

Persistence of hazard fecal bacteria for public health in a stream at the municipality of Aguazul Casanare

Persistencia de bacterias fecales peligrosas para la salud pública en un río del municipio de Aguazul (Casanare)

Traducción español-inglés a cargo de:
Julián Fernando Garnica Sepúlveda

Andrés Valenzuela-Gómez*
Juan Carlos Avella Castelblanco**
María Quisphi González***
Héctor Favio Mayor Sánchez ****

Keywords: Wastewater spill, autodepuration curve, biofilm, Escherichia coli, pollution footprint, socioeconomic consequences

Scientific research article

Palabras clave: Vertimiento aguas residuales, curva autodepuración, biopelícula, Escherichia coli, huella ambiental, consecuencias socioeconómicas

Artículo de investigación científica

Abstract: This study comes from a pollution case under an administrative point of view, but mainly this study targets to establish the spatial range which this pollution reaches on the territory, serving to set further studies on where has to be measured its socioeconomical effects. The case scenario is a punctual discharge of wastewater from the municipal depurator facility on a natural stream. The pollution was traced by the amount of fecal coliforms in water samples at ten sampling points along the stream course. Was evaluated the water suspended bacteria (planktonic coliforms) and the attached bacteria to submerged surfaces (benthic coliforms), looking for a mathematical model to describe its behavior. There along the stream was observed a decreasing trend on planktonic coliforms by a dilution effect, but also was observed an increasing trend on benthic coliforms, indicating that these bacteria can be persistent in the aquatic ecosystem under this life form (biofilm). This pollution reaches near to 7km downstream from the spill-pipe discharge, where along this course there

* Natural Sciences Professor at UNISANGIL sede Yopal. Biologist specialized on hydrobiology, licensed by Universidad Nacional de Colombia 2006. MSc Advanced Microbiology by Universitat de Barcelona 2010. Faculty of Natural Sciences and Engineering Fundación Universitaria de San Gil – Unisangil Yopal. avalenzuela@unisangil.edu.co

** UNISANGIL economics professor, economics and industrial engineer, industrial safety specialist, Director of the Research Group Scientia Karayurú. Faculty of Economics and Administrative Sciences Fundación Universitaria de San Gil – Unisangil Yopal. javella@unisangil.edu.co

*** Auxiliar de investigación, Estudiante de ingeniería ambiental unisangil, integrante del semillero de investigación investkratos y grupo de investigación Scientia Karayurú

**** goquima@gmail.com, faviomayor@gmail.com, Calle 7 #20-63 – Yopal city, Casanare

are many users of this water, mainly for crops irrigation. Furthermore was observed livestock animals grazing on the stream. All this implicates an irregular situation according to environmental normative about waters, also can causes administrative problems to the municipality because this pollution can risk public health to downstream inhabitants and workers with diseases.

Resumen: Este estudio trata un caso de contaminación hídrica bajo un marco administrativo, pero sobre todo se dirige a establecer el rango espacial que ésta contaminación alcanza en el territorio, sirviendo de base para establecer nuevos estudios sobre en dónde se tiene que medir sus efectos socioeconómicos. El escenario es un punto de vertimiento de las aguas residuales de la planta depuradora municipal en un arroyuelo natural. La contaminación fue rastreada por la cantidad de coliformes fecales en muestras de agua colectadas en diez puntos de muestreo a lo largo del curso del arroyuelo. Se evaluó tanto las bacterias en suspensión (coliformes planctónicos) como las bacterias adheridas a las superficies sumergidas (coliformes bentónicos), en búsqueda de un modelo matemático para describir su comportamiento. A lo largo de la corriente se observó una tendencia decreciente en los coliformes planctónicos por efecto de dilución, pero también se observó una tendencia creciente en los coliformes bentónicos, lo que indica que estas bacterias pueden ser persistentes en el ecosistema acuático bajo esta forma de vida sésil. Esta contaminación alcanza cerca de 7 kilómetros aguas abajo desde el vertimiento, donde a lo largo de este curso hay muchos usuarios de esta agua, principalmente para el riego de cultivos. Además se observó animales de ganadería pastando en el arroyuelo. Todo esto implica una situación irregular de acuerdo a normativas ambientales sobre aguas y ríos, y también puede causar problemas administrativos en el municipio ya que esta contaminación puede arriesgar la salud pública de los habitantes aguas-abajo y los trabajadores con enfermedades.

The urban wastewater purifying systems throughout all municipalities of Casanare predominates mostly in primary treatment process, releasing their effluents with heavy loads of organic matter and bacteria into natural waters, and ignoring the effects on near human-settlements and downstream inhabitants whom use these waters for many activities. There is a need to make a measurement of the environmental footprint size of this kind of effluents spill outs on the territory. If this pollution is traced through an indicator then the distance until where this pollution reaches can be estimated, so this information can set the area for further studies about local socioeconomic effects caused by the use of this stream's polluted water. In this case, the indicator was the presence and amount of fecal bacteria, denominated thermotolerant coliforms *Escherichia coli* (Edberg SC et al., 2000), also called simply as "coliforms", because their fecal origin is directly linked to municipal sewage system, and also these microorganisms can cause disease problems in public health.

This study focuses in evaluating how persistent are these coliform bacteria in the receiver water-body, a stream in this case, after a punctual discharge of wastewater system's effluent ("**spill-pipe**"). All this because there is a belief about these bacteria will be "diluted" with the river's water, and downstream they will "disappear", because the natural environment "consumes" them, a process called "auto-depuration".

Few direct measurements exist to confirm an auto-depuration process (Darakas, 2001) (Pascal et al., 2001) (Garrido-Perez et al, 2002). For an initial approach, this work involves sampling points along the receiver stream for surveying fecal bacteria, remarking on both life forms: those which are suspended and floating in the water column (**planktonic coliforms**) and those attached on submerged hard surfaces (**benthic coliforms**), to quantify their presence and know if these bacteria reach downstream the Unete's river mouth. With this data, it can be estimated a theoretical distance at which they "disappear" in the stream, after the discharge point (spill-pipe).

2. Objective

To evaluate the fecal coliform longitudinal persistence on the water course at the receiver stream, after a wastewater spill out from the Aguazul municipal depurator facility, for the assessment of spatial range of this microbiological water deterioration downstream.

3. Methodology

Aguazul is a little town of 25.567¹ habitants, sited in Casanare's territory within Colombia. The municipality has a wastewater depurator system, which consists mainly in oxidative pools as a primary treatment for the sewage. This facility at northeast of this town, receives all the wastewater from the urban area, and then releases the liquid phase of these settlement-ponds to a little stream nearby that is a branch of Unete river (Illustration 1).

In several visits, there were traced sample-points using a GPS while traveling in an inflatable boat and in motorcycle along the stream course. These points were selected considering the access to these areas from the road, making easier the logistic needed for the sampling process. On the illustrations 1 and 2 are maps showing the course of the stream and the marks of ten (10) sample points and other important sites.

Illustration 1. Aguazul's wastewater facility ubication and the Spill-Pipe discharge.



Spill-pipe discharge into the studied stream

1 Population of the Aguazul urban area for 2011, according to population projections from 2005 to 2020 by Departamento Administrativo Nacional de Estadística – DANE

Source: (from left to right) Secretaria de Gobierno Municipio de Aguazul-Aeroimagen Rio Unete March/2007; Satelital Picture Google Earth, 2011; Own picture, January 2011.

The weather season for this study predominates sunny without rain. The total path travelled by the water in the stream from the wastewater spill-pipe to its river-mouth on Unete River measured 6488m. The stream channel has an average width of 5,7m and a maximum depth of 0,4m. The average water-velocity was 0,97m/s. The stream crosses rice crops, oil-palm fields and prairies for cattle ranching, but the stream has some forest-coverage along its both sides. So, the study's framework is near to 7km between the first sampling point (P0) until the last sampling point (P9) (Figure 2).

Illustration 2. Receiver stream course and the sampling points



Source: Authors – Cartographic base Google Earth, 2011

Once the stream was explored, the sampling process started. There were used aseptic vessels to collect water at each point. Then, these samples were subject to bacterial colonies counting by filter's method, where filters go through an incubation process to develop bacterial colonies, in order to detect and count colonies to estimate how many coliforms are in each water-sample (APHA Standard Methods). For planktonic coliforms, samples were taken at each sample-point 1000ml of water-sample in each point traced on the stream, making three (3) replica simultaneously at each one; then, immediately at the laboratory, each sample was processed to know the quantity of coliform bacteria -expressed in Colony-Forming Units (CFU)- on the filter method incubated at 37°C by 24 hours.

On the other hand, the assessment benthic coliforms had another treatment. The process was made only for the first five sample-points (P0 to P4), and consists in a metallic base anchored to the stream bed, having attached little squares of clean ceramics (5cm X 5cm) that will be submerged into the stream, so each square serves as a new surface to be colonized by bacteria for the next 10 days. Each point had three (3) square replicas, which were surveyed every day considering the biofilm's growth measurement. On day 10, these ceramic squares were

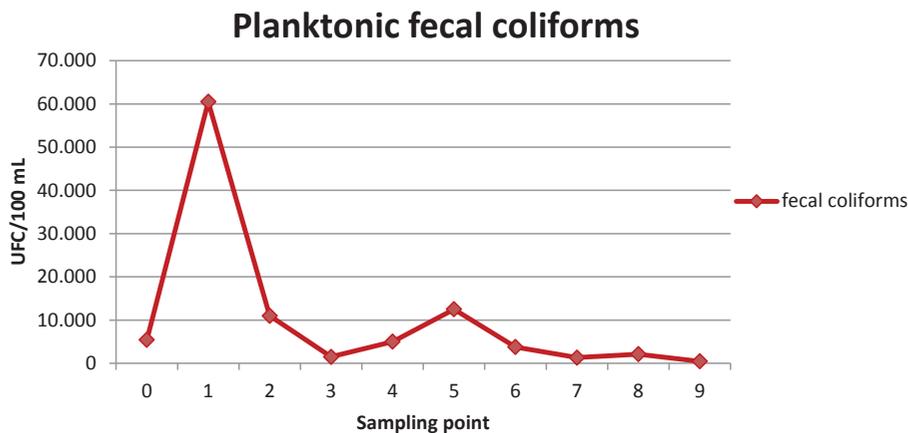
taken from the stream and transported to the lab for coliforms counting method. Each square surface was scrapped and washed with distilled water, next, this water was recovered with all the scrapped biofilm, and then this dissolution was taken to an ongoing filtration and incubation process, as the method indicates.

Then the resulted data was plotted: Coliforms-CFU vs. sampling-point metric distance from the spill-pipe; observed its behavior, looked for trends on the plotted dots and sketched a curve that can describe the persistence of the coliforms on this stream. Finally, there was plotted a trend curve adjusted on the dots, and found the curve equation, so it would let to estimate the distance at which the coliforms will be zero, or at least where coliforms have the same quantity before the discharge.

4. Results

The Figure 1 shows the amount obtained of Planktonic Fecal Coliforms at each sampling point. The data showed the expected: the water 100m before the spill-pipe (P0) is polluted in a great manner after the spill, as shows the peak on the water sampled at only 35m after the discharge of the spill-pipe (P1). Then the quantities rapidly decrease in the next points, however there is another notorious peak at P5 and a slight peak at P8.

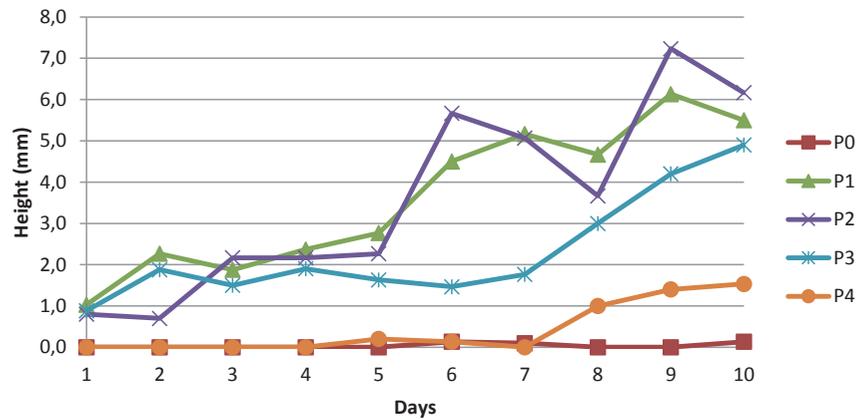
Figure 1: Planktonic Fecal Coliforms vs Sampling Points



Source: Authors

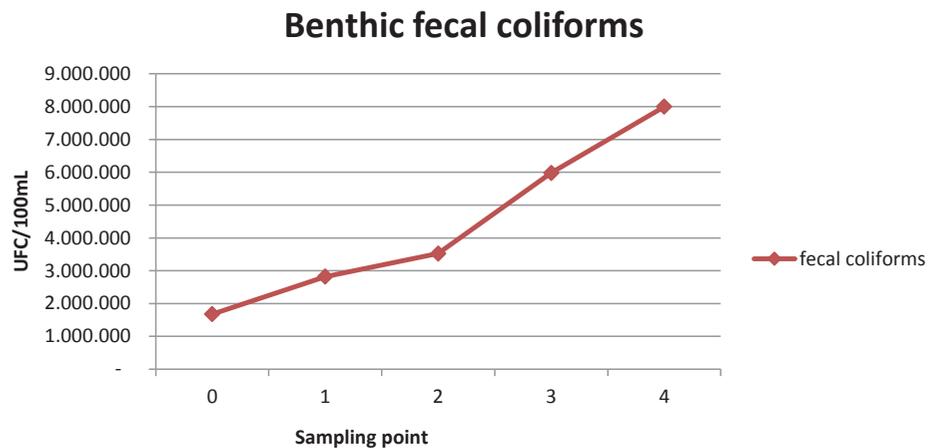
The benthic coliforms showed another pattern. The biofilm growth capability on the stream was evident by the increase of thickness along days by sampling point (Figures 2). In addition, the presence of fecal coliforms on this biofilm is confirmed by lab analysis, and has a trend to increase the number of bacteria as the sampling point is farthest from the spill-pipe (Graphics 3).

Figure 2: Biofilm growth per sampling point vs days of study (mm)



Source: Authors

Figure 3: Benthic fecal coliforms



Source: Authors

Records of water temperature (°C), conductivity (S), stream-flow (m³/s) and acidity (pH) were correlated with the quantity of bacteria at each point. The Table 1, shows the coefficient values: only the conductivity has a significant direct relation with fecal coliforms, and only the stream-flow shows an inverse relation with these bacteria.

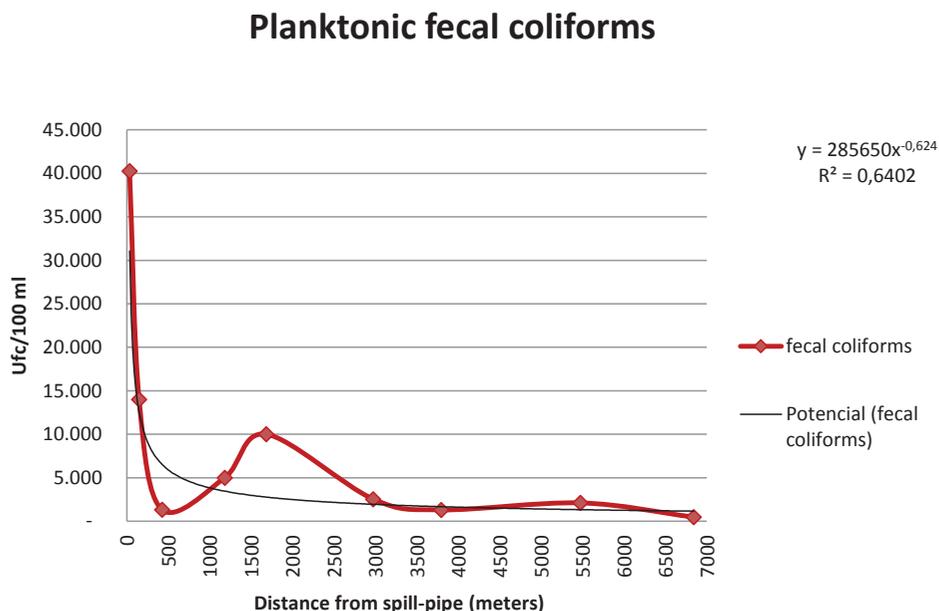
Table 1. Correlation coefficient of the quantity of bacteria at each point.

Points Parameters	0	1	2	3	4	5	6	7	8	9	Correlation CT	Correlation CF
pH	8,61	8,54	8,59	8,81	5,35	5,72	7	6,37	7,87	6,9	0,36	0,37
Temperature (degrees)	28,8	29,05	28,91	28,46	28,73	27,98	26,98	25,98	27,48	20,98	0,42	0,42
Flow (m3/S)	0,39	0,15	0,08	0,03	0,04	0,05	0,05	0,067	0,06	0,18	0,13	-0,2
Conductivity (uS)	85	178,6	144,9	139,8	117,6	115,7	95,67	83,67	147,7	54,57	0,7	0,67

Source: Authors

Finally, there was plotted the quantity of coliforms versus the distance from the spill-pipe. The dot plot shows a decreasing pattern from the spill-pipe until the final track of the stream. Then, a smooth curve that fits to all dots and can be described as an equation was sketched. The best equation was a potential equation ($R^2 = 0.6402$) which shows a decreasing trend.

Figure 4. Behavior of planktonic fecal coliform vs distance from the dumping



Source: Authors

5. Discussion

The peaks observed at P5 and P8 in Figure 1 show an increase on planktonic coliforms, due the presence of paths at these zones where cattle walk to cross the stream looking for food at either both sides. So, when cattle walk on the stream their steps stir the water and detach all the bacteria settled on the stream bed. It means that this biofilm is perturbed and releases bacteria cells to the water column, increasing the number of planktonic coliforms in these stream zones.

Despite the decreasing trend that was observed on planktonic coliforms, the benthic coliforms showed the opposite (Figure 3). It seems that the establishment of coliforms on the biofilm is better when they are far away from the spill-pipe, because the distance travelled by the coliforms allows them to settle better in zones where the stream slows down. These bacteria incorporate to the current biofilm, getting shelter and better environmental factors, so coliforms can grow at the bottom without adverse conditions for them like sunlight exposure and oxygen presence. This implicates that, while planktonic coliforms decay with the distance, the benthic coliforms can survive attached to biofilms in the stream-bed, even at long distances from their origin.

It is curious that the water conductivity is the environmental factor most correlated with the presence of coliforms at each sampling point. One possible explanation is that conductivity means a measurement of ionic molecules dissolved, so it indicates the offer of soluble minerals and nutrients that cells can use for their growth. Another observation is the quantity of coliforms in the water before the spill-pipe (~5000UFC/100ml), indicating that the water was contaminated before the discharge. This implies the need to study the course upstream in further studies.

The curve obtained in Figure 4, was a potential equation type $y = 285650x - 0,624$ showing a descendent behavior. However, if the distance goes to infinity the curve has an asymptote on $y=0$, meaning that coliforms in water, even at long distances from their source, will never be zero (0). This implies, theoretically, that the stream cannot recover its natural water quality after a discharge. This can be explained by the benthic coliforms properties described previously, because they can persist over the stream-bed by this attached life form.

The World Health Organization recommends that only water, with less than 10000UFC/100ml coliforms, be used for crop irrigation (Blumenthal et al., 2000). This applied to this case, calculating distance for this coliforms amount gives 200 meters approximately after spill-pipe, where this water can be taken for this purpose. But the actual pump of water from this stream for irrigation is placed at less distance, indicating an incorrect situation. Overall, this pollution is causing depreciation on the economic value of these lands and its touristic appealing, due to bad odors. It also decreases quality of harvested products for markets, and threatens the health of people who live and work in these fields. If this situation remains, probably the authorities are going to incur in costing health services to cure enteric diseases for these inhabitants.

6. Conclusions

There is an effect of dilution on planktonic coliforms by the stream after the spill-pipe discharge from the Aguazul's wastewater purifying facility, showing a fast decreasing pattern from the pollution source.

However, the benthic coliforms can establish themselves in biofilms formed on hard surfaces submerged into the stream, making them persistent along the stream, even at long distances from their source (spill-pipe).

The benthic life form seems to let survive fecal coliforms and be tolerant towards adverse conditions (i.e. aerobic environment) due to the protection that brings the biofilm layers. This can put at stake the concept of the stream "auto-depuration" process mentioned before.

The range of the spatial footprint of this pollution reaches 7km downstream, from the pollution source (spill-pipe), so the socioeconomic effects of this contamination must be traced downstream given this radius of contamination.

This water pollution harms not only hydric quality, also causes economic detriment when, for example, rice crops are irrigated with this water, because it can decrease value of this white rice in markets, and so on social issues when the use of this water can risk local people health with enteric diseases.

There is a need to improve this wastewater purifying system using new technologies for secondary and tertiary treatment by Aguazul authorities, in order to avoid future administrative problems on public health caused by this pollution, which currently infringes the normative (Decreto 3930 MINAMBIENTE 2010).

Referencias bibliográficas

- American Public Health Association (APHA), the American Water Works Association (AWWA), and the Water Environment Federation (WEF). *Standard Methods for the Examination of Water and Wastewater*. Edition 20
- ANDERSON, K. WHITLOCK, J. HARDWOOD, V. 2005. *Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments*. Applied and environmental microbiology vol 71 No. 6 pp 3041-3048.
- BARCINA, I. LEBARON, P. VIVES-REGO. 1997. *Survival of allochthonous bacteria in aquatic systems: a biological approach*. FEMS Microbiology Ecology 23 pp 1-9.
- BEAUDEAU, P. TOUSSET, N. BRUCHON, F. LEFÈVREAND, A. TAYLOR, H. *In situ measurement and statistical modelling of Escherichia coli decay in small rivers*. 2001. Water Research Volume 35, Issue 13, Pages 3168–3178.
- BLUEMENTHAL, U. MARA, D. PEASEY, A. RUIZ-PALACIOS, G. STOTT, R. 2000. *Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines*. Bulletin of WHO.
- COLOMBIA. Ministerio de Ambiente, Vivienda y Desarrollo Territorial. Decreto 3930 de 2010 (Octubre 25). *Por el cual se reglamenta parcialmente el Título I de la Ley 9ª de 1979, así como el Capítulo II del Título VI -Parte III- Libro II del Decreto-ley 2811 de 1974 en cuanto a usos del agua y residuos líquidos y se dictan otras disposiciones*. Diario Oficial 47873 de octubre 25 de 2010. Bogotá, D.C.

- DARAKAS, Efthymios. 2001. *A simple mathematical formula describing the survival kinetics of E.coli in natural waters*. International Journal of Environmental Studies. Vol. 58. pp. 365-372. Greece.
- EDBERG SC et al. *Escherichia coli: the best biological drinking water indicator for public health protection*. Journal of Applied Microbiology, 2000, 88: 106S–116S.
- GARRIDO-Perez, M. MERINO, A. De La Cruz-Lazaro, M.D. GARZON-Fernandez, N. NEBOT-Sanz, E. Alonso, J.M.Q. SALES-Marquez, D. *Study of the autodepuration potential of a tidal ecosystem affected by urban sewage* International Conference On Waste Management And The Environment , pp. 727-735
- GONZALEZ, J. M. 1996. *Modelling enteric bacteria survival in aquatic systems*. Hydrobiologia No. 316 pp 109-116.
- JAGALS, P. GRABOW, W. GRIESEL, M. JAGALS, C. 2000. *Evaluation of selected membrane filtration and most probable number methods for the enumeration of faecal coliformes, Escherichia coli and enterococci in environmental waters*. Quantitative Microbiology 2, 129-140. Netherlands.
- JAMIESON, R. JOY, D. LEE, H. KOSTASCHUK, R. GORDON, R. 2005. *Transport and deposition of sediment-associated E. coli in natural streams*. Water Research 39 pp 2665-2675.
- MERINO, Susana. 2009. *Análisis de Muestras Alimentarias*. Tema: Microorganismos Indicadores, pp. 22/51. Presentación de diapositivas para cátedra de Técnicas de Análisis Microbiológico en Máster de Microbiología Avanzada de la Universidad de Barcelona.
- ROLIM MENDONÇA, Sergio. 2000. *Sistemas de lagunas de estabilización*. Ed. Mc Graw Hill. Uruguay.
- WILKINSON, J. JENKINS, A. WYER, M. KAY, D. 1995. *Modelling faecal coliform dynamics in streams and rivers*. Water Research No. 29 pp 847-855.