Vol. XI, N° 31 • October 2022
ISSN: 2007-6703

Celina López González
General translation proofreader








ESPACIO I+D, INNOVACIÓN MÁS DESARROLLO

Digital Journal of the Universidad Autónoma de Chiapas
Indexed in the directory of Latindex, BIBLAT, CLASE, SIC, Actualidad Iberoamericana, REDIB, DOAJ and MIAR.

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Indexed in **Latindex, BIBLAT, CLASE, Actualidad Iberoamericana**, **Sistema de Información Cultural** of CONACULTA, **REDIB, DOAJ** and **MIAR.**

It is a digital magazine of scientific and cultural dissemination of multidisciplinary nature of the Universidad Autónoma de Chiapas (UNACH). Has a quarterly basis and record: **ISSN 2007-6703**

University Campus, Building D,
Ejido Emiliano Zapata Highway, Kilometer 8
Tuxtla Gutierrez, Chiapas; Mexico. Zip Code 29000

E-mail: espacioimasd@unach.mx

www.espacioimasd.unach.mx

Operations management

Lucía G. León Brandi • lucia.leon@unach.mx
Silvia E. Álvarez Arana • silvia.alvarez@unach.mx
Gabriel Velázquez Toledo • gabriel.toledo@unach.mx

Publication guidelines

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EDITOR'S LETTER

We're celebrating! The Universidad Autónoma de Chiapas' journal *Espacio I+D. Innovación más Desarrollo* celebrates its first ten years as a periodical publication of scientific dissemination. 31 issues, 11 volumes, and more than 286 mediated academic articles in addition to multimedia materials and digital books, all objects of science dissemination that have accompanied our publication to bring knowledge to the last corners of our geography.

On this occasion we have materials that come from strategic alliances with sister universities that collaborate with the Universidad Autónoma de Chiapas to generate knowledge, such as the one carried out with the center of animal microbiology CENID-INIFAP in the article "Epidemiological situation of bovine paratuberculosis in three economic regions of the state of Chiapas-Mexico", the one established with the Universidad Autónoma del Estado de Hidalgo with the article "In vitro control of anthracnose (colletotrichum gloeosporioides) isolated from annona muricata l. with vegetable extracts", as well as collaborations from sister universities such as UNAM "A review on birds of prey migration and toxicological effects of heavy metals", the Universidad Autónoma de Zacatecas with "Critical elements on nanotechnologies in Mexico", the Benemérita Universidad Autónoma de Puebla with "Inaction policy at Universidad Veracruzana: impact on professors", the Universidad Autónoma de Quintana Roo with "Tourist satisfaction of Temazcal in Cozumel" and the Instituto Tecnológico de Tuxtla Gutiérrez with "Osmotic dehydration of *Carica papaya var. Maradol*: Mass transfer and sensory analysis", without forgetting the collaboration that the Instituto Tecnológico Superior San Gabriel and the Escuela Superior Politécnica de Chimborazo, in Ecuador sent us, titled "Application of open-source software as a tool for systems development in maintenance management of computer equipment."

On this occasion, we have an academic document received from the Center for Applied Physics and Advanced Technology, U.N.A.M.-Juriquilla entitled "Geopolitics and innovation: The creation of value in Querétaro",

as well as the digital book "Léxico de las ciencias sociales en pandemia" that in coordination with the Universidad Autónoma de Mexico was carried out as a multidisciplinary analysis of the SARS-Cov-2 pandemic effects that our society had. In addition, we have multimedia materials that aim to disseminate information on literacy processes and what has been done in the field of culture by the Magisterial Folkloric Ballet of the State of Chiapas.

We hope that the beginning of a new cycle, in which the publication of the journal Espacio I+D aims at its consolidation as a reference of scientific dissemination in the Mexican southeast, will benefit our readers, as it has the team.

Enjoy this Space of Innovation!

"*Por la conciencia de la necesidad de servir*"
Universidad Autonoma de Chiapas

The editors

ARTICLES

Reception: **August 23, 2021** | Acceptance: **March 10, 2022**| Publication: **October 1, 2022**

Epidemiological situation of bovine paratuberculosis in three economic regions of the state of Chiapas-Mexico

Edgar Guillermo Méndez Morales[1] • racingkawa_200@hotmail.com

Horacio León Velasco[1] • holeve2001@yahoo.com

José Luis Gutiérrez Hernández[2] • Joker_jet@hotmail.com

Efrén Díaz Aparicio[2] • efredia@yahoo.com

Oscar León Velasco[1] • medicoleon@hotmail.com

1 Universidad Autónoma De Chiapas, Copainalá, Chiapas, México

2 Animal Microbiology CENID-INIFAP, Ciudad de México

To quote this article:

Méndez Morales, E. G., León Velasco, H., Gutiérrez Hernández, J. L., Díaz Aparicio, E., & León Velasco, O. (2022). Situación epidemiológica de la paratuberculosis bovina en tres regiones económicas del estado de Chiapas-México. *Espacio I+D, Innovación más Desarrollo, 11*(31). https://doi.org/10.31644/IMASD.31.2022.a01

— Abstract—

The objective of this study was to know the epidemiological situation of bovine paratuberculosis and determine the management practices that favor the presence of this disease in three economic regions of major livestock importance in the state of Chiapas. An n = 460 animals from 92 livestock production units (LU) was obtained. Samples were taken from five females and one stallion older than two years by LU. The serological diagnosis was made with a commercial ELISA IDEXX paratuberculosis Screening kit. In addition, a questionnaire was applied to the producers to detect some possible management practices in the transmission of the disease. The Relative Risk (RR) was calculated with a 95% confidence interval (CI). Based on the analysis of the management practices of this study, a RR of 0.19 to 0.30 was found for these factors (P <0.05); this implies there is a significant probability of manifesting the disease if these factors are present in the livestock production unit. The seroprevalence was calculated with the Epi Info 7. ® program, and the association between variables by the odds ratio (RR) with the Win Episcope Ver. 2.0® program. General seroprevalence was 15% (95% CI: 12.03 - 18.55%) for the Frailesca region was 12% (95% CI: 7.58 - 17.76%), while in the Istmo-Costa region, it was 17.89% (95% CI 12.72 - 24.1%) and in the Zoque Valley of 14.74% (95% CI: 8.3 - 23.49%). Poor management practices were identified such as dirty drinkers and feeders, pens with dirt floors, cleaning pens, age at weaning of calves, and stallion management. It is concluded that bovine paratuberculosis is present in cattle in the state of Chiapas and it is advisable to establish strategies that favor its control and prevention, such as the detection and segregation of positive animals, as well as the reduction of factors that allow the transmission in livestock farming.

Keywords:

Test diagnostic; Paratuberculosis; Disease.

Bovine paratuberculosis (PTB) is a chronic infectious disease caused by *Mycobacterium avium subspecies paratuberculosis* (MAP). The main organ affected by the disease is the small intestine, which causes enteritis that generates clinical manifestations such as progressive thinning, diarrhea, and, finally, death in affected animals. It is a disease of worldwide distribution and economic importance, with a prevalence ranging from 5 to 30%. Young animals (less than six months of age) are infected by the ingestion of bacteria, mainly through food, water, and nipples contaminated with feces (Castellanos, *et al*., 2010).

PTB disease may be evident after a stress period, such as childbirth or lactation. Economic losses from this disease are mainly due to premature disposal and replacement cost, decrease in milk production, low feed efficiency, infertility, decreased carcass quality, and susceptibility to other diseases.

One report estimated that the prevalence of PTB in Latin America and the Caribbean at the herd level ranges from 17 to 76% (Correa, *et al*., 2015). In Mexico, PTB in cattle is not monitored regularly, so the information about its prevalence is almost zero. In Chiapas, the study and information on PTB are of utmost importance because they are the livestock regions with the highest productivity, ignorance of this disease generates uncertainty about the sanitary status of the cattle herds of the state of Chiapas, due to the lack of productive, reproductive, and sanitary records. Undoubtedly, a disease such as paratuberculosis, with its chronic nature, could impact the herds of the state. Therefore, the objective of this study is to know the epidemiological situation of bovine paratuberculosis and determine the management practices that favor the presence of this disease, in three economic regions of the state of Chiapas.

MATERIALS AND METHODS

Study area

The state of Chiapas is in southeastern Mexico, bordered to the north by the state of Tabasco, to the west by Veracruz and Oaxaca, to the south by the Pacific Ocean, and the east by the Republic of Guatemala. North 17°59', south 14°32' from the north latitude; east 90°22', west 94°14' from the west longitude (INEGI, 2013).

The research was carried out in three economic regions of the state of Chiapas: Frailesca, Istmo-Costa, and Valle Zoque, comprising the municipalities of Villaflores, Villacorzo, La Concordia, Arriaga, Tonalá, Pijijiapan, Mapastepec, Jiquipilas, Cintalapa and Ocozocoautla (Figure 1).

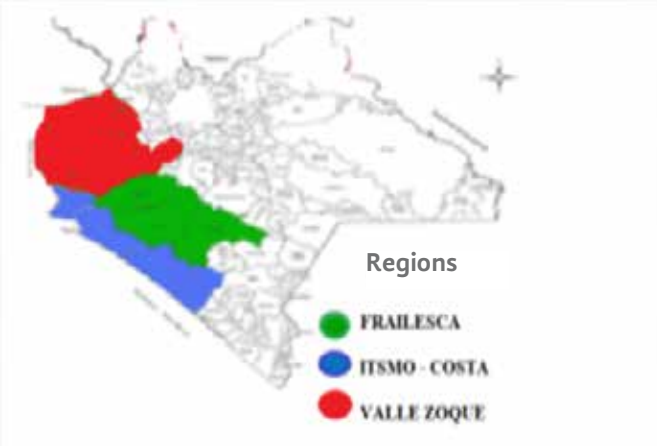

Figure 1. Main cattle regions of the state of Chiapas. Source: Own elaboration

Sample size determination

The minimum sample size was determined with the following formula (Pérez-Rivero, *et al.*, 2017):

$$n = \frac{\frac{Z^2}{E^2}\frac{q}{p}}{1 + \frac{1}{N}\left[\frac{Z^2}{E^2}\frac{q}{p} - 1\right]}$$

Where:
Z = 1.96
E = 10 %
p = 80 %
q = 20 % (1-p)
N = 14680
n = 92

A proportion (p) of paratuberculosis of 80% was considered with a confidence (z^2) of 95% and an accuracy of 10%, the study universe (N) was 14,680 primary producers of the Local Livestock Association of each of the municipalities studied in the state of Chiapas. Resulting in 92 producers for the study, with a total of 460 samples (Table 1).

Table 1
Distribution of livestock production units and animals sampled by economic region

Regions	Municipality	LU*	Male	Female
Frailesca	Villaflores	10	10	40
	Villa Corzo	14	14	56
	La Concordia	11	11	44
Istmo- Costa	Arriaga	7	7	28
	Pijijiapan	11	11	44
	Tonalá	13	13	52
	Mapastepec	7	7	28
Valle Zoque	Cintalapa	6	6	24
	Jiquipilas	5	5	20
	Ocozocoautla	8	8	32
Total		**92**	**92**	**368**

*Livestock Units (LU)

Source: Own elaboration

The inclusion-exclusion criteria that were considered for the producer to participate in the study were as follows:

- **Included**
  Adult animals (over six months of age), bellies, and active stallions of each productive unit (LU).
  Active stallions (older than six months) that were owned or lent by another producer.
  Producers belonging to the Local Livestock Association of each municipality.

- **Excluded**
  Animals of producers who do not belong to the Local Livestock Association of the municipality.

Serological sampling

The selection of the ten municipalities was made based on their livestock activity within the state, then, a database of the producers of each region was used, who were invited to participate in the study, and finally, the selection of the animals was made for convenience, following the inclusion and exclusion criteria.

Samples were taken for serological study by puncture of the coccygeal vein. The sample was taken in vacutainer tubes with serum separation gel (7ml). Blood samples were stored in refrigeration at 4°C. Once the samples were obtained, they were centrifuged at 3000 rpm for 3 min. Subsequently, the serum was taken in aliquots of 2-3 ml, which were frozen and kept at -20° C until processing.

Serological diagnosis

The detection of antibodies contained in blood serum against MAP was performed in the Laboratorio de Pequeños Rumiantes CENID-Microbiología, INIFAP, where the commercial ELISA technique IDEXX Paratuberculosis Screening (IDEXX, 2017) was used, which can detect antibodies against MAP in serum and milk from cattle, with a sensitivity of 60-80% and a specificity of 90-99% (IDEXX, 2017). This way, it will be ensured that when an animal is seropositive to a specific antigen, this response is due to field agent exposure.

The microplates are upholstered with MAP antigen. The analyzed samples were first diluted and incubated with *Mycobacterium phlei* to neutralize possible cross-reactions with any mycobacteria in the microplate wells by predilution. After incubation with *Mycobacterium phlei*, samples are deposited on the upholstered microplate. Any antibody present in the specific sample against MAP forms an antigen-antibody complex on the surface of the well. After washing, an anti-ruminant antibody attached to an enzyme is incubated in the wells. The conjugate binds to antigen-antibody complexes. After another wash, the substrate enzyme (TMB) is added to the wells. In the presence of the enzyme, the substrate oxidizes generating a blue coloration, which turns yellow when the braking solution is added. The color intensity is proportional to the concentration of specific anti-MAP antibodies present in the sample. The result is obtained by comparing the optical density (DO) of the sample with the mean of the positive control.

Epidemiological questionnaires

For the descriptive study, a survey of 15 questions was carried out simultaneously with serological sampling in each bovine production unit with questions related to sanitary problems, zootechnical management, and productive characteristics. It is important to mention that the sampling and survey were carried out by Veterinarian Zootechnicians who knew the region and the animals management extensively, and were previously trained to avoid bias in the research.

Statistical analysis

A database was developed with the spreadsheet program Microsoft Excel 2016, and data analysis with the Epi info™ version 7 program, calculating the frequencies and, where appropriate, the respective 95% confidence interval (95% CI).

To assess the strength with which the disease is associated with a certain factor (which is presumed to be its cause), relative risk (RR) was used as an indicator. Likewise, to evaluate statistical significance, the Chi-Square test was used to determine whether the proportions of the disease were homogeneous (Jaramillo & Martínez, 2010).

RESULTS AND DISCUSSION

Serological outcomes in the three economic regions: Frailesca, Istmo – Costa and Valle Zoque

Based on the results of the sampling of 460 cattle from 92 livestock production units (LU), a frequency of seropositivity of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* (MAP) of 15% (69/460) was obtained, while 85% (391/460) were negative for this disease (Table 2). In this sense, Ocepek, *et al*. (2002), who obtained a prevalence of 0.59%, analyzed serum from cattle between 6 and 24 months of age, through the indirect ELISA technique. For their part, Guamán (2017), determined the prevalence of the disease per animal at 1.72% (87/5074) in cattle between 12 and 24 months of age in four regions of Ecuador. However, Crossley, *et al*. (2005) point out that larger herds would have a higher prevalence due to population density and therefore greater environmental pollution with MAP which would promote exposure to the agent at an early age. This is consistent with Seville (2007) who cites that paratuberculosis is a disease of global distribution with variability in prevalence data in cattle.

Although in the present study the proportion found of seropositive animals is low, comparing it with seronegative animals, it does not mean that the disease is of little importance in the herd, since it must be considered that the clinical disease in addition to presenting in animals older than 24 months, there may also be young animals that carry MAP without presenting a sign that helps differentiate them from healthy animals.

Table 2

General serological MAP diagnosis using ELISE IDEXX Screening paratuberculosis

Diagnostic	Frequency	(%)	IC 95%
Negative	391	85.00	81.45- 87.97
Positive	69	15.00	12.03 - 18.55
Total	**460**	**100.00%**	

CI= Confidence Interval

Source: Own elaboration

According to the serological results by economic regions, in the Frailesca region, 175 animals were sampled; there were 154 negative cases corresponding to 88% and 21 positives corresponding to 12%. While the Istmo-Costa region sampled 190 cattle, of which 156 were negative (82.1%) and 34 cattle as positive, which corresponds to 17.9%. Finally, for the Valle Zoque region, 95 cattle were sampled, of which 85.3 % (81/95) were negative and 14.7% (14/85) were positive (Table 3). For its part, Guamán (2017), determined the frequency of four regions of Ecuador, where the highest number of positive animals was in the Costa region with 2.05% (49/2391), followed by the Sierra region with 1.64% (28/1703), the Insular region with 1.32% (2/132) and finally the East with 1.00% (8/801), concluding that the regions with the largest bovine inventory manifest more presence of antibodies to this disease. Similarly, Milian, *et al.* (2015) mention that the disease presents with a high prevalence in areas of the country that covered a higher bovine population density per km^2 of territorial extension. In this sense, the largest number of animals is found in the Istmo-Costa region, which is characterized by being one of the regions with the largest livestock inventory in the state of Chiapas as reported by the Servicio de Información Agroalimentaria y Pesquera (SIAP, 2017); the Istmo–Costa region (Arriaga, Tonalá, Pijijiapan, and Mapastepec) has 304,429 heads of cattle, unlike the Frailesca region (Villaflores, Villa Corzo and La Concordia) which has a total of 276,600 heads, and the Valle Zoque region (Ocozocoautla, Jiquipilas, and Cintalapa) has an inventory of 73,232 heads of cattle.

Table 3
Serological MAP diagnosis per region using ELISA IDEXX Screening paratuberculosis

Regions	Diagnosis	Frequency	%	CI 95%
Frailesca	Negative	154	88	82.24 - 92.42
	Positive	21	12	7.58 - 17.76
	Total	175	100%	
Istmo - Costa	Negative	156	82.11	75.9 - 87.28
	Positive	34	17.89	12.72 - 24.1
	Total	190	100%	
Valle Zoque	Negative	81	85.26	76.51 - 91.7
	Positive	14	14.74	8.3 - 23.49
	Total	95	100%	

CI= Confidence Interval

Source: Own elaboration

As for the results per livestock production unit (LU), 40 positive and 52 negative were found, which corresponds to 43.48 and 56.52% respectively. A positive LSU was considered when there was at least one positive animal (Figure 2). For its part, Guamán (2017) obtained a prevalence of LU disease of 9.5% (68/716). However, when comparing the results of the present study with those studies that report a result of seroprevalence by the herd, the prevalence result of the present study is higher than that observed by Waldner, *et al*. (2002), who found within-herd prevalence between 2 and 12.8% while Roussel (2011), observed within-herd prevalence between 2 and 12%. Another study by Roussel, *et al*. (2007) detected within-herd prevalence in most of the LU analyzed between 23 and 75%. Concerning the prevalence per herd found in the present study and in others that used ELISA as a diagnostic test, a variability in prevalence can be observed, perhaps due to the different values of sensitivity and specificity that ELISA kits have. It is also important to mention that, due to the characteristics of the state, the results obtained only show a proportion of individuals who present the disease in a certain place and time.

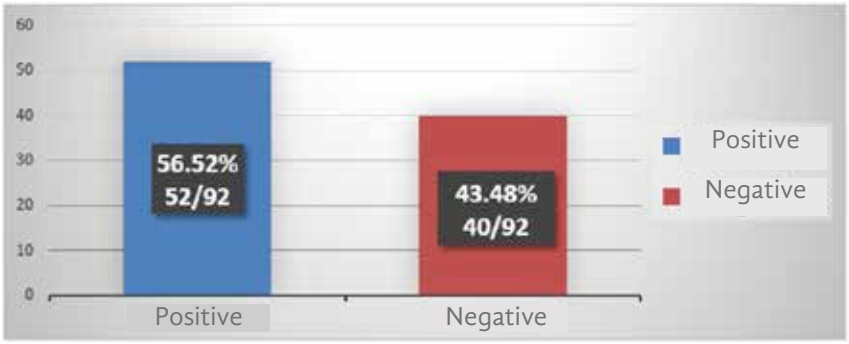

Figure 2. Results at a productive unit level against MAP. Source: Own elaboration

Regarding the results by sex per animal, 368 cows were sampled, of which 55 were positive (14.9%) and 313 negative (85.1%) for this disease. While of the 92 males sampled, 14 were positive (15.2%), and 78 were negative (84.8%) (Figure 3). Regarding the sex of the animals, a greater number of positive female animals was observed than males. These data agree with the studies conducted by Fadhel, *et al.* (2010) and Vélez, *et al.* (2016) who observed a higher percentage of seropositivity in females. The authors indicate that the difference between females and males could be due to a smaller number of males sampled. It should be noted that the system with the highest proportion in tropical regions is the dual-purpose system with 45% of the national bovine inventory and according to its production characteristics this system has two fundamental objectives: milk production and meat rearing calves at weaning, which are sold at 6-8 months of age and the disposal of cattle for the supply of meat, therefore has a higher number of bellies than stallions (González, 2018).

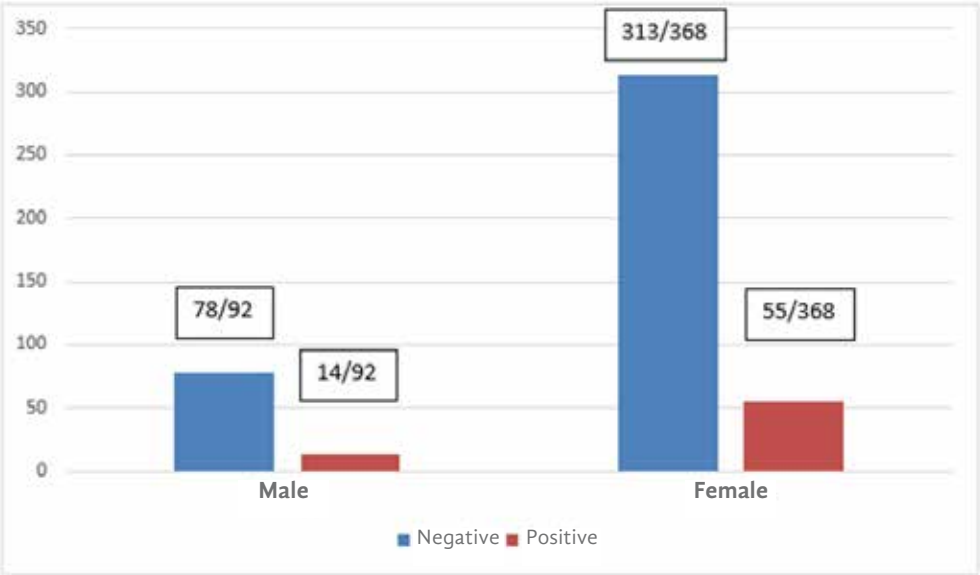

Figure 3. Paratuberculosis diagnosis results by sex. Source: Own elaboration

The ELISA technique (IDEXX, 2017), which was used in the present work, reports a sensitivity of 60-80% and a specificity of 90-99% by the manufacturer, these data coincide with the work of Martínez, *et al*. (2012), who obtained a sensitivity of 79.31% and a specificity of 82.25% in the standardization and development of an ELISA with protoplasmic antigen obtained from the MAP 3065 strain. ELISA tests are less expensive and can be applied to blood or milk samples. The ability to perform ELISA testing on collective milk samples has led to the availability of large numbers of test records in the dairy data registry.

Tobergte and Curtis (2013) mention that the indirect ELISA technique is considered the gold standard for determining the prevalence of the disease, due to the sensitivity and specificity to detect serum antibodies against the agent. However, Sánchez, *et al*. (2009) mention that this is considered a screening technique and is useful for the establishment of surveillance and control measures within the affected herds.

Therefore, it should be considered that a diagnostic test that is intended to be used as the gold standard, should have a high sensitivity, which is the ability of a test to detect a true positive case, if this test has low sensitivity, it will yield a false positive more frequently. Therefore, the ELISA-IDEXX 2017 test, which was used in the present work, should be used as a surveillance and control test for herds, because it has a sensitivity of 60-80%.

Survey results

Based on the data obtained from the 92 production units surveyed, from the three economic regions, Frailesca, Istmo-Costa, and Valle Zoque, it was observed that the main zootechnical purpose of the LU is the dual-purpose system: milk and meat production representing 92.4% (85/92), while 7.6% of producers are engaged in milk production. In addition, the production system identified in all the LU surveyed was semi-intensive with food supplementation and a basic preventive medicine program. It was also identified that 100% of the production units use water from wells for feeding livestock, cleaning facilities, and equipment. In this sense, Vilaboa, *et al*. (2009) in the state of Veracruz indicate that the dual-purpose system represents more than 77% of the production units studied. Similarly, González (2012) found that in the exploitation system in the Istmo-Costa region in Chiapas, 88% of cattle were of dual purpose. As a result, there is ample evidence that the dual-purpose cattle farming system usually predominates in the tropics.

According to the cattle breeds predominant in the animals, it was observed that the cattle Swiss-Brahman is the most predominant, followed by Brahman, Holstein, Gyr-Holstein, and in smaller numbers, cows of the Swiss American breed (Table 4). Regarding the breed of cattle, Benavides,

et al. (2016) found that in dairy herds, the Jersey breed presented higher seropositivity; however, the Holstein breed was the one with a greater proportion from the study population, finding no correlation of the sick animals with the breed variable. On the other hand, Vir Singh, *et al.* (2013) mention that breed differences play a fundamental role in the genetics of disease resistance. In this regard, it is important to mention that racial susceptibility to the disease can be confused with the popularity of the breed. On the other hand, the selection of a breed with greater susceptibility to diseases in the tropics can be a fundamental tool for the control of paratuberculosis in the state.

Table 4
Bovine breeds distribution by economic region

Regions	Breed	Frequency	(%)	CI 95%
Frailesca	Brahman	7	36.84	16.29 - 61.64
	Gyr-Holstein	1	5.26	0.13 - 26.03
	Swiss	1	5.26	0.13 - 26.03
	Swiss-Brahman	10	52.63	28.86 - 75.5
	TOTAL	19	100%	
Istmo - Costa	Brahman	17	50.00	32.43 - 67.57
	Gyr-Holstein	1	2.94	0.07 - 15.33
	Swiss	3	8.82	1.86 - 23.68
	Swiss-Brahman	13	38.2	22.17 - 56.44
	TOTAL	34	100%	
Valle Zoque	Brahman	5	38.46	13.86 - 68.42
	Swiss-Brahman	8	61.54	31.58 - 86.14
	TOTAL	13	100%	

CI= Confidence Interval

Source: Own elaboration

One of the important features for MAP transmission to susceptible animals is the accumulation of feces on the floor of the handling pen. In this sense, it was observed that in most of the management pens, the floor was 83.7% soil (77/92) and only 16.3% (15/92) concrete (firm floor).

In the Frailesca region, 20% of LU were found with concrete floor pens and 80% with soil. For the Istmo-Coast region, 10.5% of pens with concrete floors were identified, and the remaining percentage corresponds to soil. Finally, for the Valle Zoque region, 5.3% of concrete pens and 94.7% of soil pens were identified. As can be seen, the floor that has a greater frequency among the three economic regions corresponds to soil (Table 5).

Table 5
Types of handling pens in production units in the state of Chiapas

Regions	Concrete or Soil Pens	Frequency	(%)	CI 95%
Frailesca	Concrete	7	20	8.44 - 36.94
	Soil	28	80	63.06 - 91.56
	TOTAL	35	100 %	
Itsmo - Costa	Concrete	4	10.53	2.94 - 24.8
	Soil	34	89.47	75.2 - 97.06
	TOTAL	38	100 %	
Valle Zoque	Concrete	1	5.2	0.13 - 26.03
	Soil	18	94.74	73.97 - 99.87
	TOTAL	19	100 %	

CI= Confidence Interval

Source: Own elaboration

Another important item to mention is the cleaning and disinfection of pens, where 85.7% (30/35) of the producers of the Frailesca region do not clean the pens and 14.3% (5/35) do clean the waste of the animals. For the Istmo-Coast region, 100% (38/38) do not clean the pens. Finally, for the Valle Zoque region, 73.7% (14/19) do not resort to cleaning their pens, and 26.3% (5/19) clean the handling pens (Table 6).

Table 6
Pens cleaning in the three regions of the state of Chiapas

Regions	Pen Cleaning	Frequency	(%)	CI 95%
Frailesca	No	30	85.71	69.74 - 95.19
	Yes	5	14.29	4.81 - 30.26
	TOTAL	35	100%	
Itsmo - Costa	No	38	100	90.75 – 100
	TOTAL	38	100%	
Valle Zoque	No	14	73.68	48.8 - 90.85
	Yes	5	26.32	9.15 - 51.2
	TOTAL	19	100%	

CI= Confidence Interval

Source: Own elaboration

On the other hand, one of the practices that must be done in all production units is the feeders and drinkers cleaning, which is an important factor so that MAP does not spread. In the present study, it was observed that the percentage that do not clean the feeders and drinkers in the Frailesca, Istmo-Costa, and Valle Zoque was 62.8, 63.2 and 52.6%, respectively. Undoubtedly, this is a risk factor for the spread of the disease (see Table 7).

Table 7
Feeders and drinkers cleaning

Regions	Feeders and Drinkers Cleaning	Frequency	(%)	CI 95%
Frailesca	No	22	62.86	44.92 - 78.53
	Yes	13	37.14	21.47 - 55.08
	TOTAL	35	100 %	
Itsmo - Costa	No	24	63.16	45.99 - 78.19
	Yes	14	36.84	21.81 - 54.01
	TOTAL	38	100 %	
Valle Zoque	No	10	52.63	28.86 - 75.55
	Yes	9	47.37	24.45 - 71.14
	TOTAL	19	100 %	

CI= Confidence Interval

Source: Own elaboration

In this sense, it has been described that MAP transmission has as its main route the fecal-oral route (Yayo, *et al*., 2001), so the sanitary conditions that are in a productive unit are of great interest for the disease to be established or not. Therefore, the results shown about the sanitary management of the pens (cleaning in pens and the type of floor) in this study, are important, because MAP can be present in the drinkers and feeders that are not given adequate cleaning, not to mention that the pens, by having a soil floor, are more at risk, since MAP tends to stay longer on the ground due to humidity and temperature conditions. Therefore, MAP can persist in soil and water contaminated with the feces of infected animals. Some studies mention that this mycobacterium can survive in the feces of infected cattle for 152-246 days and that the survival period of MAP depends on environmental conditions such as freezing, drought, exposure to sunlight, changes in environmental temperature, and rainfall. On the other hand, Whittington, *et al*. (2000) have described that MAP can survive up to 280 days in puddles. So, it can remain viable in the environment for several months, which is undoubtedly a determining factor for the spread of infection.

Another important point is the number of viable bacilli eliminated in the feces of infected animals. Whitlock, *et al*. (2000) mention that the eliminated dose of MAP is 10^6–10^8 CFU / g, and it has been established that the infective dose is approximately 10^3 bacilli. According to this, minimal fecal contamination of the environment is sufficient to produce infection of susceptible animals.

Regarding the epidemiology of the disease, Greig, *et al*. (1999), have pointed out that some of these wild hosts, such as rabbits, could play an important role in the transmission of infection to domestic ruminants in some regions since they can release millions of MAP/hectares into their feces and domestic ruminants become infected when grazing or eating food contaminated with MAP. In addition, another possible route of infection that has been considered is predation in the case of carnivores. Greig, *et al*. (1997 and 1999) describe that the percentage of MAP isolation in predators is 62%, compared to 10% isolation of M. avium paratuberculosis in prey. Also, Stevenson, *et al*. (2009) carried out through molecular techniques the identification of the same MAP genotypes between the different wild and domestic hosts that lived in the same habitat, supporting the theory of interspecies transmission. This means that wild animals play an important role in the epidemiology of bovine paratuberculosis.

Regarding the above, it can be said that the construction of a pen with a concrete floor is of higher cost for producers, so they choose to stick to soil. However, the socioeconomic level of a LU should not justify little or no cleaning and disinfection of a pen with soil or concrete floor, as well as drinking troughs and feeders, since the frequent route of transmission for MAP in bovine animals is fecal-oral.

Regarding the internal deworming of animals, it was observed that 100% of producers deworm all their animals (Table 8). Proper deworming consists of two applications per year, i.e., every six months at least. Since the producers' conditions and economy vary every six months and every year, it was found that 71.4% (25/35) of the Frailesca region internally deworms animals each year and 28.6% (10/35) every six months. For the Istmo-Costa region, it was observed that 76.32% (29/38) do so every six months and 23.7% (9/38) deworm every year. Finally, in the Valle Zoque region, twelve producers (63.2%) deworm their animals every six months, and seven producers (36.8%) deworm them every year. It is important to mention that one of the common prophylactic practices that are done in any bovine production unit is internal deworming. However, the clinical signs of PTB are very similar to those of parasitosis, so the producer or veterinarian can give an erroneous treatment without first having the proper diagnosis.

Table 8
Deworming of animals in the three regions of the state of Chiapas

Regions	Deworming	Frequency	(%)	CI 95%
Frailesca, Itsmo - Costa y Valle Zoque	Yes	92	100	90 – 100
How Often they Deworm Them		**Frequency**	**(%)**	**CI 95%**
Frailesca	Every Year	25	71.43	53.7 - 85.36
	Every Six Months	10	28.57	14.64 - 46.3
	TOTAL	35	100%	
Itsmo - Costa	Every Year	9	23.68	11.44 - 40.24
	Every Six Months	29	76.32	59.76 - 88.56
	TOTAL	38	100%	
Valle Zoque	Every Year	7	36.84	16.29 - 61.64
	Every Six Months	12	63.16	38.36 - 83.71
	TOTAL	19	100%	

CI= Confidence Interval

Source: Own elaboration

Characteristics related to the presence of MAP in production units and its possible transmission

A series of questions were asked to livestock producers to know the presence of signs suggestive of MAP within their production units. Regarding signs in cattle, 16.7% of producers reported having had problems with submandibular swelling and decay in adult cows, and 56.5% of animals suffering from diarrhea that is treated with deworming or antibiotics and do not recover within their LU; 36.8% of producers reported having had progressive weight loss in sick cattle and 6.5% of producers had cows with hirsute hair and weakness (Table 9).

In this sense, Martinez, *et al.* (2012) describe that the signs observed are diarrhea at first intermittent and later permanent, loss of body condition, although appetite is maintained, decreased milk production, and submandibular and ventral edema caused by hypoproteinemia. Likewise, Whitlock and Buergelt (1996) mention that the clinical signs of the disease are not very evident, sometimes variable, and are mainly associated with the different stages of the disease. In this case, the clinical signs that the producers mentioned having presented in this investigation correspond to an advanced clinical phase. In this phase, the animals are slaughtered due to the reduction in milk production and loss of live weight, although sometimes some ani-

mals die from severe dehydration and cachexia. It is estimated that for each clinical case, there are 25 subclinical cases; hence the importance of having diagnostic tests that can detect infected animals in time to avoid economic losses within the LU.

Table 9
Presence of clinical signs in herds observed by producers within livestock production units

Cattle with submandibular swelling and sadness	Frequency	(%)
Submandibular Swelling and Sadness	(11/92)	16.68
Nothing	(81/92)	83.33
Cattle suffering from diarrhea, treated with deworming or antibiotics and do not recover		
Treated diarrhea	(52/92)	56.52
Nothing	(40/92)	43.48
Progressive weight loss in sick cattle		
Yes	(37/92)	36.80
No	(55/92)	59.78
Cattle with hirsute hair and weakness		
Yes	(25/92)	27.17
No	(67/92)	72.82

Source: Own elaboration

On the other hand, 84% of producers indicate that the cattle they own were bought and born in their livestock production unit. 43.5% of producers mainly introduce stallions, either purchased or borrowed from neighboring LU, and to a lesser extent (18.5%) replace females. Of the introduced cattle, 13% of producers acquire cattle within the same community, 21.7% from the same municipality, 58.7% from a different municipality, but in the same state (Table 10), and only 6.5% of producers outside the state of Chiapas.

Therefore, the purpose of introducing males and females into a dual-purpose system is done for reproductive purposes, as they are used as replacements for stallions and bellies. This agrees with González (2018), who describes that one of the characteristics of the dual-purpose production system is the rearing of calves at weaning and the disposal of cattle for the supply of meat, some calves are selected for future belly replacements, and very few bovine females are purchased externally. In this case, the future stallion is obtained outside the LU to avoid consanguinity within the herd.

Table 10
Origin of cattle from livestock production units

Characteristics		
Cattle Origin	**Frequency**	**%**
Born at the ranch	(15/92)	16.31
Bought or came from somewhere else	(0/92)	0
Born at the ranch and bought	(77/92)	83.69
Types of introduced cattle		
Stallions	(40/92)	43.48
Females	(17/92)	18.48
Stallions and Females	(35/92)	38.04
Where they bought it or Origin		
Same community	(12/92)	13.04
Same municipality	(20/92)	21.74
Another municipality of the State	(54/92)	58.69
Another State	(06/92)	6.52

Source: Own elaboration

Regarding the disposal of the stallion in productive units, 73.9% of producers declared that the stallion changes every three years, 9.8% every five years, and 16.3% have never changed it. 76% of producers lend or borrow the stallion (Table 11). Old stallions can be a risk factor for many infectious diseases within the herd. One of the ways in which PTB can be transmitted is by borrowing infected stallions used for natural riding, which represents a very common practice within dual-purpose LU, as this animal may be removing the micro bacteria through feces during the time it was lent and also consider the sanitary conditions of the facilities.

Furthermore, Roussel (2011) points out that the handling practices of calves are the biggest difference between beef and dairy cattle when considering transmission and control of paratuberculosis. The calf of milk is separated from the mother within the first 24 h. of birth, while the beef calves stay with mothers from five to six months of age. For his part, González (2018) mentions that the dual-purpose system is by breeding calves at weaning sold at 6-8 months of age. This means that the exposure of calves to adult animal manure is much higher in meat and dual-purpose farms than in milk farms, due to the close coexistence between young and adult calves.

Table 11
Characteristics that favor MAP transmission

Characteristics		
At what age weans calves from mothers	**Frequency**	**(%)**
At seven months	(54/92)	58.69
One year	(27/92)	29.34
They're not separated	(11/92)	11.95
How often they change stallion		
Every three years	(68/92)	73.91
Every five years	(09/92)	9.78
They never change it	(15/92)	16.31
Borrows or lends a stallion		
Yes	(70/92)	76.08
No	(10/92)	10.86
Another municipality of the State	(12/92)	13.04

Source: Own elaboration

Based on the analysis of management practices in this study, a relative risk (RR) of 0.19 to 0.30 was found for these factors (P<0.05). This implies that there is a significant probability of disease if these factors are present in the livestock production unit. Therefore, Nuñez, *et al.* (2006) detected that the management practices with the greatest impact were poor micro-environmental conditions, care of newborn and growing calves, handling of pregnant animals, management practices, and manure disposal. In this work, it was detected that the greatest risk factor is the cleaning of dirty drinkers and feeders, soil pens, cleaning of pens, age of weaning of calves, how long it takes to change stallions, and stallion loan. Over all, a source of permanent contagion and 90% of producers clean pens twice a year and 86% of them have soil facilities (Table 12) (Pérez-Rivero, *et al.*, 2017). However, Table 13 shows the results by economic regions of the relative risks for MAP disease. In this respect, a relative risk of 0.20, 0.23, and 0.27 was found, while the calculated values of the confidence interval (95%) were 0.13 - 0.30, 0.14 - 0.39 and 0.10 - 0.37 for the regions Frailesca, Valle Zoque and Istmo-Costa respectively.

Table 12
Calculation of relative risk

	Positive	Negative	
Exposed	a	b	a+b
Non-exposed	c	d	c+d
	a+c	b+d	a+b+c+d

$$\text{RELATIVE RISK} = \frac{\text{Incidence of those exposed}}{\text{Incidence of those not exposed}} = \frac{\frac{a}{a+b}}{\frac{c}{c+d}}$$

Source: Own elaboration

Where ***a***= exposed to the risk factor and they became ill during follow-up; ***b***= exposed to the risk factor and did not become ill during follow-up; ***c***= not exposed to the risk factor and became ill during follow-up; ***d***= not exposed to the risk factor and did not become ill during follow-up; ***a+c***= total subjects who became ill during follow-up (cases); ***a+b***= total subjects exposed to the risk factor; ***c+d***= total subjects not exposed to the risk factor.

To evaluate statistical significance, the Chi-Square test was used to determine whether the proportions of the disease are homogeneous. Therefore, the calculated value for Chi-Square in the present study was 33.96, there is a significant difference ($P<0.05$) in the proportion of disease to exposure to the risk factor.

Table 13
Calculation of risk and MAP confidence interval of the three regions

Regions	(RR)	CI=95%	X^2
Frailesca	0.20	0.13 - 0.30	5.67
Valle Zoque	0.23	0.14 - 0.39	1.61
Istmo-Costa	0.27	0.19 - 0.37	10.37

CI= Confidence Interval, X^2 = Chi-square, RR= Relative Risk

Source: Own elaboration

CONCLUSIONS

MAP infection in cattle is confirmed in the three economic regions of the state of Chiapas: Frailesca, Istmo – Costa and Valle Zoque the most impactful management practices were found to be cleaning dirty drinkers and feeders, soil pens, pens cleaning, age of weaning calves, stallion change time and stallion loan.

The ELISA technique (IDEXX, 2017) that was used in the present work, presented a sensitivity of 60-80% and a specificity of 90-99%. These types of tests are less expensive and can be applied to blood or milk samples. This technique is ideal for the identification of animals that are in a subclinical state of the disease and is considered a screening technique and is useful for the establishment of surveillance and control measures within the affected herds.

Finally, derived from the survey of producers, it was possible to identify risk behaviors that allow establishing hypotheses that in subsequent studies should be explored in depth in multidisciplinary studies to establish prevention and control strategies for this disease.

It is important to take biosecurity measures against this disease at a national and international level, which is why the creation of the national campaign against paratuberculosis is urgent. In this sense, sensitive and specific diagnostic tests are required at a good price to achieve the detection and subsequent elimination of clinical and subclinical cases of LU, so that, in this way, the exposure of young animals that are the most susceptible to infection to MAP is reduced.

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Reception: **January 11, 2021**| Acceptance: **April 27, 2022**| Publication: **October 1, 2022**

In vitro control of anthracnose (*olletotrichum gloeosporioides*) isolated from *annona muricata* l. with vegetable extracts

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Diana Acely Aguilar Pérez[1]
dianaacely@gmail.com • ORCID: 0000-0002-0471-5025

Sandra Isabel Ramírez González[2]
sanirg@yahoo.com • ORCID: 0000-0002-1563-1521

Orlando López Báez[1]
olopez@unach.mx • ORCID: 0000-0003-4200-4547

Judith Prieto Méndez[2]
jprieto@uaeh.edu.mx • ORCID: 0000-0001-5705-1704

1 Universidad Autónoma del Estado de Hidalgo, México.

2 Universidad Autónoma de Chiapas, México.

To quote this article:
Aguilar Pérez, D. A., Ramírez González, S. I., López Báez, O., & Prieto Méndez, J. (2022). Control *in vitro* de antracnosis (*colletotrichum gloeosporioides*) aislado de *annona muricata l.* con extractos vegetales. *Espacio I+D, Innovación más Desarrollo, 11*(31). https://doi.org/10.31644/IMASD.31.2022.a02

— Abstract—

Colletotrichum gloeosporioides is a disease of great importance in soursop cultivation since it can cause large production losses by being present in all the crop's phenological stages as an alternative to chemical synthesis products for its control. The in vitro effect of eight extracts in hydrolate form obtained from *Bougainvillea* spp., *Hibiscus sabdariffa* L., *Mangifera indica* L., *Carica papaya* L., *Pimenta dioica* L. and *Psidium guajava* L. were investigated using the poisoned media technique. In the first stage, the hydrolates were evaluated at 50% concentration V/V, those that achieved total inhibition of the pathogen were revaluated to calculate their minimum inhibitory concentration (MIC), the daily growth of the radial diameter of the pathogen as well as the number of total and germinated conidia. The results show that all the evaluated plants have compounds with fungistatic capacity on C. *gloeosporioides* when tested *in vitro*; in the first stage, P. *dioica* L., D. *ambrosioides* L., M. *indica* L., and *Bougainvillea* spp. inhibited the total development of the pathogen, while H. *sabdariffa* L. and P. *guajava* L. showed a minor inhibition in mycelial growth; however, they showed high antisporulant capacity (99.45% and 83.33% respectively); on the other hand, C. *papaya* showed low inhibition in both sporulation and mycelial growth. In the second stage, only P. *dioica* L. inhibited the total development of C. *gloeosporioides*, for which it achieved the lowest MIC with 40%, the other treatments failed to inhibit mycelial growth but all showed antisporulant capacity according to the comparison test Stocking by Tukey. The P. *dioica* L. hydrolate showed the lowest minimum inhibitory concentration with 40% (V/V), while for the hydrolates the D. *ambrosioides* L., *Bougainvillea* spp. (leaf, flower, and bract) and M. *indica* L., the minimum inhibitory concentration was 50% (V/V) on C. *gloeosporioides*.

Keywords:

Colletotrichum gloeosporioides; hydrolates; secondary metabolites; anthracnose; soursop; inhibition.

The soursop, *Annona muricata* L., is the most important species in the Annonaceae family, it is a crop with great economic potential, being demanded in agribusiness, the perfumery industry, and even pharmacology, taking advantage not only of the fruit but also the leaves and seeds (Vit, *et al*., 2014., León-Hernández, *et al*, 2019). Mexico has the climatic characteristics that allow it to be the country with the largest production area worldwide (National Institute for Forestry, Agricultural and Livestock Research - INIFAP, 2015; Reyes-Montero, *et al*., 2018). However, the scarce agronomic research around the problems in the crop production represent an obstacle to the development of producers, mainly small and those who carry out organic production. Among the productive problems faced by this crop, is anthracnose, caused by the fungus *Colletotrichum gloeosporioides*, a phytosanitary problem that causes large economic losses (Anaya-Dyck, *et al*., 2021).

C. *gloeosporioides* is characterized by a remarkable infective capacity, causing considerable losses in both crop production and postharvest, (Landero-Valenzuela, *et al*., 2016; Betancourt, 2019), infecting seedlings and adult plants by attacking flowers, branches, stems, leaves and fruits (Sáyago and Álvarez, 2018), causing dark brown to black lesions, falling of flowers, fruits and leaves (Hernández and López, 2018).

The traditional control of this disease consists of practices of cultural work as well as spraying of chemical synthesis products. However, these products have been used irrationally and, in many cases, erratically, which has consequently generated health and human health problems; in addition to a serious ecological imbalance favoring the emergence of pests and diseases more aggressive and resistant to certain substances that are traditionally used for their control (Gordillo, 2019).

Due to the above, agriculture is often mentioned as one of the factors that have contributed greatly to environmental pollution and climate change, so it is necessary to change the current agricultural production model to a sustainable production model that ensures the ecological harmony of agroecosystems.

On the other hand, both the production and consumption of soursop have increased in recent years at a national level (Terán-Erazo, *et al*., 2019), which presents a need for research aimed at generating strategies that allow the incorporation of soursop cultivation into a sustainable system. An interesting alternative to contribute to this is using plant extracts to replace synthetic products for phytosanitary problems management.

Previous research has shown the advantages and success of using plant extracts on different microorganisms and even insects. In this framework, extracts of *Bougainvillea* spp. have been tested on *Botrytis cinerea* (Santiago,

et al., 2019) and C. *gloeosporioides* (Hernández, 2004), obtaining inhibitory results. On the other hand, D. *ambrosioides* L. has been shown to have a positive effect on various phytopathogenic fungi (Cabrera, *et al*., 2016; Montes-Belmont and Carvajal, 1997; Rivera-Castañeda, *et al*., 2001; Vasquez, *et al*., 2014;) and even on *Spodoptera frugiperda* larvae (Chávez, 2019).

Another plant species that have reported antifungal properties is *H. sabdariffa* L., whose extract achieved a total inhibitory effect against *Alternaria solani* (Goussou, *et al*., 2010). On the other hand, extracts of *P. dioica* L. have been successfully tested on different fungal pathogens (Aguilar, *et al*., 2019; Arcos-Méndez, *et al*., 2019; Ramírez-González, *et al*., 2007). The *C. papaya* L. seed insecticidal (Figueroa, *et al*., 2011; Franco-Archundia, 2006) and fungicide capacity has been previously reported (Ramírez-González, *et al*., 2007). Likewise, authors report inhibition when evaluating extracts of *P. guajava* L. in vitro versus *C. gloeosporioides* (Baños-Guevara, 2003), while *M. indica* L. stands out for having an antimicrobial effect against bacteria of health interest (Guerra & Román, 2016).

Plant extracts, being products obtained from renewable materials, have the advantage of being able to degrade quickly, be harmless to the environment, and be selective with pests and diseases (Ibáñez & Zoppolo, 2008; Figueroa, *et al*., 2019), in addition to being economical, reducing negative impacts on the ecological balance and contributing to the development of small producers.

Considering the biological diversity of Mexico, it is interesting to explore the potential of plant extracts in the control of diseases such as anthracnose. That is why the objective of this research is to evaluate the potential use of eight botanical extracts in the control of the pathogen's growth that causes anthracnose in soursop (*Colletotrichum gloeosporioides*), taking into account the importance of the culture and the relevance of the disease in terms of damage caused, and in this way, Contribute to the resolution of one of the most important phytosanitary problems of soursop cultivation, acting within the framework of sustainable agriculture.

METHODOLOGY

The experimental process was developed in the Agrotecnologías de la Agencia Universitaria para el Desarrollo (AUDES) Cacao-Chocolate of the Universidad Autónoma de Chiapas (UNACH). The plants used for the preparation of the extracts were: *Bougainvillea* spp. (leaves, bracts, and flowers), *Dysphania ambrosioides* L. (stem and leaves), *Mangifera indica* L. (leaves), *Carica papaya* L. (seeds) fresh and dry, *Hibiscus sabdariffa* L. (flower), *Psidium guajava* L. (leaves) and *Pimenta dioica* L. (fruit).

C. gloeosporioides was previously isolated from a soursop fruit of a crop located in the municipality of Tecpatán, Chiapas; its multiplication was carried out in a PDA medium using a punch and allowed to grow for 12 days.

To obtain the hydrolates, the methodology proposed by Ramírez (2013) was followed, using the method described as hydrolates by distillation, as a solvent, the ethyl alcohol in a 10:1 ratio, as reported by Tamayo (2016), was used as the water mixture. The hydrolates obtained were stored in sterile Erlenmeyer flasks and refrigerated at 4°C for later use.

Evaluation of hydrolats at 50% concentration

The hydrolates were evaluated at 50% concentration in a PDA medium, using the poisoned media technique, a completely randomized design was established with ten treatments and three repetitions each, eight treatments corresponding to the hydrolates, a chemical control (i.a. Chlorothalonil) and an absolute control (PDA). Inoculation of the pathogen was performed using a punch. The inhibitory effect was quantified by measuring the growth of the pathogen mycelial diameter every 24 hours for 12 days. Likewise, the production of total and germinated spores was quantified using the Neubauer chamber following the methodology described by Gilchrist, *et al.* (2005). The data obtained were used to calculate inhibition percentages on growth and sporulation.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the treatments that in the first trial managed to inhibit the pathogen was determined, for this, concentrations of 40%, 30%, 20%, and 10% were evaluated, following the same methodology described above.

Statistical analysis

To determine the effects of the treatments studied, a variance analysis (ANOVA) was performed, and the 5% Tukey Mean Comparison Test was applied, using SPSS version 17.0 software for Windows.

RESULTS

Hydrolates effect at 50% concentration

Bougainvillea spp., *D. ambrosioides* L., *M. indica* L., and *P. dioica* L. hydrolates, inhibited the development of the pathogen by 100%, surpassing even the result obtained by the chemical control, which inhibited mycelial growth by 75.33%. The statistical analysis shows that these results are statistically different from each other; in the case of the sporulation variable, the chemical control inhibited by 98.53%, a result statistically identical to the treatments results mentioned above. On the other hand, hydrolates based on *H. sabdariffa* L. and *P. guajava* L. showed high antisporulant capacity with 99.45 and 83.33%, respectively, results statistically identical to those achieved by the chemical control and treatments that completely inhibited the pathogen; however, its inhibitory capacity on mycelial growth of *C. gloeosporioides* was reduced. In the case of *C. papaya* L., the results report a reduced capacity in both growth inhibition and sporulation, inhibiting below 50% in both cases (Table 1).

Table 1
Mycelial growth and sporulation of C. gloeosporioids by 50% concentrated hydrolate effect

	Mycelial growth			Conidia concentration		
Treatment	Growth (mm)	Tukey HSD[a]	Inhibition (%)	Conidias/mL *10^{11}	Tukey HSD[a]	Inhibition (%)
***Bougainvillea* spp. (leaf)**	0	A	100	0	A	100
***Bougainvillea* spp. (flower and bract)**	0	A	100	0	A	100
***D. ambrosioides* L.**	0	A	100	0	A	100
***M. indica* L.**	0	A	100	0	A	100
***P. dioica* L.**	0	A	100	0	A	100
***H. Sabdariffa* L.**	26.67	C	46.67	2.93	A	99.45
***C. papaya* L.**	47.67	D	4.67	285.33	B	46.89
***P. guajava* L.**	48.33	D	3.33	78.81	A	83.33
Chemical control	12.33	B	75.33	7.91	A	98.53
Absolut control	50	D	0	537.27	C	0

*Averages with the same letter in the same column show no statistically significant differences in the Tukey test ($p<0.05$).

Source: Own elaboration

Determination of minimum inhibitory concentration (MIC)

The treatments corresponding to *Bougainvillea* spp., *D. ambrosioides* L., *M. indica* L., and *P. dioica* L. that at a 50% concentration completely inhibited the pathogen, were evaluated at this stage in different concentrations. Only the hydrolate based on *P. dioica* L. affected the growth of the pathogen, achieving a total inhibition of the same at a 40% concentration, showing an increase in the concentration of conidia by decreasing the concentration of the hydrolate in the culture medium, similar behavior observed in all treatments (Table 2). The statistical analysis showed significant differences between the treatments corresponding to *P. dioica* L., so the MIC for this treatment was established at 40%.

On the other hand, all treatments showed antisporulant capacity, highlighting those corresponding to *Bougainvillea* spp. (bracts) at 40 and 30%, *Bougainvillea* spp. (leaves) at 40, 30, and 20%, *D. ambrosioides* L. in all concentrations, *M. indica* L. at 40, 30, and 20%, and *P. dioica* L. at 30% indicating an inhibition greater than 70%. It should be noted that all concentrations of *D. ambrosioides* were able to inhibit pathogen sporulation above 90%. Only *Bougainvillea* spp. (bracts and leaves) and *P. dioica* L. at 10% showed less than 50% inhibition. The analysis of variance showed differences between the treatments, and the comparison test of means by Tukey showed that no treatment is statistically identical to the result of the Absolute Control, so they all have antisporulant capacity (Table 2). It should be noted that there were no germinated conidia present for any treatment in the trials.

Table 2

Mycelial growth and sporulation of C. gloeosporioids at 40%, 30%, 20%, and 10% concentrated hydrolate effect

Treatment	Mycelial growth			Conidia concentration		
	Growth (mm)	Tukey HSD[a]	Inhibition (%)	Conidias/mL *10^{11}	Tukey HSD[a]	Inhibition (%)
***Bougainvillea* spp.**	50	D	0	12.77	F	86.36
***Bougainvillea* spp. (flower and bracts)-30%**	50	D	0	23.68	I	74.70
***Bougainvillea* spp. (flower and bracts)-20%**	50	D	0	40.01	L	57.25
***Bougainvillea* spp. (flower and bracts)-10%**	50	D	0	90.30	N	3.52
***Bougainvillea* spp. (leaf)-40%**	50	D	0	9.68	R	89.66
***Bougainvillea* spp. (leaf)-30%**	50	D	0	17.44	G	81.37
***Bougainvillea* spp. (leaf)-20%**	50	D	0	27.94	J	70.15
***Bougainvillea* spp. (leaf)-10%**	50	D	0	50.27	M	46.29
***D. ambrosioides L.*-40%**	50	D	0	2.27	AB	97.57
***D. ambrosioides L.*-30%**	50	D	0	4.23	BC	95.48
***D. ambrosioides L.*-20%**	50	D	0	5.95	CD	93.64
***D. ambrosioides L.*-10%**	50	D	0	8.02	DE	91.43
***M. indica L.*-40%**	50	D	0	3.15	B	96.63
***M. indica L.*-30%**	50	D	0	9.43	E	89.92
***M. indica L.*-20%**	50	D	0	21.84	I	76.66
***M. indica L.*-10%**	50	D	0	37.84	L	59.57
***P. dioica L.*-40%**	0	A	100	0	A	100
***P. dioica L.*-30%**	10	B	80	17.93	H	80.84
***P. dioica L.*-20%**	30	C	40	28.81	JK	69.22
***P. dioica L.*-10%**	50	D	0	50.52	M	46.06
Absolut control	50	D	0	93.59	N	0

*Averages with the same letter in the same column show no statistically significant differences in the Tukey test ($p<0.05$).

Source: Own elaboration

DISCUSSION

The hydrolate of *P. guajava* L., allowed the greatest mycelial growth, in contrast to these results, other authors report inhibition versus *C. lindemuthianum*; however, they do not report inhibition for *M. fructicola*, A. pisi, and *P. parasítica* (Villanueva, *et al.*, 2012). Regarding these differences in the studies, several authors mention that fungistatic activity differs between the different forms of extraction, the species of the plant, and the pathogen evaluated (Hernández, *et al.*, 2007; Sánchez, 2019). On the other hand, this treatment showed significant inhibition on the concentration of conidia, evidencing anti-sporulant qualities, this information coincides with that reported by Bravo, *et al.*, (2000), who evaluated powders of this plant obtaining an anti-sporulant effect against *Fusarium moniliforme*. This capacity may be due to the secondary metabolites present in its structure, such as phenols, flavonoids, triterpenes, and saponins, among others, which have been reported with antifungal capacity (Rodríguez, *et al.*, 2013; Mas, *et al.*, 2017).

On the other hand, *H. sabdariffa* L. showed high antisporulant capacity; information that adds to that reported by Goussous, *et al.*, (2010), who report total inhibition on A*lternaria solani*, using raw extracts from this plant, attributing these results to the presence of a polyphenol called protocatechuic acid. Similarly, the antimicrobial capacity of different extracts of this plant against bacteria such as *Salmonella enteritidis* and *Excherichia coli* has been previously tested (Castillo, 2018), thus demonstrating the antimicrobial potential of the plant.

The results obtained for the treatment with C. papaya L. seed hydrolate are partially like those obtained by Bautista-Baños, *et al.*, (2003), who tested *C. papaya* L. seed extract on *C. gloeosporioides* papaya isolate and obtained a zero inhibition both in sporulation and in mycelium growth; however, there are methodological differences in the preparation of the extracts evaluated in the different studies.

The results obtained using the hydrolate of *P. dioica* L. are added to those obtained by other authors such as Ramírez-González, *et al.* (2007), who report total inhibition of *Phytophthora palmivorausing* 50% concentration of *P. dioica* hydrolate. Duarte, in 2019, reports the use of an extract of *P. dioica* obtained by microwave at 50% concentration, obtaining total inhibition against *C. gloeosporioides* and *A. alternata*. Pepper is recognized for its remarkable antifungal activity due to the secondary metabolites present in its structure, such as essential oils, tannins, flavonoids, phenols, and terpenes (Álvarez, *et al.*, 2010; Velázquez-Silva, *et al.*, 2019).

In the case of *D. ambrosioides* L., the results of this study improve those obtained by authors such as Cabrera, *et al.* (2016), who report 79% inhibition against *C. gloeosporioides* in tests *in vitro* using ethanolic extracts. The

antifungal capacity of epazote, demonstrated in this study, is added to the many reported qualities of this plant, such as pesticide against lepidoptera and beetle (Chávez, 2019), amebicide, analgesic, among others (López, 2020). Qualities that are attributed to the different metabolites reported in their structure, such as essential oil, phenols, flavonoids, and saponins, among others (Chávez, 2019).

In the case of *Bougainvillea* spp., the results achieved in this study reveal antifungal and antisporulant capacity, both for the leaf and the bracts. Other authors report having obtained significant inhibition on the germination of spores of *C. gloeosporioides* (Hernández, *et al*., 2004), in addition to inhibition against *Botritys cinerea* in blueberry fruits (Santiago, *et al*., 2019). The uses of this plant in both agriculture and traditional medicine have been previously reported (Edwin, *et al*., 2007; Galindo, *et al*., 2009), likewise, different compounds have been found both in leaves and in the bracts responsible for antifungal activity, such as the low molecular weight proteins called defensins (Hernández, 2004), in addition to flavonoids, tannins, alkaloids, and saponins, which perform defense functions in plants (Edwin, *et al*., 2007).

The results obtained in this study verify the antifungal and antisporulant capacity of the hydrolate of *M. indica* L., thus increasing knowledge about the properties of this plant, contrasting with previous investigations that have reported low inhibition in the germination of spores of *C. gloeosporioides*, using *indica* L. leave extracts, (Hernández-Altíber, *et al*., 2006). On the other hand, the antimicrobial capacity of the plant has been reported against bacteria of health interest, such as *Escherichia coli*, *Staphylococcuss aureus*, *Listeria monocytogenes* (Ortiz, 2015), *Pseudomona aeuroginosa, Salmonella Typhimurium, Enterococcus Faecalis* (Carrillo-Tomalá, *et al*., 2019), among others. These properties present in the mango leaves extract are attributed to their bioactive compounds such as polyphenols, flavonoids, and gallic tannins, which in addition to being antimicrobial, are antiviral, anti-inflammatory, antioxidants, etc., (Carrillo-Tomalá, *et al*., 2019; Ortiz, 2015).

The results show that although not all treatments managed to inhibit mycelial growth, they do possess the ability to inhibit the production of spores of *C. gloeosporioides*, since when compared to the absolute control, a reduction of these structures is noticeable; conidia are the main source of the inoculum for the dissemination and development of the disease (Díaz-Medina, *et al*., 2019) so a reduction in the number of these structures would substantially reduce the pathogenicity of the fungus and, consequently, its ability to initiate an infectious cycle (Valdés, *et al*., 2017). The inhibition of mycelial growth produced by hydrolates in the present work may be because naturally occurring compounds cause irreversible damage to the cell structure, affecting the physiology of the fungus. Phenolic compounds affect the

active sites of enzymes and cell metabolism by reducing the growth rate of the pathogen (D´Luis, 2018). In previous studies, it is reported that plant extracts can cause alterations in the structure and shape of pathogens; Duarte (2019) reports having observed dehydration in conidia of *C. gloeosporioides* treated with extract of *P. dioica*. On the other hand, alkaloids are related to the inhibition of protein synthesis, induction of apoptosis, and inhibition of carbohydrate metabolism enzymes (Duarte, *et al*., 2021, González-Chavarro, *et al*., 2020); however, these properties, present in the extracts, although attributed to their active compounds, highlight the fact of the synergy that exists between all the components of the extract, since the effect they achieve is not due to their individual action but to several reactions that act in a certain concentration and proportion (D´Luis, 2018; Hernández, 2019, Ramírez-González, *et al*., 2016).

CONCLUSIONS

Hydrolates of *P. dioica* L., *M. indica* L., *D. ambrosioides* L., *Bougainvillea* spp. (leaf, flower, and bract), *P. guajava* L e *Hibiscus sabdariffa* L., presented an *in vitro* inhibitory effect on the growth and development of *Colletotrichum gloeosporioides* isolated from *Annona muricata* L.

The hydrolate of *P. dioica* L. showed the lowest minimum inhibitory concentration with 40% (V/V), while for the hydrolates based on *D. ambrosioides* L., *Bougainvillea* spp. (leaf, flower, and bract) and *M. indica* L., the minimum inhibitory concentration was 50% (V/V).

The hydrolates based on *H. sabdariffa* L and *P. guajava* L. showed a high antisporulant capacity. The form of extraction used to obtain hydrolates from the plants tested was shown to be an effective way of controlling the development of phytopathogenic fungi at the laboratory level.

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Reception: **December 7, 2021** | Acceptance: **June 9, 2022** | Publication: **October 1, 2022**

A review on birds of prey migration and the toxicological effects of heavy metals

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Manuel Becerril-González
manuel.becerril@cch.unam.mx
ORCID 0000-0002-0245-0756

Colegio de Ciencias y Humanidades, Plantel Sur. Universidad Nacional Autónoma de México Ciudad de México, México

To quote this article:
Becerril González, M. (2022). Una revisión sobre la migración de las aves rapaces y los efectos toxicológicos de los metales pesados. *Espacio I+D, Innovación más Desarrollo, 11*(31). https://doi.org/10.31644/IMASD.31.2022.a03

— Abstract—

In this work, the importance of migrating birds of prey as bioindicators to show the presence and concentration of heavy metals and metalloids is examined, adittionally, a general view of the toxicity of these elements is discussed. Human activities such as mining, the petroleum/oil industry, and agriculture are the principal sources of environmental pollution, the effects of these industries on the health of migrating raptors are variable, at present, studies to determine the degree to which raptors are affected are being conducted. Some heavy metals and metalloids have negative effects on biomolecules essential to cell metabolism; despite the number of written works on the subject, their results are inconclusive, and changes in natural populations of raptors are diverse.

Keywords:

Birds of prey; migration; indicator species; heavy metals; Veracruz Rio de Rapaces.

CURRENT TALKS ON THE SUBJECT

Various environmental issues have caught our attention in the last six decades, particularly those studies that account for the negative effects we have caused as a society on ecosystems, these are diverse and have caused effects of different magnitudes, however, increasing scientific evidence and new knowledge are provided to assemble the intricate environmental puzzle, with its key pieces and processes that keep it in balance.

It is worth mentioning that one of the ways we can know the impacts of environmental pollution is the presence or absence of what we know today as bio-indicator species,[1] several of them we can "evaluate" due to specific study techniques that lead us to know aspects of particular interest, for example, the toxicity that can generate an element or chemical compound, adversely affecting some metabolic function of a living being.

Activities such as mining, petrochemicals, agriculture, and industry in general, are responsible for the generation of a series of highly polluting waste or use in their processes the so-called heavy metals and metalloids (*e.g.*, cadmium, chromium, mercury, lead, zinc, arsenic, among others), these can provide us with information not only on the levels of environmental pollution, but, in addition, they allow us to evaluate the health status of indicator species, such is the case of birds of prey (Figure 1), this group of birds is classified into diurnal raptors such as eagles, hawks, sparrowhawk, kestrels, etc., and nocturnal raptors such as owls and tecolotes[2] the majority are, unfortunately, under some category as endangered species or with populations very vulnerable to decline.

Figure 1. Photograph of *Rupornis magnirostris*, juvenile individual, Roadside hawk, Papantla, Veracruz, bird of prey resident in Mexico and with wide distribution in South America. Source: Own elaboration (2018)

1 They are the species that provide us with very valuable information, and which can be analyzed qualitatively and quantitatively to know the state of conservation or alteration of ecosystems.

2 Birds of prey or raptors are made up of four Orders: Strigiformes (owls and related), Cathartiformes (New World vultures), Accipitriformes (eagles and related), and Falconiformes (hawks and related) and seven families with more than 500 species on all continents (Del Hoyo & Collar, 2014).

It is pertinent to mention that because birds of pray are part of charismatic birds, they have been studied from different points of view, among which are the study of their conservation, geographical distribution, migration, spatial modeling, the assessment of landscape conditions, and the use of geographic information systems (Rodríguez-Estrella & Bojórquez, 2004).

BIRDS OF PREY MIGRATION IN THE WORLD

The migration of birds is one of the natural phenomena of greatest interest in ornithology (i.e., the study of birds), although many things are known in this regard, there are still unknown basic and specific aspects of this extraordinary journey; at least in birds there is a record of 1,855 species that migrate annually (BirdLife, 2004a, and 2004b), these migrations can be a long or short distance, from north to south or from east to west, it is presented cyclically, and it has been said that it is the result of avoiding low temperatures, having access to food or having suitable sites for reproduction, whatever the case may be, during the movement through the various migratory routes the birds can be analyzed and thus determine their health status. There are two major birds of prey migratory routes recognized globally, one follows the migration from northeast Africa to Asia and ends in the central and northern part of Europe, this has been studied by the German Ornithologists' Society (Bairlein, 2003) and BirdLife International, and the other in the north-central-south of the American continent, analyzed by Cornell University of New York, through the Cornell Lab, the Audubon Society and in Mexico by the Asociación Civil Pronatura Veracruz since 1991.

Mexico is one of the countries that offer a unique opportunity for the observation and registration of migratory species. Some reports mention that just over 300 species can be recorded weekly, well above other tropical and subtropical sites in the world, for example, in the state of Veracruz alone there are more than 50 important sites for observation with the presence of more than 700 autumn migratory species in total (Straub, 2007). Some data shared by the National Commission for the Knowledge and Use of Biodiversity mention that in the migration of "Veracruz Río de Rapaces" only in 2005 there were 5,691,204 individuals belonging to 25 birds of prey species and four unidentified genera, which reveals the magnitude of migration in the Gulf Coastal Plain.

Migratory birds of prey present serious problems due to the change in land use for agriculture-related purposes, since it involves 80% of migratory birds of prey (Kirby *et al*., 2008), in addition to the use of organochlorine pesticides and fertilizers, it should be noted that many of the companies dedicated to the synthesis of these products present partial information and in few cases the risks to health and ecosystems are mentioned in general,

some of them apparently are not even regulated by environmental laws, just remember the use of FURADAN 350 L (*i.e.*, Carbofuran: 2-3-dihydro-2,2-dimethyl-7-benzofuranyl-methyl carbamate) to fight the nematodes "roundworms" in Africa that has caused the death of lions and as of yet, an undetermined number of vultures of various species, thus breaking the ecological balance, this product in Mexico is used in various crops (*e.g.*, peanut, coffee, sugar cane, chili, pumpkin, melon, cucumber, watermelon, strawberry, corn, potato, banana and wheat among others) especially for its availability and low price ($978.00 pesos per liter/quoted as of November 2021 in Mercado Libre) and its specific effects on wildlife are still unknown.

Undoubtedly, the health assessment of migratory raptors is of vital importance, due to the eco-physiological conditions necessary to carry out the complete migration since they face the destruction of their habitat and climate change, and if it were not enough, the same health conditions of the bird (Klaassen *et al.*, 2012), in these cases the energy needs of ATP (adenosine triphosphate, a highly energetic molecule used in cell metabolism) by aerobic and anaerobic routes are related to efficient metabolic processes and, in turn, with the health status of individuals, whether young or adult. On the other hand, a clear relationship has been observed between the absence of migratory species and the loss of ecological and evolutionary relationships.

BIRDS AS BIO-INDICATORS OF HEAVY METALS

In the specific case of birds of prey, as they are species positioned at the top of the food chain, they can be used as bio-indicator species (Figure 2), and therefore, their monitoring is a matter of high priority for experts since they are individuals who help determine the presence of heavy metals and their effect on other fauna and flora in general (Hermoso de Mendoza *et al.*, 2006).

Regarding the biology of birds of prey, it should be noted that they are part of the birds' wildlife that presents long life cycles, some travel long distances, and others are part of the "sanitary body" of nature (*i.e.*, scavengers) that are responsible for cleaning the ecosystem of dead animal bodies or trap sick animals, that does not mean that they do not feed on healthy animals (*i.e.*, hunters), but we can imagine the intricacy of the problem to be studied, some of these birds of prey feed on other birds, mammals of various sizes, reptiles, amphibians, fish and even some include insects and mollusks in their diets. This can also help to understand the different concentrations of heavy metals present and even depend on the region where the bird is distributed and the season of the year, as well as the age of the individuals and their sex.

Figure 2. Photograph of *Accipiter cooperii*, adult individual, Cooper's hawk, Mayor's Office Miguel Hidalgo, Second Section of the Chapultepec Forest in Mexico City, bio-indicative and migratory bird of prey in "Veracruz Río de Rapaces". Source: Own elaboration (2018)

The most complete studies have been carried out in the Gulf Coastal Plain since they are very close to reality; their methodology includes working with all the migratory raptors trapped throughout each year, this has been done in little more than 20 years, taking samples of blood and feathers from live birds of prey for subsequent release; these studies have been analyzed by high-level task forces, as directed in the state of Veracruz by Dr. Ernesto Ruelas Inzunza of the Universidad Veracruzana y Pronatura Veracruz, obtaining valuable information on this subject.

As a general evaluation of heavy metals and metalloids in living beings, various effects are presented at the molecular level, within which we can observe the following:

1. Blockade of functional groups in biomolecules, due to the affinity of metal cations for the sulfhydryl groups of proteins, specifically cysteine residues, which causes their denaturation (Schützendübel & Polle, 2002; Peralta-Videa *et al*., 2009).
2. Displacement of cationic sites in important enzymes, so they lose their function (Schützendübel & Polle, 2002; Smeets *et al*., 2005).
3. Formation of reactive oxygen species due to the autooxidation of metals such as Fe^{2+} (iron ion) or Cu^{+} (copper ion), resulting in the formation of H_2O_2 (hydrogen peroxide) and the OH radical (hydroxyl), in the first case, it has been observed that its accumulation considerably increases programmed cell death (*i.e*., apoptosis), while the OH radical is one of the most reactive known for its ability to initiate free radical chain reactions that cause modifications and irreversible

damage to cellular molecules such as carbohydrates, DNA (deoxyribonucleic acid), proteins and lipids (Mithöfer *et al*., 2004).

BAD MEMORIES OF PAST EXPERIENCES

For just over 50 years, there had been deaths of adult individuals and chicks of White-headed Eagles (*Haliaeetus leucocephalus*) due to lead poisoning; in those cases, the concentrations ranged from 5-61 ppm (parts per million) and 5-12 ppm respectively. This caught the scientific community's attention dedicated to dealing with environmental pollution issues by chemical substances. The most immediate thing was to detect the sources to prolonged exposure in these raptors, even when it was referred to that this only happened in underdeveloped countries in tropical and subtropical areas. Currently, the evidence suggests the opposite and establishes, unfortunately, a generality in terms of prolonged exposures and absorption of heavy metals in many developed or non-developed countries, whose environmental policies have been insufficient to curb these problems. The truth remains that birds have high and lethal concentrations of one or more of these chemical elements.

Mining in our country is one of the leading causes of environmental pollution by heavy metals due to the inadequate management of its waste known as "mining tailings" reports indicate that in Mexico, there could be millions of tons of tailings scattered in the national territory, of which its conditions and its potential to affect the environment are still unknown (Yañez *et al*., 2003; Meza-Figueroa *et al*., 2009; Mireles *et al*., 2012; Cortés-Jiménez *et al*., 2013; Ramos-Arroyo & Siebe-Grabach, 2006)

HEAVY METALS AND METALLOIDS PRESENT IN BIRDS OF PREY, RECENT STORIES WITH SCIENTIFIC EVIDENCE

For some time, the effect caused by various concentrations of heavy metals and metalloids in the liver tissue of birds of prey has been investigated, as in some areas of Spain, where "normal concentrations of As (arsenic), Cd (cadmium), Pb (lead) and Zn (zinc)" apparently have no toxicological severe effects on their health (Hermoso de Mendoza *et al*., 2006), however, these data are not entirely conclusive, it is worth mentioning that information from various sources should be checked and strict methodologies used to assess the impact on the environment. In the previous study, for example, nine birds of prey species were analyzed for a total of 85 individuals, of which three species were analyzed, three individuals of each, and of one species, 44 specimens were studied; the latter represents 51.7% of the samples analyzed, suffice to mention that only in Spain have been recorded 33 species of birds of prey.

In several wild species, including the White-headed Eagle and the Golden Eagle (*Haliaeetus leucocephalus* and *Aquila chrysaetos* respectively), blood tests have reported harmful effects on the neurological system, as well as on the reproductive system, resulting from high concentrations of arsenic, and mercury and lead (Lehner *et al*., 2013).

In species such as the Californian Condor (*Gymnogyps californianus*), which is endangered (SEMARNAT, 2010. NOM-059-SEMARNAT-2010) and whose eating habits place them within scavenger raptors, lead exposure is very high when lead munitions residues have been found in dead animals, causing harmful effects on synaptic activity and problems in bone tissue conformation (Hunt, 2012), in addition to shortening longevity and having negative effects on reproduction (*e.g*.spermatogenesis). In the same case of ingestion by meat contaminated by Pb are Golden Eagles (Iwata *et al*., 2000; Hunt *et al*., 2006; Krone *et al*., 2009), Old World Vulture and Hawks of various species (Mateo, 2009; Fisher *et al*.,2006).

In the American Kestrel (*Falco sparverius*), δ-aminolevulinic acid is a precursor in the synthesis of hemoglobin, however, its concentration can be elevated due to lead poisoning, affecting, in turn, the synthesis of porphyrins that result in hemoglobin part of the heme group not being produced properly, and therefore, the transport of oxygen to the body is not carried out properly (Pattee, 1985).

Nighat and his colleagues studied five species of the Family *Falconidae* (*falcons*), nine of *Accipitridae* (*eaglets*), and four *Strigidae* (*owls*) in Pakistan in 2013 due to the current high concentrations of heavy metals in the country to implement remedial measures. Although, the results have been mixed in terms of concentrations, in all cases they have been detected and show a devastating ecological crisis due to industrialization; particularly in South Asian countries, exhibiting, inter alia, inadequate solid and liquid waste management in urban areas (Karn & Harada, 2001; Hinrichsen *et al*., 1997; Pandey, 2006).

Arsenic

Birds of prey allow us to evaluate specific cases such as arsenic, where said element, in addition to being carcinogenic, is a bioaccumulative toxic (Hermoso de Mendoza *et al*., 2006). Currently, research is being carried out to study the food contents of various species of birds, especially raptors, because traces have been found in some stomach remains. Possibly, its use as FeAsS arsenopyrite in agricultural products as pesticides and herbicides (Londoño-Franco *et al*.2016) is one of the main routes of direct ingestion. The feeding habits of some raptors include small and medium-sized mammals found in farmland that eat heavy metals in their usual diet.

Cadmium

The case of cadmium is different since it shows recent exposure to some blood source and prolonged exposure in feathers, however, variables such as age, the time of sampling, the site where it was taken should be considered, etc. However, it is seen that juvenile individuals present lower concentrations, and adult birds of prey multiply up to ten times their concentration (Hermoso de Mendoza *et al*., 2006). The effects (as well as the presence of lead) on the endocrine system of birds can affect development and growth, feather molting, and migration (Stoica *et al*., 2000; Martin *et al*., 2003), cause respiratory diseases and inhibits egg production and shell thickness.

Cd when used as part of some fertilizers can be present in soil and water bodies due to some medications (Figure 3), we can also find it in cereals, vegetables, and tubers (Londoño-Franco *et al*., 2016) that are part of the diet of some rodents and therefore can be the route of direct ingestion by birds of prey.

Figure 3. Photograph of *Pandion haliaetus*, adult individual, Osprey, Tecolutla, Veracruz, some individuals have lethal concentrations of Cd due to their strictly piscivorous diet. Source: Own elaboration (2020)

Chromium

The presence of Cr has shown that it is an element that causes damage to genetic material (*i.e*., mutagenic agent), and in general, to eukaryotic cells of many species (Robles-Camacho & Armienta, 2000), obviously high non-lethal concentrations of chromium have been found in birds of prey, research continues as it is an essential micro-mineral in lipid metabolism, carbohydrates and proteins, however, the various results raise more specific studies in this regard.

Mercury

Blood, feather, and egg tests for White-headed Eagle (*Haliaeetus leucocephalus*) chickens, at some U.S. sites, reveal that in 95% of the samples Hg is found and that in most of the concentrations are low (0.025-0.079 mg/kg – ppm), the presence of mercury is correlated with the diet of the species. However, it is important to remember that some studies establish that in adult individuals the number of heavy metals can be ten times higher than in juvenile individuals or chickens. Interestingly, some evaluations consider that for this species between 2008 and 2010 the amount and concentration of mercury in blood and feathers has been decreasing (Mojica & Watts, 2011).

For their part, Carlson and collaborators, in 2012, found mercury in all blood samples analyzed in the same species, on average 0.28 ppm, and in chickens, it was observed that in 7.8% of cases, had high levels, greater than 0.7 ppm, this can generate in various toxicological effects in the medium and long term during the individuals' development.

In the Red-breasted Eagle (*Buteo lineatus*) the levels detected of Hg are alarmingly high in blood and feathers, both in chickens and adult individuals (Hanneman, 2021), so we should not think about generalizing these situations at any time, in some species the risks of toxicity are greater for others, as a result of their eating habits, geographical distribution or exposure times to various chemical elements, among other variables.

In our country, Campbell (2018), has found a higher concentration of mercury in blood samples in resident birds of prey such as the Short-tailed Eagle, Striped Falcon and Road Eagle (*Buteo brachyurus, Falco femoralis* and *Rupornis magnirostris*, respectively) while in migratory raptors such as Cooper's Hawk and Cinnamon-breasted Hawk (*Accipiter cooperii* and *Accipiter striatus*) the highest concentration of the same element is greater in feathers, which clearly suggests that raptors in general present contamination in their body by the presence of Hg and are exposed and ingest this heavy metal. Whatever the case, we can clearly associate it with the reproduction-nesting sites in the north of the American continent and their resting sites along the migratory routes where industrial activities and urban areas generate pollution due to the large amount of these metals, in the Mexican case it is possible that having carried out this work in the state of Veracruz, which is characterized by its petrochemical activities, the concentrations are expected to be high due to the food and its position in the food chain.

In many countries, mercury can be detected in water, such samples have been taken from natural sources such as rivers, wetlands, lakes, coastal areas, and oceans where birds of prey consume fish contaminated with various heavy metals, including mercury. In industrialized countries, water

pollution is associated with the expansion of industrial projects (Carlson *et al*., 2012), so they should be evaluated with stricter scientific criteria.

Another aspect to consider is that the adverse effects of mercury vary depending on its chemical form, concentration, and time of exposure, for example, the most toxic form of mercury is methyl mercury $[CH_3Hg]^+$ since it is absorbed by 90% compared to mercury chloride ($HgCl_2$) of which only 2% is absorbed (UNEP, 2003).

Lead

In birds of prey species that feed on rats and mice, in which case they have approached urban centers, it is more likely that the high levels of Pb detected in bones and feathers (Hunt, 2012) are related to sources of anthropic pollution. The case of the American Kestrel (*Falco sparverius*) (Figure 4) is one of the examples that are currently being worked on due to lethal concentrations of lead, especially due to the consumption of field mice and their relationship with pesticides in cultivated areas; the same happens with various species of owls (Figure 5) and tecolotes in Mexico since rodent hair that is expelled in the form of pellets (*i.e*., regurgitation of material that was not degraded in the digestive tract of the bird and that can contain: bones, teeth, hair, feathers and diverse organic matter) is indicative and cumulative of heavy metals (McLean *et al*., 2009).

In the case of the Conservation and Reintroduction Programs of raptors such as the White-headed Eagle (*Haliaeetus leucocephalus*), the solution since 2007 has been to change pellets or non-toxic bullets "lead-free" so that when accidentally ingested in carrion they do not cause harm, this is only part of the actions carried out in the legal hunting of mammals as sports trophies in the state of California USA. (Pagel *et al*. 2012).

Figure 4. Photograph of *Falco sparverius*, adult male individual, American Kestrel, Coyoacán City Hall, CD MX, the species is currently being studied for its Pb concentrations in blood, bones, feathers, and brain. Source: Own elaboration (2016)

Figure 5. Photograph of *Ciccaba virgata*, adult individual, Brown Owl, Municipality of Jalpan de Serra, Querétaro. Source: Own elaboration (2017)

Zinc

In birds such as the Osprey (*Pandion haliaetus*) (Figure 6), it has been considered that the normal physiological concentration is around 38 ppm in the liver, however, an updated record must be kept due to the high levels of Zn contamination in rivers, wetlands, lakes, and inland water bodies where this species feeds on fish probably with the presence of Zinc. Let us not forget that the availability of heavy metals is basically due to the geological history of the site where they are located or to the various anthropic activities, including irrigation with wastewater highly contaminated by heavy metals.

The high concentration of this metal causes the number of eggs per nest (*i.e.*, clutch) to decrease, and particularly there is an abnormal growth of the bones of birds known as osteochondrosis (Martorell, 2009). Finally, this element is one of the most mobiles in the environment and is bioaccumulative (Zarazúa *et al.*, 2013).

Figure 6. Photo of *Pandion haliaetus*, adult individual, Osprey, Ría Lagartos, Yucatán, may have high concentrations of Zn due to its strictly piscivorous diet. Source: Own elaboration (2014)

The Official Mexican Standard (SEMARNAP, 1996. NOM-001-ECOL-1996) establishes the maximum permissible limits of pollutants in wastewater discharges into national waters and goods, covers nine heavy metals and metalloids and the Official Mexican Standard (SEMARNAT/SSA, 2004. NOM-147-SEMARNAT/SSA-2004), which establishes criteria for determining the remediation concentrations of contaminated soils establishes 12 heavy metals; of which six were included in this documentary research work (As, Cd, Cr, Hg, Pb, and Zn) since they are part of studies related to birds of prey, the results are varied, although unfortunately an increase in the presence and concentration of heavy metals in water and soil is observed in most published works. In 90% of the verified studies, it is noted that the averages allowed for heavy metals in water and soils are well above the maximum permissible limits, this implies that there should be studies that monitor the routes followed by these chemical elements as well as their permanence in the environment and their potential effect on the health of flora and fauna.

CONCLUSIONS

In some cases, birds of prey exhibit non-lethal or sublethal levels of heavy metals, however, their continued evaluation may provide important future information and clarify long-term toxicological effects.

Although it is not a generality, various bird populations around the world are declining considerably, some of them possibly related to Climate Change or serious effects of environmental pollution.

This documentary research can be useful to analyze the migration of birds of prey from North America that converges on the route of mining

and oil states such as Chihuahua, Guanajuato, Hidalgo, Querétaro, San Luis Potosí, Tlaxcala, Veracruz and Zacatecas and where the data for soil and water contamination are well above what is allowed in official Mexican standards. It is highly likely that the mining activity developed since colonial and oil times are the reasons why there are places that can be considered as sites of high exposure by heavy metals and metalloids in birds of prey. It is worth mentioning that this is not so different in northern countries such as the US and Canada due to their mining and oil history, plus the growing industry in all areas of development, with their clear consequences for flora and fauna.

In raptors, the results indicate that some birds increased their populations, others have remained constant, while others are in clear decline, therefore, conclusive studies on the subject would be lacking.

The proximity to urban, industrial, or agricultural areas can increase the concentrations of heavy metals in birds due to the feeding habits of each species. In some cases, such as in the Lerma River in the State of Mexico, low biodiversity has been recorded (Zarazúa *et al*., 2013), possibly associated with high concentrations of heavy metals, since it is a site of discharge of water contaminated by industrial, agricultural and urban activities. It is also important to consider the persistence of heavy metals in nature and their intricate networks in ecosystems.

Undoubtedly, the information presented is a very reliable source that allows us to make decisions about heavy metal contamination in models such as birds of prey and establishes other doubts about what will happen to flora and fauna in the short, medium, and long term since their destiny is linked to our future.

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Reception: **May 27, 2022** | Acceptance: **June 10, 2022** | Publication: **October 1, 2022**

Critical elements on nanotechnologies in Mexico

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Ángeles Ortiz-Espinoza[1]
angeles.ortiz.espinoza@uaz.edu.mx
ORCID: 0000-0002-9852-8342

Guillermo Foladori[1]
12foladori@gmail.com
ORCID: 0000-0002-7441-3233

Ericka Bracamonte Arámburo[2]
ericka.bracamonte99@gmail.com
ORCID: 0000-0001-9386-8463

1 Universidad Autónoma de Zacatecas, Zacatecas, México
2 Universidad de Sonora, Hermosillo, Sonora, México

To quote this article:
Ortiz Espinoza, Ángeles., Foladori, G., & Bracamonte Arámburo, E. (2022). Elementos críticos sobre las nanotecnologías en México. *Espacio I+D, Innovación más Desarrollo, 11*(31). https://doi.org/10.31644/IMASD.31.2022.a04

— Abstract—

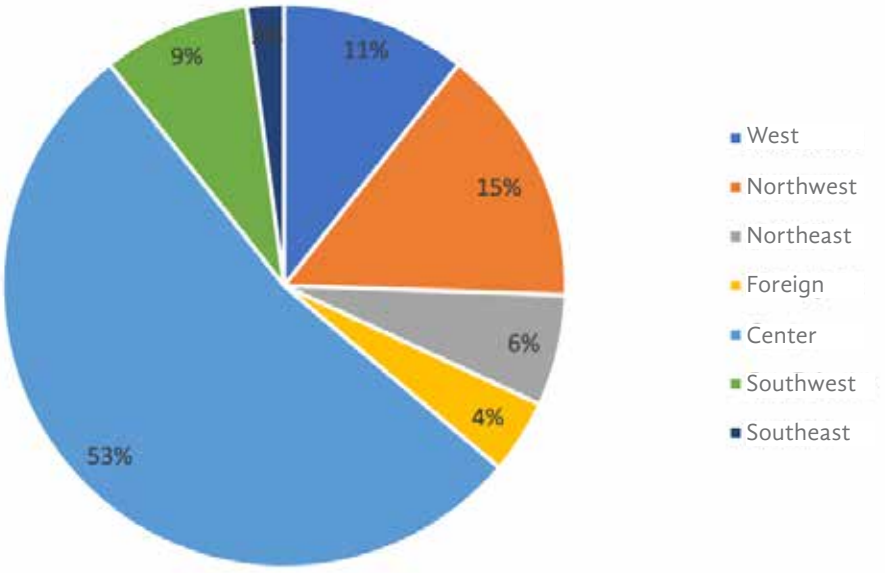
As part of the Conacyt-Ciencia de Frontera Project: A critical review of nanotechnologies development in Mexico, an exploratory electronic questionnaire was conducted on nanotechnology research for health/medicine in the country. The purpose was to examine the experts' perspectives and conduct in-depth individual interviews. Given the richness of the data and the global scope of the reflections, this text aims to present the results obtained and analyze the conditions of nanotechnologies in Mexico based on the responses. Among the main results was the generalized perspective on the need to have an updated inventory of nanotechnologies in Mexico, as well as the ignorance of researchers in some issues of standardization and regulation. Similarly, it was observed that the main investor in nanomedicine research is the State, with little collaboration from researchers with private initiatives.

Keywords:

Nanotechnologies; health; medicine; regulation; public policy.

At the end of 2021, as part of the Conacyt-Ciencia de Frontera Project 2019 No. 304320: *A critical review of nanotechnologies development in Mexico*, an exploratory questionnaire on nanotechnology research for health/medicine in the country was electronically surveyed. The purpose was to examine the topics and questions from a large number of answers, to improve it with the further purpose of conducting in-depth individual interviews. We have decided to present a systematization, given the richness of the results and the global scope of the reflections. The respondents were selected from the database of the aforementioned project on scientific publications on the topic from authors based in Mexico (Robles Belmont, 2021).

The methodology used was based on an electronic questionnaire consisting of 68 questions divided into six modules: the first of them requested information about the interviewee's profile, as well as some general data about the research carried out. Subsequent modules included the following topics: regulation, research and development, health and environmental risks, and major research areas. We obtained 47 responses from researchers, most of whom are working in the central area of the country (see Figure 1). After the survey, an analysis was carried out based on descriptive statistics of each of the modules mentioned, linking this information with the different regulations and documentation related to the topics addressed, as well as data from alternative sources to the questionnaire to reinforce the results obtained.



Note: Center: Mexico City, Mexico State, Guanajuato, Querétaro, San Luis Potosí. Northeast: Coahuila, Durango, Nuevo León. Northwest: Baja California, Baja California Sur, Sonora. West: Aguascalientes, Jalisco, Michoacán. Southwest: Hidalgo, Puebla. Southeast: Tabasco.

Although 4% of researchers live abroad, their research work is carried out in the national context.

Figure 1. Regional distribution of nanomedicine research in Mexico. Source: Own development based on the questionnaire on nanotechnology research in Mexico

The research covers different nanotechnology applications to the health/medical sector, with different degrees of potential application. The distinction between basic science and applied science, which has gained strength since the middle of the twentieth century, has been in disuse in recent decades once the term technoscience has been consolidated and with it, the distinction between science and technology disappeared. However, the distinction was kept in the questionnaire because it is still preserved in official documents and is common among natural physical scientists (Roll-Hansen, 2017). However, the above will be referred to below on how the market pressure for rapid returns of capital invested in Research and Development is what distorts the distinction between basic and applied science, something that is a greater market in countries where financing is directly or indirectly private; something that does not yet happen in Mexico.

Of the respondents, 53% considered that their research corresponds to the basic sciences category while 34% to the applied science category; the rest stated that their research corresponded to both categories. In this regard, it is essential to mention that since 2019 Conacyt has sought to direct research toward areas of the scientific frontier that have a social impact. As this general orientation crystallizes in research projects, it is possible to modify the use of concepts such as basic or applied science. For business purposes the term basic science ceased to be attractive since the nineties; it is possible that for political-social purposes something similar happens, although under different strategic interests.

Most of the research has been carried out in groups (94%). Of those who answered that they conduct collective research, 62% said they work in teams of up to three people; 24% in groups of four to five researchers, and 14%, in six or more. The important participation of undergraduate students is striking: 97% of those who claimed to do research in a group included students of this educational level in their teams, something that could be further encouraged by integrating these practices flexibly into academic curricula, as is the case in other countries and universities.

In addition to this brief introduction, which includes the main findings of the first module of the questionnaire, the present text is divided into four sections. First, review information regarding the main areas of application and knowledge. The data obtained regarding the issue of regulation of nanotechnologies in Mexico are presented below. It then shows the results in terms of the production, research, and development of nanotechnologies in the country. Finally, there are some final considerations.

AREAS OF APPLICATION AND KNOWLEDGE

Nanotechnologies constitute a wide range of technologies characterized by manipulating matter at the atomic and molecular level, conventionally between 1 and 100 nanometers. The work's purpose on the topic is to explore the new functionalities that matter can manifest in that size and that are different from those known on the macro scale. It is an area of research with accelerated growth from this century and, in many cases, requires interdisciplinary participation, both because at the atomic level, the traditional differences between chemistry and physics are erased and between the living and non-living, as well as applications may require engineering involvement (High-Level Expert Group, 2004; Roco, 2003). Moreover, if we consider the potential risks to health and the environment as well, the social and human sciences play a significant role. For these reasons, identifying an area as health/medicine is not simple. It is, for example, the case that basic research on nanoscale material properties gives rise to its potential medical application, although the starting point did not go beyond knowledge of the effect of the combination of certain materials; "a solution searching for problems" (Wilsdon, 2004, p. 16).

It is not by chance that most of the answers place their research in more than one area of nanomedicines considering the multifunctionality of nanotechnologies. However, the sector that called for more responses is biopharmaceuticals, where the production of drugs with nanotechnology (including vaccines), delivery and release within the organism is located. Both aspects constitute a significant advantage of nanometric encapsulation of drugs and their release into specific organs or cells at appropriate times. 74% of the responses, which are not mutually exclusive, consolidated biopharmaceuticals as the area of greatest interest in nanomedicine research in Mexico. Meanwhile, between 40 and 47% of the responses combined research in *implants* with other topics, highlighting the sensors. Similarly, in the *instruments* area, interest in biosensors also stands out, and in the *diagnosis* area, the emphasis is on imaging.

It is noteworthy that almost half of all research options (47%) were inclined to consider the potential risks of nanotechnologies to health and the environment, a pending issue worldwide because, as we will see below, there is no legislation treating nanomaterials as new substances to regulate their control; most nanotechnology products are not labeled with the nano components they contain; there is no liability on the part of producers for adverse effects and there are no measures to control and monitor imports. Workers in companies that manufacture nanotechnology do not have information about it, due to ignorance and confidentiality clauses. Even less is known about the effects of nanoparticles on ecosystems. The general lack of

knowledge on the subject of risks is reinforced by the postgraduate courses themselves in nanotechnology, whose programs tend to highlight the benevolent aspects of nanotechnologies, avoiding potential risks (Chemsec. org, s/f; ChemTrust, 2013).

Based on information obtained from the questionnaire, it is noted that only 36% of the replies indicated that there were projects in their institutions concerning the potential toxicity of nanoparticles existed. Considering that these are researchers in areas of health/medicine this percentage is not flattering.

Given that most respondents consider it important to have a national register of research and development, and of companies producing with nanotechnologies, an inventory can be considered; monitoring of production and marketing and developing regulatory measures are some of the pending issues in Mexico that could be supported by important nanotechnology research sectors, particularly in the heatlh area.

NANOTECHNOLOGY REGULATION IN MEXICO: RESEARCHERS' PERSPECTIVE

The regulation of materials is a recent policy in the history of the development of the chemical industry. Although the first measures in this area have been carried out in the United States and Europe since the 1970s, it was not until 2006 with the so-called European Union's regulation *Registration, Evaluation, Authorisation,* and *Restriction of Chemical Substances* (REACH) made significant progress. This regulation addresses the potential impacts of chemicals on human health and the environment and can be considered one of the most advanced worldwide.

Once in force, REACH faced a challenge: the status of nanomaterials. Since the eighties, it is known that several known materials manifest novel physicochemical properties when they are in nanometric size, for example, materials that are not reactive at a macroscale become reactive in nano size, while others that at the nanoscale are conductors or semiconductors are known to be insulators in a larger size. This is typical of the prevalence of quantum forces due to the greater relative external surface concerning its mass when the matter is in nanometric size. This functionality is the reason for the boom in the financing industry and the race to apply nanotechnologies in economic sectors since 2000 and globally (RS&RAE, 2004).

The challenge of REACH is that, as nanoscale matter manifests physicochemical properties different from macroscale, it is possible that it also develops different toxicology, a consideration that was pointed out by some scientists since the nineties. In the early 2000s, several environmental NGOs denounced the entry into the market of nanotechnology products without

adequate toxicological analysis (ETC group, 2002); Already in 2004, a meeting of experts from the European Commission's Community Health and Consumer Protection warned about the risks and impossibility of transferring matter's properties on a macro scale to the micro scale (Community Health and Consumer Protection, 2004)[1]

In general, the properties of nanoscale materials are used in the industry quickly and extensively, but risks to health and the environment are not assumed with equal speed. In addition, the industry insists that macro examinations are sufficient and replicable for the nano size of matter (*e.g.* Foladori & Invernizzi, 2021). It is until the end of the first decade of the 21st century that the European Union begins to introduce some regulatory criteria for certain nanotechnology products (Figure 2).

Table 1
Overview of the main nanotechnology regulations in the European Union

Date	Subject	Title	Main regulations
2008, dic.	Food additives	On food additives	Pre-assessment, labeling
2009, Dec.	Cosmetics	On cosmetic products	Labeling
2011, Nov.	Food Products	On the provision of food information to consumers	Labeling
2011, Jan.	Food contact plastics	On plastic materials and articles	Previous evaluation Labelling
2012, June	Biocides	On the market and use of biocidal products.	Labeling and specific information
2015, Dec.	Food Products	On Novel Foods	Specific information
2017, Apr.	Medicine	On medical devices	Special requirement for authorization
2018, Dec.	REACH review	Nanoforms or nanosubstances	• Definition of nanoform and nanoform group • Requirement for new technical analysis • Report any nanoform • Incorporate downstream users into the report

Source: Foladori (2021)

Other countries such as China, Iran, Taiwan, Thailand, and the United States also have some regulations for nanotechnologies. This is not the case in Latin America, which chooses to leave voluntary standards on the characteristics and potential risks of nanomaterials in the hands of private or semi-private organizations (Anzaldo Montoya, 2022; Anzaldo Montoya & Foladori, 2022).

1 "Panel experts were unanimous in their view that the adverse effects of nanoparticles cannot be predicted (or derived) from the known toxicity of bulk material (Community Health and Consumer Protection, 2004, p. 11)."

In academia and research, there are controversies over the need to regulate nanomaterials; while in the industrial field, the position is one of systematic opposition because regulation is perceived as a commercial difficulty and an obstacle to economic benefit. So much so that regulatory policy advances very slowly while the market entry of nanotechnology products is increasing and practically without barriers (DTU Environment *et al*., n.d.; The European Consumer Organisation, 2013; Woodrow Wilson Center, 2017).

In Mexico, there is no register of companies that use nanocomponents to produce, nor of imports; nor are there any glimpses of any kind of regulation. On the contrary, the Ministry of Economy has joined the guidelines of the United States that are among the laxest (Foladori & Záyago Lau, 2014).

Given the uncertainty of the potentially toxic effects of nanoparticles and the rapid increase in commercialized products that incorporate them and circulate in international markets, a module on the subject was included in the questionnaire. The results of the four questions of the questionnaire relating to regulatory and governance issues of nanotechnologies are summarized below.

ON THE REGISTRATION OF COMPANIES WORKING WITH OR PLACING NANOMATERIALS ON THE MARKET

Some countries, such as France and Belgium, have established as a mandatory measure a register for companies (public and private) and research laboratories that buy, sell or handle nanomaterials either in their pure state, in combinations or incorporated into other products, and that involves the minimum amount of 100 grams per year of a substance that is considered to be in the nanoparticulate state (ChemSafetyPro, 2016)[2]. In this regard, the researchers were asked about the consideration of implementing a similar registry in Mexico.

More than 90% considered an equivalent registration important or necessary in Mexico; and only 4% of respondents answered that it was not necessary to establish a register where information on research, sale, and handling of nanomaterials is consolidated, similar to that of countries such as France, Denmark, Norway, Belgium, Sweden.

2 Refers to a "substance intentionally produced at the nanometer scale containing unbound particles, in aggregate or agglomerate form, of which 50% are quem particles in the size distribution of the number, have one or more external dimensions between 1 nm and 100 nm. (This minimum proportion may be reduced in individual cases where it is justified on grounds relating to environmental protection, public health, safety, or competitiveness. However, fullerenes, graphene flakes and single-walled carbon nanotubes with one or more of their external dimensions less than 1 nm should be considered as substances in the nanoparticulate state)" (European Commission, 2011).

ON THE LABELING OF NANOTECHNOLOGY PRODUCTS ENTERING THE MARKET

Some countries began labeling nanotechnology products at the end of the first decade of the century. In December 2008, the European Union imposed labeling of food additives, in 2009 of cosmetics, in 2011 of food and plastics in contact with them, and 2012 of biocidal products (European Commission, several years). Other countries such as Taiwan, Iran, Thailand (Karim & Munir, 2014), and New Zealand (EPA, 2012) are also labeling certain products with nanotechnologies. These measures have forced countries that export to these regions to label their products, including Mexico, where some companies are already doing so (*e.g.* Nivea in certain cosmetics).

It is argued that labeling allows transparency to the consumer, however, labeling does not imply that there is a prohibition on marketing. However, some products that were early labeled are on track to be banned because they contain several chemicals considered toxic (Bergeson, *et al*., 2022). There is widespread discussion about the usefulness of regulations such as labeling.

Following the international discussion, the questionnaire asks researchers if they consider it appropriate to establish a regulation on the labeling of nano components in marketed products. The majority (87%) are in favor of labeling.

One comment that stands out is about the difficulty of labeling products with nano-sized elements when the potential toxicity is debatable. Other comments concern whether the consumer has the conditions to evaluate what nanomaterials are. Place, for example, a label that explains that sunscreens contain "TiO2 Nano" or "ZnO Nano", it does not mean that the consumer knows the codes of the chemical elements, and although the indication appears in extensive (for example nano titanium dioxide), there is no guarantee that he knows the degree of health risk of this chemical element. This type of uncertainty happens with all the labeled elements, but it is still a controversial aspect.

In that sense, other comments indicate that, if the term "nano" is repeated on labels about the health risk, a negative association could be created around these products and even generate a rejection of scientific information. Some of the researchers surveyed are concerned about the possibility of hindering research and development in general, because of complaints arising from consumer appreciation of particular products. As can be seen, labeling is a highly debatable issue that requires an official position, something that will be accentuated by the foreseeable expansion of this type of requirement in the markets of affluent countries.

THE PRECAUTIONARY PRINCIPLE IN MEXICO

The Precautionary Principle was widespread internationally after its adoption at the 1992 Earth Summit in Rio de Janeiro (UN-GA, 1992). The principle states that when there are indications of the potential risk to health or the environment, precautionary measures must be taken, even when there is no conclusive scientific evidence. Several countries have recognized the principle, being party, for example, to the European Union's chemicals regulation (REACH) (European Union Legislation, 2000) and the strategy for nanotechnologies (European Commission, 2004).

The precautionary principle is based on the fact that toxicity analyses carried out in laboratories are never conclusive, due to several reasons, such as the fact that the analysis time is reduced since there are elements that are bioaccumulative and the manifestation of toxicity only occurs years or decades later; or the reduced number of variables that can be used, which contrasts with the thousands or millions that potentially intervene in a living being, or the impossibility of analyzing the impact on an ecosystem, etc. The European Union Environment Agency compiled two volumes with examples of chemical elements that were only regulated decades after their toxicity was reported and for not applying the precaution (EEA, 2002, 2013).

In the case of nanomaterials, the precautionary principle is particularly critical. Due to their large surface area in relation to their volume, nanomaterials are more reactive and this suggests that the impact on living organisms and ecosystems has unknown effects and eventually risk.

Mexico has signed several international conventions that accept the precautionary principle, such as the Convention on Biological Diversity and the Cartagena Protocol on Biosafety. It also participates in tribunals such as the Inter-American Court of Human Rights, where the principle is included (DOF - Official Journal of the Federation, 2020), as well as in the International Tribunal for the Law of the Sea where a specialized judge has been appointed. At the domestic legislative level, this principle is part of the Law on Biosafety of Genetically Modified Organisms and the decree on glyphosate (DOF - Official Journal of the Federation, 2020); and has been used by the Supreme Court of Justice (Precautionary Measure -Transgenic Maize-, 2021).

Based on the above considerations regarding the precautionary principle, the researchers were asked about the relevance of the precautionary principle being used in Mexico to regulate nanoparticulate materials. In this regard, 85% of the responses were in favor of incorporating the principle into the regulation of nanotechnologies in Mexico. 6% were strongly opposed; some were on the basis that the precautionary principle is not based on scientific evidence, that is, laboratory analysis; or that it could hinder research and development.

Among those who supported the incorporation of the precautionary principle, there were some conditioning comments. It was noted, for example, that there is uncertainty about the definition of nanoparticle, which may lead to the application of the principle to materials that do not exhibit properties different from macroscale materials. Caution was also expressed about bureaucratization, which regulatory measures may entail.

NANOTECHNOLOGIES: VOLUNTARY STANDARDS AND/OR OFFICIAL REGULATION

Industrial standards arise from the need for the raw material to conform to criteria of quality and homogeneity for its trade. This need increased with globalization during the eighties and nineties, when industrial standards grew, such as those of the International Organization for Standardization (ISO), pushed by large corporations to promote free trade and reduce trade barriers. As they grow, they overshadow any kind of mandatory state regulation (OECD & ISO, 2017).

In 2005, ISO created the Technical Committee 229 (ISO TC-229) dedicated to nanotechnologies, to date it has published about 100 norms or standards on the subject. For their part, the National Committees reproduce those standards with minimal adjustments. In Latin America, several countries (Brazil, Mexico, Colombia, Peru, Argentina, Costa Rica, and Chile) have a national committee and have begun to reproduce nanotechnology standards (Anzaldo Montoya & Foladori, 2022).

Mexico adopted the standards on nanotechnologies in 2013, when the Ministry of Economy absorbed the standardization work that had been developed by the Mexican Institute of Standardization and Certification, A.C. and instead created the Technical Committee for National Standardization in Nanotechnologies (CTNNN) under the coordination of the National Metrology Center (CENAM). It should be noted that the latter is not a regulatory body, so the standards issued are voluntary. To date, Mexico has published 19 such standards on nanotechnology (ISO NMX-R) (Anzaldo Montoya, 2022).

One of the questions asked in the regulation section was about the consideration of whether complying with NMX standards for nanotechnology made regulation of nanotechnologies unnecessary in Mexico. The results show a lack of knowledge and understanding on the subject: 23% of respondents said they did not know about standardization and regulation, while 40% do not recognize the difference between a voluntary standard and a mandatory regulation. This suggests that a policy to inform nanotechnology researchers about some legal aspects may be important. As for the answers of "yes" and "no" in a forceful way, they reflect a percentage of 30% for each option.

The regulation of nanotechnologies is under discussion worldwide; an example of this is the updating of the regulations on chemical materials in the European Union and China, which seeks to include special chapters on nanotechnologies differentiating them from their same elements in macro size (Foladori, 2021). Nanomaterials can develop particular toxicities, both for humans and the environment, so specific regulation is considered necessary and, therefore, it is important to have the opinion of researchers and experts in the field.

PRODUCTION AND ENTERPRISES: RESEARCH AND DEVELOPMENT

The section on production and enterprises, research, and development include questions concerning the relationship of researchers with companies. In the same way, information was requested on raw materials and their origin, as well as on the technical equipment used.

40% of the researchers surveyed do not register collaboration with companies or other research centers. If we add to this percentage 26% who admit not knowing if there are such agreements in their center/project, it is assumed the need for public policies that induce institutional linkage and interdisciplinary work that allows research to be approached from different perspectives, including networks that provide external financing to the state and international agreements. The above concern is reinforced if we consider that 53% of research is directed to basic science, an area that does not generate greater interest on private companies due to the uncertain or long-term outcome. The tendency of private companies and corporations is not to direct basic research, but to follow up on the new start-ups that are successful to associate or buy or control them through conditional credits, thus avoiding investing in research stages of high financial risk (Tsarouva, 2022).

This is of great relevance considering that the latest UNESCO report, with data from 2018, indicates that Latin America invests about 0.6% of its GDP in Research and Development, a percentage lower than the previous measurement of 2015. Mexico's investment is even lower: by 2018 Mexico only reached 0.3% of GDP, even though the goal was to reach 1%. In contrast, developed countries invest around 2% of their GDP and some up to 3%. (Unesco, 2021). According to the same report, 78% of research and development expenditures in Mexico come from public sources, while private financing does not reach 18%. This differs from other countries in the region, such as Brazil, where the private contribution was 48% (Unesco, 2021).

The survey highlights in a previous section that almost 66% said that their research had funding, being that 52% of it was with public resources (mostly from Conacyt), following the pattern of Mexico where the National Expenditure on Science, Technology, and Innovation (GNCTI) is composed

mainly of public resources. In 2019 it was 89% (Conacyt, 2019). Only 10% said their research was supported by the private sector, just as only 13% of respondents said they were researching in collaboration with a private company. This is practically the norm in Latin America, where private companies do not invest in Research and Development.

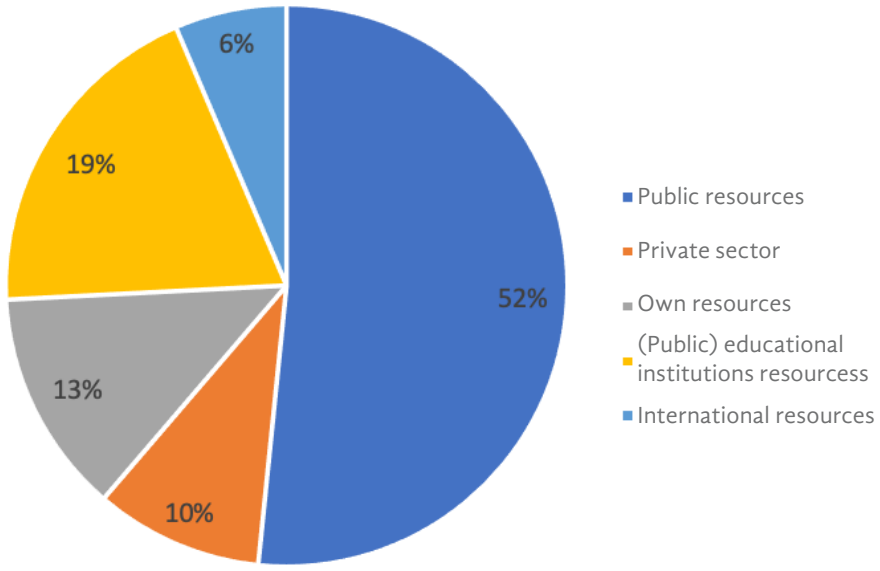


Figure 2. Sources of funding for nanomedicine research. Source: Own development based on the questionnaire on nanotechnology research in Mexico

Likewise, 34% of the responses register participation with other centers or companies and are distributed in equal percentages between public research centers and private companies. The public centers and universities with which some type of collaboration is mentioned are three Conacyt centers (CIDETEQ, CIATEJ, and IPICYT) and five universities (UNAM, IPN, UAQ, BUAP, and UASLP). Only one case was reported to be linked with a foreign public research center.

As for investigations with business collaboration, the information is briefer, since some responses indicate that they cannot disclose the names of the companies with which they work because of confidentiality agreements in the contracts. This type of contract can be considered an obstacle for the government to follow up on investigations with business collaboration in the country.

On the other hand, most ongoing research projects require nano-raw material. The production of this type of material for commercial purposes has been concentrated in large chemical corporations worldwide. Particularly in the case of carbon elements (*e.g.* nanotubes, fibers) and oxides (*e.g.* titanium, zinc, aluminum). There is little systematized information globally,

and estimates by output vary significantly between sources. A report by the European Commission in the early 2010s noted the following nano substances with new properties such as those with the highest volume of production worldwide: aluminum oxide, barium titanate, titanium dioxide, zinc oxide, cerium oxide, and carbon nanotubes (European Commission, 2012).

Some of the nano raw material requires sophisticated laboratories to produce them for industrial purposes, hence the trend toward global concentration (Scientific, 2008). The cause of this is the sophistication of the technology used, which for commercial purposes of mass production must result in exactly homogeneous products, for which there are few facilities, but it is also important to consider that the rise of nanotechnologies occurs at the beginning of the first decade of the century, when the degree of concentration of capital worldwide, after the nineties, was much higher than in previous decades and marks the difference with other technologies such as biotechnology of the eighties, in which capital did not have that degree of concentration (Foladori, 2018). Thus, for example, around 2010, estimates of the production of carbon nanotubes for commercialization worldwide recorded 66% concentrated in four chemical corporations (Patel, 2011).

Basic research may require a small amount of raw material and not necessarily with the same standards of homogeneity as that intended for industrial marketing. But, the fact that it is the starting point for research and a highly demanded resource at the international level, must be considered in terms of long-term science and technology policy. Since Mexico is the world's leading producer of silver, a diagnosis of nanotechnologies carried out by CIMAV in 2008 suggested that Mexico could become an international producer of nanosilver, a substance highly demanded in research and industrial production with nanotechnologies (CIMAV, 2008)[3].

The answers to the questionnaire place metals and alloys as the most used nano raw materials (30%), followed by polymers and dendrimers with 22% and ceramic materials with 14%: with these three types of materials 66% of the total is reached. It is necessary to remember that the question refers only to the type of raw material and not to the quantity, so it may happen that another type, with fewer users, registers higher annual volumes.

In the same way, information was obtained on the origin of the main raw material. 16% claim to have their own manufacture, but some of them combine it with purchases in the national and international markets. When

3 For the interested reader, the Conacyt project registered companies that use nanotechnology by economic sector, and its headquarters are georeferenced (Arteaga Figueroa, 2022). In some cases, it is possible to assume the main nanomaterial they use based on the product they launch on the market.

it comes to a single origin, 32% indicate international purchases and 13% consumption in the domestic market. Likely, these differences are closely associated with the type of raw material, even so, the dependence on the foreign market is significant. This situation occurs in most countries given the degree of concentration of nano raw material production as indicated above. This is exemplified by the responses included by the selling company, 75% of which correspond to purchases from Sigma-Aldrich.

Regarding the equipment used, most of the most valuable equipment is foreign-made, which is a reflection of financial and technical dependence. In this last aspect, 65% of the responses indicate that the equipment requires qualified foreign personnel for its maintenance. It is worth noting that, in other non-core countries, such as Iran, the government invested not only in research and development of nanotechnologies but also in sophisticated equipment, and today they have an important international market to which they sell.

FINAL CONSIDERATIONS

The introduction of new technologies is a daily occurrence worldwide, given the degree of historical accumulation of knowledge and technological development. This puts countries in the dilemma of how to assume global trends that are practically impossible to avoid together with the orientation towards national interests and safeguarding potential harmful effects on both human health and the environment.

The case of nanotechnologies in health/medicine, which was the subject of this work, exemplifies the previous dilemma. On the one hand, there is already a wide range of benefits that nanotechnologies can offer when applied in the sector. On the other hand, the potential risks of these and other applications are only superficially reduced. The pressure of the market to transform techno-scientific innovations into economic benefits makes it difficult for any public policy to seriously consider this type of dilemma. Countries that have fewer resources and experience to evaluate the products that enter the world market each year and that have novel chemical elements have the possibility of replicating what the most advanced countries do in terms of materials regulation. This has been the case of nanotechnologies in Mexico and Latin America, but not in terms of regulation but of voluntary codes as is of interest to large global corporations.

It is worth noting that most of the responses to the questionnaire are in favor of considering the risks of nanotechnologies and regulations in this regard, although at first glance the research projects do not include any type of requirement in terms of literature review regarding potential risks of the products with which they work. It would be worthwhile for universities and

laboratories to update the safety practices of their researchers against the manipulation of nanomaterials and derivatives.

THANKS

This research is part of the National Council of Science and Technology (Conacyt) Ciencia de Frontera Project 2019, n.° 304320 "A critical review of nanotechnologies development in Mexico according to national socio-economic priorities", funded by Conacyt and executed by the Academic Unit in Development Studies (UAED), Universidad Autónoma de Zacatecas (UAZ), Mexico.

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Reception: **November 22, 2020** | Acceptance: **June 10, 2022** | Publication: **October 1, 2022**

Inaction policy at Universidad Veracruzana: Impact on professors

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César Augusto Borromeo García
cesar.bogc@gmail.com

Jorge Alejandro Fernández Pérez
jafp58@prodigy.net.mx

Benemérita Universidad Autónoma de Puebla,
Puebla de Zaragoza, México

To quote this article:
Borromeo García, C. A., & Fernández Pérez, J. A. (2022). Políticas de inacción en la Universidad Veracruzana: Impacto en los docentes. *Espacio I+D, Innovación más Desarrollo, 11*(31). https://doi.org/10.31644/IMASD.31.2022.a05

— Abstract—

Inaction policy is a decision made by authorities when they know there is a problem but decide not to act on it, whatever the reason may be. Professors of the Languages School at Universidad Veracruzana and high-hierarchy authorities were interviewed via a qualitative study, we could notice that these policies are commonplace, and even promoted by authorities. The focus of the original research was limited to digital technology for language teaching, but results showed that inaction policies extend far beyond the equipment and technological infrastructure.

Keywords:

Public policy, Education policy, ICT, teachers .

Decisions, which are made by the authorities and are planned to be applied to a group in society, are usually planned. On many occasions, there is a work to understand the problem to be treated, the effects of implementing this decision, and an analysis of needs. In other cases, decisions are made without planning or consideration by the people these decisions will impact. At other times, the decision is not made. In other words, even when there is a problem, it is consciously decided not to decide what to do, not to act. Broadly speaking, this is called an inaction policy. It is rare to see these policies being implemented because there is no official document from the authority stating that the policy is one of inaction. However, when you analyze carefully and listen to those impacted by the policies and policymakers, you can begin to understand the extent of the problem.

This research work is derived from the doctoral thesis *Technology in language teaching: the case of the Languages School of the Universidad Veracruzana*, where the institution's educational policies role and its impact on professor's work at the School of Languages of the Universidad Veracruzana (FIUV) are analyzed, particularly on the use of Information and Communication Technologies (ICT). Unexpectedly, the results of the research were directed toward other topics, not only in the area of ICT. This article presents the preliminary results found thanks to the intervention carried out. It should be noted that the focus of the research does not consider the breadth of education policies as a whole, only those of ICT. But it is interesting that the search for understanding of the original research problem, which was to know the role of ICT-related educational policies in the FIUV, will lead to showing that the problem is not only found in ICT-related policies but in the institution's lack of action on various issues.

Given this, it is worth mentioning that the research questions from which the present article emerges are how do professors perceive the incorporation of ICT in their discipline and context? How are the technological policies of the Universidad Veracruzana impacting the language teaching subject at the Langugages School? And what are the needs of teachers concerning ICT?

Hence, the following objectives have been drafted: Analyze how the Languages School of the Universidad Veracruzana has incorporated ICT, as well as the opinion and specific needs that teachers have around ICT and analyze the perception of professors with ICT, which are implemented in their discipline and context to, in turn, explain how the ICT policies of the Universidad Veracruzana have impacted their inclusion in the language teaching subject and identify the professors' technological needs at the Languages School of the UV.

It is important to emphasize that the descriptive results reflect a portion of these questions and objectives. However, it is vitally important to know the questions and objectives of the main research to understand how the vision presented in this document was reached.

Regarding the above, this article focuses on the analysis of the second specific objective. This is related to the impact of the ICT policies of the Universidad Veracruzana, however, after the intervention, it was possible to see that the policies go beyond only the technological. That is, it is a common practice that policies impact other areas.

CONTEXT

The Universidad Veracruzana (UV) is an institution with tuition of 87,388 students, 6,235 academics, presence in 27 municipalities of the State of Veracruz through 5 campuses and 315 formal study programs (UV, 2020). The School of Languages has an enrolment of 2,753 students, including undergraduate, postgraduate, continuing education, and language courses open to the public, and a teaching staff of 206 professors. It is located in Xalapa, Veracruz, in the Humanities Unit, a space shared by the Spanish Literature, Anthropology, History, Sociology, and Philosophy Schools. There is a building called the Self-Access Center of the Languages School (CAAFI), which works for the language and external community. The rest of the spaces are shared, as are the library, computer and documentation centers, auditoriums, and some halls.

CONCEPTUAL FRAMEWORK

To understand thie work donw, it is essential to address some key terms. This section describes three concepts: public policy, education policy, and inaction policy.

Public policy is defined as all actions by an authority, governmental, school, or institutional, that try to impact the public. They are carried out through actions, inactions, planning, and results that will satisfy or affect the members of a group or society (Aguilar, 2010; Ejea, 2011). Public policies are focused on people's lives, the town, and the term itself suggests that it should be so. However, there are instances where policies are dictated from a very high hierarchical level, and the lowest levels are solely the object of policy-making. In other words, there is little consultation on the needs of the people they will impact. Thus, the adjective 'public' does not mean that they are chosen by those impacted, but that they are focused on the public, on the people, and society.

Education policies are a type of public policy whose primary focus is the field of education. Alternatively, they are called educational policies. In this article, the term educational policy(ies) will be used to refer to them. It is also important to mention that the focus of this work is higher education, so this work will discuss such educational policies. This type of policy is not far from its definition of public policies, since it is derived from them. Thus, they can be considered as decisions made by actors related to education to determine educational services' interactions with society (Kent, 2009a: 26).

These policies are dependent on public policies for two reasons: First, education serves society, mainly in the case of higher education. Decisions made within this level become important for social life because they are seen as an institution at the service of social problems (Kent, 2002). That is why the inclusion of society in decision-making is vital. Second, the head of the institution, usually the dean, is also seen as a politician (an influence in government). Although the dean is not elected through massive democratic methods, as is done with a popular representative, but by various processes of the internal government of universities, there are many deans with a political weight of importance (Kent, 2009b; Kent & Acosta, 2009). This happens because the institution has a very large enrollment, has very high funding, which is why the dean may have a history of public positions, or even the institution (or the dean) could have a relation with political figures, etc. Whatever the case, a significant number of heads of institutions, mainly public higher education institutions, are considered political figures, and for this reason, their performance must be per this vision.

After analyzing this, we can consider that educational policies are decisions that have the characteristic of being dictated from and within an educational institution. However, due to the importance of the institution or the figures representing it, they have influence even on the outside, in society. It should be added that despite the above, educational policies are not only dictated within the HEIs. Sometimes, these decisions come from governments and national and international organizations, they are adapted to each institution and context but they are 'pushed' by external entities.

Regarding policies of inaction or omission, we must emphasize that this term does not exist as such. It is a concept that is generated through a process of inaction, from which its name comes (Knoepfel, Larrue, Varone & Hinojosa, 2007). Bachrach and Baratz (1963) call it "nondecision-making" (p. 632) to all those decisions of inaction (deciding not to decide, or simply not deciding). This happens because they want to protect some personal, economic, political, or institutional interest. It can occur even when there is an awareness that there is a problem that must be addressed, but which, for one reason or another, is deliberately ignored. From here on out, the term

"policies of inaction" is used, which will try to combine the fact that it is a conscious decision; plus, the fact that it is a voluntary inaction.

These policies are implemented through the same authorities mentioned above. But contrary to the policies that act, these are the exact opposite: inaction. They are created when a given problem exists; when that problem is known among those affected, and when its existence is known at the highest hierarchical levels, but it is decided to do nothing. Knoepfel *et al*, call it a "passive attitude" or "non-action" (p. 12) on the part of policy-making actors.

METHODOLOGY

The data collection process was carried out following a qualitative model. It is a cross-sectional study, which allows us to know the current state of the problem, and to be able to compare it in the future if so desired. Data analysis was carried out following a hermeneutic-interpretative method. The data collection instrument was a semi-structured interview guide. Each of these elements is detailed below.

Research Approach

Because of the desire to understand the problem thoroughly and in-depth to reach an explanatory level, the decision was made to select a qualitative research approach. Denzin and Lincoln (2011) point out that following this approach allows a deep understanding of the problem being investigated, in addition to applying it to various fields and disciplines.

The selected model was the hermeneutic-interpretative model. This is due to the objectives set for this research, which focus on understanding the needs and opinions of the subjects involved. Originally this model was used for text interpretation (Grondin, 2008: 16-17). But the contributions of Schleiermacher, Dilthey, and later Heidegger, allow us to consolidate hermeneutics as a general understanding of life instead of one related exclusively to texts. In this model, hermeneutics uses linguistic and historical dimensions for the understanding of human phenomena (Grondin, 2008: 20). This research work takes place in the logic of contemporary hermeneutics.

Information collection instrument

The data collection technique to be used will be the survey. In an interview, the researcher has key informants (Taylor & Bogdan, 1994: 103), who are the witnesses to what it is trying to observe, which is impossible without the vision of these informants. The instrument used to conduct the interviews was an interview guide. This is the most important tool for developing the

interview and obtaining the objective and the specifics. The guide allowed the interview to flow according to a more or less defined plan, as mentioned by Keats (1992).

The guide's construction was a process based on the research documentary stage and included the development of categories, indicators, and questions. The themes that were observed as essential during the literature review were: Educational Policies, Teacher Training, Information and Communication Technologies, and Distance Learning. These topics were part of the research state. Likewise, the principles of the hermeneutic-interpretative method were considered to create the instrument according to the characteristics of the population and the research problem. This ensured that the choice of topics and interview questions had a theoretical, documentary, and methodological basis.

Key informants (participants)

The population of FIUV is 206 academics, a plant that is distributed in its programs as follows (Languages School UV, 2019): B.A. in English Language: 75 academics; B.A. in French Language: 33 academics; B.A. in English Teaching (virtual modality): 14 academics; M.A. in French Didactics: seven academics; M.A. in Teaching English as a Foreign Language: nine academics; Ph.D. in Language Studies and Applied Linguistics: ten academics; Department of Foreign Languages (DELEX): 58 academics.

The eligibility criteria for the interviewees were refined, which were: Be a professor, technician, administrative, or manager with any type of uninterrupted contract at the UV in the field of language teaching and with an assignment (current or past) at FIUV. It was intended that several professors, who are retired or in positions outside the school, but within the institution, would have the opportunity to participate in the research. It was important to consider this because there were major professors in the history of the school, whose personal history in it was of special interest, or because the time they were in it is important to understand it, but for one reason or another, they are no longer there. This also made it possible to gather information from all types of teachers: temporary, depending on subject, tenured, full-time, and all ages and seniorities.

The selection of key informants was done through a three-phase process. The first was through an intervention in the same group of teachers (Borromeo, 2016, 2017). From here emerged some names that are part of the teaching staff or decision-makers in the school and the institution. The second phase was a series of informal talks with professors and people related to the school. Here several different actors considered paramount to the development and integration of ICT in FIUV were identified. The third

phase of the selection was when some of the key informants during the interviews suggested names of people who played an important role. This is considered advisable by several authors (Izcara & Andrade, 2003; Taylor & Bogdan, 1994) since the interviews are qualitative, and this type of change is allowed if it is for the benefit and improvement of the information.

3. ANALYSIS OF MECHANICAL ELEMENTS

The determination of the resistant nominal moments was made for the reinforcement with steel, per the provisions of the NTC Mexico City (2017) for concrete beams reinforced with steel rods in its section (5.1.3) relating to Flexural strength, as well as section (5.3.3) corresponding to Shear strength resistance. While for those relating to GFRP reinforced beams, what is stated in ACI 440 1R (2015), chapter 7.2 on Flexural strength and chapter 8 on Shear stregnth were used. The doubts of interpretation on the design with GFRP were resolved supported by the work of Wainshtok Rivas, Hernández Caneiro, and Díaz Pérez (2015).

Instrument construction

Each category resulted from the construction of the state of the art and, from there, different indicators emerged. To move from topics to indicators, Cisterna's model (2005: 66) was followed, which suggests a method for creating categories and indicators that go hand in hand with questions and objectives. Following this suggestion, the indicators for each category were reached. Below is the result of the categorization, a brief description, and the indicators that emanated from it (see Table 1):

Table 1
Topics, descriptions, and indicators

Category and description	Indicators
Education policy Decisions made by the Universidad Veracruzana in the academic area. Focus on teaching and ICT. It also includes how teachers assimilate them, if they impact their teaching and professional development within the institution. It also considers faculty participation in political discussions, and the means and ways of participation. Finally, it considers the openness of the institution to receive complaints and suggestions regarding policies.	• Importance of Institutional Decisions (1.1 PE-IMPOR) • Internal discussions on needs (1.2 PE-DISCU) • Professor's participation in decisions (level, frequency, means, and opportunities) (1.3 PE-PARTIC) • Higher authorities' attention to requests (1.4 PE-ATN-AUT)
Teacher training These are the elements that shape the professors' development inside or outside the institution, and the impact it has had on the teachers' professional and personal lives. Likewise, the recent training that the teachers have undergone is also considered. Teacher training located in the institution, the relevance, frequency, quality, and opportunities offered to carry it out are considered. The economic and temporary investment turns out to be a topic of interest, as well as the modality, quality, and relevance of the training taken.	• Importance of teacher training (2.1 FD-IMPOR) • Recent training (2.2 FD-RECIENTE) • Source of training (staff/institution/collaborative) and economic investment (2.3 FD-FUENTE) • Impact of training (professional and personal life) (2.4 FD-IMPAC) • Relevance, quality, frequency, and opportunities of institutional training (2.5 FD-CARAC_FD_INSTIT) • Training modality (face-to-face, virtual, other) (2.6FD-MODE)
ICT in higher education These are the Information and Communication Technologies that have been integrated into higher education, mainly in language teaching. The minimum knowledge necessary for students, graduates, and themselves regarding ICT was considered, from the teacher's point of view.	• Essential ICT in HEI (3.1 TIC-ESSENTIAL) • Basic knowledge (of students, teachers, and graduates) (3.2 TIC_CONOC_BASIC) • ICT training opportunities (3.3. TIC-OPORT_FORM) • Quality impact (3.4 TIC-IMPAC_QUAL) • Impact on interest (students) (3.5 ICT-IMPAC_ALUM) • Impact on teaching and administrative processes (3.6 TIC-IMPAC_DOC_ADMON) • Availability of ICT (in market, institution, discipline, and faculty) (3.7 TIC-DISPON)
Distance learning It deals with non-traditional education, that is, blended (mixed) and total virtuality. The necessary elements for these educational modalities (ICT) that allow this, the availability of these modalities in the institution, the quality, and the future it may have been addressed are talked about.	• Current discipline in the DL (4.1 EaD-ACTUAL) • ICT needed and available (4.2 EaD-TIC_NEC_DISP) • Modalities used (in FIUV) (4.3 EaD-MODE) • Quality vs in person (4.4. EaD-QUAL) • Future of DL in the discipline (4.5 EaD-FUTURO)

Source: Own elaboration

For the creation of questions, the suggestions of various authors were followed (Denzin & Lincoln, 2011; Flick, 2004; Keats, 1992; Rojas, 2011; Taylor & Bogdan, 1994) for this stage. The questions that were generated covered an indicator and, at the same time, allowed the obtaining of information from one or more research objectives. The format of the final guide had two types of questions: main and supportive. It was decided to have one main question and put some support. The main one would guide the conversation and the

supporting question would be asked only when a particular topic that was of interest had not been touched by the main question.

Piloting and Interviews

A pilot stage of the interview guide was conducted to carry out modifications not foreseen in the creation phase. There were seven interviews with a population similar to the target. Through each of these interviews, the instrument was modified and details about the interview process were fine-tuned. From this phase emerged the final version of the guide used with key informants. The next step would be formal interviews with key informants.

There were twelve interviews in total, eight professors, two professors who are also decision-makers in the FIUV, and two decision-makers of a high hierarchical level (Rectory). Access to the Directorate of the Languages School of the Universidad Veracruzana was required to be able to enter to invite the professors. In the case of high-ranking decision-makers, we went to their offices in person and with a formal request for an interview. At each interview, informed consent was provided, which had to be signed to accept participation. The interview period was between November 6, 2019, and January 16, 2020. The average duration of each was one hour and 30 minutes.

Data analysis

Go Transcript's online software was used for the transcription of the interviews. This is a very easy-to-use online tool. Despite being online, the work of audio and text is completely local. This was important considering that the identity and data of the participants were protected by Informed Consent. A process of *full verbatim*, word-for-word, or full transcription was used, including interjections, errors, corrections, pauses, annotations or comments, and other nonverbal elements that were captured during the interview. Approximately every hour of interview meant between eight and ten hours of fully manual transcription. This process took approximately 150 hours of work.

For data analysis, *Atlas.Ti 6* was selected as it is easy to use in Windows 10. It was required to divide each case (interviewee) separately and thus have a separate count of how many codes were obtained and also to be able to see which categories and indicators were observed in each case. Administratively it was easier to work like this. Finally, *Atlas.Ti* provided the opportunity to work with cases, categories, indicators, and codes more independently. It should be noted that the role of this software was basically for two purposes: 1) coding and 2) efficient organization of information. No use of advanced software tools was carried out.

The objective was to achieve identification of the main themes. The ones most mentioned by the teachers and those that were, therefore, the most significant for them. It ended with the topics ordered from highest mentions to lowest mentions as well (see Table 2):

Table 2
Mention count of each indicator

Indicator	Count	Indicator	Count	Indicator	Count
1.1 PE-IMPOR	713	3.5 TIC-IMPAC_ALUM	212	4.5 EaD-FUTURO	67
1.4 PE-ATN-AUT	600	1.2 PE-DISCU	211	3.3 TIC-OPORT_FORM	66
1.3 PE-PARTIC	440	3.1 TIC-ESENCIAL	207	4.4 EaD-QUAL	65
3.7 TIC_DISPON	317	2.4 FD-IMPAC	191	2.2 FD-RECIENTE	63
3.6 TIC-IMPAC_DOC_ ADMON	259	2.1 FD-IMPOR	168	4.1 EaD-ACTUAL	61
2.3 FD-FUENTE	243	3.4 TIC-IMPAC_QUAL	123	4.2 EaD-TIC_NEC_ DISP	51
2.5 FD-CARAC_FD_ INSTIT	234	4.3 EaD-MODE	116	2.6 FD-MODE	49
3.2 TIC-CONOC_BASIC	225				

Source: Own elaboration

These results had yet to be interpreted. Therefore, a new stage of categorization (recategorization) was used. On this occasion, it would be based on interpretation, on understanding what is considered the main comments. It would no longer be organized by aprioristic categories, now it would be considering experience, documentary research, and, in what was verbalized by teachers, to understand them in context.

The second result was that 62 emerging categories were obtained, which are considered topics of importance, and which were described in full in the thesis work. In this work, however, we focus on some of them. The results section then discusses these topics.

RESULTS

Once the analysis was concluded, we reached a series of interesting result, which can be observed more clearly when considering an crucial axis: the policy of inaction. This was something that permeated without exception, all the topics explored, and that was mentioned equally by all professors, professors/decision makers, and which was confirmed (although not orally, but through the interpretation of circumstances and answers) by high hierarchy decision makers of the Universidad Veracruzana. In this way, when a policy of inaction or omission is considered a guideline of the institu-

tional authorities, it is possible to understand how the lack of action or, as we call it, the inaction policy impacts teachers. Through a mention of the different examples where there is inaction or an omission, we wish to present that one of the institutional policies is precisely that of not addressing known problems. Three areas in which they were found are mentioned: technological infrastructure, physical infrastructure, and personnel recruitment. These topics were ordered according to the importance given to them by the teachers, either expressly through direct questions or an interpretation.

Technology infrastructure

The most mentioned topic that impacts teachers is technological infrastructure. This includes the equipment available to them at FIUV, as well as existing or missing means and/or resources to use the equipment within the faculty or institution.

First, the teachers mentioned that the equipment is necessary. That their job teaching English is not very fruitful if audiovisual media are not implemented. This means that they require using audio and video as almost daily tools. While some can cope with the use of these tools by implementing their equipment (computer, projector, and speakers, mainly), some others cannot and must not use the limited equipment found in FIUV. This, of course, being limited and having so many teachers who require it, is usually complicated, even more so in the busiest hours of the day.

In response to this, a problem that has plagued teachers for many years (decades, according to some professors), FIUV carried out a purchase of projectors, which were installed in 2019. With this, the School Management seeks to facilitate access to the most complicated and expensive equipment for professors to obtain. Many of them already have computers, and speakers turn out to be more easily accessible peripherals, as well as being inexpensive. For this reason, it was sought that the investment, which exceeded 200,000 Mexican pesos, was authentically significant for its activities. Although the teachers appreciate the School Management's action, they point out that many did not agree with the plan, did not initially support it, and considered that other needs could be covered with that investment.

The previous initiative was part of an action plan of the School Management for its administration, which at the same time was part of the Dean's plan, which, likewise, is per the General Development Plan 2030 of the Universidad Veracruzana (UV, 2018). That is, this action of installing equipment is a decision already made for teachers. Aguilar (2010) and Ejea (2011) suggest that this is normal, that is, decisions are made from higher hierarchical levels. For this reason, it is considered that the institution

follows the established paradigm that does not directly consider those impacted by the policies that will be imposed on them.

This is key because the professors expressed that the technological infrastructure, including the lack of computers and particularly the Internet network that constantly fails, needs to be improved. It is a topic that had already been mentioned six years ago (Borromeo, 2016) but for some reason has not been addressed. This means a lot for professors, but even if they have their equipment, it is difficult for them to use it without Internet since they require online tools, such as translators, dictionaries, audiovisuals, or databases. This is even more problematic when considering the teachers who are part of the FIUV virtual program for their needs.

When asked if connectivity is good, teachers mentioned that it is limited and of low quality. This was very pointed out by those who are part of the virtual program since their activities require a stable and fast connection. On some occasions, because they do not have access to the institution's networks, for whatever reason, they have had to resort to using mobile data from their mobile phones to connect to the platform, make video calls for advice, and even for degree exams. And this, although it is not a problem that occurs daily, it is an economic, administrative, and procedural burden for those involved. As an example of the above, a testimony of a worker who was interviewed is presented:

> For example, we all have cell phones. And the vast majority of us have our cell phones services with Telcel. If there's no Internet in Humanities, I can... I could use my data with Telcel. With three, or four clicks on my phone. You ask a professor about that, and they don't know [...] when they talk [...] that infrastructure is lacking because everyone wants a PC when everyone brings a laptop. Yes. Or brings a cell phone. (Mayte, personal communication)

With this response, it's deduced that there is a lack of equipment and that the infrastructure is deficient. But authorities predict that teachers will solve this problem, even if it means an economic burden that should be borne by the institution itself. In addition, there is a lack of interest from General Management for Information Technologies (DGTI), which did not respond to the request for information on the institution's role in this matter. The lack of response to the request through formal means (one of them never answered and the other outside legal time) only shows that, in fact, the institution, in this case through the DGTI, has a policy of inaction regarding the issues that are known to exist.

Physical infrastructure

Before proceeding to the next two sections, it is necessary to emphasize that the Dean's office is aware of these needs. Through the Dean's Plan (UV, 2018), it was required that all the institution's study programs be accredited or re-accredited by an independent entity. By the end of 2018, the institution had covered almost 100% of the accredited programs. This means that the problems of the various programs and faculties in its five regions are known, as accreditations are usually presented with suggestions and comments from those in charge of the work. FIUV provided accreditations from the Council for the Accreditation of Educational Programs in the Humanities (COAPEHUM) for its three bachelor's degrees, which include a series of recommendations. Thus, we can consider the Dean's office of UV aware of the needs of the FIUV. Now, we discuss the issues that have been ignored.

Through interviews, it became clear that teachers require physical space. There is a lack of sufficient space for all FIUV member teachers to work, either independently, in groups with colleagues, or with students. The FIUV, being located in a shared unit with various faculties and being the largest in the number of professors and enrollment, requires a large number of spaces. Its growth has been steady, both in its undergraduate and graduate programs, continuing education, and language courses to the public. This adds to the need for spaces in the FIUV.

Due to this growth, teachers lack cubicles, some must share them with up to four teachers, while others do not have one assigned. Nor are there sufficient spaces for these teachers to work with colleagues or students. There are some cubicles available for teachers to schedule, but their use is limited to counseling that students require from teachers at the Language Self-Access Center. That is, not all teachers can use it and they cannot use it if they are not accompanied by the student requesting the advice.

This lack of space has an impact on teaching and administrative work. The FIUV must borrow spaces for some courses from the other faculties of the Humanities Unit. Sometimes it's easy to get, sometimes it's complicated. In any case, it becomes a debt of favors that the other schools use when they require a similar favor. And although there is no hostility, the reality is that when the available spaces are distributed (as when Pedagogy was moved from headquarters), an old grudge about the Self-Access Language Center being built in common spaces, comes to light, and the spaces that are distributed are generally distributed in equal numbers among the faculties, not based on their tuition. This means that the new spaces, either because they are left by another faculty or because they are created from scratch, are distributed equally, and remain insufficient.

The institution is aware of the lack of space since these needs are indicated in the accreditations recommendations of the study programs. However, for more than a decade, the construction of new spaces is practically non-existent, they have been limited to modifications and/or renovations of spaces. It can be believed that economic problems have a role in this problem, however, UV invested about 30 million pesos in the construction of a building for the faculties of Mathematics and Artificial Intelligence, although the project has been stopped for years, which means that this money was not invested to make it work actively. Instead, it became two unfinished works: a metal structure for a building, and a building in an incomplete condition that cannot be used. The plan is to continue this project, but with works that have lasted six years, this investment is beginning to be seen more as an expense, being that there are spaces where its impact would have been immediate, as in the Humanities Unit.

Finally, it should be noted that the authorities criticize that the investment of resources is stopped, and they claim that the investment must continue so that it does not become an expense and mention it this way in the interview granted for this article:

> "When you plan, you must know that if you invest a peso, you gain it back. Because in Mexico we are used to making proposals and not thinking about the money that is being thrown away, again and again. And a clear example is CONACYT. We have scholarships, and out of nowhere they say, "No, there's no more money for scholarships." "Sorry, wait. You've been investing in so many students and such." Now more or less, they react and suddenly they take it back, just because, sorry... even if you paid a year, you paid a year. And you're paying a year in England or France. How many million pesos are those? "Well, do you know what? You tighten your belt and finish the investment. Because that's what's going to ensure you the product. And that's when you plan." (Mayte, personal communication)

Although this authority is not directly responsible for the administration of movable property, real estate, or financial resources, it does have a great weight in the decisions that these actors make in this regard. When we add that the Dean's Office is aware of this lack of spaces in the Humanities Unit, since they requested accreditations where they indicate these needs, we can observe a policy of inaction, in this case, on physical infrastructure. This affects teachers and students and is believed to not only reach the Humanities Unit but may be a widespread problem in the institution.

Recruitment

Finally, there is the issue of staff recruitment. In this area, it is also important to remember the COAPEHUM accreditations that were made to the FIUV bachelor's degree programs. They also mention that the faculty requires more full-time teachers to be recruited. These accreditations have been carried out since 2010, so the problem has an omission of at least ten years, but it can be even older.

Between 2010 (previous accreditation of the bachelor's degrees in English and French) and 2017 (most recent re-accreditation), the number of full-time professors at the FIUV remains the same: 23, being 39% of the total teaching staff in 2010 and 28% in 2017 (a decreasing trend). But subject teachers, substitutes, and those assigned to other academic programs increased from 35 (61%) to 59 (72%) between the same periods. We understand that the number of full-time professors did not decrease in absolute numbers, but did in relative numbers.

This means that in regards to teacher improvement, the UV's plan from 2010 to the present has been to improve this aspect. But the reality is different. The policy of UV has been to talk about what would be ideal (increasing the academic base), but the most convenient or the least damaging to institutional finances is what gets done (hiring more temporary professors). Hiring a Full-Time Teacher means providing statutory employment benefits, as well as translating into a long-term commitment. In addition to this, we must mention that not all the hours of a full-time teacher are charged to teach, but all the essential tasks that UV considers must be covered: teaching, tutoring, linking, and dissemination. When hiring teachers temporarily and doing so through semi-annual competitions, it is ensured that there is no long-term contractual relationship and that the hours that are paid are directed exclusively to teaching, being that extra-work activities (tutoring, bonding, and dissemination) are not paid, but are the ones that count the most at the time of competing for base hours or a full-time position.

This results in an omission. UV knows it requires more full-time teachers. But it is less problematic (in economic matters for the institution) to hire temporary teachers and to pay them only for teaching hours, adjusting the competition scores to obtain base hours and full-time places so that extra-work activities have a great weight. Teachers will obtain these bases only when they carry out these tasks on their initiative. Instead of following the recommendations that would return to their stronger educational programs, they go on the side of saving economic funds. The School of Languages has made continuous requests for the assignment of full-time positions to the institution's Academic Secretariat, as indicated in the Transparency document 242/2020 of the Mkatsiná UV system. However, for nine years

they have not received positive responses, beyond reassigning vacancies by retirement. The Academic Secretariat, being responsible for this issue, has carried out a policy of inaction. The reasons for the refusal were given by FIUV itself, which were related to the institution's lack of financial resources. At the time of writing this article, and with the pure intention of following up on the problem (since it is not the focus of the main research that gives rise to this article), no response has been obtained from the Academic Secretariat regarding how many full-time positions have been created from 2010 to date (folio 241/2020 of the Mkatsiná UV system). However, hiring more temporary professors and facilitating their entry, while making it difficult to allocate time bases and full-time places, can be considered to run counter to the school's needs. In addition, no progress is being made to comply with the teacher improvement plans established in the 2030 General Development Plan.

CONCLUSIONS

Regarding what was found in this research, in at least three areas the Universidad Veracruzana has carried out a series of policies of inaction or omission, so it is worth asking: In what other aspects and topics is a policy of inaction or omission adopted? What actions and activities have been affected by the institution's refusal to act as a result of the problems that, as we have shown, are known? How many issues, moreover, are not known because there are no mechanisms for the care of teachers if an institutional hierarchical path is not followed? All these questions are genuinely intriguing and are the result of research. For this reason, and because of their scope, it is not possible to attempt to answer them correctly. We can make an evidence-based assumption and venture to think that the problems are many.

Finally, it is necessary to mention that the fact of omitting decisions jeopardizes the operation of the institution itself in terms of quality. It becomes undeniable that leaving out key issues raises doubts among those involved regarding their chances of achieving better interaction and attention in the institution. This is the same for students, teachers, administrators, and society at large. In this way, the fact that the institution has inaction policies becomes a systemic and social problem, in which the confidence that is held is being lost and, therefore, the quality is decreasing. This accumulation of incidents is only increasing, which produces more deep-rooted and difficult problems to deal with in the future, a problem that is reflected in countless public institutions in the country.

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Reception: **May 19, 2021** | Acceptance: **June 17, 2022** | Publication: **October 1, 2022**

Application of open-source software as a tool for systems development in maintenance management of computer equipment

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Samantha Elizabeth Trujillo Morocho[1]
elizabeth.trujillo.ec@gmail.com

Ángel Polivio Huilca Loyola[1]
gelohl@hotmail.com

Richar Neboth Muyulema Erazo[2]
rimuer1@gmail.com

1 Instituto Tecnológico Superior San Gabriel, Riobamba, Ecuador

2 Escuela Superior Politécnica de Chimborazo, Riobamba, Ecuador

To quote this article:

Trujillo Morocho, S. E., Huilca Loyola, Ángel P., & Muyulema Erazo, R. N. (2022). Aplicación del software libre como herramienta para el desarrollo de sistemas en gestión de mantenimiento de equipos informáticos. *Espacio I+D, Innovación más Desarrollo, 11*(31). https://doi.org/10.31644/IMASD.31.2022.a06

— Abstract—

In recent years, computer equipment in higher education institutions has become a key element for access to information and services; both hardware and software are essential elements to maintain the information, becoming an asset to companies and institutions. The present investigation has allowed the development of an inventory and maintenance record system for the computing equipment for the Instituto Tecnológico Superior "San Gabriel" of the city of Riobamba-Ecuador. It was developed through waterfall methodology, Java programming language, and the MYSQL database manager. With the system's implementation, 90% of the maintenance plans were fulfilled in the department of information and communication technologies (ICT) of this institution.

Keywords:

Computer system; Maintenance; Open-Source Software.

Maintenance is an activity that every company or institution must carry out according to established standards and techniques (Cantos, 2018) to avoid risking the loss of information and devices, and to preserve the equipment throughout its useful life, with the greatest possible availability at the lowest cost, hand in hand with the manufacturer's recommendations.

Suárez *et al.* (2015) and Andreu (2018), agree that Java programming language is widely used in the business environment, highlighting the programming of desktop and web apps. These apps have been extended for their easy deployment to customers, their not excessively complicated development, easy update, and their security, both for the customer and the server.

For Boasberg *et al.* (2019), the measures and actions that are taken to keep computer equipment in proper operation, without memory dump occurring or emitting error messages frequently, can be one of the two types of maintenance such as preventive and corrective, when the first thing that must be determined is the time of use and technological delay thereof.

The purpose of the Instituto Tecnológico San Gabriel is to provide quality services to the student sector of the province of Chimborazo. According to reports of the Department of Information and Communication Technologies, the institution has a network of computer equipment consisting of 40 computers, both desktop and personal; in addition, they have five printers distributed throughout the institution. The problematic situation that is identified by direct observation is that the institution has a maintenance plan, but this is not fulfilled with total normality due to different factors.

Based on the above, the present work aims to develop a system for the inventory and maintenance record of the computer equipment of the Instituto Tecnológico Superior "San Gabriel" in the city of Riobamba, Ecuador.

MATERIALS AND METHODS

Study Area Location

The present research was developed in the department of information and communication technologies (ICT) of the Instituto Tecnológico Superior "San Gabriel" located in the Veloz parish, canton Riobamba, province of Chimborazo, Ecuador.

Methodology

For the development of this system, two proposals were handled within the research part, a preliminary proposal in which the process of information collection was carried out, the research process begins at theoretical, scientific,

and legal levels, where we reviewed sources from Scielo, Scopus, Latindex, where authors such as Ferro (2020) or Rosenfeld & Irazábal (2016) stand out, which specify, from a judicial point of view, the protection of hardware. Through this synthesis, the understanding of the study variables (maintenance plan, registration of computer equipment) was achieved, and that is the basis that determines the extent to address the problem. On the other hand, the use of the Unified Process methodology proposed by García & Vásquez (2019), in which the focus of the research begins in the Conception phase, where the current management and the obtaining of basic operational information are evaluated, to then begin with the Elaboration of the maintenance system, its Construction, and Transition. According to Zumba (2018), these processes allow the development of systems quickly. In our programming we used the Java language, the MySQL database manager, and Netbeans as an integrated development environment (IDE); it should be noted that all the applications used are open-source software.

Interviews were applied to six users who are in charge of computer equipment within the institution to determine how often they encounter a malfunction in terms of hardware and software. We also registered on the files whenever a user called to request help for an inconvenience in the equipment.

RESULTS AND DISCUSSION

For the development of the IT equipment maintenance management system, the stages of Beginning, Development, Construction, and Transition were planned.

Beginning Stage

At this stage, the scoping study of the project was carried out where the costs, profitability, and feasibility were evaluated. The feasibility study resulted in a general description and list of use cases such as registration of computer equipment assets, managers, users, assignment of assets to users, equipment to be maintained, user who carries it out, and reports.

Development Stage

This stage allowed us to generate a specific solution based on the use cases of the previous stage, resulting in a detailed design plan such as interfaces, and buttons like Save, close, return, search, and print.

The messages that will appear in Windows window format were defined according to the following:

- When the user has entered the wrong username and password.
- When no information has been entered in the forms.
- By entering the data and sending it to save.
- When a new confirmation username and password are created, it is not the same as the main one.
- When a new person in charge is created and the ID card is incorrect.

Based on the above, the interfaces for the Windows environment were made because it is a desktop application, so it was designed based on menus, labels, text boxes, buttons, and radio buttons, among others, thus forming a friendly interface. The work environments were Netbeans as an integrated development environment (IDE), Java as a programming language, and MySql as a database manager. Based on the above, the main screen would be established according to Figure 1.

Figure 1. Computer Equipment Maintenance Management System Main Window. Source: Own elaboration

Construction Stage

At this stage, several iterative loops were created in which use cases are merged sequentially based on project risk factors. For example, this approach allows for older versions of the system that satisfy the primary use case. Changes to requirements are not merged until the beginning of the next iteration.

Transition Stage

At this stage, the first version of the system was developed and culminated with a system in production. Later the final product was sent to the users of the ICT department (see table 1), once the system was implemented the first test was acceptance, which allowed determining if the software complies with what was defined in the beginning stage, the requirements, once they passed this phase we proceeded to other validation tests.

Below are the results of the maintenance software validation through surveys applied to the personnel of the ICT area. The evaluation was applied through the software technical evaluation guide (Largo, 2005), in table 2, you can see the criteria on which it is based.

Table 1
Teachers and faculty board

Institution	Teachers	Faculty Board	Total
Instituto Tecnológico "San Gabriel"	5	1	6

Source: Own elaboration

Table 2
Software Evaluation

Scope	Description
Utility	Aspects related to what it fulfills
Interface	Aspects related to the interface
Technical aspects	Aspects related to functionality

Source: Adaptation (Medina-chicaiza, 2018)

The evaluation proposed for this system allows us to know its usefulness, whether or not it meets the established requirements, if the interface is intuitive, if it contains a high degree of usability, and if it facilitates the transmission of information and interaction to its maximum. The technical field is the systems' characteristic that allows verifying that the system is light, with low hardware requirements, easy to install, and scalable. Below is the score range handled by the user acceptance test managed by software quality metrics according to López *et al.* (2016), in which 18 questions covering the adaptation of table 2 are handled; the evaluation criteria in this metric range from 1 to 3 respectively.

Table 3 shows the evaluations obtained from all the staff of the ICT area of the institution, teachers, and managers, to draw up work guidelines and improve them in terms of preventive and corrective maintenance of computer equipment.

Table 3
Evaluation of ICT personnel

	Rating from 1 to 3 (3=Excellent, 2=good, 1=bad)						
QUESTION	**1**	**2**	**3**	**4**	**5**	**6**	**Average**
1. Are there terms mixed in different languages?	3	3	3	3	3	2	2.83
2. Is the vocabulary used simple?	3	2	3	3	3	3	2.83
3. Is there enough time to perform keyboard inputs?	3	2	3	3	2	3	2.67
4. Is there any assistance for users who are using the system for the first time?	3	3	3	3	2	3	2.83
3. Is the system easy to operate for someone who was not trained in its operation?	3	2	3	3	3	2	2.67
6. Can the interface and its content be understood?	3	2	3	3	3	3	2.83
7. Is it easy to identify an object or an action?	3	3	2	3	3	2	2.67
8. Is it easy to understand the outcome of an action?	1	3	2	3	3	3	2.50
9. Is the interface designed to facilitate the efficient performance of tasks in the best possible way?	3	2	1	3	2	3	2.33
10. Are the messages presented by the system appropriate?	3	2	3	1	3	3	2.50
11. Does the system act in the prevention of errors?	2	3	3	3	2	3	2.67
12. Does the system report on the errors presented?	3	2	3	2	3	3	2.67
13. Are descriptive messages and texts used?	3	2	2	3	3	3	2.67
14. Does it allow convenient navigation within the product and easy exit from it?	2	3	3	3	2	3	2.67
15. Is the user allowed to customize the interface?	3	2	3	1	2	2	2.17
16. Is visual information provided of where the user is, what are they doing, and what can they do next?	2	2	2	3	2	2	2.17
17. Are there keyboard shortcuts?	3	3	3	3	3	3	3.00
18. Is the user presented only with the information they need?	2	3	3	3	3	3	2.83
Total Average							2.64

Source: Adaptation by Medina-chicaiza (2018) and López *et al*. (2016)

According to the scores obtained from the evaluation measured through the parameters, usefulness, interface, and technique, a good evaluation was obtained with an acceptable quality, which makes it easy to install, access, and manage for any user in the ICT area.

CONCLUSIONS

The evaluation technique applied to the development of systems in maintenance management of computer equipment allows us to appreciate the quality and acceptability of the system developed through open-source software.

All kinds of management and administration software are considered as a software quality model in our country, in addition to that, it allows to obtain an evaluation score for academic accreditation purposes by educational entities, therefore, the software developed for maintenance control fits within the established considerations, allowing the processes to improve continuously with its implementation.

The Unified Process methodology with its four stages, iterative, structured, and adaptable, allowed the development of the system according to the needs of the institution. The result is an incremental and modular system for possible future changes.

By developing the system through open-source software, it is possible to know that Java as a programming language is an optimal platform for the development of desktop applications, and with excellent work in its graphical interfaces, the functions and source code that were used are simple so that other users can understand and modify it, in case it is necessary for subsequent maintenance of code or modular increments.

This research contributes to the importance of adaptable methodologies use for developing software with specific organizational requirements, the use of open-source software as a development language for management systems that serve as a quantitative evaluative means in educational accreditations in our country, the implementation of software metrics adapted by current authors and, finally, in being a tool for registration of computer equipment and maintenance plans in the ICT department of our Institution.

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Reception: **April 4, 2022** | Acceptance: **August 20, 2021** | Publication: **October 1, 2022**

Tourist satisfaction with Temazcal in Cozumel

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Jesús López Ruiz

Romano Gino Segrado Pavón

María de Jesús Moo Canul
romanogino@hotmail.com

Universidad de Quintana Roo, Cozumel Quintana Roo, México

To quote this article:

López Ruiz, J., Segrado Pavón, R. G., & Moo Canul, M. de J. (2022). Satisfacción del turista de Temazcal en Cozumel. *Espacio I+D, Innovación más Desarrollo, 11*(31). https://doi.org/10.31644/IMASD.31.2022.a07

— Abstract—

In Mexico, the temazcal bath is part of the historical uses and customs and is also promoted as a tourist activity for relaxation and stress release. The general objective of this study was to reveal the most important factors associated with tourist satisfaction after the temazcal experience on the island of Cozumel. The quantitative method was applied, with a survey of temazcal users on the Cozumel island, in 2018. As a result, the contrasting combination allows for achieving a high level of satisfaction. Marketers will be able to design and promote appropriate strategies for this market segment.

Keywords:

Temazcal; tourism; experience; satisfaction; Cozumel.

Satisfaction is relevant for people because regardless of the positive or negative character it implies, it influences present and future decisions. In the tourism sector, positive experiences contribute in multiple ways to the people's satisfaction and psychological well-being; they are a competitive advantage for destinations, make visitors repete their stay, they help recommendation to spread word of mouth, get a greater probability of loyalty, and commercial benefits.

Measuring the level of satisfaction is crucial for the success of the tourism service (Chen *et al.*, 2011; Meng *et al.*, 2008), especially in a context of competition between destinations, since it allows prioritizing strategies and actions linked to the strengths and weaknesses detected, to contribute to the established objectives. For Alegre and Cladera (2009), it is an important factor for the consolidation of long-term commercial relationships because it contributes to the attractive image of the destination and reduces investment in tourism promotion.

In the case of SPA tourism [Latin acronym *Salus Per Aqua* (Frost, 2004)], it is considered a new trend in the international market (Mak *et al.*, 2009), with wide popularity and accelerated growth worldwide (Kamata and Misui, 2015; Okech, 2014) due to demographic changes, longer life expectancy, and social aging. In this sense, evaluating satisfaction allows us to quickly improve products and services, to achieve more memorable and pleasant experiences.

Within this market segment, in Mexico, the temazcal (literal translation "house of sweat") is a form of traditional spa that combines the steam bath with aromatic herbs and aboriginal rituals, integrating contrasting stimuli for the five senses in a healthy and pleasant environment. According to Cohen and Bodeker (2008), the main attraction is the experience of relaxation, stress release, or general well-being.

This tourist activity has been incorporated in various destinations, among which is Cozumel, as part of the transition towards the provision of services that involve experiences (Gilmore and Pine II, 1998) to complement the main tourist products and services. However, despite the breadth of research regarding the satisfaction associated with the spa as a tourist activity (Anaya-Aguilar *et al.*, 2021; Meng *et al.*, 2008; Perić *et al.*, 2018), for the temazcal, there are few studies linked to the satisfaction of tourists.

The service of a temazcal is more than simply the steam bath since it involves providing a rewarding and joyful experience, as well as a bodily utility, which can be difficult to achieve due to the multiple factors involved, cultural aspects, language, experiences, expectations, beliefs, among other aspects related to tourists, where the level of satisfaction is an indicator of effective and efficient service, as well as a reference to retain or attract new users, while dissatisfaction may cause lack of loyalty, the breakdown of the

business relationship or the absence of recommendation for the service or the company. Thus, market segmentation through the most important characteristics for a specific segment can be part of a strategy refined for marketing, and meet tourists' needs comprehensively and with a higher level of quality.

Determining the main attributes that contribute to tourists' satisfaction also helps to have the elements to provide a differentiating image and consolidate a brand value that can be associated with the destination (Tung and Ritchie, 2011). It will also facilitate the products and services' design for the market segment and will allow influencing the return decision (Huh *et al*., 2006). Therefore, the research question was: What are the most important factors associated with tourists' satisfaction after the temazcal experience on the Cozumel Island?

The study's overall objective was to reveal the most important factors associated with tourists' satisfaction after the temazcal experience on the Cozumel Island. Therefore, this study contributes to the academic literature on tourism with the analysis of tourism satisfaction in an innovative segment, in addition to the fact that for business decision-making, it provides information for the design of marketing strategies to create the conditions that facilitate a high level of satisfaction and increase visits.

TOURIST SATISFACTION

There is no unanimous definition of the concept of "satisfaction" (González *et al*., 2007) due to the subjectivity that is inherent in the phenomenon, but there is consensus that it is a personal evaluation or interpretation, partial or total, usually loaded with a positive or negative meaning, related to an emotional or external stimulus, linked to a product, service or experience, which can be combined with personal history, social and cultural tourists.

Multiple theoretical models have been proposed to explain tourists' satisfaction (Bigné and Andreu, 2004; Kozak *et al*., 2005; Maunier and Camelis, 2013), although the application of the cognitive-affective model is the most widespread in the scientific literature (Weaver y Lawton, 2011; Žabkar *et al*., 2010). It explains the phenomenon based on the product or service efficiency or performance and the combination with affective (emotional) psychological factors that imply a judgment or evaluation whose result is positive (conformity or satisfaction) or negative (dissatisfaction or dissatisfaction) after the experience.

To summarize, this model suggests that satisfaction arises as a combination between tourists' knowledge and emotions at the end of an experience related to the product or service linked (Kotler and Keller, 2012). Thus, factors such as price, colors, time, distance, and quantity, are

combined with feelings, memories, impressions, and details, for a multidimensional approach, in which the result must be considered, but also each of the parts and all as a whole. The context described recognizes the paradox of a high level of satisfaction that arises from poor performance or poor service, or vice versa, attributable to the eminently psychological and personal character of the associated judgment.

In the case of the spa, according to Campón-Cerro *et al.* (2020) and Lee *et al.* (2012), tourist experiences linked to water correlate positively with satisfaction, by stimulating feelings of well-being, relaxation, and rejoicing. For Alén (2018), satisfaction is a partial or indirect predictor of behavioral intent, while dissatisfaction is a motivator to switch products or services and negative word-of-mouth comments (Su and Hsu, 2013). On the other hand, positive satisfaction influences the intention to visit again (Han *et al.*, 2017), favorable comments, and the perception of value in the experience (Abubakar and Mavondo, 2014; Maunier and Camelis, 2013).

The subjective, individual character and the environment of the experience make measuring satisfaction a process without a single scale, due to the combination of attributes such as cleanliness, kindness, trust, communication, activities, and amenities, which are common in spa and temazcal centers, although proposals for systematic measurement have been made in this regard (Lagrosen and Lagrosen, 2016; Lo *et al.*, 2015; Silvestri *et al.*, 2017) according to the bibliographic review carried out.

Several studies have been published that analyzed the factors that attract tourists to spas. An example is the research of Rodrigues *et al.* (2020), who performed a content analysis, using specialized software, of 1254 comments from tourists on web pages. As a result, the key attributes for satisfaction are the steam room, the staff, the room, the location, and the pool.

Another example is the research of Han *et al.* (2018) which analyzed the relationship between loyalty, experiences, and satisfaction in the spas of Thailand, through a survey of 558 visitors. A factor analysis was performed with factors such as price, variety of services, treatments, therapists, and collaborators, facilities. As a result, it was established that product characteristics, experiences, and satisfaction are related and contribute to satisfaction and loyalty to the destination.

A study in Greece (Weaver and Lawton, 2011) evaluated 207 clients from ten spa centers related to the variables that influence satisfaction. The survey was designed based on the SERVQUAL model, and a factor analysis was applied whose result establishes that attention, service confidence, food, comfort, honesty, and empathy are the aspects that allow to better predict the level of satisfaction. Similarly, the study of Tsai *et al.* (2012) revealed that service, trust, and especially the environment, influence satisfaction and recommendation.

Consequently, to understand tourists' satisfaction of their temazcal experience, we designed a questionnaire, adapted the items and their wording so that they could reflect the products and services' characteristics associated with the destination of Cozumel.

METHOD

The case study was applied in two temazcales of Cozumel, whose main economic activity is tourism, which in 2019 showed an economic spillover of $ 1,272,200,000 US dollars (SEDETUR, 2020). The tourist infrastructure of this destination includes an international airport, three international cruise terminals, a maritime terminal for vehicle ferries, and another maritime passenger terminal. There are 66 registered lodging establishments, with an offer of 4 701 rooms available (SEDETUR, 2020). There are also more than 800 companies providing tourist services, 423 craft shops, and 225 food and beverage service establishments [restaurants, cafes, ice-cream shops, nightclubs, discos, and bars, among others] (INEGI, 2017). In the health tourism sector, there are five temazcales (field observation, 2019) that provide services.

The research was descriptive, with quantitative data collection between 2017 and 2018. The technique used consisted of a survey through the application of an anonymous and confidential questionnaire, written in English, to determine the level of satisfaction, in a negative or positive sense, of the aspects involved in the temazcal bath. To avoid bias, the information collection was carried out following a standardized procedure, so that each person surveyed responded on equal terms.

A pilot test was carried out with 31 questionnaires, which allowed some minor corrections in some sentences. As a second pilot test, the questionnaire was applied in other temazcales of Cozumel, so slight modifications were made to generalize it and adjust it to the environment and facilities of the temazcales. The questions were carefully designed and drafted to avoid discomfort or incur any lack of morality, considering intercultural issues.

The questionnaire was designed to be applied quickly and at the end of the service to avoid delaying appointments and rejections since tourists usually have established schedules for different activities during their stay on the island. In all cases, verbal consent was always requested from the participants and the reason for the questionnaire was explained.

The exploratory factor analysis (EFA) orthogonal of maximum variance was applied to the results, which is frequently used for the development and validation of tests, especially in social areas (Méndez Martínez and Rondón Sepúlveda, 2012) due to its versatility in the exploration of the set of latent variables or common factors that explain the answers to the items of a test

(Lloret-Segura *et al.*, 2014). The KMO validation test was 0.731, and Barlett's sphericity was 0.0000, confirming the validity of the EFA.

The Cronbach's Alpha test was performed, which is a measure of validation of answers related to the same topic (Domínguez-Lara and Merino-Soto, 2015), whose result was 0.726 with ten items of Likert scale, so the set of items is considered valid. Data were analyzed with the statistical processing software JASP 0.14.

RESULTS

Of the 164 tourists who answered the questionnaire in 2018, 63% were women. The age range was between 18 and 66 years of age, predominating young people from 27 to 34 years, with 66 people (40%). 78% came from the United States, while the remaining 23% came from various countries, including Mexico and Canada. 50% have visited Cozumel on two or more occasions. Regarding the previous experience in the temazcal, 37 tourists (22%) indicated that they had previously participated in at least one session, although only 5% were in Cozumel.

Participants were asked about their motivations for participating in the temazcal session, and 71 people (43%) indicated that it was for "Detoxification and purification", 26 people (16%) chose the "Try something different" option, 12 (7%) people marked "Holistic spiritual activities" and 11 people (7%) pointed to "Release tension and relaxation." The rest of the responses were widely diverse. Participants were also asked about meeting expectations, and 99 tourists (60%) responded that "Exceeded my expectations", while 16 people (10%) indicated that they were "Met widely", while 45 people (27%) chose the option "Met expectations."

Regarding the tangible elements of the experience, 80 people (50%) answered that "the guide" is the prominent factor, while 35 responses (21%) pointed to "heat and steam" and 34 responses (21%) indicated "the environment." On the other hand, among the intangible elements the option "Aromas (copal and herbal tea)" received 47 responses (29%), while "Feeling of rebirth or rejuvenation" obtained 45 (27%). The "Feeling of relaxation" had 26 responses (16%), and "Exercises and dynamics during the session", had 21 responses (13%).

The explanation that the collaborators gave to the tourists before each of the experiences of the temazcal, was the aspect with the highest rating among the criteria to measure satisfaction descriptively, since it obtained a sum of 4.95/5, while the in-person service resulted in 4.93/5. Third, the guide, with 4.92/5 (see Table 1).

Table 1
Descriptive results of the temazcal satisfaction questionnaire

Item	Valid	Mean	Desv. Standard
Environment	164	4.762	0.441
Facilities	164	4.494	0.622
Guide	164	4.921	0.399
Explanation	164	4.951	0.364
Internet service	164	4.817	0.860
In-person service	164	4.939	0.652
Duration of experience	164	4.659	0.488
Fruits and beverages	164	4.860	0.365
Man of Fire	164	4.695	0.668
Road	164	4.671	0.792

To evaluate the underlying structure of the descriptive analysis, the EFA statistical test was applied, and tangible aspects (time, food, facilities, environment) were the most important factor (17%). As a second factor were complementary activities like the road and man of fire (14%). The third factor was the spirit of service, with personalized and online assistance (13%). The fourth factor was confidence, represented by the temazcal guide (12%). Together the four factors accounted for 56% of the total variance explained.

Table 2
AFE of the temazcal satisfaction questionnaire

Items	Factor 1	Factor 2	Factor 3	Factor 4	Uniqueness
Duration of experience	0.712				0.378
Fruits and beverages	0.601				0.634
Facilities	0.552				0.617
Environment	0.520				0.595
Road		0.972			-0.004
Man of Fire		0.486			0.557
In-person service			0.894		0.188
Internet service			0.424		0.754
Guide				0.983	0.003
Explanation					0.714

Note: made with Varimax rotation.

Finally, to know the participants' perceptions about the temazcal bath, the following question was asked: "How would you describe your expe-

rience after your temazcal session?" 37 people (23%) expressed the option "Reconnection with my interior and the environment"; 36 people (22%) marked "Deep Relaxation"; another 29 (18%) chose "Intense purification", while 22 users (13%) indicated "Difficult to describe in words".

DISCUSSION

In 2018, 164 questionnaires were carried out with a rating of 100%, to reveal the most important factors associated with tourists' satisfaction, after their temazcal experience on the Cozumel Island, through an exploratory factor analysis, which determined that some factors are more important from the tourist's perception and contribute more to their general satisfaction.

According to the results, the three main factors that influence satisfaction are tangible attributes (time, food, facilities, environment), complementary attributes (road and man of fire), and the attribute of the spirit of service (in-person and online assistance). As an isolated variable, the facilities received the lowest level of satisfaction, although it did not imply dissatisfaction on the part of tourists. These factors deserve careful monitoring to consolidate the favorable aspects and, in the counterpart, improve those attributes where deficiencies are perceived.

As a tourist attraction linked to well-being or health, temazcal is somewhat difficult to understand by most foreign tourists, since it is unknown to them, there is little information on the Internet about it, and, as in some cases, it has been distorted by turning it into a form of practices and spiritual beliefs to diversify the tourist offer.

As a theoretical contribution, the study expands the very limited literature on temazcal and satisfaction, by providing an explained understanding of the general factors that can be considered important in different aspects of a product or service (Petrick *et al*., 2001), although the results should be interpreted with caution, since the convenience sample does not allow generalizations, but the results are useful and can influence the temazcales for their management strategies and marketing.

Among the practical implications for health tourism managers in temazcal, the results will help increase the satisfaction and loyalty of tourists, as well as increase the level of quality, by prioritizing and applying resources and capacities to improve the most critical factors. At the level of microenterprises, adequate and comfortable facilities, training for communication, commitment to service, and personalized treatment, are very important in the satisfaction level.

As a limitation, when analyzing attributes that are combined in time and space for a group of people, the possibility that there are interactions between the factors must be recognized, a situation that was not evaluated

in this research, since no shared variance was observed. However, in the practice of temazcal, one attribute might influence other attributes, such as, for example, the heat of the sauna and the taste of refreshing drinks. As a strength, it is highlighted that the exhibition was constituted by real tourists who had the complete experience of the temazcal, as well as with the entire team of collaborators who provided the services. Also, unlike other highly structured satisfaction questionnaires, this study emphasized simple and natural language, with descriptions adjusted to the uses and customs of the temazcal bathroom service.

For future research, it is advisable to evaluate the level of satisfaction, considering attributes such as expectations, price, value, service, quality, physical effects, enjoyment, the intention of repeting the experience, and the motivation to choose the activity. The validity of the SPAQUAL questionnaire for the temazcal bath could also be evaluated, with the adaptations corresponding to this activity.

CONCLUSION

This research provides knowledge about tourists' satisfaction of temazcal, and the results allow us to affirm that the activity represents a satisfactory experience for the users, where the most important factors that intervene are linked to the tangible attributes. Companies should use these features to increase quality and maximize satisfaction, support competitiveness, and foster loyalty.

The categories identified as significant for the satisfaction of the temazcal market segment provide information and help the systematic understanding of this experience at the theoretical level, while, at the practical level, management and facilities conditions can be improved, as well as strategies to expand the attractiveness of this activity and direct expectations towards the characteristics of the service.

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Reception: **August 31, 2021** | Acceptance: **August 20, 2022** | Publication: **October 1, 2022**

Osmotic dehydration of *Carica papaya* var. Maradol: Mass transfer and sensory analysis

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Cyntia María D´Aquino de los Santos[1]
cyntia.daquino.san@cobach.edu.mx • ORCID: 0000-0002-2768-5033,

María Celina Luján-Hidalgo[2]
maria.lh@tuxtla.tecnm.mx • ORCID: 0000-0002-5720-9652

Lucia María Cristina Ventura-Canseco[2]
lucia.vc@tuxtla.tecnm.mx • ORCID: 0000-0001-6983-0430

Miguel Abud-Archila[2]
miguel.aa@tuxtla.tecnm.mx • ORCID: 0000-0002-4509-7964

1 Colegio de Bachilleres de Chiapas, Tuxtla Gutiérrez, México

2 Tecnológico Nacional de México/Instituto Tecnológico de Tuxtla Gutiérrez, División de Estudios de Posgrado e Investigación, Tuxtla Gutiérrez, México.

To quote this article:
D Aquino de los Santos, C. M., Luján Hidalgo, M. C., Ventura Canseco, L. M. C., & Abud Archila, M. (2022). Deshidratación osmótica de Carica papaya var. Maradol: Transferencia de masa y análisis sensorial. *Espacio I+D, Innovación más Desarrollo, 11*(31). https://doi.org/10.31644/IMASD.31.2022.a08

Carica papaya var. Maradol is an important fresh produce grown in Chiapas, Mexico. However, their shelf life is very short so they should be processed to increase their useful life. The objective of this work was to evaluate the effect of the temperature and sucrose concentration of the osmotic solution on water loss and solute gain during the osmotic dehydration of papaya slices. For this, slices of 5 mm thick were dehydrated by osmosis in sucrose solutions at 40, 50, 60, and 70°Brix kept at 50, 60, and 70°C for 6 h, keeping a solid: solution ratio of 1:5 (weight: volume). Water loss and solute gain were adjusted with the Azuara equation to obtain the effective diffusivities of water and sucrose. Sensory analysis of the samples was carried out using a nine-point hedonic test. The results were analyzed using a variance analysis, and the means were compared with Tukey's test ($p < 0.05$). The average diffusivities for water varied between 4×10^{-10} and 7.2×10^{-10} $m^2 s^{-1}$; while for sucrose they were 3.62×10^{-10} a 8.4×10^{-10} $m^2 s^{-1}$. Sensory analysis showed that osmotic dehydration significantly influenced the acceptance of papaya. Osmotic dehydration using an osmotic solution at 50°C, at 50°Brix for 6 h allowed obtaining papayas with a water loss of 49%, a sucrose gain of 14%, and a good level of acceptance. These processing conditions increase the shelf life of papaya and could be used for industrial purposes.

Keywords:

Effective diffusivity; water loss; solute gain; hedonic test.

Drying is a preservation method commonly used in the food industry, whose primary objective is to increase its shelf life by evaporation of water. At present, the process of dehydration of fruits and vegetables is carried out mainly using hot air and freeze-drying. Dehydration by freeze-drying largely preserves the quality of food, however, it is a process that is very expensive compared to other dehydration processes. Conversely, drying or dehydration with hot air can cause several important changes in food such as changes in color (enzymatic and non-enzymatic reactions) and taste; as well as changes in its texture and nutritional quality, to mention a few. Hot air drying significantly decreases the acceptance of dehydrated papaya compared to fresh fruit (Abud-Archila *et al*., 2002). These sensory characteristics are important, as they are what define the product's degree of acceptance by the consumer (Radojˇcin *et al*., 2022).

In recent decades, osmotic drying, at atmospheric or vacuum pressures (Saleena *et al*., 2021) is an alternative for the processing of perishable products to preserve, to a large extent, the quality of the final product. This consists of the removal of water by immersion of the food in an osmotic solution, such as a solution with high concentrations of sugar or NaCl. Osmotic dehydration is a complex process where various parameters influence: the type and concentration of the osmotic agent, the temperature and agitation of the osmotic solution, the immersion time, the fruit: osmotic solution ratio, as well as the shape, size, and structure of the tissue (Bashir *et al*., 2020), however, the temperature and concentration of the agent are paramount in the mass transfer.

Osmotic dehydration improves food's shelf life, and the products obtained will present, depending on the conditions of the process, attractive sensory characteristics and "similar" to the original product before processing. During food processing, the color and texture of food have been studied mainly as sensory attributes, with color being one of the most influential in the acceptance of a product, without forgetting the taste. Lopez *et al*. (2021), point out that the osmotic agent plays a very important role in the sensory and physical attributes of the product. In addition, several studies were reported where osmotic drying prevents undesirable color changes, as in the case of bananas and apples (Krokida *et al*., 2000a), papaya (Islam *et al*., 2019), as well as in vegetables, such as potatoes and carrots (Krokida *et al*., 2000b). While osmotic drying prevents color changes, the processing time is also important. In that sense, the grapes' loss of color was minimized when the time of osmotic dehydration was short, as reported by Nsonzi and Ramaswamy (1998). Regarding the degree of global acceptance of a product, Romero-Bello (1995) and Madamba and López (2002) reported that osmotic drying allows obtaining products with a good degree of global acceptance for the case of pineapple and mango, respectively. During osmotic dry-

ing, the mass transfer between the fruit and the osmotic solution can be identified (Saleena *et al*., 2021). Mass transfer (water loss and solid gain) during osmotic dehydration has been modeled by several authors (Azuara *et al*., 1992; Lazarides *et al*., 1997; Waliszewski *et al*., 2002; Islam *et al*., 2019). Models based on diffusion theory (Fick's law), irreversible thermodynamics, multicomponent diffusion, and hydrodynamic flow have been thoroughly discussed by Shi and Le Maguer (2002).

The objective of the present work was to determine the effect of temperature, osmotic solution concentration, and impregnation time on water loss, solid gain, and hedonic test acceptance of papaya slices and to model water loss and solid gain during osmotic dehydration.

MATERIALS AND METHODS

Raw Material

Maradol papaya fruits (*Carica papaya*) of the same size and without any post-harvest treatment were provided by the company AGROMOD SA de CV of the San Juan ranch in Villa de Acala, Chiapas, Mexico. Fruits, with about 90% yellow-orange color, were washed with soap and water and peeled manually. Subsequently, after the removal of the seeds, slices of 25 x 20 mm with 5 mm thickness were obtained. The initial humidity of the fresh papaya was determined in a vacuum oven at 60°C for 48 h or up to constant weight.

Osmotic dehydration

The papaya pieces were immersed in a temperature-controlled sucrose solution maintaining a fruit: osmotic solution (p:v) ratio of 1 to 5. The papaya pieces were kept immersed in the sucrose solution in continuous stirring for 6 h at a controlled temperature with the help of a heating grill with magnetic stirring. Subsequently, sampling was carried out every 30 minutes to monitor the dehydration kinetics. Which, approximately 10 g of papaya were extracted from the system every 30 min and the weight (0.001 g) was determined with the help of an electronic scale. The samples were then washed with distilled water to remove the surface sucrose, and the wastewater was removed with a paper towel. The moisture content of the samples was finally determined in a vacuum oven at 60°C for 48 h or until the weight variation was not greater than 0.001 g.

During osmotic dehydration kinetics, the water loss (WL) of the sample was calculated using equation (1):

$$WL = \frac{P_o X_o - P_t X_t}{X_o} \quad (1)$$

where P_o is the initial papaya's weight; Pt is the papaya's weight (in grams) at time t, X_o is the initial moisture content (g water / g initial, wet base) and, X_t is the papaya's moisture content at time t (g water / g initial, wet base).

Solid gain during osmotic dehydration was also determined with the help of equation (2):

$$SG = \frac{W_o MS_o - W_t MS_t}{X_o} \quad (2)$$

where MS_o is the initial fraction of dry matter (initial g/g) and MS_t is the dry matter fraction at time t (g/g initial).

The effect of the temperature and sugar concentration of the osmotic solution was evaluated using an experimental factorial design with three repetitions. The sugar concentrations studied were 40, 50, 60, and 70°Brix, while the temperature of the solution was 50, 60, and 70°C. A total of 36 treatments were performed.

Mathematical model

The osmotic dehydration kinetics (water loss and solid gain) of papaya was modeled using an empirical model (equation 3) as reported by Azuara *et al.* (1992) and Solgi *et al.* (2021).

$$\frac{WL}{WL_\infty} = \frac{s_1 t}{1 + s_1 t} \quad (3)$$

$$\frac{SG}{SG_\infty} = \frac{s_2 t}{1 + s_2 t}$$

where WL and SG are water loss and solid gain during osmotic dehydration, t is time, WL_∞ and SG_∞ are water loss, and tissue solid gain in equilibrium; and s_1 and s_2 are the empirical parameters of the model (equation 3) to be identified.

These empirical parameters (s_1 and s_2) were identified for each kinetic, using the modified Simplex method (Van Nievuwenhuijzen *et al.*, 2001) through the minimization of the objective function, called error (equation 4):

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} \left(WL_{exp} - WL_{sim}\right)^2}{n}} \quad y \quad \sigma = \sqrt{\frac{\sum_{i=1}^{n} \left(SG_{exp} - SG_{sim}\right)^2}{n}} \quad (4)$$

where n is the number of values, the subscript "exp" correspond to experimental values, and the subscript "sim" corresponds to the values simulated by the model.

Finally, the diffusivity (Di) of water and solids, as a function of empirical parameters(s), was calculated using equation 5 (Waliszewski *et al*., 2002):

$$D_i = -\frac{4L^2}{\pi^2 t}\ln\left\{\frac{\pi^2}{8}\left[1-\frac{s_i t}{1+s_i t}\right]-\frac{1}{9}\left[\frac{\pi^2}{8}\left(1-\frac{s_i t}{1+s_i t}\right)^9\right]\right\} \quad (5)$$

For each kinetic, values s_1 and s_2 were identified, which were used to calculate instantaneous diffusivity (Di) at time t. The effective diffusivities of water and sucrose were eventually expressed as an average of instantaneous results (Azuara *et al*., 1992).

Evaluation of acceptance of osmotically dehydrated papaya by hedonic test

After osmotic drying, all samples were stored in refrigeration (approximately at 5°C). Before the sensory test, the samples were left to balance at room temperature (approximately 30°C) for 2 h. Subsequently, a nine-point structured hedonic test was used to determine the level of acceptance of the samples according to Wichchukit and O'Mahony (2022). Due to the number of samples to be evaluated (12 treatments), the sensory analysis was carried out in three sessions on different days to avoid consumer fatigue. All samples were evaluated by 80 untrained judges. The sensory evaluation was carried out in a supermarket in the city of Tuxtla Gutiérrez, Chiapas, Mexico. The results were analyzed with a bidirectional analysis of variance ($p < 0.05$), and the means were compared using the Turkey's test with the help of the Statgraphics plus XV1 program.

RESULTS AND DISCUSSION

Water Loss and Solid Gain

Water loss and solute gain have been identified as the main factors that modify mass transfer during osmotic dehydration. The papaya's water loss and solid gain were influenced by processing time, temperature, and sucrose concentration (Figures 1 and 2). Water loss and solute gain increase rapidly in the first two hours, but after three hours, these values remained almost constant until the end of the process (6 h) for all processing conditions. These results were consistent with the literature (El-Aouar *et al*., 2006). The results also show that water loss (after 6 h of processing) varied between

34% and 70%; while the variation of solid gain was from 10% to 25%, depending on the sucrose concentration and the temperature of the solution (Figure 1).

Water loss (Figure 1a) and solid gain (Figure 2a) increased with solution temperature. This could be explained because by increasing the temperature of the solution, the permeability of the papaya tissue possibly increased, facilitating water loss. In addition, water loss decreased when a low sucrose concentration was used (40°Brix, Figure 1b). This could be explained because the sucrose concentration gradient between the papaya and the osmotic solution was lower. However, solid gain increased when a low sucrose concentration was used (40°Brix). The driving force for moisture transport from tissues to the solution is provided by the higher osmotic pressure of the concentrated solution (Radojˇcin *et al*., 2022).

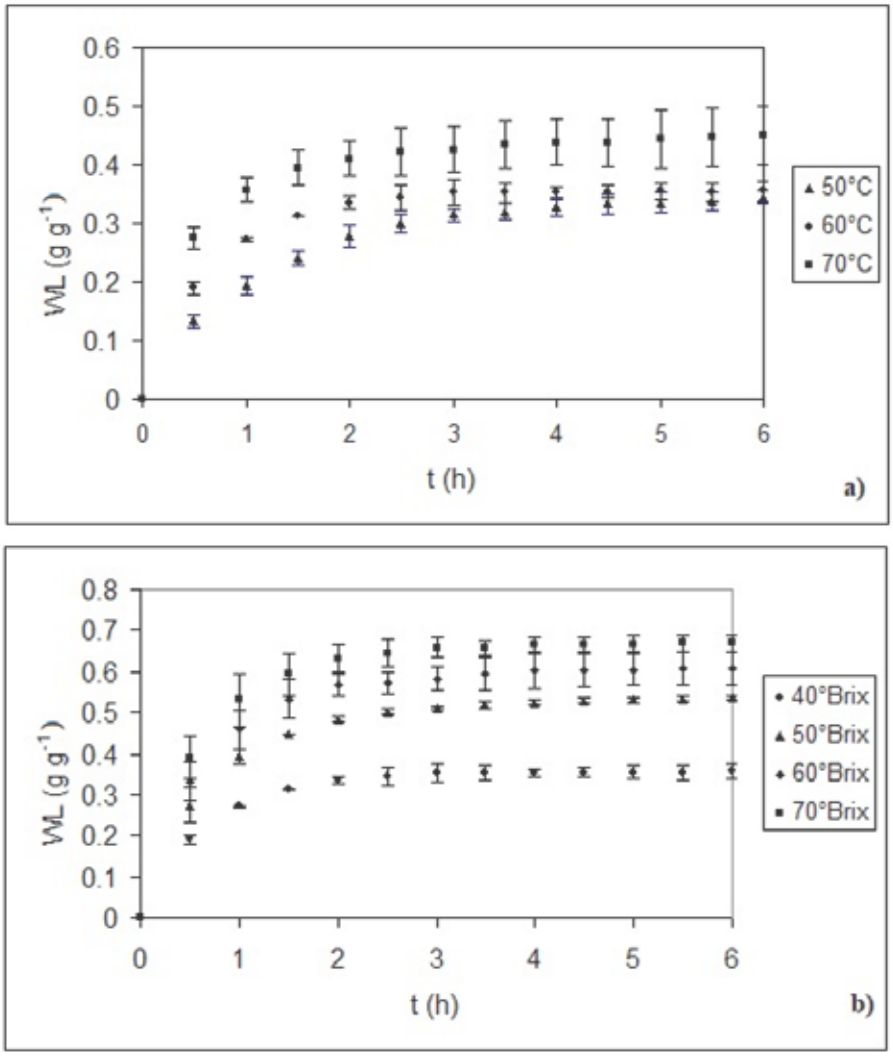

Figure 1. Water loss (WL) during osmotic dehydration of papaya at 40°Brix (a) and 60°C (b).
Source: Own elaboration

On the other hand, when the concentration of sucrose in the solution is higher, the impregnation rate probably increases faster in the first minutes of the process causing sucrose to accumulate outside the sample. In this case, the accumulation of sucrose on the surface of the papaya probably formed a semipermeable film in the papaya, preventing the exit of water and the entry of sucrose. Saputra (2001) and Waliszewski *et al.* (2002) found similar results to ours with a pineapple osmotic dehydration. But different results for papaya were reported by Rodrigues *et al.* (2003) who published that mass transfer during osmotic dehydration of papaya increases with the temperature and concentration of the osmotic solution. The differences could be attributed to the additives (citric or lactic acid and sodium lactate or calcium chloride) used by these authors in the osmotic solution, compounds that were not used in this work. In addition, the differences found could also be attributed to the variety of the fruit, as well as to the soil and environmental conditions where the papayas were grown.

On the other hand, the water loss (WL) and solute gain (SG) reported in this paper are higher than those published by Jain *et al.* (2011), who found for a papaya that the WL was 28% and the SG was 4% when dehydration was performed at 60oBrix, 37°C and 4.25 h of osmotic dehydration. The differences may be because these authors used 37°C in the dehydration process, which resulted in decreased mass transfer. In addition, these authors used a syrup volume: fruit weight ratio of 4:1 (mL:g), and the osmotic dehydration time was 4.25 h.

During osmotic dehydration, sucrose is impregnated in the papaya, and at the same time, the papaya loses water. So, when a low syrup: fruit ratio (v:w) is used, for example, 4:1, i.e. 4 mL of solution per gram of fruit, the osmotic solution is diluted by the loss of water from the papaya in the first hours of the process causing a lower sucrose gradient between the solution and the papaya. This causes a decrease in the rate of impregnation and dehydration of the fruit.

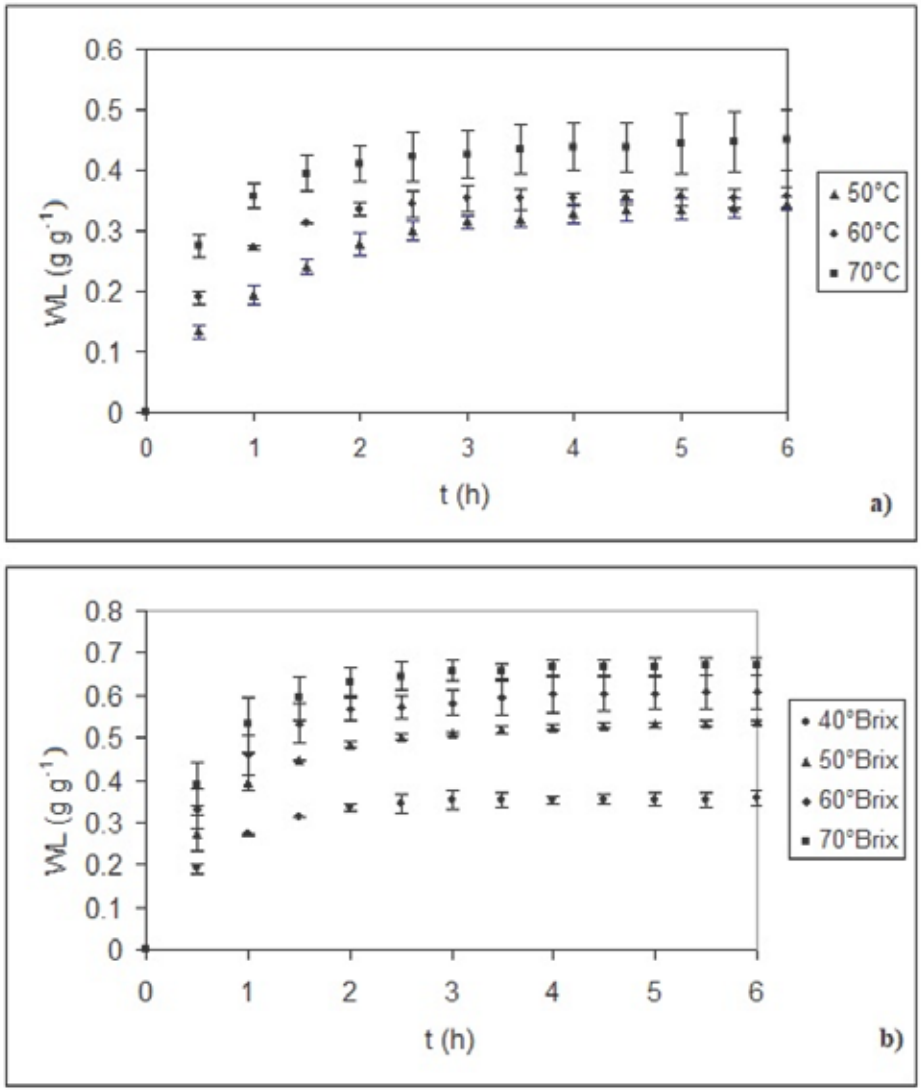

Figure 2. Solid gain (SG) during a papaya osmotic dehydration at 50°Brix (a) and 60°C (b).
Source: Own elaboration

Water Loss and Solid Gain Modeling

The parameters s_1 and s_2 of the Azuara model, identified for each kinetic, are shown in Table 1. Results ranged from 1.91 to 5.05 for water loss with a maximum error of 3.7% and between 1.5 and 5.86 for solid gain with a maximum error of 1.7%. The quality of the fit can be seen in Table 1, represented as the prediction error (σ), as well as in the graphs in Figure 3, where the model (equation 3) simulated very well the kinetics of osmotic dehydration in terms of water loss and solid gain. The graphs show that the increase in temperature causes an increase in the speed of water loss

and solid gain, especially during the first 2 h of the process. Subsequently, the curves tend to behave *quasi*-stable, which is probably because, during the first hours of the process, the mass transfer increases. Afterward, the speed of movement of solutes is reduced, to such a degree that the solids accumulated on the surface no longer allow for further water loss.

Table 1

Values of s_1 and s_2 for water loss and solid gain respectively to the empirical model (equation 3)

Sucrose concentration (°Brix)	Temperature (°C)	s_1	σ (Error)	s_2	σ (Error)
40	50	1.91	0.0226	1.61	0.0109
40	60	3.90	0.0206	1.58	0.0146
40	70	4.16	0.0136	1.50	0.017
50	50	3.18	0.0261	3.42	0.0082
50	60	3.26	0.0268	3.04	0.0108
50	70	5.05	0.0177	3.06	0.0087
60	50	3.52	0.0273	4.88	0.0042
60	60	3.77	0.0303	3.23	0.0087
60	70	3.91*	0.0256	2.66	0.007
70	50	3.29	0.0372	3.44	0.007
70	60	4.35	0.0316	5.86	0.0059
70	70	4.82	0.0244	3.63	0.0063

σ was calculated with equation 4

Source: Own elaboration

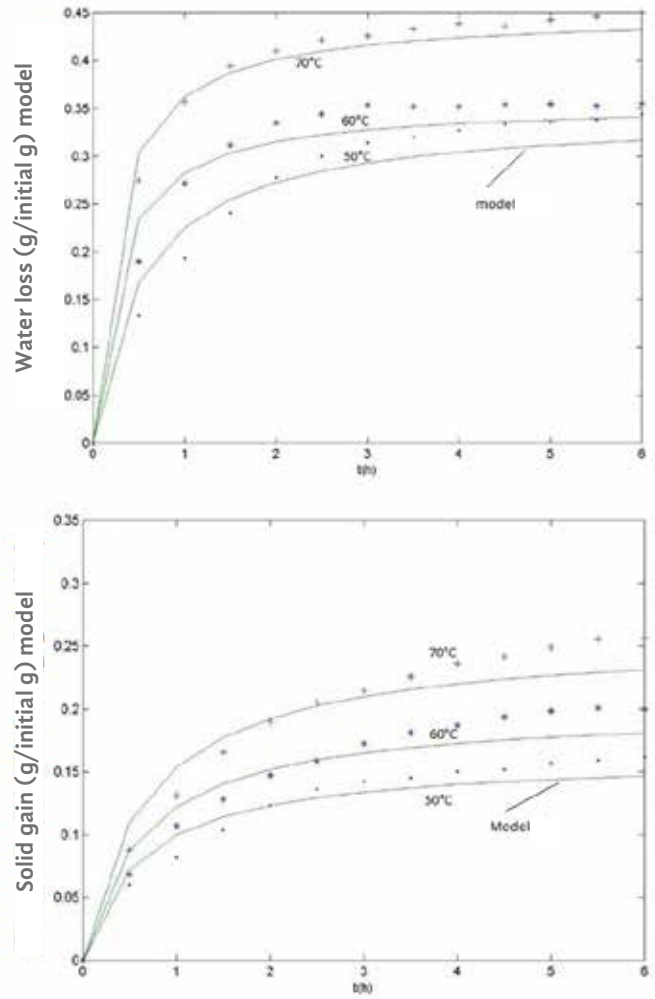

Figure 3. Water loss and solid gain experimental and predicted during the papaya osmotic dehydration at 40°Brix and different temperatures (•50°C, * 60°C, + 70°C, - model). Source: Own elaboration

The values of effective diffusivity of water and solids (sucrose) were obtained using equation 5. Diffusivity values increased with sucrose concentration, however, these decreased at high concentrations (60 and 70°Brix). This could be because a layer of sucrose formed on the papaya's surface, preventing the diffusion of water and sugar as explained above. The mean effective diffusivities calculated with equation 5 were between 4 x 10^{-10} and 7.2 x 10^{-10} $m^2\ s^{-1}$ for water loss and between 3.62 x 10^{-10} and 8.4 x 10^{-10} $m^2\ s^{-1}$ for solid gain. These values are like those reported by Solgi *et al.* (2021) for osmotic dehydration of *Ziziphus jujuba*, with effective diffusivities between 2.7 and 5.96 x 10^{-10} $m^2\ s^{-1}$. However, these values are ten times lower than those reported by Islam *et al.* (2019), who reported average effective diffusivity values for water loss and solute gain of 2.25 × 10^{-9} to 4.31 × 10^{-9} $m^2\ s^{-1}$ and 3.01 × 10^{-9} a 5.61 × 10^{-9} $m^2\ s^{-1}$, respectively during the papaya osmotic dehydration.

The differences could be attributed to the fact that they used a fruit:solution ratio of 1:4 (w/v), and the duration of the osmotic process was 240 min, in addition to the fact that they used another variety of papaya. Mendoza and Schmalko (2002) found an effective water diffusivity approximately two times greater (13 x 10^{-10} m^2 s^{-1}) than those found in this research for slices of papaya with 10 mm thickness as opposed to the 5 mm thickness used in this work. In the case of sucrose diffusivity, Mendoza and Schmalko (2002) reported a diffusivity of 34.7 x 10^{-10} m^2 s^{-1} for papaya. The difference could be attributed to the fact that these authors used slices 10 mm thick, and the mass transfer was on a single side of the slice, as well as those authors used another variety of papaya.

Sensory Evaluation of Papaya Osmodeshydrated

The hedonic scale is a test commonly used to determine the acceptance degree of a product as reported by Guadalupe-Tapia (2022) and López-Quevedo (2022). The variance analysis of the hedonic test results of the osmotically dehydrated papaya is shown in Table 2. In Table 2, the calculated distribution value F is greater than the F value of the tables, which indicates that there is a significant statistical difference ($p < 0.05$) between the treatments and the judges who performed the evaluation. These results are different from those reported for the sensory analysis of the osmotically dehydrated pineapple (Romero-Bello, 1995) and the osmotically dehydrated mango (Madamba and López, 2002), whose authors found that there was no statistically significant difference between their treatments. This difference is attributed to Romero-Bello (1995) using a 5-point hedonic test, and we used a 9-point test, while Madamba and López (2002) carried out the acceptance analysis with only ten judges.

Table 2
Variance analysis for the degree of acceptance of the osmotically dehydrated papaya at different sucrose concentration conditions and temperatures

Source:	GL	Sum of squares	Var	F-ratio	F (0.05)
Treatment	11	71.053	6.459	3.3	1.8
Judges	79	228.3073	2.8899	1.476	1.3
Residuals	869	1701.0302	1.957		
Total	959	2000.3906			

Source: Own elaboration

The Tukey's test, represented in Table 3, shows that the products with the highest acceptance were samples F (50°Brix, 70°C) and B (50°Brix, 50°C), and of lower acceptance was the sample H (60°Brix, 60°C).

Table 3
Tukey's test for the average degree of acceptance of the osmotically dehydrated papaya to different processing conditions

Code of the Treatment	Sucrose concentration (°Brix)	Temperature (°C)	Average degree of acceptance	
F	50	70	7.1875	a
B	50	50	7.0625	a
J	40	70	6.8875	abc
A	40	50	6.875	abc
D	60	70	6.825	abc
I	50	60	6.7375	abc
C	70	60	6.7125	abc
E	40	60	6.5625	abc
G	70	50	6.525	abc
L	70	70	6.4875	abc
K	60	50	6.3125	bc
H	60	60	6.2625	c
		LSD	0.10	

Equal lowercase letters next to the value of the acceptance degree mean that there is no statistically significant difference between treatments. LSD= least significant difference.

Source: Own elaboration

For samples F and B, a score higher than 7 were obtained, that is, the judges rated it as "I moderately like it"; and for the H sample "I kind of like it". However, in the same Table 3, it is observed that the difference between the treatments is *only* from a point of the hedonic scale, which indicates that all the samples had a good acceptance and that, probably, the consumer judge has difficulty identifying if there are differences between the treatments. This suggests that the product has a very good degree of acceptance. Tukey's test for judges was not conducted because the judges were not trained. From the above results, it is recommended to dry the 5 mm thick papaya for 6 h at 50°Brix and 50°C, which will cause a water loss of 49% and a solid gain of 14%, obtaining a product with a reasonable level of acceptance with a view to the commercialization of an osmotically dehydrated papaya product with a shelf life of not less than three months at room temperature. In addition, this treatment is the one that will allow the lowest energy consumption since less heating will be required to keep the process at 50°C.

CONCLUSIONS

The results show that water loss and solid gain from papaya slices during osmotic dehydration were affected by process time, sucrose concentration and solution temperature. The mathematical model simulated water loss with an average error of 3.7% and 1.7% for solid gain. This model could be used to predict the osmotic process to other conditions. The average diffusivities for water varied between 4 x 10-10 and 7.2 x 10-10 $m^2 s^{-1}$; while for sucrose they were 3.62 x 10-10 to 8.4 x 10-10 $m^2 s^{-1}$. The results showed that the temperature and sucrose concentration of the solution during osmotic dehydration of papaya affected the sensory acceptance of Maradol papaya. The best treatment that increased water loss and decreased solid gain was 50°Brix with 50°C during 6 h of process.

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A C A D E M I C
P A P E R

Reception: **March 14, 2022** | Acceptance: **April 4, 2022** | Publication: **October 1, 2022**

Geopolitics and innovation: The creation of value in Querétaro[i]

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Víctor M. Castaño
meneses@unam.mx; vmcastano@ai.org.mx

Centro de Física Aplicada y Tecnología Avanzada, U.N.A.M.-Juriquilla, Academia Mexicana de Ciencias, Academia de Ingeniería Academia Nacional de Medicina

To quote this article:
M. Castaño, V. (2022). Geopolítica e innovación: La creación de valor en Querétaro. *Espacio I+D, Innovación más Desarrollo, 11*(31). https://doi.org/10.31644/IMASD.31.2022.a09

GEOPOLITICS AND DECISION-MAKING

Geopolitics is "the science that, through Political Geography, Regional Studies, and History, studies the spatial causality of political events and their future effects." In other words, it allows us to understand, from a global perspective (spatially and temporally speaking), the origins of the incomprehensible contemporary global phenomena, such as the crisis in Ukraine, the conflicts in the Middle East, the presence of the Zetas in the Gulf of Mexico or the industrial dynamics in Querétaro and Bajío. Even more relevant, how these "isolated" incidents, which may seem distant or disconnected from each other, will come to affect a political, economic, social, or even technological decision anywhere in the world and, thus, be able to make the relevant decisions in a local or regional context[1,2]. The "Theory of the Small World"[3,4] reaffirms the incredible connectivity that people, events, and geographical locations are constantly generating and modifying, through dynamic networks in very varied areas of knowledge and economic development[5-6].

From a planning and decision-making point of view, Geopolitics is very relevant since it allows us to generate a vision, which leads us to move from a scenario of a "forced future" to one of a "desired future", in which a country, a state, a society or a company is enabled to design not only where they want to reach, but how and when. Geopolitics, in short, represents a powerful weapon of planning and decision-making, global and historically grounded.

Science, Technology, and Innovation in 21st-Century Geopolitics

Modern geopolitics was born, as a science, towards the end of the nineteenth century, coinciding, in an interesting way, with the emergence of science and technology as engines of economic development, a phenomenon that had begun with the Industrial Revolution. The twentieth century thus witnessed both enormous growths in science and technology, unprecedented in the history of mankind, and unprecedented geopolitical changes.

Open innovation vs. Closed innovation

While the term "innovation" has become almost a cliché in geopolitical decision-making today, the reality is that it continues to represent an important concept that is constantly being adapted and improved. Innovation as a development strategy is already part of global macroeconomic patterns, and there is a tremendous interest in generating alternative innovation models. One of these models, which is becoming very important in recent times, is that of "open innovation"[7], in contrast to traditional innovation, which would be "closed". The primary difference in open innovation is that it considers that generating innovation internally in the market sector where it is operated is not fundamental but to have access to it, through agreements, alliances, joint ventures, and all the types of partnerships in networks that can be imagined. The basic philosophy is that we don't need to possess all the talent, but we do need to have access to it, wherever it is. This, apparently very simple, is representing a revolution in the field of business, technological intelligence, and decision-making.

Open innovation emphasizes the importance of having, rather than physical assets (buildings, equipment, human and material resources, etc.), highly dynamic and accessible networks that allow access to resources and where they are located. What is relevant, then, is not necessary to create hardware but to invent software that articulates resources efficiently and openly.

The Local Innovation System (LIS) Program

In this sense, about a decade ago, the Massachusetts Institute of Technology (MIT) created an interesting program (LIS)[8] along the elements that were outlined in the previous paragraphs, with a regional development aspect. The basic questions that this program raises are:

1. What is the role that innovation plays in boosting regional competitiveness and development?
2. How can Higher Education Institutions (HEIs) and Public Research Centers (PRCs) promote regional innovation capacities?

Taiwan, and Norway, which have overthrown several myths associated with the academia-industry relationship, such as, first, that HEIs and PRCs do not have, in practice, economic significance, only in highly developed economies. The second myth, very popular, is that patent licensing is the mechanism for HEIs and PRCs to achieve economic impact. The third myth is that the transfer of technology from academia to industry takes place, preferably, through intellectual property protection instruments. As a counterproposal to these myths, the LIS proposes four actions that have demonstrated, at least where appropriate, economic effectiveness: education (with emphasis

on competencies), the generation of spaces (physical and virtual), the resolution of problems proposed by and for the industry and the constitution of reservoirs of knowledge. The implementation of these actions led MIT to create another dependency, also very successful, the Industrial Performance Center (IPC)[9] that performs four specific tasks:

1. Local creation of new industries
2. Transplanting industries from other regions to the locality
3. Diversification of local industries
4. Modernization of mature industries

Open innovation in Querétaro

Based on the theoretical elements described above, it is now worth reflecting on the relevance of creating a "Prospective and Innovation Center" in Querétaro, which could be integrated into MIT's LIS. The first point to emphasize is the risk of making decisions based on isolated data, such as the number of companies located in the State, how many researchers reside in Querétaro, the nations that are creating similar centers, etc. Such a potentially important step must be based on a geopolitical analysis as complete as possible, for which an interesting starting tool is the technological Road Maps[10] whose effectiveness has been proven in several successful cases of planning national and regional technological development in various fields of knowledge[11].

The second lesson to remember is that, in addition to hardware, a State Innovation System[12-13], preferably "open"10,12 must ensure the availability of software that manages to instrument the resources that are being put into play. Additionally, the evaluation criteria must be different for science, technology, and innovation, which leads, necessarily, to the creation of relevant measurement instruments on a case-by-case basis. Another important aspect to consider is innovation governance, from a global perspective[14], and how knowledge networks linked to innovation networks can be generated, which is not always the case[15].

Finally, the profiles of innovators imply skills that neither a technologist nor a scientist possesses and that, rather than adding pressure to the evaluations to which the current actors of the State Innovation System, turn into the articulation of networks and collaboration and competitiveness clouds. The question, in a word, is not what, but how.

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i To further expand the topic, you can consult:
J.L. Lucio y M. Torres (Coords.). (2017). Presente y Futuro de la Ciencia en México. Retos y Perspectivas de la Física. Academia Mexicana de las Ciencias. Ciudad de México. http://ccciencias.mx/librospfcm/presente_futuro_retos.pdf