PATHOGENS AND PHARMACEUTICALS POLLUTANTS AS INDICATORS OF CONTAMINATION AT THE NORTHEASTHERN AQUIFER OF QUINTANA ROO

[PRESENCIA DE PRODUCTOS FARMACÉUTICOS Y PATÓGENOS POTENCIALES EN LA PARTE NORESTE DEL ACUÍFERO DE QUINTANA ROO]

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SUMMARY

The objective of this research project was to sample groundwater from touristic to non-touristic wells and sinkholes along the Riviera Maya region, to determine the occurrence, source and extent of the fecal contamination, and establish if the recreational activities of this zone is rising as an anthropogenic contamination source. *Escherichia coli* (*E. coli*) is one of the most common bio-indicators to asses the bacteriological water quality its presence is related with pathogenic health problems associated to anthropogenic influence such as leaks from septic tanks, and raw wastewater. Despite the limitations and problems associated with the detection of fecal bacteria, this bio-indicator is still present in several water policies around the world. The study zone was located at Puerto Morelos Quintana Roo, where seven groundwater samples were collected from sinkholes and wells. Bacteriological analysis were performed by the chromogenic substrate technique to determine total coliforms and *E. coli*, molecular microbiology technique allowed for identification of *Vibrio* spp., and chromatography method was performed to determine the presence or absence of caffeine. At the field, pH, dissolved oxygen and temperature were determined at the collection of the sample by a multiparametric sonde. Alkalinity, nitrate, nitrite, total nitrogen, phosphate, total suspended solid and chemical oxygen demand (COD) were determined at laboratory. The results reveal that fecal contamination in groundwater at the touristic and non-touristic sampled sites is occurring. However the detection of the *Vibrio* spp. and the determination of caffeine makes evident that only in the touristic sites the extent of the contamination implies a human source. The others sources of bacteriological contamination can be linked to the type of coastal ecosystems presented along the area of study.

Key words: Fecal contamination; Caffeine; Karstic Aquifer.

RESUMEN

El objetivo de este estudio fue determinar la presencia, extensión y fuentes de contaminación fecal de muestras de agua subterránea de pozos y cenotes en sitios turísticos y no turísticos en la Riviera Maya y establecer si las actividades recreativas que van en aumento, son fuente de contaminación antrópica en esta zona. *Escherichia coli* (*E. coli*) es uno de los bioindicadores mas común para evaluar la calidad bacteriológica del agua, su presencia está relacionada con problemas de salud por patógenos asociados a influencia antrópica tales como fugas de tanques sépticos y aguas residuales sin tratar. A pesar de las limitaciones y problemas asociados a la detección de bacterias fecales, este bioindicador continua presente en las diversas políticas de calidad de agua en el mundo. El estudio se desarrolló en el área de Puerto Morelos, Quintana Roo. México, colectándose siete muestras de agua subterránea de pozos y cenotes. Se efectuaron análisis bacteriológicos mediante las técnicas de sustrato cromogénico para la determinación de Coliformes totales, *E. coli* y de microbiología molecular para la identificación del genero *Vibrio* spp.; la detección de cafeína se efectúo mediante técnicas cromatográficas. Los parámetros de campo (pH, oxígeno disuelto, conductividad eléctrica y temperatura), fueron determinados al momento de la colecta de muestras mediante una sonda multiparamétrica. Los parámetros de alcalinidad, nitratos, nitritos, nitrógeno total, fosfato, sólidos

suspendidos totales y Demanda Química de Oxigeno se determinaron en laboratorio. Los resultados revelaron la presencia de contaminación fecal en agua subterránea en sitios turísticos y no turísticos. La presencia del género vibrio, y la detección de cafeína confirmaron que la alteración de la calidad del agua en algunos de estos sitios tiene una conexión directa con actividades antrópicas de tipo turístico. La información colectada en este estudio además de servir a la comunidad científica puede ser adecuada para entender la importancia del manejo de las políticas del agua y la importancia de la evaluación del impacto ecológico de las actividades turísticas.

Palabras clave: Contaminación fecal; Cafeína; Kárstico Acuífero.

INTRODUCTION

The touristic activity has become an important economical activity worldwide, in México this represent the third economical force. The Cancun-Riviera Maya in Quintana Roo; México; due to its natural resources: beaches, jungles, sinkholes, and reef, is one of the main Mexican touristic poles. It has reached a great development and growth in Latin America with 20-25% growth per year (SECTUR, 2009). Moreover, this region underlies a vulnerable karstic coastal aquifer at the Yucatán Peninsula, which is considered a groundwater dependent ecosystem (GDE) completely reliant on aquifers for supplies of fresh water. High permeability fractured limestone, such as the one underlying the Cancun-Riviera Maya, allows rapid transport of microbial and chemical contamination resulting in a significant potential increase of pollution affecting the ecosystems. As this activity rises, there is an increase in service and new influx of new inhabitants. In 2005 the overall net migration (immigrants into the state versus emigrants out of the state) in Quintana Roo of persons that have lived in the state for five years or more recorded an influx of 72,306 individuals (INEGI, 2005). In that same year the percentage of immigrants in the state in respect to the rest of the population was estimated at 8.1% (INEGI, 2005). Compared to the influx estimated in 2002 at 5.3% the overall migration into Quintana Roo is on the rise (Hausman, 2009).

Hydrogeologic setting

The Yucatán Peninsula is a large, emergent carbonate platform with developed karst features. Dissolution of carbonate material along fractures is common, promoting the karstic features such as sinkholes, karstic caves of varying width and depth. The study zone present most of the characteristics discussed by some authors such as Perry *et al*., (2002): 1) beneath almost the entire northern region fresh water lens is underlain by a marine saline intrusion that is in close hydraulic contact with the ocean; 2) the fresh water lens is approximately 10 m thick near the coast and reaches a depth of about 60 m depth; and 3) precipitation across Quintana Roo range 1,500 mm annually to 1,100 mm annually.

The unconfined aquifer of the Yucatán Peninsula serves as the sole source of fresh groundwater throughout Quintana Roo. Rainwater is the main source of aquifer recharge with 15% of the total precipitation infiltrating down to the water table (Moore, 1992).The precipitation rapidly penetrates a surface calcrete layer through fractures and moves through a highly permeable vadose zone to the water table. As consequence there are no surface streams more than few hundred meters long in the northern and northeast peninsula. The high permeable fractured limestone of the Yucatán Peninsula allows for a rapid transport of contaminants. The extreme population growth and the increase in touristic activities have created high potential for microbial and pharmaceutical pollution to occur.

The hydrogeologic characteristics of the Caribbean coast of Quintana Roo allow for the rapid downward infiltration and equally rapid lateral movement of water flow within the subsurface (Back and Hanshaw, 1978). The water table at the northeast part of the Yucatán Peninsula is located at 2.5 meters above sea level at 46 km from the coast, the hydraulic gradient decrease and the flow of the groundwater discharge at the coast (Perry et al., 1989; 1995; Marin 1990). There are no rivers or streams present throughout northeastern Yucatán due to the relative lack of soil and the presence of highly permeable, karstic limestone. An important karstic feature is a sinkhole or "cenotes", which are major tourist attractions for the Riviera Maya industry. Cenotes (water-filled sinkholes) are direct conduits to the underlying aquifer.

Contamination bacteriologic indicators

Several water quality studies had focused in pathogens and its human health relation. (Seiler *et a*l., 1999; Boehm *et al*., 2003; Buerge *et al*., 2003; Siegener *et al*., 2002; Aquality Env. Co. 2005; Pacheco, 2005). Bacterial enteric pathogens associated with drinking water include *E. coli, Shigella spp, Campylobacter spp,* and *Legionella spp* (Maier *et al*., 2000). Enteric pathogens are those agents that are derived from the intestines of living organisms. Waterborne pathogenic microbes' reservoirs include animals, humans and the environment. Infection of new hosts can occur via two pathways: fecal contamination of water and

recreational water activities. Indicator organisms are exactly that and their presence indicates the possible presence of the pathogenic organisms associated with the particular origin, coliforms serve as indicator organisms of fecal contamination of water sources.

Coliforms are typically harmless bacteria that can be found in the intestines of all warm-blooded animals. Enteric bacteria can reach the water environment mainly by three pathways: direct contact with surface waters, infiltration through terrestrial media, and contamination from wastewater. Bohem *et al.* (2003), mentioned that to assess water quality throughout the world Fecal Indicator Bacteria (FIB) are used as surrogates for pathogens because they are present in high concentrations in sewage and urban runoff. The FIB groups most commonly used as indices of water quality are total coliforms, fecal coliforms, *Escherichia coli* (*E. coli*), and *Enterococcos* sp.

The fecal coliform is used worldwide as a microbiological water quality indicator due to three characteristics: 1) they behave as pathogens, 2) its abundance and 3) the methods of detection and quantification are quick and easy to follow through. Moreover, detection of fecal coliforms and *E. coli* could be associated with pathogens that could represent a higher health risk, such as: *Salmonella* sp., *Shigella* sp., *Yersinia enterocolitica, Y. pseudotuberculosis, Leptospira* sp., *Francisella tularensis, Pseudomonas* spp., *Vibrio* sp., *Legionella* sp. (Gerba and Keswick 1981). However, there are certain disadvantages in the evaluation of the fecal microbial organisms, some studies mentioned that fecal coliform can survive in tropical areas; they survived attached to sediments and maintained activity to grow an increase the population (Vasconcelos and Swartz 1976; Perez-Rosas and Hazen, 1989; Hernandez-Delgado *et al*., 1991; Carrillo *et al*., 1985).

Fecal coliforms and *E.coli* persistence in the water column is highly dependent on temperature, salinity, turbidity, and nutrient availability. Total and fecal coliforms have a short survival time in the natural water environment (dependent on temperature and nutrient supply) and have greater susceptibility to water treatment (Fujioka, 1997). It has also been recorded that *E. coli*, when attached to a surface, has significant increase in its resistivity to chlorine (Maier *et al.*, 2000). Yet, despite this FIB do serve as an indication of fecal contamination being present in the water supply; the absence of coliforms indicates that the water is bacteriologically safe for consumption and use. Environmental Protection Agency USEPA has set standards concerning the level of coliforms concentrations that are tolerable in drinking water supplies (MCL = 0); in México the drinking limit (NOM-SSA-127-1994) also is set at same value. The NOM-ECOL-003-1996 has set a maximum contaminant level of fecal coliforms of wastewater treatment at 240 MPN/100 mL as direct use and 1000 MPN/ 100 mL for indirect use. This standard has been adopted by many countries across the world. In addition, clear anthropic origin of caffeine makes it as a good indicator for human sewage because caffeine is present in beverages, foods and probably the mostly widely consumed drug in the world (Seiler *et al*., 1999).

Pacheco *et al.*, 2000 reported high densities in total and fecal coliforms (7,320 to 12,989 MPN/100 mL) in the groundwater of the Yucatán Peninsula, these authors suggested that the sources of the microbial activity of groundwater samples collected at the north of City of Merida is related to the leaching of animal and human waste in a rural area of the Yucatán Peninsula.

Some studies indicated that caffeine has been used as a tracer to elucidate if the fecal contamination is due to anthropic contamination, due to its relative high concentration detected in surface water (Seiler *et al*., 1999; Buerge *et al.*, 2003; 2006; Jingming *et al*., 2008). Buerge *et al.*, (2003; 2006) indicated that caffeine has being used as an anthropic marker for wastewater contamination of surface waters in Switzerland; and as marker for untreated domestic wastewater. Moreover, Peeler *et al*., (2006) also, indicated that caffeine has being employed for tracking anthropic inputs in rural freshwater and urban marine systems; Jingming *et al*., (2008) explored the correlation among caffeine, fecal bacteria and other pharmaceuticals compounds in surface waters to identify sewage contamination. Pacheco (2005) used caffeine as environmental indicator for assessing urban aquatic ecosystems; these studies indicated the suitability of caffeine as anthropogenic marker to differentiate pollution sources is based in the use of caffeine in drinks, pharmaceutical enhancers. Some researchers pointed out the importance to distinguish the different bacteriological sources of contamination and have an indicator that can correlate the detection of pathogens with anthropic activity. Thus, the major source of caffeine in domestic wastewater comes from unconsumed coffee, tea, soft drinks, or medication moving through ineffective on-site wastewater treatment systems (septic tanks). Crowded areas of septic systems present a high risk of microbial fecal contamination to groundwater and general degradation of water quality.

METHODOLOGY

Study zone and groundwater sampling

Seven groundwater samples were collected form wells and sinkholes from Puerto Morelos area, located at the Riviera Maya 21° 22’ – 20° 43’ North Latitude 86°44’

87° 19' West Longitude (Figure 1). The region preponderant ecosystems are wetlands, dunes and rainforest. At the north and south area present geological depressions that allow for flooding during rain season, this water infiltrates and reach the dunes and coastal area through underground channels. There is a groundwater current directly from the sinkholes that are located along 35 km west from the coast (SEDUMA, 2008).

The samples were collected through July to August 2009 from five wells and two sinkholes the sites are for touristic and domestic use (Table 1). Parameters were set for pH, specific conductivity, redox (ORP), depth and temperature in the field by a multiparametric sonde (Hydrolab DS5). This equipment was calibrated each morning of sampling to ensure precision of data collection. A Van Dorn sampling bottle of 2.2 L was used to collect the samples in a range of 0.5 to 6 m depth depending of the possible depth in each site. The bacteriological sample was the first to be collected to ensure the analysis. The chromogenic substrate (Idexx-Colilert ®) was performed to quantify total coliforms and *E. coli*; in triplicate of volumes of 10 mL, 50 mL, and 100 mL.

The extraction of caffeine was performed from 1 L of sample collected in an amber glass container. The analysis was performed by HPLC and UV detector (Thermofinnigan Spectra System-UV2000) using a CP10-10 cm column with methanol/water 35/65, 1 mL/min flow rate, 50 µL were injected and the UV detector was set at 295 nm. (Modified method of Cavazos *et al*., 2001).

Parameters of Chemical Oxygen Demand (COD), Total Suspended Solids were performed in triplicate, following the Standard Methods (APHA, AWWA, WEF 1998) and HACH techniques (HACH techniques, 1997).

Molecular microbiology

Vibrio spp. assays were used to determine the presence/absence of potential contamination of the sampling sites and their correlating depths. Enrichments for *Vibrio* spp. were performed by the membrane filtration technique (APHA, 1998). Water samples were filtered trough 0.22-µm-pore-size filters (Millipore) and enriched using APW (1-3 % NaCl, pH 8.5) at 35± 2 °C for 18 to 24 h (Baumann *et al*., 1984). Aliquots of each culture were grown in TCBS at same conditions for colony isolation of vibrios. ADN was extracted from bacterial isolates by using DNAzol kit (Molecular Research Center, Inc.) and according to the manufacturer's instructions. PCR analysis was conducted using primers (Popovic *et al*., 1994) and PCR conditions (Hernández-Zárate and Olmos-Soto, 2006) previously described. Positive and negative controls were included for each set of reaction mixtures, including 16S rADN amplification as reference gene. The PCR products were analyzed in agarose gels, stained with ethidium bromide (0.5 µg/mL). The expected molecular weight of amplicons was confirmed by comparison to a known DNA size marker (HyperLadder IVTM, Bioline), under ultraviolet light.

RESULTS

Field parameters

The lowest alkalinity value was detected at P_4 were the temperature is the highest detected value of 29.06 ºC; at this site the conductivity shows the highest value of 4.20 mS cm^{-1}. The combination of these parameters could indicate saline intrusion. Confirmed by the highest value of TDS of 2.70 mg L^{-1} at site P_4. On the other hand, P_7 shows the highest value of alkalinity of but the lowest value of 0.90 mg L^{-1} TDS with a conductivity of 1.40 mS cm^{-1}.

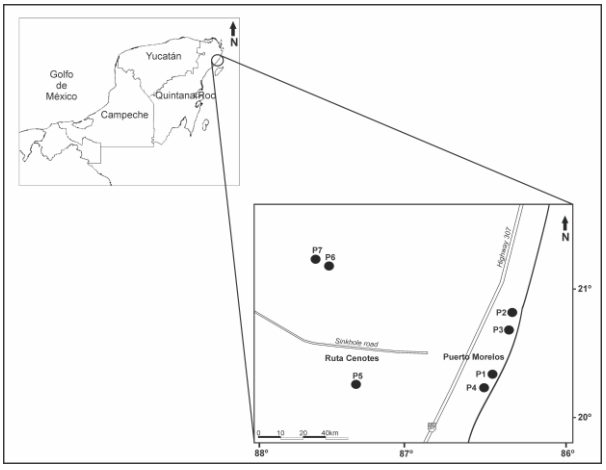

Figure 1. Site of study, showing the studied sites.

Bacteriologic Indicators

The results of the microbiology analyses showed that total coliforms are present in all sites, the detection range in 298.7 to 4.1 MPN/100 mL. The highest values of 298.7 and 206.4 MPN/100 mL correspond to P_1 (Well) and P_3 (well); and the lowest values of 4.1 and 6.3 MPN/100 mL correspond to P_6 (sinkhole) and P_2 (well). Presence of E. *coli* was detected in three of the sites (P_1, P_3 and P_5) where P_5 showed the highest value of 85 MPN/100 mL; whereas the lowest value was detected at P1 of 10 MPN/100 mL (Table 3).

Vibrio spp. detection

Vibrio species were detected by PCR in only one sampling site (P_5); this was a sinkhole for recreational use. Agarose gel electrophoresis of PCR products showed that amplicons were of the expected size and, non-specific amplification was not observed in the PCR reactions. No well (evaluated at different depths) showed the presence of vibrios with the primers used in this study. Table 3 shows the results obtained by PCR analysis.

Caffeine

Results from caffeine analyses show the presence of this pharmaceutical compound at sites P_3 y P_5 (Table 3). P_3 corresponds to a domestic well located close to compost filed of a dry-toilet system of a hostel. On the other hand P_5 corresponds to sinkhole of touristic emphasis (recreational activities). The parameter was identified as presence and absence of the peak that identify the caffeine at the chromatogram.

Chemical Oxygen Demand (COD), Total Suspended Solids (TSS) and nutrients

Table 4 shows the Chemical Oxygen Demand (COD), the result for P_6 is almost cero and its Total Suspended solids (TSS) showed a value of 182 mg L^{-1}. This site correspond a sinkhole without any anthropological activity in this case the TSS could be of inorganic origin (minerals) since the COD showed a value of 3 mg L^{-1} which indicate that the TSS value has not relation with the organic matter. In the case of P_3 the COD total value is 10.5 mg L^{-1} and the TSS result is 17 mg L^{-1}. In the case of P_5 COD total value is 14.0 mg L^{-1} and the TSS result is 12 mg L^{-1}. Total nitrogen is 4mg/L at site P_2 and for the rest of the sites the value is zero (0 mg L^{-1}). The highest value for nitrates was 3.1 mg L^{-1} for P_1 and the lowest value of 0.4 mg L^{-1} for P_4. Nitrite ranged from 1 to 2 mg L^{-1} where sites P_1 and P_2 showed the highest value. Finally phosphates ranged from 1.05 mg L^{-1} to 0.3 mg L^{-1}, where the highest value corresponded to P_3 and the lowest value corresponded to P_6.

Table 1. Identification and description of studied points

CODE	Location	Description	TYPE
P_1	86° 52' 23.7" N 20° 51' 8.9" W	Well	Public distribution
P_2	86° 52' 10.1" N 20° 51' 51.7" W	Well	Public distribution
P_3	86° 52' 40.3" N 20° 52' 17.7" W	Well	Public distribution
P_4	86° 52' 35.7" N 20° 50' 50.8" W	Well	Public distribution
P_5	87° 03' 57.2" N 20° 51' 37.6" W	Cenote (Sinkhole)	Recreational
P_6	87° 05' 27.5" N 20° 53' 43.4" W	Cenote (Sinkhole)	Not in use
P_7	87° 05' 28" N 20° 53' 45" W	Well	Public distribution

Table 2. Field parameters collected with a parametric sonde

Code	Sample date	Alkalinity as HCO_3	Temp.	pH	SpCond	ORP	TDS
		mg L^{-1}	°C		mS cm^{-1}	mV	mg L^{-1}
P1	29/07/09	343.40	27.54	7.10	1.65	326.00	1.05
P2	29/07/09	370.65	27.38	7.08	2.17	206.42	1.39
P3	29/07/09	389.71	27.81	7.50	2.50	80.50	1.60
P4	29/07/09	328.90	29.06	7.01	4.20	97.54	2.70
P5	03/08/09	384.81	27.71	7.20	1.40	328.43	0.90
P6	03/08/09	343.21	25.13	6.71	0.80	338.86	0.50
P7	03/08/09	391.81	25.02	6.97	1.40	335.20	0.90

SpCond=Specific electrical conductivity TDS= Total dissolved solids

Table 3. Bacteriological and pharmaceutical indicators

	Total Coliform	*E. coli*	Caffeine	*Vibrio* spp.
Code	NMP/100 mL	NMP/100 mL	Presence/Absence	Presence/Absence
P_1	206.4	10	A	A
P_2	6.3	0	A	A
P_3	143	31	P	A
P_4	93.2	0	A	A
P_5	298.7	85	P	P
P_6	4.1	0	A	A
P_7	49.6	0	A	A

DISCUSSION

The objective of this project was to assess the impact of the tourist activity along the Cancun-Riviera area (particularly in Puerto Morelos), the collected groundwater samples range from non-touristic to touristic activities to determine the occurrence, source, end extent of fecal contamination. All samples tested positive to the presence of total coliforms, and the presence of *E. coli* indicates fecal contamination, however this is not indication its only source is anthropogenic. The percent of sites that tested positive for *E. coli* indicates that bacteriological quality could be related to water-borne disease pathogens. A portion of this contamination may be naturally occurring as other researchers had been indicated. Once in the environment a pathogen can survive for extended periods of time depending on the organism and its ability to acclimate to the environmental conditions (Fujioka *et al.*, 1981; Lopez-Torres *et al.*, 1988; Jimenez *et al*., 1989). The caffeine method was implemented to determine if a source of the fecal contamination was an anthropic origin. Geographically fecal contamination has been found to be occurring throughout the touristic areas; as the area continues to increase its tourism sector there is a rise in the potential for this type of contamination.

As was mentioned at the results the P_6 does not actual anthropic activity neither recreational nor any touristic relation. The actual microbial activity detected is limited to the natural presence of the geological material. This indicates that P_6 can be used as a reference point in this evaluation. On the other hand, P_3 y P_5 showed the highest bacteriological in this study. At site P3 nitrates and nitrites could be related to the compost field that is 10 m up gradient of the well. P4 shows total absence of *E. coli* and caffeine indicating not an anthropic source of contamination; however the results from COD, SST, conductivity and the highest reported value of temperature should indicates anaerobic conditions where the saline intrusion is a possible source of contamination. The COD and TSS confirm that the conditions promote the microbial activity which is correlated to the anthropical activity of area by presence of caffeine in both sites and the detection of *Vibrio* spp. at P_5. Despite the lower number of sites that tested positive for the presence of these microorganisms the presence of *Vibrio* spp. could be linked to the increased human activity.

Table 4. Chemical parameters

PARAMETERS	P_1	P_2	P_3	P_4	P_5	P_6	P_7
TOTAL COD (mg L^{-1})	9	11	10.5	103.5	14	3	0
TS (mg L^{-1})	838	1008	983	2228	772	407	672
TSS (mg L^{-1})	8	33	17	105	12	182	22
Total Nitrogen (mg L^{-1})	0	4	0	0	0	0	0
Nitrates (mg L^{-1})	3.1	0.55	1.25	0.4	0.75	0.6	1.0
Nitrite (mg L^{-1})	2	1	2	1	1	1	1.5
Phosphates (mg L^{-1})	0.55	0.4	1.05	0.4	0.4	0.3	0.35

COD= Chemical oxygen demand TS= Total solids TSS= Total suspended solids

CONCLUSIONS

The results reveal that fecal contamination in groundwater at the touristic and non-touristic sampled sites is occurring. However, the detection of the pathogens such as: E. *coli* or *Vibrio* spp. and the determination of caffeine makes evident that only in the touristic sites the extent of the contamination implies a human source. The others sources of bacteriological contamination can be linked to the type of coastal ecosystems presented along the area of study. Geographically fecal contamination has been occurring throughout the Yucatán Peninsula, Cancun-Riviera Maya and even in Puerto Morelos the conditions is persistent. As the area continues to raise its tourism sector, there is an increased factor of recreational and touristic activities in the area. The tourist impact resulted in a common characteristic of the sites with highest microbial activity where caffeine detection probed to be a useful indicator of the predominant anthropic activity in the zone. The information of this project could assist scientist and members of the communities along this coastal karstic aquifer to understand the importance of water management protocols and assessment of ecological impact by the tourist activity.

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REFERENCES

Aquality Environmental Co. 2005. Lac La Nonne Water Quality Report 2004: Nutrients, Bacteria and Caffeine. The Lac La Nonne Enhancement and Protection Association, Canada

APHA, AWWA and WEF. 1998. Standard Methods for the Examination of Water and Wastewater. 20th ed., Washington, D. C. USA.

Back, W., and Hanshaw B. B. 1978. Hydrogeochemistry of the northern Yucatán Peninsula, México, with a section on Mayan water practices. In: Ward W. C. and Weidie A. E., (eds). Geology and hydrogeology of northeastern Yucatán. New Orleans Geological Society, New Orleans, Louisiana. pp. 229–261.

Baumann, P., L. Furniss and J. V. Lee. 1984. Genus I. Vibrio. In: Krieg N.R., Holt J. G. (eds.). Bergey's manual of systematic bacteriology. The Williams and Wilkins Co., Baltimore, Md. pp. 518-538.

Boehm A.B., Fuhrman J., Mrsÿe R.D. and Grant S.D., 2003. Tiered approach for identification of a human fecal pollution source at a recreational beach: Case study at Avalon Bay, Catalina Island, California. Journal of Environmental Sciences and Technology. 37: 673-680.

Buerge I. J., Poiger T., Muller M.D., Buser H.R. 2003. Caffeine, an anthropogenic marker for wastewater contamination of surface waters, Journal of Environmental Sciences and Technology. 37: 691-700.

Buerge I.J., Poiger T., Muller M.D., and Buser H.R. 2006. Combined sewage overflows to surface waters detected by the anthropogenic markers. Environmental Sciences technology 40:4096-4102.

Carrillo M., Estrada E., and Hazen T.C. 1985. Survival and enumeration of fecal indicators *Bifidobacterium adolescentes* and *Escherichia coli* in a tropical rain forest watershed. Applied Environmental Microbiology. 50:468-476.

Cavazos R.N., Zarate R.L., and Torres de Navarro E. 2001. Phosphorus and caffeine determination and quantification in soft cola drinks. Educacion Quimica 12:116-120.

Chen Z., Pavelic P., Dillon P., Naidu R. 2002. Determination of caffeine as a tracer of sewage effluent in natural waters by on-line solid-phase extraction and liquid chromatography with diode-array detection. Water Reserch. 36:4830-4838.

Fujioka R.S. 1997. Indicators of marine recreational water quality. Walter M.V ed. Manual of Environmental Microbiology. ASM Press. Washington DC. Pp 176-183.

Fujioka R.S., Hashimoto H.H., Siwak E.B., Young R.H. 1981. Effect of sunlight on survival of indicator bacteria in seawater.Applied Environmental Microbiology 41:690–696.

Gerba. C.P. and Keswick B.H. 1981. Survival and transport of enteric viruses in groundwater. In: Quality of Groundwater. W. Van Duijvenbooden, P. Glasbergen and H. Van Leyveld, eds. Elsevier, Amsterdam. 511-515.

Hach. 1997. Hach Analysis Handbook. 3rd Edition. Hach Company

Hausman H. 2009. Responsible Development in Tulum, México: Considering Water Quality and Subaqueous Cave Locations. Masters project submitted in partial fulfillment of the requirements for the Master of Environmental Management degree in the Nicholas School of the Environment of Duke University, 55p.

Hernandez-Delgado E.A., Sierra M.L., and Toranzos G.A. 1991. Coliphages as alternate indicators of fecal contamination in tropical waters. Environmental Toxicology and Water Quality. 6:131-143.

Hernández-Zárate, G. and Olmos Soto, J. 2006. Identification of bacterial diversity in oysters (*Crassostrea gigas*) by FISH and PCR. Journal of Applied Microbiology, 100: 664-672.

Jimenez L., Muniz I., Toranzos G.A., and Hazen T.C. 1989. Survival and activity of Salmonella typhimurium and Escherichia coli in tropical freshwater. Journal of Applied Bacteriology 67:61–69.

Jingming W., Junqi Y., Ruikang H., Zhaoguang Y., and Lifeng Z. 2008. Use of caffeine and human pharmaceutical compounds to identify sewage contamination. Proceedings of world academy of science, engineering and technology. 34: 438-442.

INEGI. 2005. Instituto Nacional de Geografia e Informatica. Anuario estadístico del estado de Quintana Roo, 1st Ed. Aguascalientes, México. vol XI, 42.

Lopez-Torres A.J., Prieto L., Hazen T.C. 1988. Comparison of the in situ survival and activity of Klebsiella pneumoniae and Escherichia coli in tropical marine environment. Microbial Ecology. 15:41–57

Maier, R.M., Pepper I.L., and Gerba C.P. 2000. Environmental Microbiology. Academic Press, San Diego, CA. 562p.

Marín, L. 1990. Field investigations and numerical simulation of ground-water flow in the karstic aquifer of northwestern Yucatán, México. PhD. Thesis. Department of Geology. Northern Illinois University. Dekalb, Illinois, USA.

Moore, Y.H., Stoessell R.K, and Easley D.H. 1992. Freshwater/sea-water relationship within a ground-water flow system, northeastern coast of the Yucatán Peninsula. Ground water. 30:343-350.

NOM-127-SSA1-1994. Norma Oficial Mexicana, Salud ambiental, agua para uso y consumo humano-límites permisibles de calidad y tratamientos a que debe someterse el agua para su potabilizacion, Diario Oficial de la Federación.

NOM-ECOL-003-1996. Norma Oficial Mexicana, Establece los límites máximos permisibles de contaminantes para las aguas residuales tratadas que se reusen en servicios al público, Diario Oficial de la Federacion. 9p

Pacheco J., Cabrera, and Marin L. 2000. Bacteriological contamination in Karstic Aquifer of Yucatán, México. Geofisica Internacional. 39:285-291.

Pacheco F.A. 2005. Caffeine as an environmental indicator for assessing urban aquatic ecosystems, Cad. Saúde Pública, Rio de Janeiro. 21:1884-1892.

Peeler K.A., Opsahl S.P, and ChantonI J.P. 2006. Tracking anthropogenic inputs using caffeine, indicator bacteria, and nutrients in rural freshwater and urban marine systems, Environmental Sciences and Technology. 40: 7616-7622.

Perry E., Smith E., Gamboa J., Reeve A., Sanborn R., MarinL., and Villasuso M. 1989. Geologic and environmental aspects of surface cementation, North Coast, Yucatán, México. Geology.17: 818-821.

Perry E., Marin L., McClain .J and Velazquez G. 1995. Ring of Cenotes (sinkholes), northwest Yucatán, México: Its hydrogeologic characteristics and possible association with the Chicxulub impact crater, Geology. 23: 17-20

Perry, E. C., Velazquez-Oliman, G., Marin, L. 2002. The Hydrochemistry of the Karst Aquifer System of the Northern Yucatán Peninsula, México. International Geology Review 44:191-221.

Perez-Rosas and Hazen T.C. 1989. Survival of Vibrio cholerae in tropical marine environments. Applied Environmental Microbiology. 55: 495-499.

Popovic, T., Fields P. I. and Olsvik Ø. 1994. Detection of Cholera Toxin Genes. In: Wachsmuth I. K., Blake, P. A., Olsvik Ø. (eds). *Vibrio cholerae* and Cholerae: Molecular to Global Perspectives. American Society for Microbiology, Washington, D. C. USA.. 41-41.

Seiler R.L., Zaugg S.D., Thomas J.M., Howcroft D.L. 1999.Caffeine and pharmaceuticals as indicators of wastewater contamination in wells. Ground Water. 37:405-10.

SECTUR. 2009. Secretaria de Turismo. Principales indicadores en hoteles y moteles de Quintana Roo, Serie Anual: 2006 a 2008. http://datatur.sectur.gob.mx/portalDatatur2/reporteador desplegado (NOV.19, 2009).

SEDUMA. 2008. Secretaria de Desarrollo Urbano y Medio Ambiente Actualización del programa de desarrollo urbano del centro de población de Puerto Morelos, Municipio de Benito Juárez, Quintana Roo 2008-2023. http://seduma.qroo.gob.mx/PDUs/22.-ACTUALIZACIONPPDUPUERTOMORELOSPDF(MAYO2009)/ActualizacionPDUCentrodePoblacionPuertoMorelos.pdf

Siegener R. and Chen R.F. 2002. Caffeine in Boston Harbor seawater. Marine Pollution Bulletin 44:383-7

Vasconcelos G. J. and Swartz R. G. 1976. Survival of bacteria in seawater using a diffusion chamber apparatus in situ. Applied Environmental Microbiology. 31: 913-920.

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