Article

Atoyac River pollution Dynamics. Case Study: Emilio Portes Gil, Puebla, México

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Abstract: The Atoyac River crosses the metropolitan area of Puebla, Mexico, and presents a condition of severe degradation that has been poorly studied. The research was conducted in the year 2016 and analyzed the space-time dynamics of the water quality of the river, the increase in pollution in the period 2011–2016, and the water quality of the Atoyac River used for agricultural irrigation and human consumption in the population of Emilio Portes Gil, Ocoyucan, based on official Mexican standards (NOMs). The anoxic state of the river was demonstrated (~1.47 mgO_2/L) and the high organic pollution, particularly in drought, as well as the presence of large populations of coliform bacteria, and 11 enterobacteries of pathogenic importance. The pollution recorded an average increase of 49% in the period 2011-2016, and the values of Fe, Al, Pb, and Cd in variable percentages. It was evidenced that water for irrigation and wells is contaminated with fecal bacteria (104–549 NMP/100 mL), including pathogenic. In wells, the concentration of heavy metals was 5 times higher in drought. These results represent a serious threat for the population of Emilio Portes Gil and the environment in the metropolitan area of Puebla.

Keywords: Atoyac River; water pollution; heavy metals; coliforms; Emilio Portes Gil; Puebla

1. Introduction

The pollution of surface water bodies is an important environmental problem in the world [1]; Mexico is not the exception [2,3,4]. Many of the central and western rivers of the country are severely affected, such is the Balsas River case [5,6], one of the most important in Mexico due to the extension of its watershed (6% of the national territory) that includes the states of Morelos (100%), Tlaxcala (75%), Puebla (55%), México (36%), Oaxaca (9%), Guerrero (63%), Michoacán (62%) and Jalisco (4%) [7]. The Balsas hydrological region is composed of 15 watersheds, and between these, the Alto Atoyac watershed, where the Atoyac River is located. The Alto Atoyac watershed comprises from where the drainings of the Atoyac River are born, until the “Manuel Ávila Camacho” dam (Valsequillo); It has an contribution area of 4,135.52 km^2, and includes part of the states of Puebla (22 municipalities) and Tlaxcala (47 municipalities) [7,8]. At present this hydrological region presents negative availability and significant deterioration in the quality of the surface currents [5,8]; in the metropolitan area of Puebla-Tlaxcala (ZMPT), the fourth largest human concentration in Mexico (~ three million inhabitants), where the Atoyac River passes in a ~ 80% of its route [10].

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The Atoyac River degradation is historical and complex. Several researchers have studied the Atoyac River water as a reference for economic, political and social development of the Puebla city in different periods of its history [11-16]. During the seventeenth, eighteenth and nineteenth centuries, the anthropogenic activity did not pollute and the rivers had the capacity to self-purification [17] they were kept in ecological equilibrium; the circulation natural cycles of currents and nutrients and migratory movements of the species were not altered. In the seventies with the installation of factories related to petrochemical, metal and automotive, joining the textile, the Atoyac River degradation begins and increases in the nineties by demographic expansion and economic development in the Region [8,18,19].

The human pressure that is generated over the Atoyac River is very strong. This river crosses or encircles 16 municipalities of the ZMPT; the river enters to San Martín Texmelucan, second most populated city of Puebla (152 051 inhabitants), where is located the Industrial corridor Quetzalcoatl [10]. In Tlaxcala, the river receives noxious substances from the industries of three industrial corridors, Quetzalcoatl, Ixtacuixtla and Huejotzingo, of the "Independencia" petrochemical complex and more than 30 companies of the maquila of Intraurban Denim [20]. Also, it receives large amounts of organic pollutants by poorly treated municipal discharges [8,21], and chemical pollutants of pesticides and fertilizers [8,20,22,23]. So there is a large heterogeneity in the region, coexisting agricultural, livestock, industrial activities and residential areas [8,24].

To the punctual and diffuse pollution of the Atoyac river, is added the pollution coming of the River Zahuapan (tributary of the Atoyac River), in whose area of influence, approximately 59% of inhabitants of Tlaxcala live [3]. The Atoyac River encircles the municipalities of Cuautlancingo, San Andrés Cholula and Ocoyucan, which comprises 35% of the total population of the Puebla state (2 140 231 inhabitants) in a territory that represents less than 3% of the state surface [10,23]. In this urban area, in addition to concentrating the highest population density, there are fourteen industrial zones that include factories of different turns [23]. In this section also discharges waters of three treatment plants of the Puebla city, which in addition to contain municipal waters, include waters of the Cuautlancingo and Camino a San Lorenzo industrial parks. Also, it receives industrial pollutants of the Atenco Stream and also the San Francisco River, which are tributaries of the Atoyac River as well as direct discharges of automotive, textile, metalworking, metallurgical, pharmachemical, food Industries, among others [21]. As a result, the Atoyac River is among the most polluted currents in the country, detecting more than 50 pollutants in the water, compounds and carcinogenic elements for the human. [8,19,21,24,25,26,27].

As a government strategy to improve the river conditions, and because of NOM-001-SEMARNAT-1996 limitations to achieve the required quality, was issued in 2011 the legal Technical Instrument "Declaration of Atoyac and Xochiac or Hueyapan classification, and its tributaries" which contains parameters that pollutants discharges must keep, the periods and water quality aims of the river [25]. Studies after the date of delivery of the same, show persistence in pollution and water high toxicity, as pointed out Arellano-Aguilar (2015), Martínez-Tavera *et al.* (2017) [28,29] and the National Monitoring Network (NMN or RNM by its initials in Spanish) 2012-2015 [6].

Many agricultural populations, nearby to the Puebla urban area, depends on the Atoyac River polluted water for irrigation of their crops, as well as Emilio Portes Gil (EPG) population in Ocoyucan. In this municipality is registered a high degree of marginalization and social backwardness, there are serious problems in the water service, and occupies the place 215 in water supply, of 217 municipalities that has the Puebla state [30]. This situation explains why the population uses water from the Atoyac River for irrigation and domestic use from particular wells and a spring. The Atoyac River water is also used for agricultural purposes in Atlixco, Izúcar de Matamoros and some municipalities of Tlaxcala as well as in the region of Tecamachalco-Tehuacán (17 municipalities) that use the Atoyac river water reserves (Valsequillo dam) for the irrigation of 21 322 ha (17 municipalities) [8,31,32,33,34,35].

For the above, the work purpose is to evaluate the pollution spatial-temporal dynamics of the Atoyac River, in the Puebla city, in rainy season and drought, during 2016, to determine the increase of the pollution in the region, based on information of the Declaration of Atoyac and Xochiac or

Hueyapan classification, and its tributaries (2011), as well as, analyze the water quality for agricultural irrigation and domestic use in the population of EPG based on the official Mexican standards (NOMs).

2. Study area

The Atoyac River belongs to the Alto Atoyac watershed, Balsas River sub-watershed [7]. It originates from the Iztaccíhuatl volcano thaw, in the state of Puebla, at an altitude of 3250 meters above sea level; it has a length of 84.97 km, a cross section between 15 and 60 meters, and a depth of 1 to 3 meters [29]. In its descent, the current passes through the state of Puebla (~ 30 km of travel), Tlaxcala (~ 22 km), and again through Puebla (~ 32 km) where it is accumulated in the Valsequillo dam [25, 29]. The climate of the region is temperate subhumid with rain in summer [23]. The average annual temperature and rainfall are 17.2 ° C and 969.1 mm and rainfall increases between June and October [36].

The study area has an approximate length of 25 km; starts in north-west of the Puebla municipality, in the Covadonga dam (Z1) (19° 08' 15.3'' N and 098° 13'27.6''W), continues at the Echeverría Dam (Z2) (19 °) 01'07.6 "N and 098 ° 13'58.0" W) and ends in the irrigation channel located in the town of EPG (Z3). The neighboring municipalities are: Puebla to the east, and to the west Cuatlancingo, San Andrés Cholula and Ocoyucan. The Atoyac River, in this area, receives discharges from three macro-plants of the Puebla municipality (El Conde, San Francisco, and Atoyac Sur) and the tributaries of the Atenco and Rabanillo streams that transport discharges from textile and spinning industries, and the San Francisco and Zapatero Rivers (Fig.1). In its margins there are 14 industrial zones, which discharge their waters directly and indirectly into the channel [21].

The EPG town belongs to the Ocoyucan municipality. It is an ejidal colony of 522 inhabitants, its main economic activity is the agriculture [37]. The Atoyac River water, flows through a channel (6.39 km) and a tunnel (4.72 km) to the Portezuelo I hydroelectric plant, where it generates electricity, and then joins to the EPG main irrigation channel (18 ° 55'32.4 "N098 ° 20'40.4 "W) and finally discharge to the Nexapa River [33]. Homes, mostly (> 90%), have piped water [37] from a spring, and as the supply is not periodic, they have wells (Figure 1).

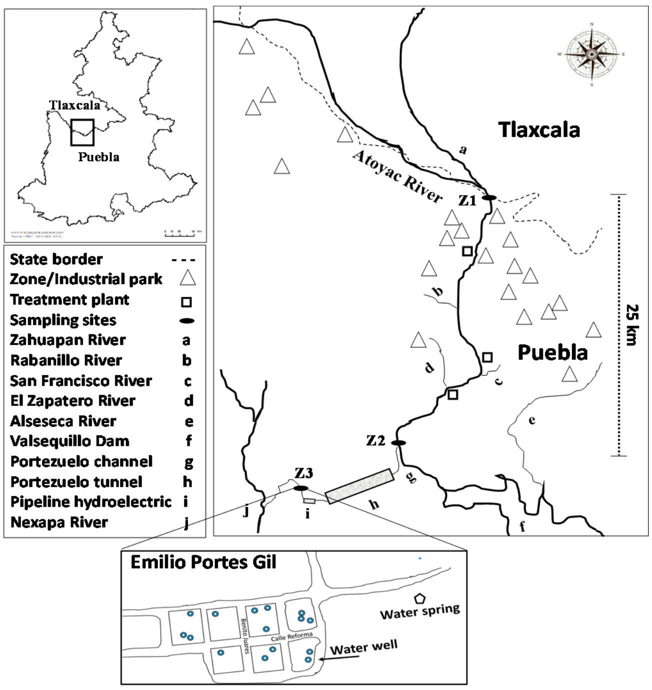

Figure 1. Study area map. Z1: Covadonga dam, Z2: Echeverría dam, Z3: irrigation channel in EPG.

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3. Materials and Methods

During year 2016, samplings were carried out, in the rainy season (June, August, October) and drought (May, November), in three zones: Covadonga Dam (Z1), Echeverría Dam (Z2), and irrigation canal in E Portes Gil (Z3) (Figure 1).

The methodology used for the water analyzes was based on APHA-AWWA standard methods [38]. In the field, the surface water temperature, pH (thermometer and field pH meter), and dissolved oxygen (Winkler) were measured in triplicate. Water samples were collected in triplicate for the analysis of parameters: DQO, DBO_5, bacteria, turbidity (TURB), color and heavy metals (Al, Fe, Cu, Pb, Cd, Zn, Co, Ni, Cr). In the laboratory, DQO was determined with a Spectroquant kit (range 25-1500 mg/L), and a Nova 60 Spectroquant Merck photometer, which also measured turbidity and color. For the DBO5 determination was used an OxiTop Control OC 100 to measure oxygen consumption on fifth day. The heavy metal analysis was carried out using an Atomic Absorption Spectrophotometer of double ray flame "Varian 55B"and hydride generator (VGA 77), and a coupled digester stove (Mars press) as indicated by NMX-AA-051-SCFI- 2001 [39]. The detection and coliform bacteria counting was carried out using the Most Likely Number (MLN or NMP by its initials in Spanish) in multiple tubes according to the NMX-AA-042-SCFI-2015 method [40]. In the presumptive and confirmatory test of Total Coliforms (TC), the lactose culture medium and bright green bile were used and for the confirmatory test of Fecal Coliforms (FC), EC-MUG was used like a selective medium. In the aerobic mesophilic bacteria counting, the trypticase and soy agar (TSA) cultivation medium was used. Bacteria taxonomic identification was carried out by biochemical tests for the API 20E Biomerieux^R system.

In the E. Portes Gil locality, physical-chemical and bacteriological parameters previously mentioned were determined, except DBO_5 and DQO, in collected water samples of 15 wells and a spring. Wells selection was random (three wells per month).

In the study, a total of 1230 physical-chemical analyzes were carry out, and 555 analyzes corresponding to the bacteria study (TC, FC, mesophilic bacteria, API, and complementary tests). An inquiry per house (110 total) was applied to gather information on water supply and sanitary measures.

To data obtained, the Kruskal-Wallis test, Tamhane's multiple comparison test was applied. In addition, Principal Component Analysis (PCA) and correlation matrix (Kendall coefficient) were carry out using Statgraphics Centuriun software and IBM SPSS statistics. Values obtained were compared with the Declaration of Atoyac and Xochiac or Hueyapan classification, and its tributaries [25] based on the (NOMs) CE-CCA-001/89 [41], NOM-127-SSA1-94-Modification (2000) [42] and NOM 001-SEMARNAT-1996 [43]. Monthly rainfall data were obtained from the National Meteorological Service website [36] (SMN, Servicio Metereológico Nacional, 2016), and data from DBO_5, DQO, and faecal coliforms from the RNM-CONAGUA (2016) [44]. To compare between official declaration records of the Rio Atoyac (2011), and the present study (2016), declaration data are expressed in mg/L.

3. Results

3.1. River pollution during the dry-rainy season

Hydrological characteristics of the river, showed marked variations between drought and rain (Figure 2 and Table 1). The organic contamination measured through the DQO, doubled in the dry season (260.60 mg/L), achieve values that classify the water body as "highly contaminated" (DQO> 200 mg / L), according to the Classification Scale of Water Quality (ECCA, by its initials in Spanish) from CONAGUA (Comisión Nacional del Agua) [45]. The other two pollution indicators, DBO_5 and Total Coliforms (TC), also registered an increases in drought, around 50%. The Principal Components Analysis (PCA) (Figure 3) shows the positive relationship between these three indicators (DQO, DBO_5, and TC), as well as the inverse relationship between these, with respect to the variable Precipitation.

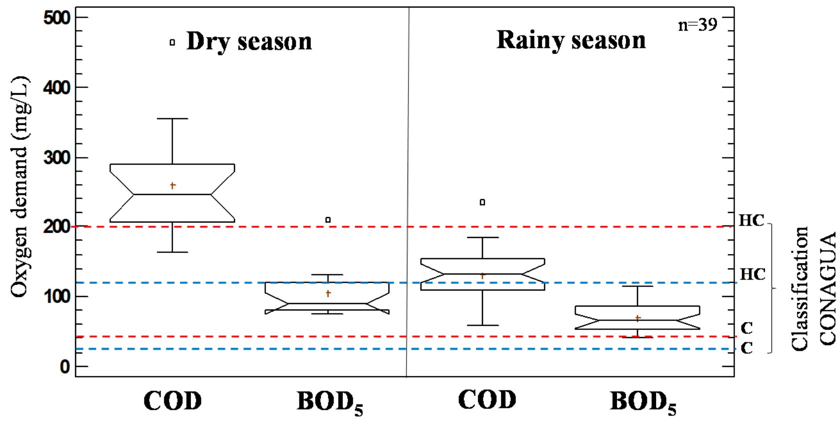

Figure 2. Average values of COD and BOD_5 in the Atoyac River, in dry and rainy season. *HC=Highly Contaminated, C=Contaminated. Limits for COD in red lines, and BOD_5 blue lines.

A marked seasonality was also observed with other physical-chemical parameters. More murky, colored, and relatively more oxygenated waters were recorded in rain (Table 2), at which time the river is more abundant and turbulent. Three variables (color, turbidity, O_2) were positively associated, but only turbidity and color registered a significant correlation. Although O_2 concentrations in surface waters showed relatively higher values in rain (up to 3 mgO_2/L), the average was lower than 2 mg/L and is below the Maximum Permissible Limit (MPL) for the Aquatic Life Protection (ALP) (5 mg/L) according to EC-CCA-001/89 [41]. Aerobic Mesophilic Bacteria (AMB) correlated positively with turbidity, color and O_2, coinciding with their peaks in rain (Table 2). The Atoyac River water was characterized by its alkalinity (pH ~ 8) and temperatures that fluctuate, more in dry season, around 20°C. Positive correlation was recorded between the pH and TC values.

In heavy metals terms, Fe and Al recorded values higher than the MPL for the Aquatic Life Protection (ALP) (MPL: Al = 0.05 mg/L and Fe = 1 mg/L), according to CE-CCA- 001/89, in drought and rain, and for agricultural irrigation (AI) (MPL Fe = 5 mg / L) in the Fe case in the rainy season (~ 5.92mg/L). The Cd and Ni show significantly higher values in drought, but these, like metals rest, did not exceed the MPL according to the NOMs. Based on the PCA, the Cd and Ni were positively related to Cr and Zn, showing an increasing trend in the drought season. The same was observed with Al, Fe and Pb in rain.

Table 1. Descriptives statistics and P-values of the Kruskal-Wallis test in rainy and dry season; Atoyac River.

Parameters	Rainy season			Dry season			K.Wallis
	Mean	Min-Max	SD	Mean	Min-Max	SD	P-value
TURB (UNT)	234.41	106 – 460	115.50	102.20	59 – 136	26.70	0.00*
Color (1/m)	35.42	16.40 – 65	14.70	19.40	14.30 – 24.20	3.80	0.00*
T (°C)	20.25	19 – 22	0.97	19.94	17.8 – 23	2.21	0.29
pH	8.02	7.60 – 8.40	0.21	8.21	7.80 – 8.70	0.28	0.00*
O_2 (mg/L)	1.47	0 – 3.80	1.40	0.27	0 – 1.90	0.60	0.05*
BOD_5 (mg/L)	69.79	40 – 115	22.52	105.00	75 – 210	34.89	0.00*
COD (mg/L)	130.91	58 – 234	39.52	260.60	163 – 464	75.42	0.00*
CT	246 868	44 497 – 6.00×10^5	1.8×10^5	357 539	83 557 – 6.00×10^5	2.0×10^5	0.10

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AMB	$1.08x10^8$	$2.4x10^7 – 4.09x10^8$	$1.5x10^8$	$2.96x10^7$	$6.0x10^6 – 6.56x10^7$	$8.6x10^7$	0.07
Al (mg/L)	4.807	0 – 21.16	7.13	2.131	0 – 20.72	5.21	0.50
Fe (mg/L)	5.928	0.77 – 16.5	4.84	3.388	0.93 – 16.5	4.19	0.00*
Zn (mg/L)	0.056	0 – 0.156	0.04	0.067	0 – 0.133	0.03	0.16
Ni (mg/L)	0.007	0 – 0.056	0.01	0.023	0 – 0.1	0.027	0.03*
Pb (mg/L)	0.019	0 – 0.07	0.02	0.008	0 – 0.03	0.009	0.28
Cr (mg/L)	0.015	0 – 0.067	0.022	0.028	0 – 0.178	0.046	0.26
Cu (mg/L)	0.017	0 – 0.089	0.020	0.014	0 – 0.067	0.022	0.38
Cd (mg/L)	0.000	-	-	0.004	0 – 0.011	0.005	0.00*

n=39; SD= Standard desviation/ *$p<0.05$ significant differences between seasons. CT, CF in NMP/100mL y BMA in UFC/100mL.

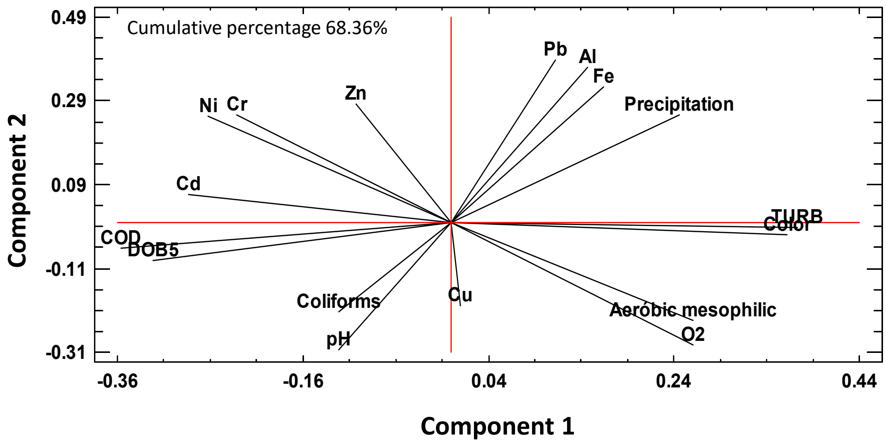

Figure 3. Principal Component Analysis considering the physical, chemical, bacteriologic variables of Atoyac River, and level of the precipitation for 2016.

Table 2. Correlation matrix of physicochemical and bacteriologic parameters in Atoyac River (Kendall Rank).

(n=39)	DBO_5	DQO	O_2	TURB	Color	BMA	CT	pH	T°C	Al	Fe	Cr	Pb	Ni	Zn	Cd	Cu
DBO_5	1.00																
DQO	0.73*	1.00															
O_2	0.06	-0.20	1.00														
TURB	-0.33	-0.60	0.60	1.00													
Color	-0.33	-0.60	0.60	1.00*	1.00												
BMA	-0.20	-0.46	0.73*	0.86*	0.86*	1.00											
CT	0.20	0.20	0.33	0.20	0.20	0.33	1.00										
pH	0.20	0.20	0.06	-0.06	-0.06	0.06	0.73*	1.00									
T°C	0.33	0.33	0.20	0.06	0.06	0.20	0.33	0.06	1.00								
Al	-0.33	-0.33	0.06	0.46	0.46	0.33	-0.06	-0.33	0.33	1.00							
Fe	0.06	0.06	-0.06	0.06	0.06	0.20	0.06	-0.20	0.73*	0.60	1.00						
Cr	0.46	0.46	-0.20	-0.33	-0.33	-0.20	0.20	-0.06	0.60	0.20	0.60	1.00					
Pb	0.00	0.00	-0.13	0.27	0.27	0.13	-0.27	-0.55	0.41	0.41	0.55	0.41	1.00				
Ni	0.07	0.35	-0.50	-0.35	-0.35	-0.21	0.21	-0.07	0.21	0.21	0.50	0.64	0.29	1.00			
Zn	0.06	0.06	-0.06	0.06	0.06	0.20	0.33	0.06	0.46	0.60	0.73*	0.60	0.27	0.64	1.00		
Cd	0.18	0.36	-0.73	-0.73	-0.73	-0.73	-0.18	0.00	-0.36	-0.18	-0.18	0.18	-0.28	0.49	0.00	1.00	
Cu	0.07	-0.07	0.50	0.35	0.35	0.50	0.78*	0.50	0.21	0.21	0.07	0.21	-0.37	0.07	0.35	-0.19	1.00

*Significant correlation ($p<0.05$)

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3.2. Pollution in the sampling zones

Table 3 shows the increase pattern in DBO_5 and DQO organic contamination and bacteriological from the Covadonga Dam (Z1) to EPG (Z3); however, spatial differences or between sampling zones were not significant, which indicates that the flow is contaminated in all its extension, including the tributary that is diverted to EPG. In the study, average TC populations were recorded between $1.86x10^5$ to $3.02x10^5$ NMP/100 mL, achieve up to $2.6x10^5$ NMP/100 mL of fecal coliforms (FC), a figure that substantially exceeds the MPL for agricultural irrigation (<1000 NMP/100 mL) according to CE-CCA-001/89. We identified 11 species of gram-negative opportunistic pathogenic bacteria, belonging to families: 1) Enterobacteriaceae (*Morganella morganii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Hafnia alvei*, *Kluyvera* sp., *Pantoea* sp.), 2) Pseudomonadaceae (*Pseudomonas aeruginosa*, *Pseudomonas* sp.), and 3) Xanthomonadaceae (*Stenotrophomona maltophilia*).

Table 3. Descriptives statistics and P-values of the Kruskal-Wallis test between sampling sites; Atoyac River.

Parameters	Zone 1 Covadonga Dam	Zone 2 Echeverría Dam	Zone 3 Irrigation channel	K.Wallis	Tamhane-test
	Mean	Mean	Mean	P-Value	Differences
TURB (UNT)	151.06	190.20	203.66	0.172	-
Color (1/m)	25.23	29.79	32.27	0.053	-
T (°C)	19.17	20.97	20.26	0.004*	Z1≠Z2,Z3
pH	8.04	7.98	8.14	0.000*	Z3≠Z2,Z1
O_2 (mg/L)	0.52	0.49	2.32	0.000*	Z3≠Z2,Z1
BOD_5 (mg/L)	87.00	78.66	94.58	0.128	-
COD (mg/L)	179.33	180.60	215.5	0.429	-
CT	$1.86x10^5$	$2.84x10^5$	$3.09x10^5$	0.092	-
AMB	$3.03x10^7$	$6.69x10^7$	$8.05x10^7$	0.031*	Z3≠Z2,Z1
Al (mg/L)	1.11	7.73	1.62	0.039*	Z2≠Z1,Z3
Fe (mg/L)	2.96	7.50	4.00	0.128	-
Zn (mg/L)	0.038	0.088	0.050	0.007*	Z2≠Z1,Z3
Ni (mg/L)	0.006	0.025	0.005	0.029*	Z2≠Z1,Z3
Pb (mg/L)	0.008	0.028	0.003	0.004*	Z2≠Z1,Z3
Cr (mg/L)	0.013	0.033	0.011	0.081	-
Cu (mg/L)	0.006	0.019	0.026	0.063	-
Cd (mg/L)	0.002	0.002	0.000	0.354	-

n=39; *p<0.05 Significant differences between sampling sites. CT, CF in NMP/100mL and BMA in UFC/100mL.

It is observed that turbidity, color, pH, O_2 and BMA, show an increase from Z1 to Z3, and the increase in Z3, for pH, O_2 and BMA, is statistically significant. The pH, oscillated around 8 units and indicates the water basic condition. The surface water temperature registered lower values in the Covadonga Dam Z1 (19.17 °C), with respect to zones, Z2 and Z3. Of the eight metals that were detected in the water, six had higher concentrations in the Z2 (Echeverría Dam), for Al, Zn, Ni and Pb, with increases of 468%, 100%, 355% and 409% respectively, the Al exceeded the MPL for the ALP (Aquatic Life Protection) and RA according to CE-CCA-001/89 (Table 3).

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3.3. Water quality in the EPG population

EPG houses, have a domestic water supply that comes from the spring at 1940 meters above sea level. Inhabitants also obtain water from wells (70% of houses). Wells have a depth of 10 to 20 m, the water temperature varies between 20-25 ° C, pH between 7-7.8, and O_2 between 2-4.5 mg/L. 88% of houses do not apply treatment for the water purification from wells, which is used in the food preparation and personal hygiene. Half of houses boil the water. TC densities in Wells, ranged around 1000 NMP/100mL (Table 4) and bacterial species were identified from the Enterobacteriaceae family (*Serratia fonticola*, *Citrobacter freundii*, *Citrobacter braakii*, *Enterobacter* sp., *Enterobacter cloacae*, *Enterobacter amnigenus*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella oxytoca* and *Klebsiella pneumoniae*), and of the family Aeromonadaceae (*Aeromonas hydrophila*, and *Aeromonas* sp.).

Table 4. Average values, P-values of the Kruskal-Wallis test between rainy an dry season, and reference values; Water well and Water spring.

Parameters	Waterwell (n=15)			Waterspring (n=1)			NOM-127[+]	CE
	Rainy	Dry	P-Value	Rainy	Dry	P-Value		
							-	-
T (°C)	20.70	22.56	0.001*	21.33	23	0.006*		
pH	7.30	7.6	0.000*	7.20	7.37	0.240	6.5 – 8.5	5 - 9
O_2 (mg/L)	2.98	3.42	0.181	2.00	1.83	0.108	-	4
CT	1378	1078	0.290	16	0	0.121	2	-
CF	549	104	0.308	15	0	0.317	No D	1000
AMB	114734	57067	0.404	314	374	0.486	-	-
Al (mg/L)	0.016	0.108	0.150	0	0.014	0.102	0.20	0.02
Fe (mg/L)	0.107	0.682	0.010*	0	0.418	0.001*	0.3	0.3
Zn (mg/L)	0.004	0.033	0.009*	0	0.022	0.001*	5	5
Ni (mg/L)	0.003	0.024	0.019*	0	0.003	0.102	-	0.01
Pb (mg/L)	0.000	0.012	0.000*	0.003	0.013	0.142	0.01	0.05
Cr (mg/L)	0.001	0.002	0.721	0.002	0.011	0.363	0.050	0.05
Cu (mg/L)	0.001	0.030	0.000*	0	0.036	0.001*	2	1

n=35; *$p<0.05$ Significant differences between seasons. CT, CF in NMP/100mL and AMB in UFC/100mL. [+]NOM-127 (Modified in DOF 2000).

In the spring water, FC were detected only in August, and the presence of two bacterial species reported as opportunistic pathogens, *Citrobacter freundii* and *Serratia ficaria*. Mesophilic bacteria were detected (314-374 CFU/100mL). No significant differences were observed in relation to bacterial densities between wells of the dwellings, according to their spatial location, or between the drought season and rainy season.

It was detected in wells and spring, the presence of 7 heavy metals from 9 evaluated. In wells in drought season, concentrations higher than 200% were registered with respect to the rainy season, being statistically significant, Fe, Zn, Ni and Pb values. In the drought season, average concentrations of Fe and Ni doubled the MPL for human consumption (0.02 mg/L) according to CE-CCA-001/89, while the Al (0.108 mg/L) exceeded more than 4 times to the MPL (0.02 mg/L). The Pb registered average values, slightly higher (0.012 mg/L) to the MPL for human use and consumption according to NOM-127-Modified 2000 (MPL = 0.01 mg/L); in some of samples, concentrations were measured up to 0.052 mg/L, that is, five times higher than the MPL. In the rest of metals (Zn, Cu, Cr) concentrations were kept below the MPL. Regarding the spatial comparison,

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no significant differences were registered between wells, in relation to metals concentration, or rest of parameters.

At the spring, the same pattern of increase in the drought season was observed for all detected metals, being significant for Cu, Fe and Zn. The Fe in the spring, registered concentrations higher than the MPL, like the Pb, particularly in drought, according to the NOMs.

4. Discussion

A the study demonstrates the marked seasonality in Atoyac river hydrological conditions: increase in Ni, pH, Cd, DBO_5 and DQO levels in the drought season, and in rainfall, in turbidity, color, Fe and O_2. In Martínez-Tavera *et al.* (2017) [29] the same trend is reported with respect to the parameters DQO, DBO_5 and O_2, for 2013-2014 period in the Atoyac River; that is, higher values of DQO and DBO in drought, and O_2 in rain. Results indicate that organic pollutants measured through the DQO and DBO_5, are more concentrated in the low precipitation season, possibly due to the decrease in the river flow, which obeys a typical behavior for this type of ecosystem where changes in precipitation and flow affect values of many physical-chemical parameters of water by the dilution and turbulence effect [29,35,46,47]. In Silva-Gómez *et al.* (2002) [35], Atlixco rivers, registered DBO and DQO higher concentrations in drought, and registered higher values of turbidity, pH and Fe in rains, behavior that coincides with this investigation; However, Fe values recorded by these authors oscillated around 0.10 mg/L, a much lower value than the obtained in the present study (5.92 mg/L), which suggests that there is a greater discharge of chemical elements in the urban area [48].

It is confirmed that the Atoyac River in the Puebla metropolitan zone, presents a high deterioration degree, based on the detected organic and bacteriological contamination, as well as the surface water anoxic condition. With the current O_2 levels (~ 1.47mg / L), the development or survival of aquatic fauna is not possible, except for anaerobic or facultative bacteria, or with low O_2 requirements, since values lower than 5 mg/L they generate an adverse effect in fish and other aquatic organisms [49,50]. According to Huang *et al.* (2017) [51] the dissolved oxygen decrease in urban rivers is increasing in developing countries, which causes strong stresses in aquatic ecosystems. Decrease is due to the bacterial decomposition process in presence of a large number of organic compounds [46,49,52]. In the Atoyac River case, in the Puebla metropolitan area, these come mainly from discharges of three municipal treatment macro-plants (El Conde, San Francisco, and Atoyac Sur), and effluents of hundreds of industries of different turns (automotive, pharmaco-chemistry, metalworking, food, paint, etc.) that do not have efficient purification systems [21,22]. The organic matter also comes from municipal waters that has not been subjected to treatment due to hydraulic infrastructure lack, which is discharged directly into the river, or ravines [8,19,20,21,53,54]. To the organic load, in this river section, is added the one coming from Tlaxcala and Puebla municipalities, located in the watershed upper area, where the Quetzalcoatl industrial park is located, which has been criticized and linked to problems of health by different NGOs [53] as well as industrial corridors of Ixtacuixtla, Huejotzingo, the independent Petrochemical Complex, and municipal sewage networks waters, which also receive domestic effluents and sewage from laundries denim [53,55].

When comparing the Atoyac River with other urban rivers of other countries, that flow by metropolitan zones [56-63] it is observed that the Atoyac River contamination in Puebla city is severe, in DQO, DBO_5, TC, FC terms and the highly pathogenic bacteria presence, which represents a public health threat, taking into account that water resources are used to irrigate crops. This suggests that the typical self-cleaning process of these ecosystems (dilution and assimilation) does not occur, possibly due to the residual effluents amount that are discharged along the river that exceeds the natural buffering capacity of the aquatic ecosystem [52,64], so the oxygen concentration in the water remains low, independently of the river area or year season. Although seemingly, sewage is not discharged into the Echeverría-EPG river section, the water does not present a better quality in the irrigation channel, probably due to the fact that the deviated effluent, flows through a channel covered in concrete. In opposite case to the one reported by the FAO (2013) [65], which

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indicates that the water quality for irrigation in Tula Valley, coming from Mexico City, improves in its route due to the fact that the canal is not covered, that favors the natural processes of organic matter degradation.

The water contamination by fecal bacteria, for irrigation, has been detected in varied studies [35,66,67]. TC high amounts and Enterobacteria presence in the river and irrigation canal, manifest the deficiency in municipal waters treatment, since many of identified bacteria are part of the human intestinal flora [68,69], and enter in aquatic system through municipal discharges mainly. Rodríguez and Morales (2014) [8] point out that, since 2010 there is insufficiency in the municipal plants capacity, because the population growth and the wastewaters amount that is generated. The situation regarding to the municipal waters treatment is critical in all of Mexico; which explains why high loads of bacteria coliform are a constant in Mexican rivers that transit through some rural-urban zone [3,48,70]. According to the Statistics and Geography National Institute of Mexico [54], of 217 municipalities that make up the state of Puebla, only 34 have a treatment process applied to the municipal wastewater, the rest (183) pour their waters to the river, lake, dam, or canyon, without any treatment.

Regarding to heavy metals, spatial differences were observed in their concentration; registering a significant increase of more than 100% in the Echeverría zone (Z2) of Al, Zn, Ni, Pb, which is explained by the great variety of effluents that are discharged in the Covadonga-Echeverría section, especially considering that 50% of the total flow that is discharged directly to the Atoyac River comes from the San Francisco treatment plant, that discharges its waters to 7 km from the Echeverría Dam [21]. In this section, they also unload two other municipal plants (Barranca del Conde, and Atoyac Sur) and directly and indirectly 14 industrial zones [21, 25, 71, 72], which are heavy metals sources and other chemical compounds [21].

In view of this situation, since year 2011 it had been indicated in the river classification declaration [25], that the water body did not admit additional contaminants load. Five years later, the condition is even more serious. It is shown that the concentration of DQO, DBO_5, TC, Fe, Al, Pb, Cd, Zn and Cr is higher in 2016 (in all or some of sampling stations) compared to the value calculated in 2011 Also, it is confirmed that 5 of these parameters (DQO, DBO_5, TC, Fe and Al) show values well above the river quality goals that had been set for 2015 for the corresponding section, surpassing the assimilation capacity and dilution of the river, for them. When comparing the values of DBO_5 and DQO of 2011 with those obtained in 2016, by both the National Monitoring Network (NMN) of the government [6], and the study, it is highlighted that the organic pollution measured through the DBO5 it remained practically constant through time (<3%), while the increase in DQO was relatively higher, between 22% (study) and 71% (NMN). These results suggest that, to date, larger amounts of wastewater are discharged without adequate treatment, particularly those of industrial nature (given the minimum increase in DBO_5), loaded with organic matter that is not very biodegradable. Also recorded increases for the 2011-2016 period in the Al concentration of 885%, and in the Fe concentration above 1000%, exceeding the MPL for the aquatic life protection (CE-CCA-001/89), like the MPL for agricultural irrigation in the Fe case in rains. Although these chemical elements are found in nature, high levels are a consequence of minerals processing, metal parts production, alloys, etc. [21]. Al is also used as a constituent of antacids, astringent, aspirin with enteric coating, food additives, deodorants, etc. [73] The rest of metals did not exceed the MPL, however, there is an increase of 5% in the Pb and 815% in the Cd in 2011-2016 period, which indicates the additional contribution of the same, through time.

The DQO and heavy metals increase can be related to industrial growth in the region, since the number of manufacturing factories increased by 35.7% from 2009 to 2016 [71,72]. Bonilla *et al*. (2015) [32] point out that industrial development and sanitation lack in the region have caused pollution problems in recent years. Currently, there are 45 143 manufacturing industries [71] and only 67 (at 2015) private treatment plants that do not include tertiary treatment processes [23], the process where specific pollutants, usually toxic or non-biodegradable compounds, are removed, including the removal of contaminants not extracted in the secondary treatment [74]. Although there are no updated records at date, IMTA in year 2005 [21] reported that 78% of industries did not comply with NOM-001-SEMARNAT-1996 provisions for some of basic contaminants such as heavy metals. At the

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national level, 49% of municipal wastewater is treated, and only 14% of pollutants load from industrial discharges is removed [5], which explains the chemical contaminants presence in water bodies, such as heavy metals, as well as bacteriological contaminants from municipal wastewater.

The trend at national level shows a similar pattern in terms of increase in the contamination degree of water bodies, since, monitored stations number by the NMN in 2012-2014 period classified like a contaminated and heavily contaminated, according to the DQO and DBO_5 increased 33%, concentrating on 4 hydrological regions, including the Balsas river (IV), whose watershed contains the Atoyac River [75].

According to Casiano *et al.* (2016) [53] discharges and water quality standards, established by rivers classification are still far from being achieved, which is evidenced in the present study. In coliforms fecal case, the MPL in the declaration is 200 NMP/100 mL and the aim for 2012-2015 was <1000 NMP/100mL; however, current FC populations are very large, with 265 631 NMP/100 mL FC values in the study, and in the NRN densities> 24 000 NMP/mL for year 2016. In terms of O_2 dissolved in the water, it should be noted that the declaration does not include this parameter, however, the O_2 data recorded in year 2007 by Sandoval *et al.* (2009) [10], indicate that to that date the O_2 concentration had higher values (5.9-6.1mg/L) in located stations in the same section, while in the evaluation conducted by Martínez-Tavera *et al.* (2017) [29] in 2014 and the present, average values <2 mg/L are recorded, which is a river affectation manifestation.

Among associated problems with the Atoyac River contamination is the water quality deterioration in wells and supply sources due to the pollutants infiltration into groundwater bodies [8]. It is likely that this infiltration process is occurring in EPG. Evidence of this is wells contamination by fecal bacteria, the presence of Fe, Al and Pb high levels, with respect to the NOM, and increases in heavy metals values of groundwater in drought, in whose season the water requirement for irrigation, coming from the Atoyac River, is higher. In addition to these indicatives, the wells depth of EPG (10-20 m) shows that it is an aquifer or also known as the water table (underground water), which are more exposed to contamination [76].

The aquifers affectation is a growing problem in Mexico. The Mezquital Valley aquifer is the most emblematic case, since the metropolitan area of Mexico City, discharges ~ 60 m3 / s of without processing wastewater in the valley, and a substantial part of this, infiltrates the regional aquifer [66,77,78,79]. In a study by Lesser-Carillo *et al*. (2011) [66] it was found that the main water input to the Mezquital Valley aquifer, corresponds to the untreated wastewater infiltration from Mexico City, through irrigation channels and returns, which indicates that the aquifer recharge is made with part of the wastewater that lead irrigation channels, registering in 40% of wells contamination by FC, in concentrations by the order of 8 NMP/100mL, notably lower than those estimated in this study (~ 438 NMP/100mL). The wells contamination in the Mezquital Valley due to the residual water infiltration, is manifested through the fecal bacteria presence and there is no increase in heavy metals, however in EPG, the Fe, Al and Pb, present higher values than the MPL for human consumption.

In 2016, technical studies results of national subterranean waters of the Mezquital Valley aquifer, Mexico Valley waters, Hydrological-Administrative Region, were published in the Official Gazette [78], as follows: "with the intensive use of wastewater from Mexico city and its conurbated areas, which are used for irrigation, the aquifer receives a very significant induced recharge of wastewater, which has caused chemical and bacteriological pollution problems in the groundwater". This situation can be presented in EPG, since all wells evaluated showed a high presence of mesophilic aerobic bacteria ($1x10^5$ CFU/100mL ± $1.14x10^5$) and faecal contamination to a lesser or greater degree with the presence of pathogenic important species [68,69,80] as: *E.coli*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae*, which represents a threat to the population, considering that the water used for human consumption and personal hygiene must be free of pathogenic microorganisms [69], and bacteria were isolated in wells that produce infections in the central nervous system, digestive (*Escherichia*), in the lower respiratory tract, bloodstream and in the urinary tract (*Klebsiella*, *Enterobacter*, *Escherichia*), and in 88% of houses, they do not perform the chlorination of wells.

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In Silva-Gómez *et al.*(2002) [35] the bacteriological pollution of all wells and springs evaluated in the Atlixco region is demonstrated, with FC densities in the order of 60 NMP/mL in wells, while in springs, comparable densities to the irrigation channel, authors indicate, that the pollution is due to the contaminated water filtration, towards them. Authors also record Al values higher than the MPL for human consumption in a well near the Nexapa River, in 0.23 mg/L concentrations. Al and Fe high values, and in general of other metals in water sources of this region, can be explained by the presence of these in the soils, since according to Méndez-García *et al.* (2000) [81], and Méndez-García *et al.* (1997) [82], the accumulation of heavy metals in soils has increased due to irrigation with increasingly polluted waters from the Atoyac River; these soils have been subjected to irrigation with water from this river, for more than 30 years. Méndez-García *et al.* (2000) [81] mention that the Fe concentration in soil stands out in parcels irrigated with waste water from channels.

The low microbiological quality of rural water is an extended problem in developing countries; and in Mexico its magnitude is uncertain [68]. More and more studies show pollution evidence by chemical components of wells water in the country [83,84,85], as well as fecal bacteria [68,86,87,88,89]. The EPG situation is not favorable; water pollution affects the population well-being, well the pollutants exposure includes at least two routes: water intake and contaminated food, increasing the acquiring diseases probability.

5. Conclusions

The Atoyac River in the Puebla metropolitan area is severely affected and the water does not accomplish with Aquatic Life Protection standards and for agricultural irrigation according to CE-CCA-001/89 and NOM 001-SEMARNAT-1996. The deterioration is caused by the dumping of huge amounts of wastewater poorly treated. The aquatic system shows a marked seasonality in water quality and in the pollutants concentration. Results indicate an increase in the Atoyac River pollution in last 5 years, which is transferred to the irrigation channel in the EPG town, and possibly, of these, toward wells by infiltration. The pollution problem is serious and complex, and deserves attention, because health threats increase in correspondence with industrial and municipal pollution.

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