Phosphorus Pesticides and Nitrates Pollution in The San Pedro Mezquital River, Nayarit, Mexico.

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Licence PRO GENA

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1. Introduction

Agriculture is one of the main sources of pollution; it contributes to greenhouse gas effect. Also, agricultural, forestry and fisheries methods are the main causes of biodiversity loose **(FAO, 2002).** Agriculture causes pollution because of the discharge of pollutants and sediments in surface and groundwater. Agriculture is the main user of fresh water; almost 70% of world fresh water is used in agriculture. Agriculture pollute water with nitrates, phosphorus and pesticides, it means agriculture is the main pollutant and victim of water pollution **(FAO, 2002).**

In Mexico, river pollution is a generalized problem **(CONAGUA, 2014)**, the most emblematic cases of water pollution are the Atoyac River (Puebla, Tlaxcala and Oaxaca) and the Lerma-Santiago River (Mexico State, Guanajuato, Michoacan, Jalisco and Nayarit) **(GREENPEACE, 2012).**

The coast region is part of the state of Nayarit; it covers portions of the municipalities Ruiz, Rosamorada, Tuxpan and Santiago Ixcuintla. This municipalities together have a surface of 3 000 km^2 it means almost 16% of the state surface and a population of 31 000 people **(Consejo de Cuenca de los Ríos Presidio al San Pedro, 2006)**

The coast region is mainly agricultural; here we can find crops as bean (11%), grain corn (2%), tobacco (23%), grain sorghum (8%), rice, vegetables and fruits **(WWF, 2015).**

The San Pedro Mezquital River is the seventh largest river in Mexico by volume; it starts at Durango and ends at Nayarit, it discharge in the Pacific Ocean. About 800 000 people live along the San Pedro River, there can be found ethnic groups like Mexicaneros, Wixarikas, Coras and Tepehuanes of the south. This river is 540 km long; 2 763 406 hectares is the surface around the river, of which 279 648 ha are used for agriculture. The main economic activities are animal husbandry, agriculture, forest skidding and shrimp and oysters production in the coast region.

Agriculture uses 68% of underground water and 58% of superficial water **(WWF, 2015).**

This project aims to analyze the impact of aquatic pollution generated by agricultural practices in the river surrounded by fields and orchards. This was explored by the measure of nitrates in water samples; using a nitrate meter HORIBA. It was also measured via the activity of the target enzyme of organophosphorus and carbamate pesticides, namely acetylcholinesterase, a protein involved in the nervous system functioning, in native fishes of the family Poecillidae.

2. Presentation of the Host Institution

The University is located in the state of Nayarit, it is a public institution of higher education. Its address is Ciudad de la Cultura "Amado Nervo" Tepic, Nayarit. Mexico. C.P. 63155.

Since 1925 the University of Nayarit has the fist juridical records; it was 1927 when the high school of Nayarit was reopened (it was closed because of lack of funds). In 1930 was the first record of University. Since then the Universidad Autonoma de Nayarit has had a lot of juridical and administrative changes. **(Figure 1)**

Figure 1 Universidad Autonoma de Nayarit Logo

2.1. Mission

Its mission is to attend the necessities of high and higher education, from a critical perspective, purposeful and plural, with social compromise. It participates in the integral and sustainable development of the state of Nayarit through its entailment and its services extension.

2.2. Vision

Its vision is being an institution with national and international recognition. Being leader in the generation and application of knowledge, and educating students with social compromise, capable to transform their surroundings and elevate the life quality of their families and their society.

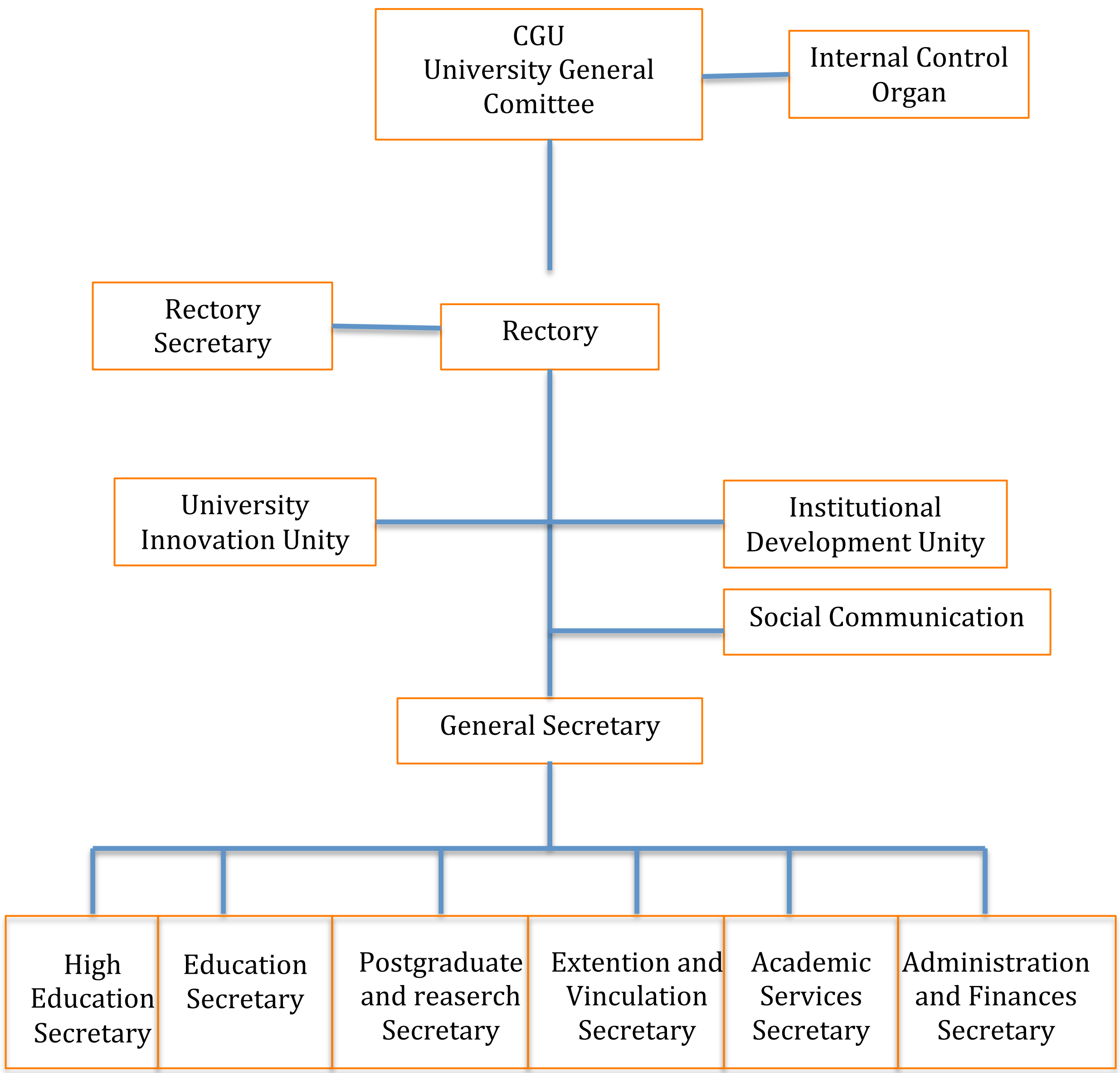
2.3 Educative Offer

The Universidad Autonoma de Nayarit has 13 614 students in higher education level. It offers high school, higher education and postgraduate education. It offers 33 programs in different areas of education.

- Arts area.
- Biologic, agricultural and fishery area.
- Administrative and economic sciences area.
- Basic and engineering sciences area.
- Health sciences area.
- Human studies and social sciences area.

2.4 Organization Chart

This university as many others has an Comitte General, chosen by the academic members. This university has not a student participation in the process of choosing Rector, or other members of the organization chart. However, students have an organization named FEUAN (Students Federation of the Universidad Autonoma de Nayarit), it is similar to a syndicate.



3. Literature Review

3.1 Water Pollution

Water is one of the most important commodities which man has exploited. Most of the water in the planet is in oceans and ice caps. The quantity of utilizable water is very much limited in earth **(Goel, 1997).** Any of the human activities may produce a quantity of pollution that could pollute water.

In a dictionary the word pollution can be found as:

"The presence in or introduction into the environment of a substance, which has harmful or poisonous effects"

Water is polluted when it gets changed in its quality or composition so as to become less suitable for human activities, wildlife and other uses. A water pollutant is any physical, chemical or biological factor causing aesthetic or detrimental effects on aquatic life and those who consume water **(Goel, 1997).**

The effects of water pollution can be summarized as:

- Aesthetic: visual nuisance caused, e.g. litter, discoloration and smells.
- Temperature: usually heat.
- Deoxygenation: lack of oxygen in the water.
- Toxicity: acute or chronic toxicity causing damage to aquatic or human life.
- Sublethal toxicity: such as endocrine disruption or changes in biodiversity.
- Acidity/alkalinity: disturbance of the pH regime.
- Eutrophication: nutrients giving rise to excessive growths of some organisms. **(Harrison, 1983).**

Chemical water pollutants can be divided into two categories, the relatively small number of macropollutants, it includes nutrients such as nitrogen **(Gruber and Galloway, 2008)** and phosphorus species **(Filippelli, 2008)** as well as natural organic constituents **(Jorgenson, 2009)**.

With respect to human health, the most direct and most severe impact is the lack of improved sanitation, and related to it is the lack of safe drinking water, which currently affects more than a third of the people in the world. Additional threats include, for example, exposure to pathogens or to chemical toxicants via the food chain or during recreation. **(Schwarzenbach *et al,* 2010)**. The provision of fresh water and sanity is a significant factor for human health; it is crucial to reduce mortality and mobility in children; decrease in hydric transmission diseases (viral hepatitis, typhoid, cholera, dysentery and other causes of diarrhea), as well as diseases resulting from consumption of chemicals (arsenic, nitrates or fluorine) **(CONAGUA, 2014).**

3.2 Water Pollution due to Agriculture

Freshwater is also the end point for biological waste, in the form of human sewage, animal excrement, and rainwater runoff flavored by nutrient-rich fertilizers from yards and farms. On average, 99 million pounds (45 million kilograms) of fertilizers and chemicals are used each year **(National Geographic, 2015)**.

Water pollution can be of different kind of source types; it can be diffuse, point or a mix of both. Agricultural pollution is diffuse **(Schwarzenbach *et al,* 2010)**. Pollution from diffuse sources is normally less immediately apparent and more difficult to control than that from point sources **(Harrison, 1983).** Agriculture uses 70 % of fresh water in earth, but it is also the main water pollutant; agriculture pollutes water throwing nitrates, phosphorus and pesticides **(FAO, 2003).**

Each year, several million tons of chemicals products are consumed by agricultural production to maintain and increase crop yields. A pesticide is a chemical product used in the battle against pests, diseases and weeds **(FAO, 2015)**.

From 1940 to 1995, the pesticide consummation in the world has increased near to 11% each year, reaching 5 millions tons in 1995. Experts estimate that only a 0.1% of these products achieve its species objective, the rest can pollute soil, water and biota. **(Carvalho *et al*, 1998).**

There are multiple causes of pollution generated by pesticides; these can be an inherent use of the component, for wrong manipulation and negligence **(Orta, 2002).** The impact produced by agrochemicals to environment depend on four criteria: quantity of active ingredient applied and its place of action, its concentration in air, soil and water, the grade and speed of compound degradation and finally the toxicity depending on species **(Hayo, 1996).**

3.2.1 Water Pollution in Mexico

In Mexico, river pollution is a generalized problem **(CONAGUA, 2014)** the most emblematic cases of water pollution are the Atoyac River (Puebla, Tlaxcala and Oaxaca) and the Lerma-Santiago River (Mexico State, Guanajuato, Michoacan, Jalisco and Nayarit) **(GREENPEACE, 2012).**

To evaluate water quality **CONAGUA** evaluate: oxygen biochemical demand (DBO_5), oxygen chemical demand (DQO) and total suspended solids (SST). DBO_5 indicates quantity of organic biodegradable material; DQO evaluates organic material quantity; and SST has its origin in blackwater and soil erosion. According to the results of quality evaluation for three indicators applied in 2013, all 260 sites are classified as strongly polluted according to two or all indicators **(CONAGUA, 2014).**

The sites strongly polluted are:

- Descanso Los Médanos
- Guadalupe
- Rio Colorado
- Río Mayo 3
- Rio Juchipilan
- El Salado
- Rio Turbio
- Presa El Niagara
- Rio Lerma 5
- Lago de cuitzeo
- Rio Papagayo 4
- Rio Quetzala
- Rio Blanco
- Rio Necaxa
- Rio Alto Atoyac
- Xochimilco
- Texcoco
- Ciudad de Mexico
- Rio Cuautitlan
- Rio Salado
- Rio San Juan
- Rio Toliman

Mexico has no information about agricultural impact in water; but CONAGUA has indicated that agriculture, forestry and a wrong garbage management are responsible of 70% of water pollution **(Perez, 2015).**

3.2.1.1 Government Answers to Agricultural Water Pollution

Mexico has multiple environment and water politics; but those politics do not concern to pollution caused by agriculture. The main preoccupations are: freshwater provision, blackwater treatment and recollection, pluvial and polluted water management, adequate access to public data and climate change impact **(SEMARNAT, 2015).**

Reasons that can explain this emptiness are:

- Absence of systematic and large research.
- Lack of basic information to design environmental action and to make decisions to solve the problems.

- Considering agriculture from a general point of view without taking into account its particularities.
  (Perez, 2015)

3.3 The San Pedro Mezquital River

The San Pedro Mezquital River is one of the most important in Mexico; it is located in the states Durango, Zacatecas and Nayarit. It is 540 km long and is the seventh largest river by volume in Mexico **(WWF, 2015). (Figure 2)**

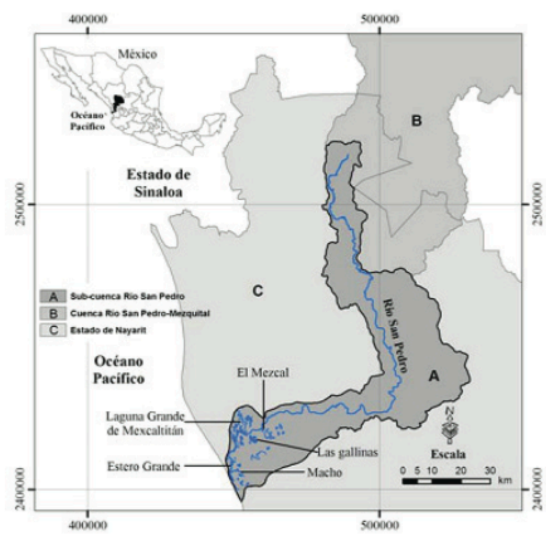


Figure 2 The San Pedro Mezquital River Localization in Nayarit

Source: Instituto Nacional de Ecología, 2005.

It has elevations between 0 and 3 340 AMSL. The union of the rivers La Sauceda, El Tunal and El Santiago Bayacora form it; it starts in the mountain chains of Durango; it traverse the mountain chain a cross the Mezquital Canon and continue through the Coastal Plains of Nayarit **(WWF, 2015).**

3.3.1 Environmental Importance

It has multiple climates, desert in Durango, template in the half part and tropical in the Coastal Plains. It has an average annual precipitation of 634 mm; in the high part it has a precipitation of 300 mm and in the lower part it has a precipitation of 1450 mm **(WWF, 2015). (Figure 3)**

Figure 3 The San Pedro Mezquital River and Its vegetation

It supplies water to Marismas Nacionales; the most important wetland in Mexico. It has almost 133 000 hectares; 20 % of the total mangrove of the country is located here. Marismas Nacionales is considered one of the most productive in the northwest and it is one of the most important areas of bird conservation in Mexico and in the world **(CONANP, 2013).**

3.3.2 Social Importance

About 800 000 people live along the San Pedro River, divided in 1 766 towns; it can be found ethnic groups like wixarikas (547) and coras (14 235) in Nayarit; mexicaneros (1 000) and tepehuanes of the south (18 146) in Durango. The main economic activities are animal husbandry, agriculture, forest skidding, shrimp and oysters production in coast region **(WWF, 2015). (Figure 4)**

Figure 4 A Riverside house

3.3.2.1 "Las Cruces" Dam

"Las Cruces" Dam is a hydroelectric project that has as main objective contribute to satisfy the electric energy demand for Mexico occident, maintaining the adequate levels; It will provide 780 GWh. The project is in 22° 05' 19" N and104° 57' 03" W geographic coordinates. The investment necessary to make this project is estimate in $ 639.6 millions USD; It has been approved by government in 2014 and it will be necessary 49 months for it construction. It will create 5 000 of directs jobs and other 5 000 indirect jobs **(AIDA, 2015).**

This project has had a lot of social and environmental critics. A press release issued by **AIDA, 2014** exposes violations to indigene rights; the press release explains that The "Las Cruces" Dam has not been consulted nor approved by the affected indigenous population. Finally, its construction will impact endemic riverside ecosystems.

4. Materials and Methods

4.1 Sampling Design

Four sampling point were made along the coast region (last 105 km) of The San Pedro Mezquital River, all of them were separated equidistantly **(Figure 5)**. These points were named according to the nearest town. The fist sampling was located in San Pedro Ixcatan, its geographic references were 22º 2’ 25.9’’ N and 104º 56’ 3.0’’ W; its altitude was 95 AMSL. This place was chosen as the less polluted by agriculture that’s why here were taken the fish used as control.

Figure 5 Sampling Points Repartition Along the River

The second sampling point was located in Laguna del Mar, its geographic references were 21º 2’ 22.72 ‘’ N and 104º 55’ 58.18’’ W, its altitude was 43 AMSL. Pineapple, coffee and corn were the main crops seen along the river.

The third sampling point was located in Peñitas. Its elevation was 8 AMSL; its latitude and longitude were 21º 56’ 53.8’’ N and 105º 13’ 26.0’’ W respectively. This point was the less agricultural but the most urbanized one.

Finally, the fourth sampling point was located nearby El Mezcal, it was the most agricultural, the main crops seen along the river were sorghum, corn and pumpkin; 8 AMSL was it elevation and 21º 56’ 18.2’’N and 105º 21’ 35.0’’W its geographic coordinates.

Two sampling campaigns were carried out, the one before the rainy season (end May) **(Figure 6)** and the other one during the rainy season (half July) **(Figure 7)**.

Figure 6 Fish Collect Before Rainy season

Figure 7 Fish Collect During Rainy season

Sampling points were chosen according to their access roads, and separation along the river. At first, other two sampling points, located at the coast were chosen; they were Mexcaltitan and Boca de Camichin, but these points has seawater mixed with fresh water, this did not permit an homogeneity of the fish samples and the sampling points were ignored.

4.2 Measure of the Amounts of Nitrates in Water Samples

Water samples were collected from the four sampling points; each one was taken in a half litter amber bottle. After that they were taken to the UAN Unidad Academica de Agricultura soils Laboratory for their analysis. Water was maintained under refrigeration until the nitrates determination.

Nitrate amounts were determined using a HORIBA nitrates meter with two points of calibration. After calibrate, some drops of water sample were added to the sensor; lecture was direct to mL/L unities **(Figure 8).** Lecture was made at ambient temperature (30ºC).

Figure 8 Measures of the Nitrates Amounts

The measure of the nitrates amount training was in charge of Dr. Gelacio Alejo Santiago.

4.3 Fish Collect

Fish of the family Poecillidae, **(Figure 9)**. With them, the activity of the enzyme acetylcholinesterase was measured. Dr. Leonardo Martínez Cárdenas determined fish taxonomy.

The collection was made with the help of Dr. Candelario Santillán Ortega and Erik Jesus Guzman Castañeda.

Figure 9 Fish Used to measure Acetylcholinesterase Activity

From each sampling point were collected 30 fish (minimum) using a greenhouse web **(Figure 10)**, fish were put into a plastic bag with zipper **(Figure 11)**. After that, samplings were put into a cooler with ice and transported to the Immunology laboratory of the Universidad Autonoma de Nayarit, for their later analysis.

Figure 10 Fish Collect In San Pedro Ixcatan

Figure 11 Fish in plastic bag

4.4 Enzymatic Analyses of Fish Sampling

The impact produced by agriculture to the animal communities of the San Pedro Mezquital River was estimated by measuring locked acetylcholinesterase activity in some fish species. This was performed in the Immunology laboratory of the Universidad Autonoma de Nayarit. The responsible of the training were Dr. Manuel Ivan Girón Pérez and Gladys Alejandra Toledo Ibarra.

The Acetylcolinesterase activity was measured using the method described by **Ellman (1961)**

4.4.1 Measure of Total Protein

The samples fish were put on ice; using tweezers and scissors caudal fin and head were cut. Internal organs were removed pressing lateral line.

It was done this way for methodological reasons; because of the fish size, it was impossible to use only the liver as is commonly done in the immunology laboratory; additionally, muscle has less marge of error caused by butyrylcholinesterase or pseudocholinesterase; which is present mostly in the liver and plasma **(Habig and Di Giulio, 1991)**.

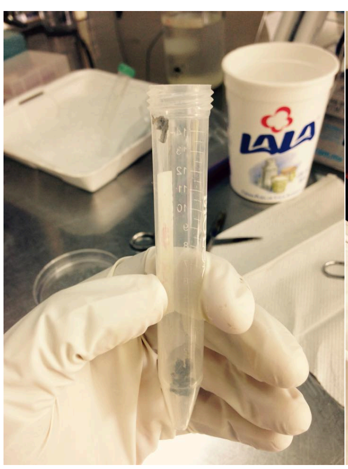


Figure 12 Centrifuge Tube with fish pieces

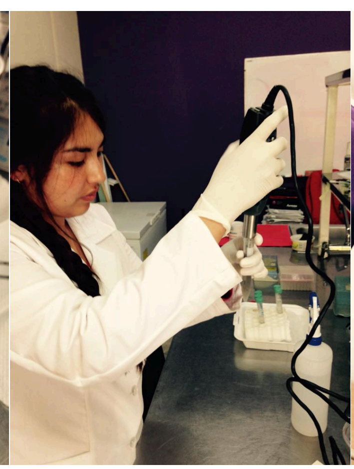


Figure 13 Homogenization

Figure 14 Centrifuge with samples

Fish was cut in little pieces; those pieces were put in a digital analytical balance. After that, the pieces were cut a second time and put into a tube for centrifuge **(Figure 12)**; 10 mL of PBS (Phosphate Buffered Saline is a solution that used to buffer pH in biochemical processes) were added to the tube. A homogenizer was used during 30 seconds to homogenize the muscle tissue **(Figure 13)**. This process was made 3 times by sample point.

Subsequently the tubes were put into a centrifuge during 30 minutes at 4 500 revolutions per minute **(Figure 14)**. When time has passed, supernatant was taken from the tube and put into another. All this process was made taking care of maintain the samples into ice.

Bradfrod is a protein assay is a spectroscopic analytical procedure used to measure the concentration of protein in a solution. It is elaborated based in a known concentration of a protein, using the values of absorbance and the known quantity of the protein a formula is obtained.

Formula used to calculate total proteins:

$$Tproteins = 0.0404(abs) + 0.0124$$

Afterwards, 20 μ L of the fish solution were put in a microwell plate, and 180 μ L of Bradford solution were added **(Figure 15)**; after 15 minutes, the microwell plate was put in a spectrophotometer (spectrophotometer the reflection or transmission properties of a material) to measure total protein. Spectrophotometer filter absorbance was 545 nm **(Figure 16).**

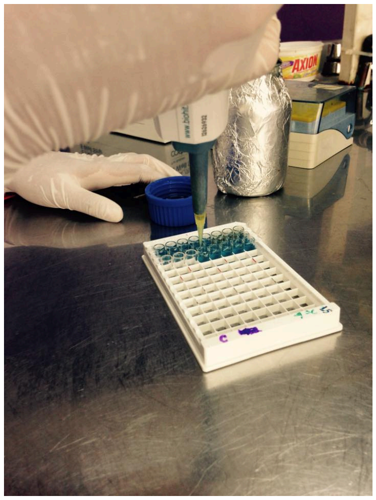


Figure 15 Microwell plate Fill

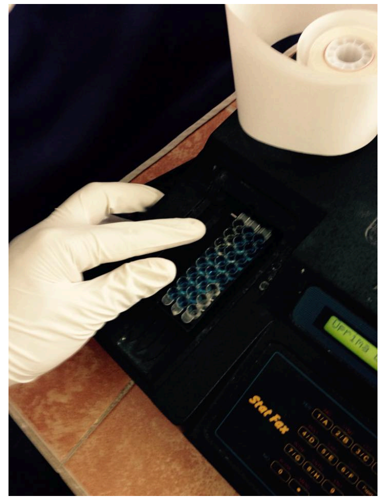


Figure 16 Measure of Total Proteins in Spectrophotometer

4.4.2 Measure of the Acetylcholinesterase Activity

Once total proteins were calculated the measure of the acetylcholinesterase activity was done. First, it was calculated what amount of the fish solution was needed to have 0.1 mg/L of protein using a cross-multiplication.

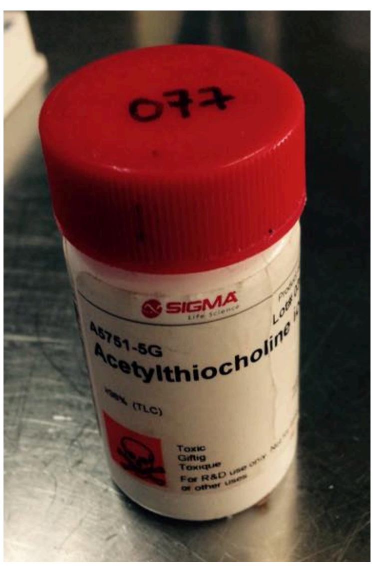


Figure 17 Acetylthiocholine

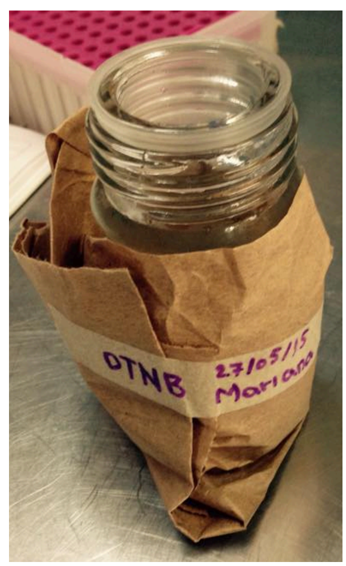
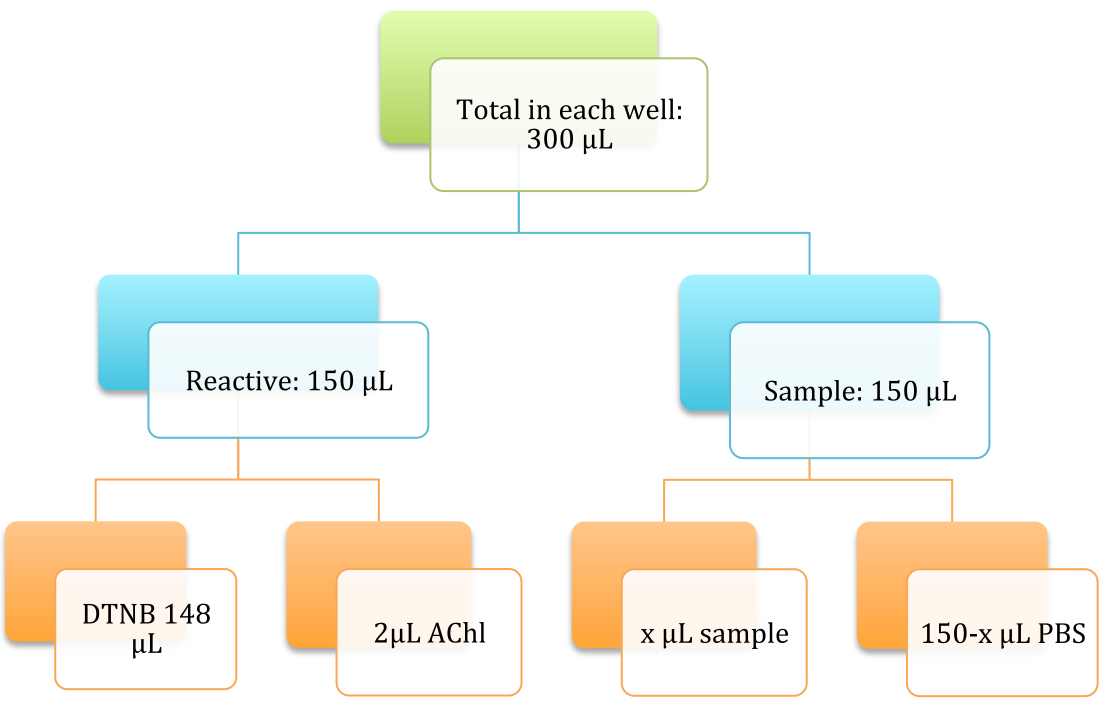


Figure 18 DTNB

Buffer solution of DTNB (2-nitrobenzoic acid, is used to quantify the number or concentration of thiol groups in a sample) was made, 75 mL PBS were put in a 250 ml glass bottle 9.9 mg of DTNB and 3.75 mg of sodium bicarbonate. The bottle was covered with brown paper to avoid solar light **(Figure 18)**.

Next, 0.03 g of acetylthiocholine (used as a substratum) **(Figure 17)** were mixed with 518 μL of distilled water; using 50% of the stock solution and 50% of distilled water an acetylthiocholine solution 1mM was made.

The microwell plate was filled the following way:



Once the wells were filled this way **(Figure 19)**, the microplates were put in the spectrophotometer; absorbance filter was 405 nm. Measures were made in the 0 and in the 5th minute **(Figure 20)**.

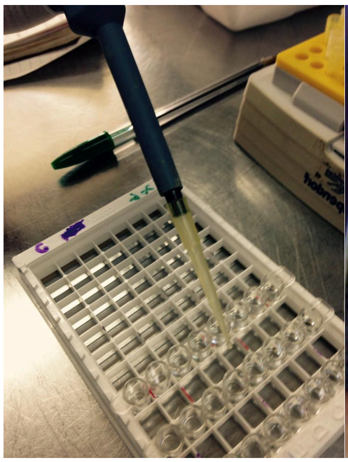


Figure 19 Microwell Plate Fill

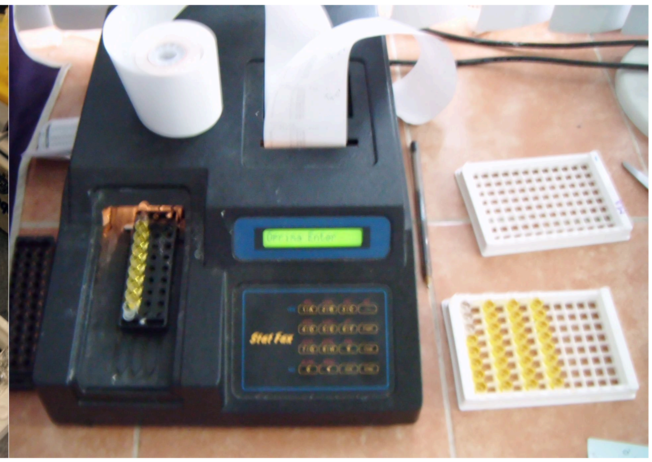


Figure 20 Measure of Acetylcholinesterase Activity in the Spectrophotometer

4.4.3 Controls Positive and Negative

From the first sampling point, the one we considered the less polluted, 200 fish were collected alive and transported to the Immunology laboratory of the Universidad Autonoma de Nayarit; there they were maintained 15 days under depuration in two 30 litters fishbowl filled with well water; they were fed with fish food brand BIOMAA **(Figure 21)**.

Figure 21 Super Flakes: Food used to feed The Fish

Figure 22 DIMETOATE (38.5%)

Subsequently, only 30 fish of 2-2.5 cm long were maintained in each fishbowl.

Positive control was exposed to DIMETHOATE (38.5 %) **(Figure 22)** sublethal dose 1 mg/L during 96 hours **(Frasco *et al.;* 2002)**, fish were maintained in a static system and they were not fed since two days before exposure. Negative control has had the same handling.

After 96 hours or 5 days, fish were put into a zipper plastic bag. Analyze was made

4.5 Statistic analyze

The experiment results were submitted to a variance analysis, followed by a median comparison by the Tuckey test (α = 0.05); the results were ordered descending.

5. Results and Discussion

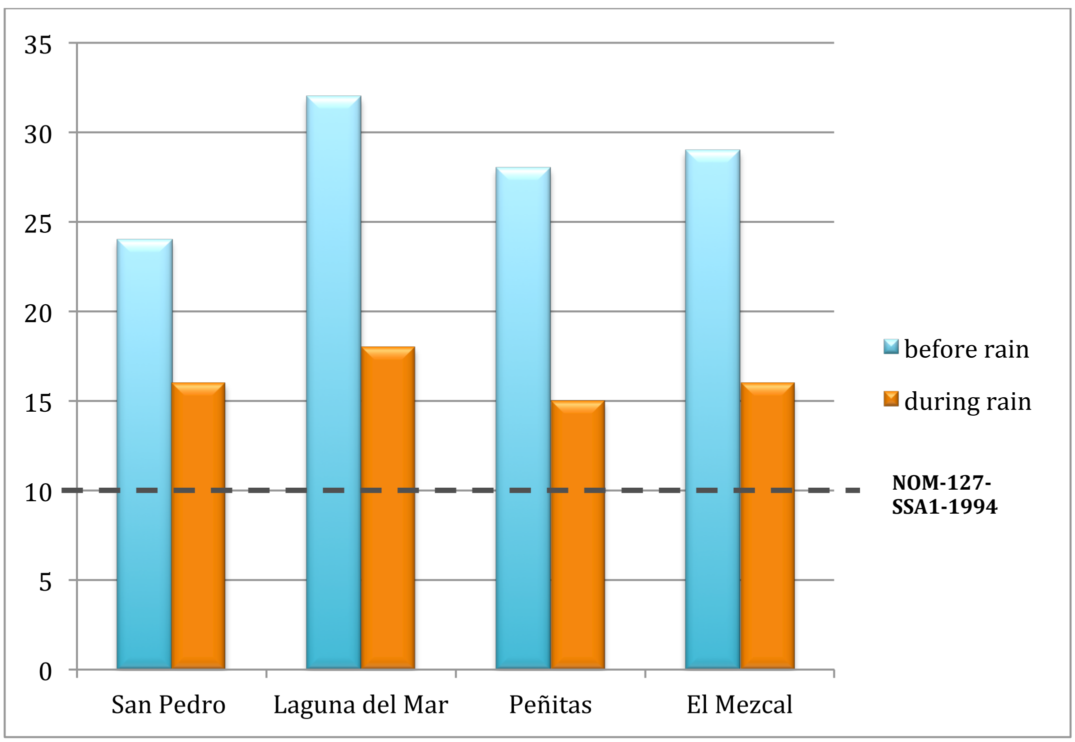
5.1 Nitrates Results and Discussion

Nitrates results of the four sampling points, before and during rain season, are presented below.

Table 1 Amount of Nitrates Found in the river water in mg/L

Place	Before Rainy Season NO_3 mg/L	During Rainy Season NO_3 mg/L
San Pedro	24	16
Laguna del Mar	32	18
Peñitas	28	15
El Mezcal	29	16

Figure 23 Amount of Nitrates in the river water.



Note: In the Pointed line, it can be seen the maximum amount of Nitrates in water for human use.

The nitrates results show a sharp difference between samplings; the first sampling made just before rainy season has higher quantities of nitrates. Due to rain water levels in river increases, it could cause the dilution of nitrates in water, and this way decrease its concentration.

According to the statistic analysis, all sampling points have the same amount of nitrates in water.

The horizontal line in the bar chart **(Figure 23)** marks the limits of Nitrates in drinkable water; the results are all above this limit. On the contrary **Rojas (2011)** has reported results under 1 mg/L Nitrates in the same river.

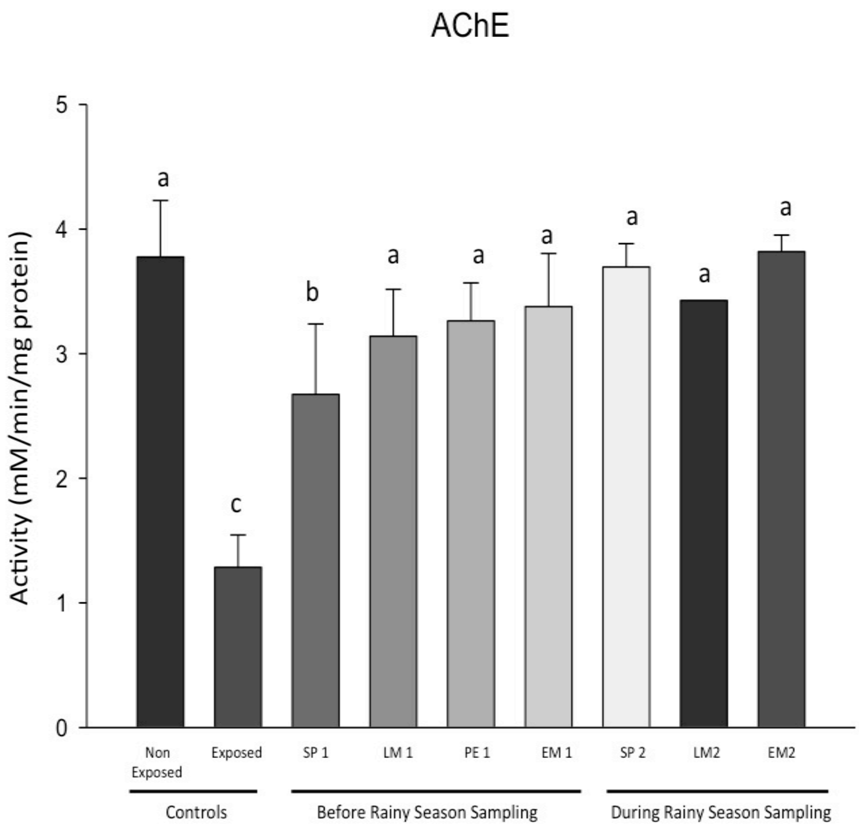
5.3 Acetylcholinesterase Activity Results and Discussion

Acetylcholinesterase Activity results of the four sampling points, before and during the rain season, are presented below.

Table 2 Acetylcholinesterase Activity in Fish found in The San Pedro Mezquital River. Activity is presented in mM/min/mg protein

Place	Activity (mM/min/mg protein)
Exposed	3.78
Non Exposed	1.29
San Pedro (Before Rainy Season)	2.67
Laguna del Mar (Before Rainy Season)	3.14
Peñitas (Before Rainy Season)	3.26
El Mezcal (Before Rainy Season)	3.38
San Pedro (During Rainy Season)	3.70
Laguna del Mar (During Rainy Season)	3.43
Peñitas (During Rainy Season)	No Data
El Mezcal (During Rainy Season)	3.82

Figure 24 Acetylcholinesterase Activity Results



Note: SP (San Pedro Ixcatán), LM (Laguna del Mar), PE (Peñitas) and EM (El Mezcal). There is not data from the Peñitas Sampling Point because it was impossible to find any fish. Letters a,b,c represents statistic equalities and differences; ex. exposed (b) and non exposed (a) are statistically different, but LM 1 (a) and SP 2 (a) are statistically equal.

Frasco *et al.;* 2002 reported that the dose of 1 mg/L of DIMETOATE was not a lethal dose for *Poecillia reticulata*; it was neither for *Poecilliopsis*.

The experiment results show a smaller impact than expected. Acetylcholinesterase activity in samplings is almost the same as the control non-exposed; this maybe because a river is not a static system, water is moving all time. Another explanation of this phenomenon is that there was not a big agricultural influence, even if this region has multiple crops fields along The San Pedro Mezquital River.

Gonzales *et al.;* 2010 reported that in spring and summer (when this project was performed) herbicides are the most sold in the region. While in autumn and winter, insecticides are the most sold. This could mean that the best season to perform this kind of studies is during autumn and winter.

6. Conclusion

According to the official Mexican NOM-127-SSA1-1994, environmental health, for human consumption and uses, water from The San Pedro Mezquital River cannot be used directly from the river and it has to pass a process to make it drinkable. Agriculture fertilizers may be the cause of the quantity of Nitrates in water.

Organophosphorus coumpounds do not seem to occur in sufficient amount to affect the acetylcholinesterase of native fishes in the genus *Poecilliopsis*. It means agriculture is not a source of organophosphurus pesticides pollution in The San Pedro Mezquital River native fishes.

This project has had important results and may offer important arguments for the implementation of future research.

7. Difficulties

Sampling points and laboratories were too far from each other; 2 hours were necessary to reach each sampling point. It is not something that can be changed, but it was really hard to transport living fish to the immunology laboratory.

There was nobody with fishing experience in the sampling team, when we did the fish samples.

Measure Acetylcholinesterase activity in the exact time, was quite difficult due to the available spectrophotometer model; it was capable to process only three lines of a microwell plate.

The second sampling campaign, during rainy season, was too hard to obtain even 30 fish, due to the increase of the water in river; there was a large amount of mud, and the river steam was strong, it made the fish collection a difficult work. Temperatures were always over 40 ºC.

Despite the interesting results of the project, it will be necessary to make deeper water analyses; such as pH, EC, or biologic pollution due to agriculture.

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9. Annexes

Annex 1. Activities Plan

	may		june				july				augost				sept	
Activity/week	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2
Team reunion (UAN)	■															
Sampling Design	■															
Acetylcholinesterace determination training		■	■													
Before rainy season Sampling		■	■													
Before rainy season water analysis				■												
Before rainy season fish analysis					■											
Controls performance and analysis						■	■									
During rainy season Sampling								■	■							
During rainy season water sampling analysis									■							
During rainy season fish sampling analysis										■						
Final Report writing											■	■				
Context, Probleme, justification (7 july)							■									
Prelecture of final report (15-25 augost)													■	■		
Final report (5 september)															■	
Oral presentation															■	■

Annex 2. Before Rainy Season Sampling Proteins Calculation

Sample	abs	Average	abs-white	Protein (mg/ml)	0.1 PROTEIN	Sample	PBS
0	0.421						
0	0.455						
0	0.432	0.4	0.0	-0.3			
1	0.584						
1	0.572						
1	0.595	0.6	0.1	3.3	29.9	30.0	120.0
2	0.602						
2	0.594						
2	0.542	0.6	0.1	3.2	30.9	31.0	119.0
3	0.545						
3	0.556						
3	0.537	0.5	0.1	2.4	41.4	41.0	109.0
4	0.563						
4	0.559						
4	0.567	0.6	0.1	2.8	35.3	35.0	115.0
5	0.554						
5	0.539						
5	0.525	0.5	0.1	2.3	44.4	44.0	106.0
6	0.547						
6	0.574						
6	0.549	0.6	0.1	2.7	37.3	37.0	113.0
7	0.528						
7	0.564						
7	0.546	0.5	0.1	2.4	41.4	41.0	109.0
8	0.581						
8	0.591						
8	0.596	0.6	0.2	3.5	28.7	29.0	121.0
9	0.556						
9	0.539						
9	0.563	0.6	0.1	2.6	38.7	39.0	111.0
10	0.575						
10	0.562						
10	0.56	0.6	0.1	2.9	34.5	34.0	116.0
11	0.576						
11	0.544						
11	0.553	0.6	0.1	2.7	37.0	37.0	113.0
12	0.558						
12	0.583						
12	0.553	0.6	0.1	2.9	34.7	35.0	115.0

Annex 3. Before Rainy Season Sampling Acetylcholinesterase Activity Calculation

Place	# Sample	ABS time (min)		TIME (average nABS)		TIME((AVERAGE nABS-white)-ABS 0')/MIN/PROT
		0	5	0	5	5
white	0	0.236	0.231			
white	0	0.244	0.237			
white	0	0.26	0.251	0.246666667	0.239666667	0
SanPedro1	1	1.811	2.828			
SanPedro1	1	1.788	2.829			
SanPedro1	1	1.78	2.765	1.793	2.807333333	2.042666667
SanPedro1	2	0.994	2.594			
SanPedro1	2	0.911	2.604			
SanPedro1	2	1.4	2.786	1.101666667	2.661333333	3.133333333
SanPedro1	3	1.419	2.809			
SanPedro1	3	0.97	2.848			
SanPedro1	3	1.834	2.811	1.407666667	2.822666667	2.844
LagMar	4	1.434	2.841			
LagMar	4	0.936	2.391			
LagMar	4	0.778	2.646	1.049333333	2.626	3.167333333
LagMar	5	0.535	2.6			
LagMar	5	0.997	2.714			
LagMar	5	1.213	2.662	0.915	2.658666667	3.501333333
LagMar	6	1.642	2.842			
LagMar	6	0.982	2.69			
LagMar	6	1.624	2.82	1.416	2.784	2.75
Peñitas	7	1.402	2.793			
Peñitas	7	1.14	2.818			
Peñitas	7	1.285	2.81	1.275666667	2.807	3.076666667
Peñitas	8	1.273	2.695			
Peñitas	8	0.95	2.622			
Peñitas	8	1.138	2.67	1.120333333	2.662333333	3.098

Peñitas	9	1.11	2.782			
Peñitas	9	0.632	2.664			
Peñitas					2.73966666	
	9	1.075	2.773	0.939	7	3.615333333
ElMezcal	10	0.885	2.78			
ElMezcal	10	0.707	2.769			
ElMezcal	10	1.051	2.84	0.881	2.796333333	3.844666667
ElMezcal	11	1.449	2.817			
ElMezcal	11	1.448	2.848			
ElMezcal	11	1.033	2.757	1.31	2.807333333	3.008666667
ElMezcal	12	1.002	2.785			
ElMezcal	12	1.172	2.8			
ElMezcal	12	1.31	2.798	1.161333333	2.794333333	3.28

Annex 4. During Rainy Season Sampling Proteins Calculation

Sample	abs	Average	abs-white	Protein (mg/ml)	.1 PROTEIN (µl)	SAMPLING(µl)	PBS(µl)
0	0.44						
0	0.401						
0	0.438	0.426333333	0	-0.306930693			
1	0.524						
1	0.526						
1	0.56	0.536666667	0.110333333	2.424092409	41.25255276	41	109
2	0.562						
2	0.56						
2	0.542	0.554666667	0.128333333	2.869636964	34.84761357	35	115
3	0.571						
3	0.559						
3	0.539	0.556333333	0.13	2.910891089	34.3537415	34	116
4	0.562						
4	0.546						
4	0.571	0.559666667	0.133333333	2.99339934	33.40683572	33	117
5	0.579						
5	0.576						
5	0.563	0.572666667	0.146333333	3.315181518	30.16426083	30	120
6	0.566						
6	0.537						
6	0.565	0.556	0.129666667	2.902640264	34.45139284	34	116
7	0.558						
7	0.565						
7	0.557	0.56	0.133666667	3.001650165	33.31500825	33	117

Annex 5. During Rainy Season Sampling Acetylcholinesterase Calculation

		ABS time (min)		TIME (average nABS)		TIME((average nABS-white)-ABS 0')/MIN/PROT
Place	**# Sample**	**0**	**5**	**0**	**5**	**5**
white	0	0.248	0.269			
white	0	0.249	0.27			
white	0	0.248	0.27	0.248333333	0.269666667	0
SanPedro2	1	0.864	2.843			
SanPedro2	1	0.871	2.814			
SanPedro2	1	0.935	2.785	0.89	2.814	3.805333333
SanPedro2	2	0.772	2.8495			
SanPedro2	2	0.893	2.911			
SanPedro2	2	1.266	2.939	0.977	2.899833333	3.803
SanPedro2	3	1.107	2.934			
SanPedro2	3	1.181	2.916			
SanPedro2	3	1.194	2.913	1.160666667	2.921	3.478
LagMar2	4	1.197	2.925			
LagMar2	4	1.099	2.798			
LagMar2	4	1.078	2.856	1.124666667	2.859666667	3.427333333
ElMezcal2	5	0.618	2.895			
ElMezcal2	5	1.128	2.969			
ElMezcal2	5	1.12	2.915	0.955333333	2.926333333	3.899333333
ElMezcal2	6	1.048	2.964			
ElMezcal2	6	1.066	2.94			
ElMezcal2	6	1.136	2.908	1.083333333	2.937333333	3.665333333
ElMezcal2	7	1.117	2.966			
ElMezcal2	7	0.904	2.886			
ElMezcal2	7	0.87	2.94	0.963666667	2.930666667	3.891333333

Annex 6. Controls proteins calculation

Sample	abs	Average	abs-white	Protein(mg/ml)	.1 PROTEIN (µl)	Sampling(µl)	PBS(µl)
0	0.439						
0	0.444						
0	0.432	0.438333333	0	-0.306930693			
1	0.555						
1	0.536						
1	0.518	0.536333333	0.098	2.118811881	47.19626168	47	103
2	0.544						
2	0.555						
2	0.534	0.544333333	0.106	2.316831683	43.16239316	43	107
3	0.548						
3	0.534						
3	0.558	0.546666667	0.108333333	2.374587459	42.11257818	42	108
4	0.557						
4	0.553						
4	0.571	0.560333333	0.122	2.712871287	36.86131387	37	113
5	0.535						
5	0.543						
5	0.523	0.533666667	0.095333333	2.052805281	48.71382637	49	101
6	0.534						
6	0.533						
6	0.535	0.534	0.095666667	2.061056106	48.51881505	49	101

Annex 7. Controls Acetylcholinesterase activity calculation

	# Sample	ABS time (min)		TIME (average nABS)		TIME ((average ABS-white)-ABS 0')/MIN/PROT
		0	5	0	5	5
white	0	0.244	0.249			
white	0	0.257	0.26			
white	0	0.272	0.273	0.257666667	0.260666667	0
CONTROL+	1	1.583	2.926			
CONTROL+	1	1.392	2.893			
CONTROL+	1	1.548	2.889	1.507666667	2.902666667	2.784
CONTROL+	2	2.055	2.888			
CONTROL+	2	2.151	2.806			
CONTROL+	2	2.233	2.933	2.146333333	2.875666667	1.452666667
CONTROL+	3	1.955	2.915			
CONTROL+	3	1.796	2.933			
CONTROL+	3	1.631	2.98	1.794	2.942666667	2.291333333
CONTROL-	4	0.572	1.664			
CONTROL-	4	0.544	1.328			
CONTROL-	4	0.541	1.322	0.552333333	1.438	1.765333333
CONTROL-	5	0.418	1.321			
CONTROL-	5	0.465	1.482			
CONTROL-	5	0.489	1.465	0.457333333	1.422666667	1.924666667
CONTROL-	6	0.393	1.25			
CONTROL-	6	0.406	1.114			
CONTROL-	6	0.385	1.359	0.394666667	1.241	1.686666667

Abstract

Any human activity can induce pollution to the environment. From this point of view, agriculture as one of the oldest human activities is also one of the most pollutants. Agriculture is the main water pollutant; it pollutes throwing pesticides, nitrates and phosphorus to water.

In Mexico river pollution is a generalized problem; but the water pollution politics do not concern pollution generated by agriculture.

This project had aimed to analyze the impact of aquatic pollution generated by agricultural practices in the river surrounded by fields and orchards. This was explored by the measure of nitrates in water samples. It was also measured via the activity of the target enzyme of organophosphorus and carbamate pesticides, namely acetylcholinesterase, in native fishes of the family Poecillidae.

The results of nitrates amount expose that agriculture has impacted throwing fertilizers. But, results expose that there is not organophosphorus pesticides blocking acetylcholinesterase activity in native fishes.