4 months; subsequently, at the end of its use, the second interview was implemented, in which the same questions from the initial interview were answered again, but focused on the use of the SISCAP-DOCACU electronic system.

For the analysis of the information, the interviews were transcribed into text, with the support of the <Taguette> platform, and based on an analysis of the content, the information data was labelled (Table 1).

Box 1

Table 1

Categories and subcategories of analysis used for the Systematisation of Experience

Types of registers used	Limitations for record formats	Innovation for new registration systems
Description of the formats.	Material resources.	Structure of the electronic format.
Reasons for use.	Difficulties with formats. Consequences of record-keeping limitations.	Quality of records.
Number of formats.	Structure of the manual format.	Satisfaction with the use of the electronic system.

Source: Own elaboration

Participation was voluntary and all nurses signed a letter of informed consent. The data collected were used for research purposes only. The ethical principles of respecting autonomy and ensuring confidentiality of information were safeguarded. The project was approved by a certified and registered

Ethics Committee in the State of San Luis Potosi, Mexico, with registration number: HNM/03-2015-024. Authorisation by the institution with registration number: DG///DSR/OF 02768/2023. The electronic system has the copyright registration (INDAUTOR) with number: 03-2019-101510192400-01.

Results

The age range of the participants was 28 to 51 years. The seniority was from 2 months to 4 years and the experience in years taking cervical cytology smears was from 4 to 13 years considering that before working in their current workplace they already had that experience (Table 2).

Box 2

Table 2

Demographic and employment data of the nursing staff in the women's care unit.

No.	Age in years	Post	Seniority in the health centre	Years of experience with cervical smear tests	Work Schedule
1	28	Operational Nurse	10 months	5 years	Evening
2	30	Operational Nurse	11 months	10 years	Morning
3	36	Operational Nurse	4years	11 years	Morning
4	37	Service Manager	13 years	13 years	Morning
5	51	Operational Nurse	16 months	11 years	Morning
6	35	Service manager	4 years	8 years	Morning
7	33	Operational Nurse	2 months	10 years	Evening
8	37	Operational Nurse	9 months	4 years	Afternoon

Source: Interview with nursing staff

Types of records conventionally used in the cervical cytology collection process

The formats currently used within the DOCACU programme are manual formats due to institutional norms, in which the clinical records of the users who come for screening for the timely detection of cervical cancer are made.

[*The manual formats, because they are the ones we use in the unit and we do not have or know of any database that we can use, so we do it manually. The patient's data is recorded in several formats, one for HPV detection, a manual format for cervical cytology and a format for breast examination.* [...] (Nurse 1).

Limitation in formats for records (readability, completeness and accuracy)

The nurses identified some factors that limit the current recording of client data on manual forms. They commented on the criteria of readability, completeness and accuracy.

[*Sometimes the limitation is that we do not have the forms, they are finished and we do not have them to make the detections in the women's care module. The space on the forms is very limited and the legibility of the information received with respect to the results is incomprehensible.* [...] (Nurse 1).

[...] *What happens is that in the formats perhaps we already do them in a very routine and very manual way and we say to ourselves: we already know them, we already know what's on here and we just fill them out, fill them out and fill them out, but we do have that situation, at the same time, when we review the form, I realise that maybe there is a piece of information missing, or maybe in terms of the handwriting, that maybe you have a lot of patients, so you write very quickly and you think that it was an 's' and in reality it wasn't, I mean, things like that, that you say: oh, it's a bit complicated, not all of them have very legible handwriting.* [...] (Nurse 4).

The nurses identify some errors or difficulties in daily practice in recording the cervical cytology results request and report sheet, such as the following:

[...] *I think that the time factor is a disadvantage because you have to fill out a form, sometimes as one of the colleagues mentioned, sometimes on the run, that is when we omit some data, then you have to pass it to a diary, which is also again repeating some data. The reality is that sometimes they are incomplete and sometimes we have not even put our own identity register and when they arrive at the central offices we do not know who took the cytology, because we omitted that information* [...] (Nurse at management level).

[...] *The manual format that we have is a bit incomplete, there are times when one observes other things on the cervix that are not on the sheet, so as one of the colleagues commented, it cannot be put in manually, so it is also a limitation that it does not have the characteristics to register what one sees at the time of the examination.* [...] (Nurse 6).

[...] *The academic level of the population we have also has an influence, because if a patient has a degree, she gives you all her data and mail, so it depends on the academic level of the population.* [...] (Nurse 7).

Therefore, when there are problems in the registers with the legibility, accuracy and completeness of the data of the clients, the staff reports that there are consequences that affect the patients themselves with the delivery of their results:

[...] *In our unit, it is a bit complicated to locate patients with a positive result because they go with us and we do not attend only to people in our area of responsibility, we are not taking from different areas, so we have a location that is, I don't know, almost two hours away, and there is no good reception to locate them by phone.* [...] (Nurse 4).

[...] *Besides, there are also patients who give us an address and sometimes we can't find them there, so how are we going to find them, I mean, there are patients who go and rent houses, but as they are in our area, in our area of responsibility, well, we go and look for them and no, it turns out that they don't live there because they rent, so we've already lost them* [...] (Nurse 6).

[...] *It just happened to us in December, she was a positive patient, this one and well, we went to the address and there were two identical addresses and nobody knew them and the second house was abandoned and nobody had lived there for years and well, we put out a publication and well it turned out, it was found, but the lady was from Mexico City and well she went to the unit, we offered it to her, we took it and she left the address but it was fictitious, well if it existed but she did not live there* [...] (Nurse 7).

Innovation for new registration systems

Currently, information and communication technologies (ICT) represent a great opportunity for the improvement of the processes that are carried out in the health sector, what we currently know as e-health.[9] In this sense, the health personnel express acceptance of the incorporation of technological tools within the health institutions as a strategy for improving the processes of the DOCACU programme.

[...] *Yes, well, definitely yes, using an electronic medium definitely optimises time, allows us to be precise in the data and does not allow us to continue and more than many platforms is what they do, they leave these locks and this, also for the result I mean, finally, the fact that the unit has the data there and that we can capture it, also helps to correct any errors, because finally it is the nurse who is doing it and can have the data at hand to record it correctly, and if there is a relationship with the pathologist, then also.* [...] (Managerial level nurse).

[...] *Yes, I consider it a good strategy, I think it could help with the timing, because previously we did have someone specifically for cytology, but there were many, but it was because of the PROSPERA programme (government programme) that they were obliged, now that it is voluntary, few people say - 'I go because I am really interested in my health' - it could be something new to attract users to have their cytology done* [...] (Nurse 7).

Perspective of data recording with the electronic system SISCAP-DOCACU

After using the electronic system, health personnel report satisfaction with its use and, as a result, refer to the advantages and benefits obtained with its implementation in the health facilities.

I found it very easy to record data electronically and I found the electronic format to be very complete, since it is possible to make observations regarding the patient's gynaecological-obstetric history and the examination during the cytology examination. [...] (Nurse 2).

[...] *Well, I found the system to be very efficient, more than anything else to save time in the results, because, of course, it asked for the same data, the same as the physical format*. [...] (Nurse 7).

Structure of the format and quality of the records with the electronic system

Based on their experience, the structure of the electronic format for the registration of patient data, the nursing staff reported that it is adequate, a logical and organised structure, with sections that allow for the entry of observations.

[...] *Yes, I think the structure is clear and simple, I had no problem in recording the data.* [...] (Nurse 1).

[*... The sheet as such in the system appears well because if it comes what we commonly handle, there is only one result, and then it says that it was reviewed by the pathologist and gives us the result, but as I said we do not know the date on which it was carried out by one of them, with this system we don't, we have the two diagnoses, it even allows us to see what one said and what the other said (cytotechnologist and pathologist), in the times when it is being reviewed and everything, so yes, it is complete, no data is missing*. [...] (Nurse 4).

[...] *In fact, the physical format does not have a record of the history or the use of hormones or so on the electronic one it does specify, it does tell you what specifically is being used, and on the other one there is only the use of hormones with a yes or no answer option. So yes, if it is more complete*. [...] (Nurse 3)

[...] *Yes, because we had the space to put some observation about why one is asking for it in an urgent or normal way. In addition, in the physical format there is no section to put an observation of what is observed during the examination and in the electronic format there is, there we would write something that we looked at when we took the sample and we would put it and justify it with the urgent taking of the sample in that section, because we looked at something important for the patient.* [...] (Nurse 7).

Another element of great relevance is that the nursing staff considers that the completeness, legibility and accuracy of the records are more adequate with the electronic system, as opposed to the manual format where there are greater limitations during the process of taking the cervical cytology.

[...] *Well, in this case all the basic data that they ask us for is included, there is a little bit of a difference in terms of the format that we have in physical format because of the gestures that this electronic format asks for it and the other one does not, that is what we did not have the same, but at the end of the day I think it is fine.* [...] (Nurse 4).

[...] *There, it would just be like a mistake on our part, if we were to enter something wrong, it would obviously be reflected there, but in terms of legibility, yes, because it's not the same when we do it by hand, we all write differently or some of us write differently because of haste or something, but it is clear*. [...] (Nurse 6).

Interconnection with the pathology department and delivery of results

The experience of the nursing staff in having an interconnection with the Pathology Department for the reception of the patients' results was satisfactory, and one of the most relevant factors was the timeliness in the delivery of results, which favoured a quicker intervention in the case of patients with positive results.

[*Of course, of course yes, because it took too long for the lab results to arrive in the jurisdiction, then from the jurisdiction here to be sent to us here, so now, the moment I tell the patient to come for her results after a month or less, that's very effective for the patient*, isn't it? [...] (Nurse 6).

[...] *And the fact that you are checking the system and you see that you already have the result there, it is very fast, so it is very good. Previously, it took two months or more to get the results to the patients*. [...] (Nurse 5).

[...] *As we've been advancing and doing and doing, now we've kind of shortened the times a little more, but now if you tell me that a patient is coming for cytology, then I go directly to the platform, open it and start to enter the data, but now the times have shortened a little more*. [...] (Nurse 3).

[...] *Yes, in the conventional way the delivery of results took up to three months, but no matter how quickly they arrived, they took at least two months, and with the electronic system it was less than a month, and the patients themselves even told us, when we called them, they said, 'is it because something came out wrong or why are you talking to me so soon', and then when they arrived we explained to them, so, I tell you, it no longer went through the jurisdiction, it arrived directly at the health centre in the system, and it was faster*. [...] (Nurse 7).

Satisfaction with the electronic system

The perspective of the health personnel with the electronic system format was gratifying and with favourable results.

[...] *I think it is a good strategy to implement, because it saves us time with not only first time patients, but also patients who come to the health centre subsequently. In addition to the interconnection with the pathology department to get the results faster. I found it satisfactory, and with the previous training I found it very easy to use.* [...] (Nurse 2).

[...] *Yes, I think it is very useful both for us as operational nurses and for the patients to receive their results, it would only be necessary to make some improvements in the platform to make it a little more agile, but in general I think it helps us a lot*. [...] (Nurse 6).

[...] *Yes, it was very useful, we were uploading all the patient's data into the system, but to say, all those who are already registered, we only searched for them through their population registry (CURP), which is in the case of those who were inadequate for some reason, we only registered data from the patient's interview section, we no longer had to fill in all the data again, so that was an advantage*. [...] (Nurse 7).

[...] *In terms of the use of the system, I think it was easy, the time for the process of taking the cytology with the patient was 20 minutes, for example, if a patient came in a year's time and we continued with the same system, well, obviously we optimised a lot of time, the patient's entire record is already discharged with her CURP*. [...] (Nurse 8).

Finally, the training of the nursing staff prior to the use of SISCAP-DOCACU had an impact on the satisfaction and performance of the nurses in achieving the objectives set with the incorporation of the technology in the DOCACU programme.

[...] *Yes, it is very practical because it also prevents us from making more mistakes, if in practice situations may appear that perhaps did not appear in the training and then different situations appear and mistakes are made, but the training was very good*. [...] (Nurse 5).

[...] *Yes, the system was available all the time, one could see if the patient's result was there or consult the patient's identification data at any time and to print out the delivery reports. In addition, we trained our colleagues with the system ourselves, it was easy, they didn't struggle.* [...] (Nurse 8).

[...] In *addition, we trained our colleagues with the system ourselves, it was easy, they did not struggle, with two patients that we started to enter and by the next one they did it independently, well in my case, I would arrive here and I would say - 'hey I am going to enter this patient' - and they would tell me - 'explain to me' - and we did it with two patients and the next ones they entered, so they had no difficulties either* [...] (Nurse 7).

Discussion

The organisation of the cervical cancer screening programme is of great relevance to the incidence and mortality rates of cervical cancer in each country. Cervical cytology or Pap smear and the test for HPV infection are the most important tests for the early detection of cervical cancer, which is why both the quality of the specimen collection and the records kept during this procedure are of great importance for detection and timely treatment.

Health information systems enable innovations and process improvement, patient tracking, faster collection and delivery of results by facilitating communication between different departments within an organisation. Currently, electronic health records can generate quality information and facilitate the exchange of data to contribute to the efficiency of health systems, **(***Kruse CS, et alt., 2018)*, such is the case of the DOCACU programme, which as a priority requires trained staff and tools with the potential to improve the quality of care (*Cifuentes et alt., 2015)*.

Among the countries that consider informatics and the use of technological tools as an essential competence for nursing professionals, the following stand out: United States, Canada, New Zealand, England, Finland, Australia and recently Peru; *(World Health Organization, 2021)*, so it is important to promote and train the development of digital and informatics skills necessary for professional performance, according to the recommendations of international bodies, because service providers must be at the forefront in the process of transformation of health care, especially of the most vulnerable population. (*Capellari Fabrizzio G, et alt., 2021)*.

According to the authors Echeverry et al., implementing a systematisation of experiences implies an intentionality of transformation and as a product, research questions can be raised to guide specialists in the production of new knowledge at the service of practice and the interests of social transformation; it also promotes positive changes in attitudes, practices and relationships based on experiences that have a significant impact on people's lives (*Echeverry Velásquez ML, 2021)*.

As a result of the analysis and reflection carried out with the support of the participants in this study, during the interviews and before implementing the electronic system, it was found that there are difficulties with the quality of the records they make, since they indicate that they use a wide variety of formats for recording the clinical data of the users of the cervical screening module, and they also indicate that the identification data of the users are repeated in several formats, thus generating difficulty in locating the users.

The application form does not have adequate space to make observations about the visualisations of the cervix, the form does not have the options of ordinary or urgent delivery, there is an excess of information and limitations in the distribution of data, which can lead to confusion.

Another problem with the manual forms in this study is that they are visually very saturated with information, and they also report that the forms have become wet, stained or lost, which makes it difficult to identify the patient. On the other hand, there are limitations in the agility and speed of the care processes, as well as prolonged times in the delivery of results, and the use of an excess of manual formats does not favour environmental sustainability, as well as the use of material and economic resources of the health institutions. The training was a central axis in the implementation of the electronic system, as it implies the sensitisation of the participants to the importance of innovation as a strategy to improve the quality of care for users by favouring the capacities and skills of the nurses through the application of a programme with a structured methodology to facilitate the teaching-learning process.

According to Torres-Flórez, the training of human resources is a human management practice that must be carried out in an orderly and structured manner in order to obtain the impact of the desired results, previously considering an analysis of various factors such as training needs, an implementation plan, as well as carrying out the evaluation and measurement of the impact (*Torres Flórez, D., 2019)*.

During the implementation of the electronic system, the participants showed difficulty in the transition from paper to digital recording, however, in a short time and with the training provided, it was possible to adopt the system on a daily basis in their work area.

On the other hand, other limitations identified were the following: lack of computer equipment in some areas, stability in the Internet, errors in some fields, some limitations in the skills they had to perform the procedure of taking cervical cytology smears and their records, and fear of the use of technology, which coincides with what Basaéz et al, This coincides with what*Basaéz et* al. points out, since it refers that the irruption of technology in society begins with a feeling of scepticism, doubt, fashion, contingency, opportunism, favourable and unfavourable, and when it passes through social, collective and cultural filters, the adoption of the new technology can be successful, becoming fluid and natural during its daily use (*Basáez E, M. J., 2022).*

At the end of the training and the application of the electronic system, the nursing staff were very satisfied with the content of the training programme and the innovation of the process implemented. They indicated that SISCAP-DOCACU could be a good strategy for optimising time and providing timely diagnosis and treatment to patients, as well as being a first step towards advancing and improving processes within the DOCACU programme with the use of technology and thus moving from manual to electronic formats, They observed benefits such as improved inter-institutional communication, a reduction in the time taken to register and send requests, as well as the ecological benefit of saving paper, due to the organised structure of the electronic system compared to the official format.

Learning about the experiences of the nurses made it possible to identify areas of opportunity to improve and increase the effectiveness of their work, as Villa-Holguín refers, where she points out that the experiences acquired in daily practices become collective spaces for feedback, scenarios that facilitate the autonomous decision-making of the subjects in the search for transformation in the personal and collective sphere *(Villa Holguín, 2019).*

Because human error can be common in all processes, it is necessary for institutions to link appropriate training to incorporate technology, with periodic, systematic and quality training, allowing the growth of workers, both in their personal and professional development (*Arias Galvis, D., 2015).*

Conclusions

The nursing staff developed skills and competencies in the implementation of electronic health records.

They expressed acceptance of the electronic system due to the visualisation of the benefits acquired, among which the following stand out: user-friendly system, easy access and adaptability, optimisation of time during the process of taking cytology, reduction in time in the delivery of results to users, improved communication and interconnection with the pathology department, as well as the readability, accuracy and completeness of the data.

Training is the backbone for the successful implementation of technological innovation projects in health services, so it is necessary to strengthen training strategies for operational staff in the use of the systems and in the performance of cervical cytology screening.

The main limitations of the study were the limited time for training and the limited culture of evaluation and change of working methods, especially in the departments with which the system requires interconnection. For future studies, it is suggested to have a fully face-to-face training programme, to increase the awareness of health personnel about the importance of the inclusion of technologies as working tools and to have more time available to practice with the new technologies.

Declarations

Conflict of interest

The authors declare that there were no conflicts of interest in the execution of this work.

Authors' contributions

Gutiérrez-Enríquez, Sandra O: original project idea, design of the electronic system, project management, supervision of methods and techniques, and correction of the manuscript.

Rodríguez-Solís, Cintia N: drafting of the manuscript, execution of the project, corrections to the manuscript.

Terán-Figueroa, Yolanda: correction of the manuscript and supervision of style:

Acuña-Aradillas, Jorge Martín: collaboration in the design of the electronic system, implementation and control of the system and evaluation of results.

Availability of data and materials

All data and bases are available.

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Abbreviations

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SISCAP-DOCACU	Capture system for the timely detection of cervical cancer
TIC	Information and Communication Technologies

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In vitro fertilization in small ruminants: a review

Fecundación *in vitro* en pequeños rumiantes: una revisión

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Key Handbooks

This document is a bibliographic review on in vitro fertilization (IVF) in small ruminants, describing the different procedures for in vitro embryo production in sheep and goats, as well as the factors that can influence the results of IVF. This research contributes to the strengthening of theoretical knowledge on the in vitro fertilization technique applied in small ruminants. In order to use this reproductive biotechnology, it is necessary to have an equipped laboratory and to have knowledge about the physiology of reproduction in small ruminants and about the elaboration of the necessary means for the production of embryos.

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Abstract

In vitro fertilization is a biotechnology that helps to increase animal genetic improvement by producing embryos from donors with superior production characteristics. It also shortens the generation interval by favouring the obtaining of oocytes from prepubertal animals. In addition, it is a technique that can be used to activate reproductive function in animals that do not respond to superovulation treatments, sick animals and those that are sent to slaughterhouse, but that have great genetic potential. To perform this procedure, the oocytes can be obtained from the living animal by laparoscopic ovum pick-up (LOPU) or collected either post-mortem from slaughtered females. Subsequently, the oocytes that will continue the *in vitro* maturation process are selected, the *in vitro* fertilization itself and finally the *in vitro* culture of the zygotes obtained.

***In vitro* fertilization in small ruminants: a review**		
Objectives	**Methodology**	**Contribution**
To describe the procedures required for *in vitro* production of embryos in small ruminants.	A literature search was conducted on the procedures for *in vitro* embryo production, from the recovery of oocyte directly from the follicles to zygote culture and the factors that influence the success of *in vitro* fertilization (IVF).	This review provides the necessary information to perform IVF in sheep and goats. It is a document that can serve as a guide to implement this reproductive biotechnology in animal reproduction laboratories.

Oocytes, Embryos, Reproduction

Resumen

La fecundación *in vitro* es una biotecnología que ayuda a incrementar la mejora genética animal al producir embriones a partir de donantes superiores en sus diferentes características productivas. También acorta el intervalo generacional al favorecer la obtención de ovocitos de animales prepúberes. Además, es una técnica que se puede utilizar para activar la función genésica en animales que no responden a los tratamientos de superovulación, animales enfermos y los que son enviados a rastro, pero que tienen un gran potencial genético. Para llevar a cabo este procedimiento, los ovocitos pueden obtenerse en el animal vivo mediante laparoscopía o pueden obtenerse a partir de ovarios de hembras sacrificadas en el rastro, posteriormente se debe realizar la selección de los ovocitos que continuarán el proceso de maduración *in vitro*, la fecundación *in vitro* propiamente dicha y finalmente el cultivo *in vitro* de los cigotos obtenidos.

Fecundación *in vitro* en pequeños rumiantes: una revisión		
Objetivo	**Metodología**	**Contribución**
Describir los procedimientos necesarios para la producción *in vitro* de embriones en pequeños rumiantes.	Se realizó una búsqueda bibliográfica sobre los procedimientos para la producción *in vitro* de embriones, desde la recuperación de los ovocitos hasta el cultivo de los cigotos y los factores que influyen en el éxito de la fecundación *in vitro* (FIV).	Esta revisión aporta la información necesaria para realizar la FIV en ovinos y caprinos. Es un documento que puede servir de guía para implementar esta biotecnología reproductiva en los laboratorios de reproducción animal.

Ovocitos, embriones, reproducción

Introduction

In vitro fertilization (IVF) represents one of the most important reproductive technologies in North and Latin America. During the last decade its use has significantly progressed worldwide (Martínez, 2022).

The reproductive efficiency of small ruminants depends on applied biotechnologies, as they play a key role in animal reproduction, making it possible to increase production rates through genetic improvement programmes. Reproduction in sheep and goats is important both for the production of lamb for slaughter and wool and for the production of breeding stock. In order to achieve the above mentioned production goals with high profitability, it is important to achieve significant reproductive efficiency. Currently, IVF has been proven to help increase this efficiency in small ruminant reproduction, leading to higher economic results in sheep production units. IVF has proven to have a great positive impact as a technique for obtaining a high number of useful embryos in sheep and goats, achieving good results both commercially and scientifically. IVF has made it possible to make the most of females in different reproductive stages, such as long-lived, infertile, pre-pubertal, anestrus, sick and even dead females, as they are oocyte donors for *in vitro* fertilization (Cuéllar, 2020).

IVF has demonstrated valuable advantages, such as: offspring with high genetic quality, good quality embryos at a low cost, increased production rate, as well as benefiting females with infertility problems, structural or functional alterations in the genital tract (Herradón *et al*., 2007). IVF reduces the dependence on hormonal treatments that are necessary for superovulation in the case of multiple ovulation production, which reduces costs and mitigates the possible side effects associated with hormonal therapies used in females. In addition, in *vitro* maturation of oocytes eliminates the need for the donor animal to undergo superovulation, which can sometimes lead to poor responses and lower embryo quality (Sharma *et al*., 2024). It is worth mentioning that IVF increases efficiency in selection programmes (Herradón *et al*., 2007) and reduces the risk of transmission of many pathogens (Menchaca, 2023).

IVF involves fertilising an artificially matured oocyte with cryopreserved sperm under controlled conditions. Oocytes are usually obtained by different collection methods, such as follicular aspiration or slicing. IVF has proven to be a useful tool in sheep and goat reproduction, producing a large number of embryos synchronised to a specific stage of development. It is important to know in detail this novel assisted reproduction technique to appreciate the value and impact opportunities that can be generated at a professional and/or business level, as IVF creates alternatives for the storage of genetic resources increasing reproductive efficiency and generating economic benefits (Hernández-Marín *et al.*, 2018). Therefore, this review will detail the procedures to perform IVF in small ruminants.

Oocyte collection methods

Oocyte collection techniques can be performed in two ways: by the follicular aspiration method or the slicing method.

Oocyte collection by follicular aspiration

The follicular aspiration technique involves the aspiration of follicles between 2 to 5 mm in diameter with the help of previously sedated ewes. This procedure consists of making three holes in the caudal part of the female's abdomen, previously disinfected. The holes are made with the help of a trocar, in the left hole the endoscope is introduced, in the central hole a probe with a needle is fixed, the probe is usually approximately 1 cm long to be able to carry out the aspiration of follicles with a diameter greater than 2 mm, the external part of the probe is connected to a tube containing the aspiration medium and the other end is connected to the aspiration pump, while the clamp that allows immobilizing and fixing the ovary is placed on the right side at the time of the aspiration. At the end of the aspiration process, the ovary is irrigated with saline and heparin to prevent the formation of adhesions (Guerrero, 2020).

Oocyte collection by the cutting or slicing technique

While the slicing technique is carried out post mortem. Oocytes are collected and brought to the laboratory in physiological saline, some authors note that antibiotics such as penicillin or gentamicin may be added.

The transport temperature should be considered, the recommended temperature is 30°C, since, if the temperature is low, the *in vitro* maturation capacity is compromised. The cutting technique is carried out with the aid of a sterile scalpel, making several transverse and longitudinal cuts along the surface of the ovary. These cuts guarantee the rupture of the follicles to obtain the follicular fluid, and finally the ovary is washed to obtain the collection of the cumulus-oocyte complex (Martínez, 2013).

Oocyte selection

When selecting oocytes, it is important to take into account three morphological criteria, which are: oocyte diameter, cytoplasmic appearance and the morphological characteristics of the cumulus surrounding the oocyte.

This stage is important because the selection of high quality oocytes will allow better results in *in vitro* fertilization, therefore several methodologies have been considered to classify and evaluate the oocyte quality, based on the critical analysis of the oocytes according to the morphological aspects already mentioned. The classification of oocyte quality can be done in four categories (Mamani, 2017):

- Quality 1 or quality A: These are those oocytes where its cytoplasm is homogeneous and its granulations are fine. It comes to present a large series of compact layers of cumulus cells and is considered an oocyte of excellent quality.
- Quality 2 or quality B: These oocytes have a homogeneous cytoplasm, but small areas of irregular pigmentation can be seen, while their cumulus is compact, but smaller in size with approximately five complete layers, despite this it is considered a good quality oocyte.
- Quality 3 or quality C: They are considered irregular as they present a complete but vacuolised cytoplasm, the zona pellucida (ZP) is covered with approximately three layers of cumulus cells, presenting smaller bare areas.
- Quality 4 or quality D: They have a completely heterogeneous pigmented cytoplasm, their cumulus is partially absent or expanded, they are considered bad oocytes of no quality.

Thanks to this classification, it is possible to recognise that A and B quality oocytes are suitable for use in the *in vitro* maturation process for subsequent fertilization.

It should be noted that the selection of oocytes with a complete cumulus is of great importance for the maturity of the oocytes. This cumulus connects with the somatic cells through cytoplasmic extensions of the granulosa cells, allowing the entry of amino acids and other nutrients that will help to complete the maturity of the oocyte. It has been shown that only oocytes with a compact and dense cumulus are able to complete their development. Likewise, the ability of oocytes to reach maturation *in vitro* depends not only on the size of the follicle or the stage of the oestrous cycle, but also on the presence of a complete cytoplasm, which is considered important to complete maturation. However, the size of the follicles from which the follicles are extracted is still an important point to consider (Jácome *et al*., 2014; Paramio & Izquierdo, 2016).

Factors affecting oocyte quality

Many of the factors affecting oocyte quality are often specific conditions that relate to the environment in which oocytes mature. These conditions limit their ability to be fertilised and usually include factors such as osmolarity, pH, temperature and the time with which the oocytes are transported, as times longer than five hours often affect and compromise the oocyte's ability to mature; culture volume and incubation time are also factors that play an important role in obtaining optimal results in oocyte quality. The oocyte selection stage is also important for proper oocyte development. It is important to know the characteristics of the cytoplasm, the nuclear status, the morphological characteristics of the corona radiata and the expansion of the cumulus cells, as these are conditions that determine the oocyte's maturation capacity (Perea *et al*., 2017).

It has also been pointed out that the presence of corpora lutea or cystic follicles in the ovaries can be a problem for obtaining good results, as they alter the quality of the oocyte, therefore, it is recommended that they are not used in selection programmes (Hernández *et al.*, 2018).

In vitro oocyte maturation

In vitro maturation (IVM) is an important step for successful *in vitro* fertilization. Its results have been of great relevance for reproduction, as it has been able to meet needs and solve existing problems in livestock production. IVM allows the result of potentially good gametes to achieve optimal results in *in vitro* fertilization (IVF). Moreover, IVM is a useful and important tool in the study of oocyte physiology. IVM is the process following oocyte collection and is considered the stage at which the female gametes become capable of being fertilized. The gametes complete their development during IVM by reaching the Metaphase II stage, where they fully mature upon completion of nuclear and cytoplasmic competence.

The success of IVF is highly dependent on IVM, which is why this stage is crucial, as the efficiency of IVM can be compromised by several factors such as: incorrect oocyte collection, transport and temperature, among many others. IVM consists of reaching a certain number of oocytes selected by a sorting method that will allow the selection of oocytes of excellent quality (Fernández *et al*., 2007b).

The IVM process can be carried out in different ways. In the case of obtaining oocytes from sacrificed females, the ovaries are transported to the laboratory in physiological saline solution at a temperature of 35 °C. They are then washed again with saline at a temperature of 37 °C. The oocytes are then collected with the help of a sterile scalpel blade, cutting the follicles which can have a diameter of between 2 to 6 mm. Once the follicle is cut, it is washed with TCM-199 maturation medium supplemented with heparin, gentamicin and HEPES, which is a cell culture medium that helps to buffer the physiological pH. After this, the follicular fluid together with the washing medium is placed in a Petri dish and left to stand for 15 minutes so that the cells settle to the bottom of the dish. After 15 minutes, the search for cumulus-oocyte complex (COCs) is started using an inverted microscope with a 4X objective. Oocytes with three or more layers of cumulus cells and homogeneous cytoplasm are selected (Cuéllar, 2020).

The commonly used maturation medium is TCM 199, which can be supplemented with pyruvate, glucose, hormones (FSH, LH, 17β-estradiol), complex fluids such as fetal bovine serum (FBS), heat-treated serum or follicular fluid recovered from healthy non- atretic follicles, as well as antibiotics such as penicillin and streptomycin (Paramio & Izquierdo, 2014). These culture media must be sterilized through filters and with the help of Millipore pores, which are equilibrated before use in boxes, under a humid atmosphere and a temperature of 38.5 °C for approximately 22 hours. However, in the case of goat oocytes, it has been recommended to use goat serum as it has been shown to correctly promote nuclear maturation and the acquisition of competence at the meiotic level, while in the case of sheep oocytes, SFB is recommended (Andino, 2014). IVM of sheep and goat oocytes is performed in groups of 25 or 50 oocytes and incubated at 38-39 °C in humidified atmosphere of 5% CO2 in air for 24 to 27 hours (Paramio & Izquierdo, 2014).

Maturation media

One of the most important factors limiting efficiency in IVF programmes is IVM, since after careful selection of oocytes, only a little more than a third of oocytes reach full cytoplasmic maturation. The commonly used maturation medium is TCM-199, which may be supplemented with serum of animal origin or derivatives thereof. In this respect, this maturation medium has been designed for somatic cell culture and is considered one of the most widely used media in IVM processes in sheep and goats. TCM-199 is composed of different components such as: hypoxanthine, phosphates and glucose (Ugalde *et al*., 2021).

In sheep, the maturation medium HECM-6 has been used, obtaining a maturation rate of 65%. Likewise, there are other treatments that have been used in maturation programmes such as: SFB (Soberano, 2011) or Synthetic Oviductal Fluid (SOF) (Herrick *et al.*, 2004, Shabankareh *et al.*, 2012).

In vitro fertilization

In vitro fertilization (IVF) is a procedure that involves the interaction and union of gametes, and the subsequent activation of the oocyte to produce a zygote. IVF is defined as the process in which the previously matured oocyte is penetrated by a capacitated spermatozoon outside the genital tract of the female, imitating the interaction of the female gamete with the male, forming pronuclei and carrying out syngamy under controlled conditions (Cuéllar, 2020).

The penetration of the sperm into the zona pellucida of the oocyte occurs within 5 to 15 minutes, with the acrosome reaction occurring before or after the sperm head attaches to the glycoprotein receptors of the zona pellucida. This penetration is important to achieve a correct fusion of the gametes, this penetration is mediated by enzymes that allow the sperm to move into the oocyte (García *et al.*, 2017).

The IVF process is complex, allowing in *vitro* matured oocytes to be incubated with viable spermatozoa in a medium supplemented with energy sources such as lactate, pyruvate and serum albumin. This requires adjustments to heparin concentrations and total sperm count for each individual ejaculate. In IVF, matured oocytes are co-cultured with spermatozoa in specific media and under a controlled environment with culture ovens for 5 to 24 hours, depending on the protocol, sperm concentration and quality of the semen used (Fernández *et al.*, 2010).

The IVF process can be carried out as follows: once IVM has been completed, the oocytes are denuded using a pipette and a constant flow at a pressure of 180 µl. Once the oocytes are denuded, they must be washed twice in order to be placed in a culture medium (Ugalde *et al.*, 2021). The most commonly used medium in sheep is SOF and in goats is Tyrode′s Albumin Lactate Pyruvate (TALP), the medium must be sterilized and equilibrated in an incubator at a temperature of 38.5 °C. At the end of washing, denuded oocytes should be placed in an incubator in 50-100 µl drops of SOF or TALP to be fertilized by spermatozoa at a final concentration of $1x10^6$/ml (Cánovas & Coy, 2008; Paramio & Izquierdo, 2014; Ugalde *et al.*, 2021). Fusion depends on successful nuclear and cytoplasmic maturation of the oocytes. It has been shown that oocytes reach penetration by cryopreserved sperm within three hours of initiating fusion (Cánovas & Coy, 2008)**.**

Sperm capacitation

Sperm capacitation is a process in which sperm undergo changes such as: protein phosphorylation, removal of cholesterol from the plasma membrane and elevation of intracellular calcium. With this capacitation, the sperm gain a vigorous flagellar movement pattern, known as hyperactivation.

Once capacitated, the sperm will be able to penetrate the zona pellucida of the oocyte to successfully fertilize it (Cerdo, 2019).

This process aims to promote the structural and biochemical changes that the sperm undergo. It is an important process for successful IVF. In capacitation, the sperm undergoes the acrosomal reaction that occurs at the plasma membrane level, which facilitates the penetration of the oocyte walls (Gimeno *et al.*, 2017).

Sperm capacitation can be understood as the phases in which the acrosome reaction proceeds, promoting alterations in motility patterns. This capacitation can be achieved by exposing the spermatozoa to concentrations of caffeine and/or heparin, substances that will help stimulate the capacitation process in the spermatozoa, preparing them for interaction and fertilization of the ovum (Jácome *et al.*, 2014).

Gamete fusion

Gamete fusion concludes the IVF process. This stage involves mechanisms of cell recognition and interaction of both gametes. The interaction is carried out from an extensive system of receptors that complement each other, these receptors prove to be a key part in the recognition of the gametes. The zona pellucida undergoes a change where it expands and once the acrosome reaction is initiated, the sperm head is attached with the help of receptors that act as binding proteins on the zona pellucida, subsequently enzymes are released that help in the motility of the sperm to penetrate into the oocyte (Hafez & Hafez, 2000).

Oocyte activation

After gamete fusion, oocyte activation is triggered, which consists of a succession of cellular transformations that are induced by the spermatozoon, thus preparing the oocyte for the transformation of the zygote. After gamete fusion, the release of an oocyte activating factor begins, this occurs in the ooplasm and contributes to induce events of the activation cascade (Castañeda, 2009).

When the sperm penetrates the oocyte, an intracellular calcium release occurs and persists for 3 to 4 hours, causing a reaction in the cortical granules in the oocyte, which releases enzymes that alter the conformation of the zona pellucida. This alteration causes a decrease in the affinity of the spermatozoa for the oocyte, thereby blocking the entry of additional spermatozoa, which is important to prevent polyspermy (Olivera *et al*., 2006).

When the process of oocyte activation begins, the cortical granules begin to line up under the plasma membrane, thus with the activation of the oocyte the membrane fuses and the process of exocytosis begins (De Paola, 2016).

In this process, there is also a series of signalling pathways that complete the second meiotic division in the oocyte and with this the expulsion of the second polar body (Cerdo, 2019).

Chemical activation of the oocyte is carried out by culturing in a medium, e.g. SOF-HEPES containing Ca for approximately 4 minutes at a temperature of 39 °C and then rapidly cultured with another medium in 100 µl drops, e.g. SOF-IVC medium supplemented with bovine serum albumin (BSA) and other compounds for five hours in a humid atmosphere with 5% CO2 in the air at a temperature of 38.5 °C (Benavides, 2012).

In vitro embryo culture

In vitro embryo culture is carried out in species of zootechnical interest, being a biotechnology that plays a role in the productive improvement of herds. This stage comprises a sequential process of three important events carried out under controlled conditions in the laboratory: *in vitro* maturation , *in vitro* fertilization of matured oocytes and *in vitro* culture of fertilized oocytes (Fernández *et al.*, 2007a).

The process begins when the embryos have more than four cells resulting from *in vitro* fertilization, at which point they are removed from the maturation medium to continue their cell division process in a special embryo culture medium, where they will complete the division of eight, sixteen and thirty-two cells to become morulae and blastocysts. Upon reaching these stages, the embryos are transferred to recipient females in two ways: fresh or frozen. In this process, energy sources such as glucose and some amino acids can be used. *In vitro* embryo culture is usually the longest stage of the whole process, where the overall efficiency of the programme is determined and the quality of the process is demonstrated by the embryos obtained (Ugalde *et al*., 2021).

After IVM, only approximately 90% of the immature oocytes that are cultured reach metaphase II and expel the first polar body. Of the total number of oocytes, 80% reach fertilization and begin to divide. However, only 25-40% reach the blastocyst stage. Somatic cell culture or somatic cell conditioned media supplemented with serum is often used in order to avoid developmental blockage, which leads to embryo death. However, it has also been shown that the addition of serum to the culture media is the main factor responsible for the development of fetal over-volume syndrome, as well as affecting the resistance to cryopreservation of the embryos obtained, causing low resistance (Tarazona *et al.*, 2010).

Culture conditions

Culture conditions are biophysical parameters and organic elements that must be controlled in the culture media, these parameters are usually: osmolarity, pH, most mammalian embryos cultured *in vitro* develop a neutral or slightly alkaline pH and bovine or ovine oviductal fluid which are characterized by minimal levels of Na and high levels of K (Salgado & Lopera, 2020).

As for the organic elements that must be taken into account are: the source of energy, the most commonly used in culture media are lactate, pyruvate and glucose. The source of protein should also be taken into account, such as amino acids, which are relatively important elements as they are involved in the regulation of embryo development (Mucci *et al.*, 2006).

Oxygen tension is one of the culture conditions that must also be taken into account, most mammalian embryos in the reproductive tract handle an oxygen tension of 3.5 to 8%, therefore, embryos cultured *in vitro* must use a similar oxygen tension.

This culture condition has several benefits, some of which are that it improves the rate of embryo production, increases cell number and reduces the production of free radicals. The use of serum free amino acids in *in vitro* culture media increases embryo development, due to its antioxidant action and the control it exerts on pH. It should be taken into account that the use of amino acids should be replaced every three days due to the high concentrations of ammonium in the medium (Benavides *et al*., 2015).

Culture media

Culture media are intended to increase metabolic efficiency; therefore, these media must be an excellent source of energy. In sheep, the medium known as synthetic oviductal fluid (SOF), which was developed by Tervit, comes into use. Culture media are generally solutions supplemented with components such as magnesium, calcium, phosphates, sulphates and bicarbonate. As well as water is one of the main components providing a higher proportion in the formulation of various types of culture media (Ortega *et al.*, 2022).

Protein sources are also often important in culture media, amino acids are important elements in regulating embryo development (Ramírez, 2020).

Evaluation of *in vitro* fertilization

In vitro fertilization can be assessed by observing the segmentation rate at 18 and 48 hours post-fertilization; the morula and blastocyst stage at 6 to 8 days or it can be assessed after a 20-hour incubation period. The oocytes are usually washed with 3% sodium citrate, observed under a microscope, denuded and fixed with the aid of an ethanol-acetic acid mixture over a period of about 24 hours at a temperature of 4°C. After this, they are stained with 1.1 % acetate-orcein and classified as follows:

- Normally penetrated: these are those that present two pronuclei in the cytoplasm of the zygote, one female and one male; certain morphological characteristics may also appear, such as a sperm tail or the decondensed head of the spermatozoon.
- Asynchronous: these are oocytes that are usually penetrated by only one sperm. One of their characteristics is that an alteration in the development of the two pronuclei can be observed.
- The third group are oocytes that remain arrested in the telophase II stage, although they are usually activated oocytes.
- The last group are the polyspermic zygotes, whose characteristics are very peculiar as they usually have more than two pronuclei and two polar corpuscles. Another of their characteristics is that more than two tails or heads of spermatozoa can be observed decondensing (Báez *et al*., 2010).

***In vitro* fertilization abnormalities**

In many cases, oocytes develop abnormalities mainly in the IVM process. Sometimes these abnormalities are due to a lack of maturity in the oocyte cytoplasm or due to cytoplasmic deficiencies that manifest themselves in the formation of pronuclei. During maturation *in vitro*, nuclear processes, germinative vesicle rupture and polar corpuscle formation appear to proceed normally, however, it should be noted that fertilization, division and early embryo development are often unsuccessful due to developmental disruption resulting from incomplete cytoplasmic maturation, which is why some authors mention that oocytes mature better *in vivo* than *in vitro*. However, it is said that certain hormonal or follicular factors are required to improve cytoplasmic maturation and thus have results with successful fertilization rates and embryo development (Guzmán *et al.*, 2016).

The ability of the zona pellucida to be penetrated may be compromised, if maturation conditions are not adequate, *in vitro* matured oocytes may not acquire full competence to be fertilized (Salgado & Lopera, 2020). It has been observed that the formation of the pronucleus of oocytes matured *in vitro* is not only minimal compared to oocytes that mature *in vivo*, but that the formation of both pronuclei becomes asynchronous. These results lead to the conclusion that in *vitro* matured oocytes may lack the male pronucleus growth factor, resulting in the formation of only one or neither pronucleus. Asynchrony of pronucleus formation is defined as a delay in the development of the male pronucleus after the normal formation of the female pronucleus following fertilization. This presence of a single pronucleus in oocytes may be related to a probable deficiency of key molecules that help regulate the blockage of meiotic division and thus become involved in the control of later stages of cell cycle progression in the mature oocyte (Martínez, 2018).

On the other hand, polyspermy is a very common anomaly that affects mature oocytes fertilized *in vitro*, accounting for 80% of the most frequent fertilization problems. Some authors have shown that a large proportion of the oocytes with this problem are usually cases of dyspermia. Although the exact process involved in blocking polyspermy is not yet known, a wide variety of causes have been explained, mainly involving cortical granules (Martínez, 2018).

Factors influencing the success of *in vitro* fertilization

There are a large number of factors that drastically influence the variability of *in vitro* fertilization, these factors can be technical, biological or in some cases genetic. Some factors that come into consideration during the IVF process are the inseminant concentration, the treatments given prior to fertilization and the sperm source. On the other hand, technical factors such as the skill, technique and experience with which the technical team carries out the stimulation and oocyte aspiration, as well as the entire remaining process, must be taken into account (Salgado & Lopera, 2020). There are also other factors that influence the success rates of IVF, for example; the age of the female, as pre-pubertal ewes or goats respond better than adults. Meanwhile, the selection and classification of oocytes is also critical for IVF success, because IVF is highly dependent on IVM (Lopera, 2011; Martínez, 2018).

Some seminal alterations can also hinder fertilization, which is why it is essential that the number, motility and morphology of the spermatozoa are appropriate. On the other hand, the conditions under which the Cumulus-Oocyte Complexes (COC's) are transported can also affect the competence of IVF, as well as the presence of corpora lutea and cystic follicles in the ovary at the time of oocyte aspiration, which is why it is not recommended to use this type of ovaries that compromise oocyte fertilization. It is important to take into account the characteristics of the ewes from which the ovaries come, because if they are not in the right conditions they can affect the oocyte quality and thus compromise the capacity for *in vitro* development. Environmental factors, such as the physical conditions of the medium in which oocyte maturation takes place (pH, osmolarity and ionic composition of the medium; temperature and CO_2 and O_2 tension of the incubator; culture volume and incubation time), as well as the maturation medium can also influence the oocytes, compromising oocyte quality. For this reason, each stage of *in vitro* embryo production must be carried out carefully, to decrease the likelihood of IVF being affected (Martínez, 2018).

Conclusions

This research contributes to the strengthening of theoretical knowledge on the *in vitro* fertilization technique applied in small ruminants. *In vitro* fertilization is gaining momentum in these species, providing valuable opportunities for reproduction and genetic improvement, as well as having a positive impact on scientific progress, helping to expand knowledge about the stages involved in the complex phenomenon of reproduction.

Declarations

Conflict of interest

The authors declare that there is no conflict of interest.

Author contribution

Sagastume-Dulce: Did the research and writing of the manuscript.

Tabarez-Abigail: Contributed to the research idea, revision of the research and editing of the manuscript,

G*arcez-Nora and Alarcón-Marco:* Did the revision and editing of the manuscript.

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Background

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Knowledge about compost in students of the Health Sciences Area

Conocimiento sobre la composta en estudiantes del Área de Ciencias de la Salud

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Subdiscipline: Soil science

Key Handbooks

This research is very important in science and technology, since it contributes to the knowledge about the perception that students in the Health Sciences Area have about compost; This is of interest to us because in recent years the pollution problem is serious for the planet. Based on the above, it should be noted that there are key aspects to apply to the generation of universal knowledge, such as: open access to research journals, the link between communities of universal access to knowledge, common objectives, epistemic solidity, linkage with local and regional communities, exchange of knowledge and resources, generation of synergies in networks, addressing strategic issues. This work concludes that students in the area of health sciences do know what compost is, but only 50% of those surveyed separate organic waste, so some strategy must be considered so that the student population becomes aware. about environmental pollution

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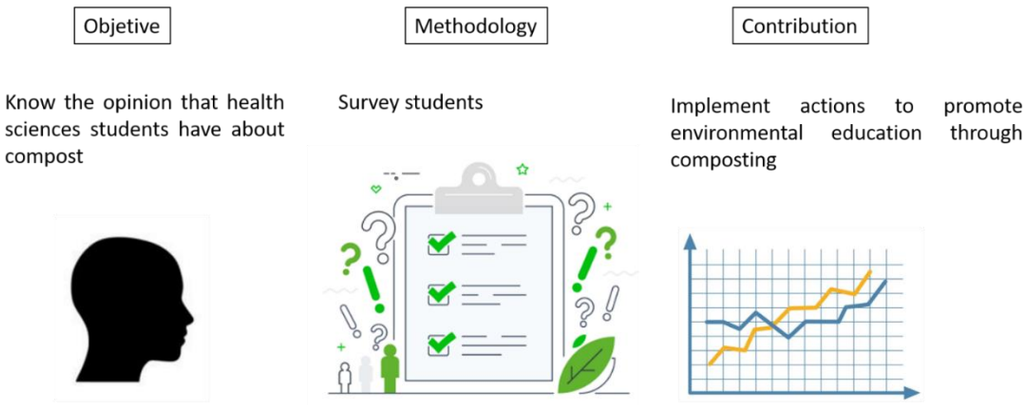
Handbook shelf URL: https://www.ecorfan.org/handbooks.php

Abstract

Pollution is an emerging problem that affects human life, for this reason, solutions are sought to reduce the pollution problem. As a sustainability action, composting is a good alternative to reduce environmental problems. Making use of organic waste for soil fertilization is very important for the environment. The objective of this research was to know how much health sciences students know about organic waste and composting. Based on the above, a questionnaire was applied in order toobtain said information. The results showed that although the majority know that it is organic waste, only about 50% separate it. Likewise, a large number of respondents know that it is compost and the majority would also like to make it as a sustainability action.

Knowledge about compost in students of the Health Sciences Area



Compost, Organic waste, Sustainability

Resumen

La contaminación es un problema emergente que afecta la vida del ser humano, por tal motivo, se buscan soluciones para reducir el problema de contaminación. Como una acción de sustentabilidad, la elaboración de composta es una buena alternativa para reducir los problemas ambientales. Hacer uso delos residuos orgánicos para la fertilización del suelo es muy importante para el medio ambiente. El objetivo de esta investigación fue saber que tanto conocen los estudiantes de ciencias de la salud sobre los residuos orgánicos y la elaboración de composta. En base a los anterior, se aplicó uncuestionario con la finalidad de obtener dicha información. Los resultados mostraron que aunque la mayoría sabe que es un residuo orgánico, solo cerca del 50% los separa, así también, gran número de los encuestados sabe que es una composta y a la mayoría también le gustaría elaborarla como una acción de sustentabilidad.

Conocimiento sobre la composta en estudiantes del Área de Ciencias de la Salud

Composta, Residuo orgánico, Sustentabilidad

Introduction

1. Compost as a sustainability action

Due to the high environmental pollution that prevails in our world today, the need has arisen to propose solutions and/or strategies to fully manage biodegradable solid waste. One of these treatment methods is compost, which consists of transforming organic solid waste by biological means and under controlled conditions (commonly called composting) to obtain organic fertilizer at the end of the process (Montes, 2021).

Organic fertilizers, also known as biomass fertilizers, represent a great economic and sustainable alternative to improve plant nutrition and improve crop soil conditions. These fertilizers, mainlyderived from materials such as manure and plant residues, offer a series of significant benefits compared to chemical fertilizers. Firstly, these fertilizers significantly improve the structure of the soil by increasing its porosity and capacity to retain water, promoting the growth of plant roots and beneficial microbial activity. In addition, compost releases a large amount of nutrients over a long period of time, ensuring plant nutrition (Alarcón, 2024). Recently, it has been shown that climate change has mainly affected cropland located in forest or grassland areas (Smith, 2021).

2. Composting phases

Composting is a biological process, which occurs under aerobic conditions (presence of oxygen). With adequate humidity and temperature, a hygienic transformation of organic remains into a homogeneous material that can be assimilated by plants is ensured.

It is possible to interpret composting as the sum of complex metabolic processes carried out by different microorganisms, which in the presence of oxygen, take advantage of the nitrogen (N) and carbon (C) present to produce their own biomass. In this process, additionally, the microorganisms generate heat and a solid substrate, with less C and N, but more stable, which is called compost (Azim et. al, 2018).

By decomposing C, N and all initial organic matter, microorganisms release measurable heat through temperature variations over time. Depending on the temperature generated during the process, three main stages are recognized in composting, in addition to a maturation stage of variable duration. The different phases of composting are divided according to temperature into:

2.1. Mesophilic phase. The starting material begins the composting process at room temperature and in a few days (and even hours), the temperature increases to 45 °C. This increase in temperature is due to microbial activity, since in this phase the microorganisms use simple sources of C and N, generating heat. The decomposition of soluble compounds, such as sugars, produces organic acids and therefore the pH can drop (to about 4.0 or 4.5). This phase lasts a few days, between two and eight days (Sánchez et. al, 2019).

2.2. Thermophilic or Sanitization Phase. When the material reaches temperatures higher than 45°C, the microorganisms that develop at medium temperatures (mesophilic microorganisms) are replaced by those that grow at higher temperatures, mostly bacteria (thermophilic bacteria), which act by facilitating the degradation of sources. more complex C, such as cellulose and lignin. These microorganisms act by transforming nitrogen into ammonia, which raises the pH of the medium. In particular, from 60 ºC onwards, bacteria appear that produce spores and actinobacteria, which are responsible for decomposing waxes, hemicellulose and other complex C compounds. This phase can last from a few days to months, depending on the starting material, weather and site conditions, and other factors. This phase is also called the sanitization phase since the heat generated destroys bacteria and contaminants of fecal origin such as Eschericha coli and Salmonella spp. This phase is important because temperatures above 55°C eliminate cysts and helminth eggs. , spores of phytopathogenic fungi and weed seeds that can befound in the starting material, resulting in a sanitized product. (Sanchez et. al, 2021)

2.3. Cooling or Mesophilic Phase II. Once the carbon sources and, especially, the nitrogen in the

composting material are exhausted, the temperature drops again to 40-45°C. During this phase, the degradation of polymers such as cellulose continues, and some fungi visible to the naked eye appear. When lowering below 40 ºC, the mesophilic organisms restart their activity and the pH of the medium decreases slightly, although in general the pH remains slightly alkaline. This cooling phase requires several weeks and can be confused with the maturation phase.

2.4. Maturation Phase. It is a period that takes months at room temperature, during which secondary reactions of condensation and polymerization of carbon compounds occur to form humic and fulvic acids. (Agreda and Deza, 2018).

3. Physicochemical parameters in composting

The composting process will depend largely on the environmental conditions, the method used, the raw materials used, and other elements, so it must be under constant surveillance of some parameters called physicochemical and can always be within an optimal range, not However, depending on the composting process some parameters may vary. Considering that composting is a biological process carried out by microorganisms, some physicochemical parameters that affect their growth and reproduction must be taken into account. These factors include oxygen or aeration,carbon dioxide, color, substrate humidity, temperature, pH, and C-N ratio. (Cerda et. al, 2019). The parameters and their optimal ranges are indicated below.

3.1.Oxygen

Composting is an aerobic process and adequate aeration must be maintained to allow microorganisms to breathe, in turn releasing carbon dioxide (CO2) into the atmosphere. Likewise, aeration prevents the material from compacting or becoming waterlogged. Oxygen needs vary during the process, reaching the highest consumption rate in the thermophilic phase. (Montoya et. al, 2019)

3.2.Carbon Dioxide (CO2)

In any aerobic or aerobic process, whether in composting or even in human respiration, oxygen serves to transform (oxidize) the Carbon present in raw materials (substrate or food) into fuel. Through the oxidation process, Carbon is transformed into biomass (more microorganisms) and carbon dioxide (CO2) which is a source of carbon for plants and other organisms that carry out photosynthesis. However, CO2 is also a greenhouse gas, that is, it contributes to climate change. (Uribe, 2020)

3.3.Color

Mature compost has a dark brown or blackish color, a forest or earthy aroma, the final product; It improves the physical, chemical and biological properties of the soil, and also allows the reduction of traditional fertilization without significantly affecting the yield of agricultural crops. (Crespo et al., 2018)

3.4.Humidity

The optimal humidity for compost is around 55%, although it varies depending on the physical state and size of the particles, as well as the system used to carry out composting. If the humidity drops below 45%, microbial activity decreases, without giving time for all the degradation phases to complete, causing the product obtained to be biologically unstable. If the humidity is too high (>60%) the water will saturate the pores and interfere with the oxygenation of the material. A simple way to monitor the humidity of the compost is to apply the “fist technique” (Ansorena et. al, 2024).

3.5.Temperature

Composting begins at room temperature and can rise to 65°C without the need for any anthropic activity (external heating), to reach room temperature again during the maturation phase. It is desirable that the temperature does not drop too quickly, since the higher the temperature and time, the greater the rate of decomposition and greater sanitation. (Campitelli et. al, 2020)

3.6.Ph

The pH of composting depends on the source materials and varies in each phase of the process (from 4.5 to 8.5). In the early stages of the process, the pH is acidified by the formation of organic acids. In the thermophilic phase, due to the conversion of ammonium into ammonia, the pH rises and the medium becomes alkaline, finally stabilizing at values close to neutral. When the pH is below 4.5 it is due to excess organic acids, however, if the pH is above 8.5 there is an excess of nitrogen. (Rodríguez-Torres et. al, 2020)

3.7.Carbon-Nitrogen (C-N) Ratio

The C-N relationship varies depending on the starting material and the numerical relationship is obtained by dividing the C content (% total C) by the total N content (% total N) of the materials to be composted. This ratio also varies throughout the process, being a continuous reduction, from 35:1 to 15:1, if the ratio is greater than 35:1 there is an excess of carbon, however, if it is less than 15:1, has an excess of nitrogen. (Castro et. al, 2019)

Methodology

The methodology that was carried out was a longitudinal, transversal and participatory research. The study sample was chosen at random with a total of 171 students from the health sciences area of the Autonomous University of Zacatecas. The time period of the study was between January-July 2024. The sample was taken at the UAZ Siglo XX campus of said University. A dichotomous questionnaire was carried out to obtain numerical data on knowledge of organic waste, waste separation, identification of compost, knowledge of how to make compost and finally the pleasure of making compost.

Results

As can be seen in the following figures, of the total number of students surveyed, 159 know what organic waste is and only a total of 12 do not; Regarding the separation of organic waste from inorganic waste, only 80 of the total separate waste, while 87 of them do not; 150 of them identify what compost is; 114 have knowledge of how to make compost and 122 students would like to make compost, the rest do not.

The questions asked were the following:

1. Do you know what organic waste is?
2. Do you separate organic waste from inorganic waste?
3. Do you identify what compost is?
4. Do you know how to make compost?
5. Would you like to know how to make compost?

Box 1

Figure 1

Knowledge about organic waste

Own elaboration

Box 2

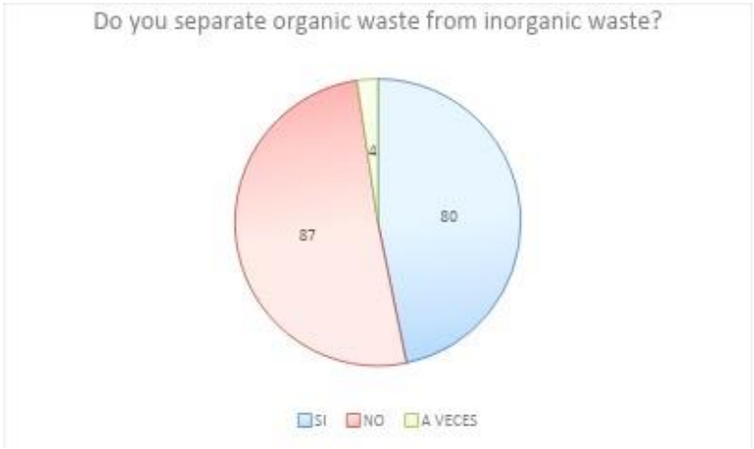


Figure 2

Separation of organic and inorganic waste

Own elaboration

Box 3

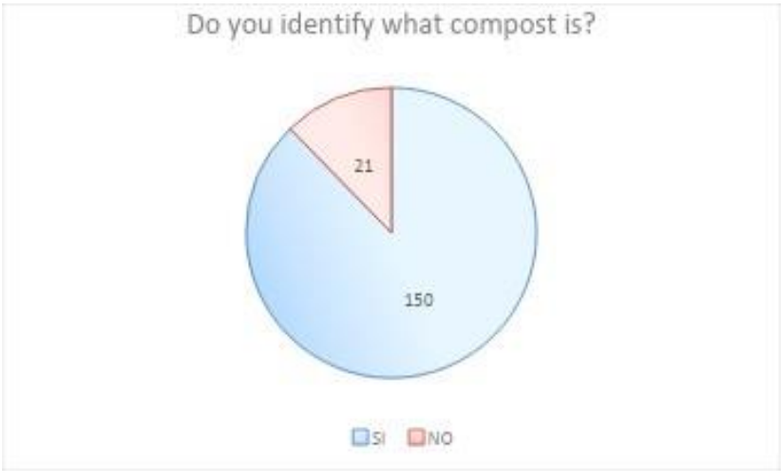


Figure 3

Compost Identification

Own elaboration

Box 4

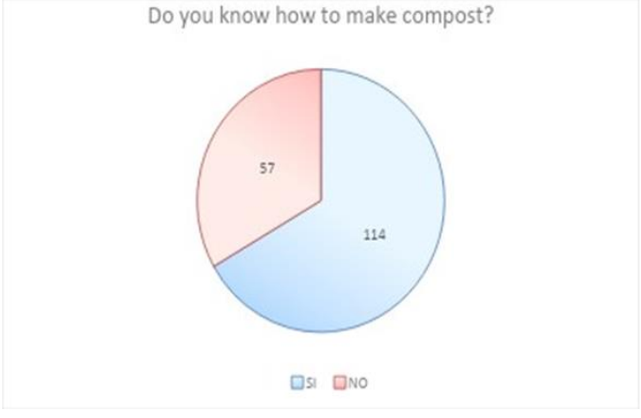


Figure 4

Knowledge of how to make compost

Own elaboration

Box 5

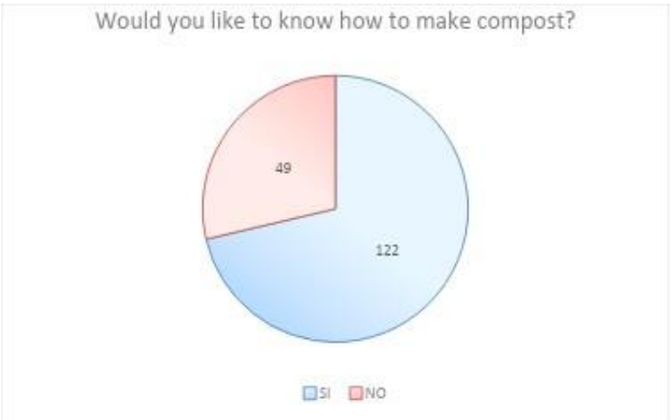


Figure 5

Like to make compost

Own elaboration

Conclusions

It is important to mention that a sustainability action today is the production of compost through the useof organic waste. The health sciences students at the Autonomous University of Zacatecas have knowledge about what organic waste is and whether they would like to make compost, since a large number of those surveyed know what compost is. It would be very interesting to carry out this type of actions in the different academic units that have to do with health, this with the aim of being sustainable and having a better planet, since it can be environmentally educated.

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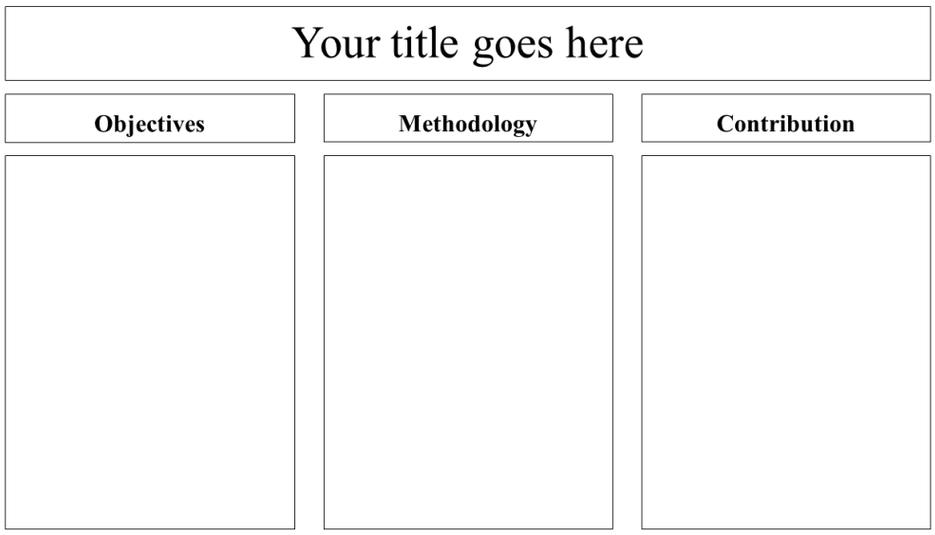

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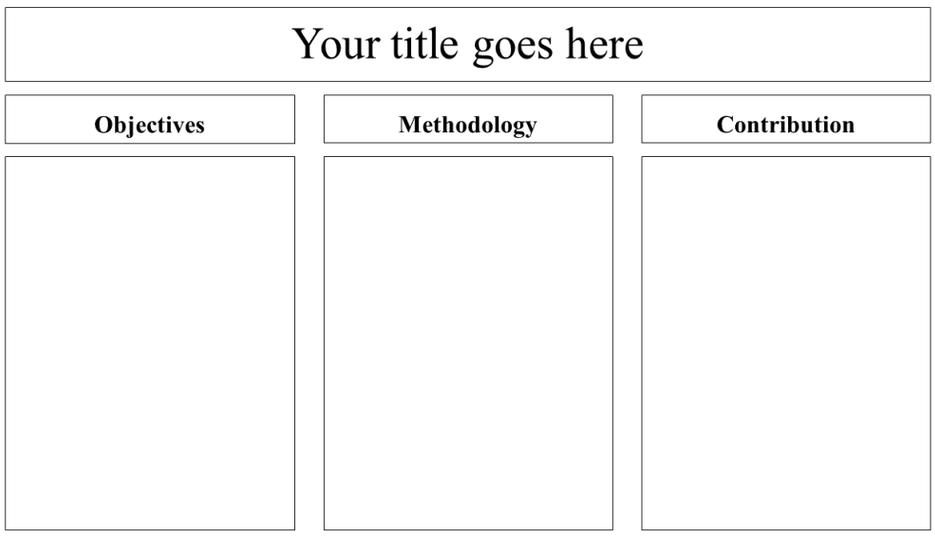

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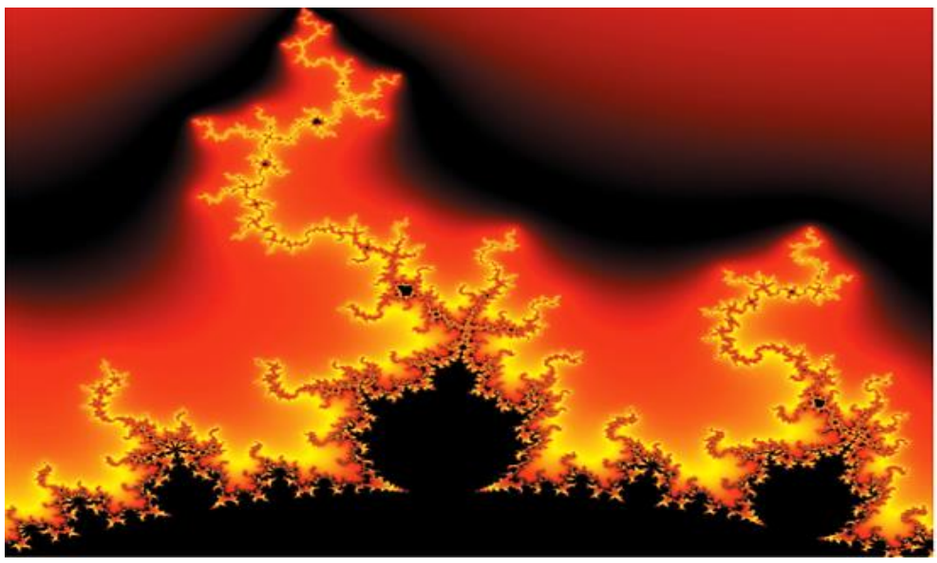

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