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## EVALUATION OF THE MUTAGENIC ACTIVITY OF WATERS COLLECTED FROM THE CAUCA RIVER IN THE CITY OF CALI, COLOMBIA BY USING THE *SALMONELLA*/MICROSOME ASSAY

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### Resumen

La actividad mutagénica de las aguas del río Cauca en el área urbana de la ciudad de Cali, Colombia, se evaluó mediante la aplicación de la prueba de Ames con las cepas TA98 y TA100 de *Salmonella typhimurium* con y sin el activador enzimático S9. Los compuestos orgánicos y metales pesados fueron extraídos de las muestras de agua utilizando las resinas XAD4 y XAD16 respectivamente. Los extractos se diluyeron con DMSO y se almacenaron a  $-20^{\circ}\text{C}$  hasta los ensayos. Los resultados mostraron actividad mutagénica en cuatro de los cinco sitios evaluados con ambas cepas y aumento de mutagenicidad con el activador enzimático S9. El mayor índice de mutagenicidad ( $MI = 8,0$ ) se obtuvo en la estación seca con la cepa TA98 en el puente de Juanchito. En la temporada de lluvias, un alto índice mutagénico ( $IM = 7,1$ ) también se obtuvo con la cepa TA98 y el activador S9 en la desembocadura del canal colector sur. Estos resultados indican una posible susceptibilidad de los mamíferos a la acción mutagénica de los compuestos presentes en las aguas del río Cauca. Los análisis químicos de los extractos se realizaron mediante GC/MS y espectrofotometría de absorción atómica. Los resultados indicaron la presencia de un gran número de compuestos orgánicos después de la desembocadura del canal colector sur. Los plaguicidas diazinón, lindano, malatión, clordano, clorpirifos y  $\alpha$ -endosulfan se encontraron en el rango de 0,271-0,003 ppb. Los metales pesados Pb, Hg y Cr fueron encontrados con concentraciones de hasta 48, 38 y 30 ppb respectivamente. Este estudio es el primero de su clase en las aguas del río Cauca.

**Palabras claves:** Mutagenicidad, test de Ames, pesticidas, metales pesados, río Cauca, Cali.

### Abstract

The mutagenic activity of the Cauca River waters in the urban area of the city of Cali, Colombia, was evaluated by applying the Ames test using the TA98 and TA100 strains of *Salmonella typhimurium* with and without the S9 enzymatic activator. Organic compounds and heavy metals were extracted from water samples using the resins XAD4 and XAD16 respectively. The extracts were diluted with DMSO and stored at  $-20^{\circ}\text{C}$  until the assays. The results showed mutagenic activity at four of the five sites evaluated with both strains, and increased mutagenicity with the S9 enzymatic activator. The highest mutagenicity index ( $MI=8.0$ ) was obtained in the dry season with strain TA98 at the

Juanchito Bridge. In the rainy season, a high mutagenic index (MI=7.1) was also obtained with the strain TA98 and the S9 enzymatic activator at the mouth of the southern collector channel. These results indicate a possible susceptibility of mammals to the mutagenic action of the compounds present in the waters of the Cauca River. The chemical analyses of the extracts were performed by GC/MS and atomic absorption spectrophotometry. Results indicated the presence of a large number of organic compounds below the mouth of the southern collector channel. The pesticides diazinon, lindane, malathion, chlordane, chlorpyrifos and  $\alpha$ -endosulfan were found in the range of 0.271-0.003 ppb. The heavy metals Pb, Hg and Cr were found with concentrations up to 48, 38 and 30 ppb respectively. This study is the first of its kind in the waters of the Cauca River.

**Keywords:** Mutagenicity, Ames test, pesticides, heavy metals, Cauca River, Cali.

## 1 Introduction

It is well documented that the world's largest rivers are highly polluted by chemicals that can cause genetic damage in living organisms [1, 2]. For example, Ohea *et al.* [3] point out that different rivers in the world, especially in Europe, Asia and South America, are contaminated with toxic chemicals that cause mutations and base pair changes in the reading frame by direct or indirect action; there are reports that these rivers have been contaminated by petrochemical industries and agricultural and urban wastewaters. The main pollutants include pesticides, heavy metals, polyaromatic hydrocarbons, heterocyclic amines, etc. [3, 4, 5, 6, 7]. In other studies [8, 9], it has been shown that these genotoxic substances from industrial and household wastes reach surface waters, and Houk [9] and White [10] noted that the high values of genotoxicity in wastewater coming from the waste of factories producing organic compounds such as the chemical industry, paper mills, metal refining, and petroleum refining. Pollution also affects rivers in Colombia as a result of industrialization and the lack of rigorous controls on industrial effluents. The Cauca River is the second largest in Colombia and provides water for about 10 million people. The river has suffered a great damage caused by dumping of industrial waste and agricultural and domestic sewage [11]. The deterioration of the Cauca River is a concern, since the river provides water for human consumption. The city of Cali uses the river as a drinking water supply for 80% of its population (2 million people). Although there have been reports of birth defects among residents near the Cauca River [12, 13]. However, no studies have been conducted to ascertain whether the pollutants dumped into the Cauca River generate damage at the genetic level in human population.

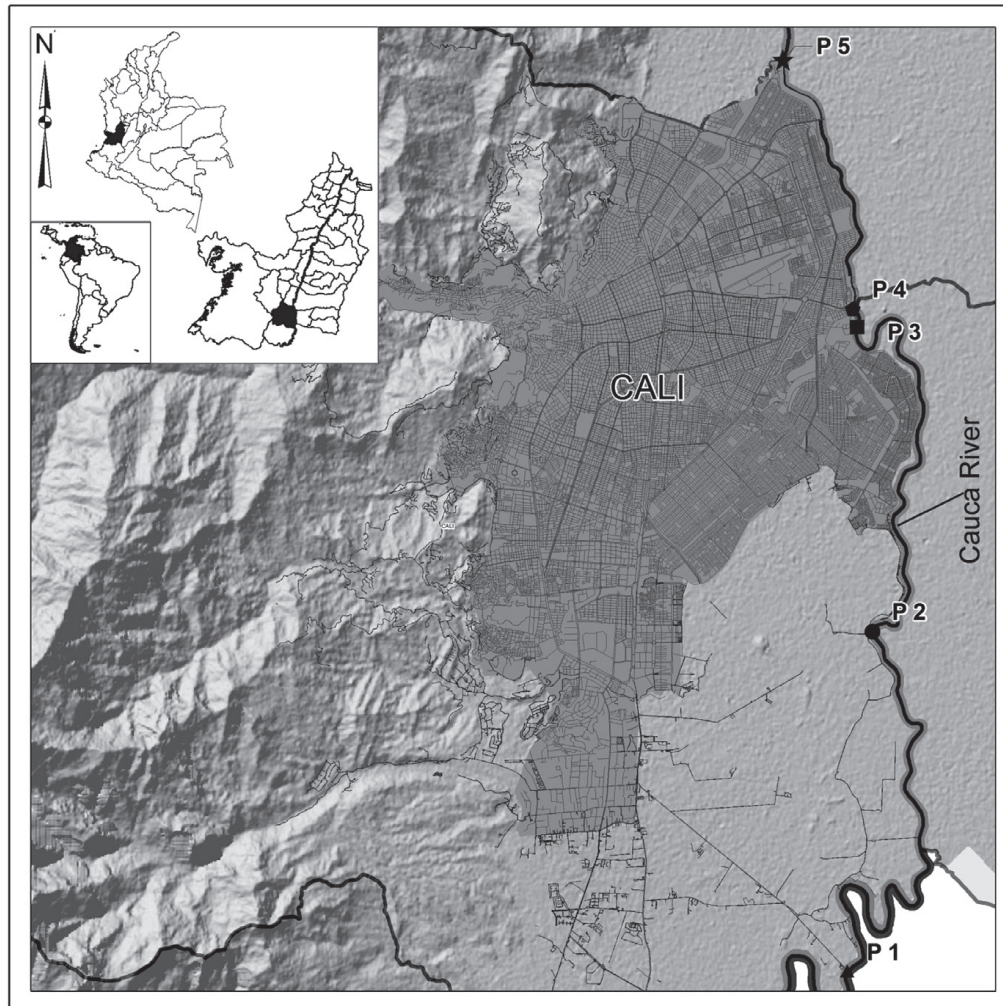
The purpose of this research was to determine the mutagenic potential of the Cauca River waters in the urban zone of the city of Cali by using the well known Ames test, and also identify and quantify some of the most dangerous pollutants such as pesticides and heavy metals.

## 2 Materials and methods

### 2.1 Area of study

Five sampling points were selected in the urban perimeter of the city of Cali as shown in Fig. 1: (P1) The Hormiguero Bridge in the suburbs of Cali (3° 18' N, 76° 28' W), (P2)

Mouth of the southern channel (collector of residual waters of the southern part of the city (3° 22' N, 76° 28' W), (P3) intake of water for the treatment plant at Port Mallarino (3° 2' N, 76° 28' W), (P4) The Juanchito Bridge (3° 27' N, 76° 28' W), and (P5) Mouth of the Cali River (3°31' N, 76° 28' W).



**Figure 1.** Map of the city of Cali, showing the sampling sites

## 2.2 Sample collection

Samples were collected in the center of the river at a depth of 50 cm. At each sampling point 200 L of water were taken. The samples were passed through columns of XAD4 resin for extraction and concentration of organic compounds [14]. The retained compounds were eluted from the resin at 1-2 mL/min using 200 mL of a hexane:acetone solution (85:15). Finally, the recovered fractions were rota-evaporated at 40 °C and pressure of 225-381 mm Hg. When the organic extracts had large amounts of water, the temperature was increased to 45 °C. For the extraction of the heavy metals, 250 mL of water were passed through columns of XA16 resin. The extracts obtained in both cases were dissolved in 10 mL of dimethyl sulfoxide (DMSO) and stored at -20 °C until mutagenicity assays were carried out [15].

### 2.3 Determination of mutagenic activity of the Cauca River waters

The mutagenic activity was determined by applying the Salmonella/microsome test, known as the Ames test, using TA98 and TA100 strains of *S. typhimurium*, with and without the addition of S9 enzymatic activator (Moltox, Inc.). The S9 enzymatic activator is used in the Ames test to simulate the effect of organic compounds in the liver of mammals. In several cases, metabolic processes transform harmless substances into mutagenic compounds [16]. The positive control without S9 for strain TA98 was 4-nitroquinoline and for TA100 was sodium azide. The positive control with enzyme activity in both strains was 2-aminofluoreno. Each sample was plated in triplicate on nutritive agar (Oxoid No.1) and followed by incubation for 2 days at 48 °C with the strain to be tested and S9 enzyme extract. After this period, revertant colonies were counted and taken as a criterion for mutagenic response when the average of colonies on plates doubled compared with the controls (mutagenic index (MI)  $\geq 2.0$ ). Slight mutagenic response was considered when the average MI was in the range of 1.0-1.9, and no mutagenic response when MI was less than 1.0 [16, 17, 18, 19].

### 2.4 Determination of pesticides

For determination of pesticides in the waters of the Cauca River, 4 L of water were taken in amber bottles and stored at 4 °C. Samples for GC/MS analysis were filtered through a membrane of 45 mm and the pH was adjusted to 2 with H<sub>3</sub>PO<sub>4</sub> [20]. The extraction of pesticides was performed using solid-phase Speedisk SIMPLE (JT Baker) preconditioned with ethyl acetate, methylene chloride, methanol, and HPLC grade water (Merck). The elution of retained compounds was done with 5 mL ethyl acetate followed by 5 mL of methylene chloride, and finally with two portions of 3 mL of a mixture (1:1) ethyl acetate/methylene chloride using vacuum. In the extraction of organophosphorus pesticides, the samples were used without acidification. The extract was concentrated with a Kuderna-Danish device up to 0.5 mL. For the determination of organic compounds, 2  $\mu$ L samples were injected into a GC/MS Agilent 6890 N MS detector Agilent 5973 N Agilent 7673 automatic injection. A DB-5MS capillary column 30 m long, 250  $\mu$ m in diameter and 0.30 mm film thickness, J & W Model No. 122-5532 was used. The parameters for analysis were: maximum column temperature of 325 °C, constant flow of 1 mL/min, and detector temperature of 250 °C. The splitless mode was used with quadropole temperature of 150 °C and source temperature of 230 °C. The initial oven temperature was 50 °C, and was increased at 25 °C/min to 100 °C and then at 5 °C/min to 300 °C for 5 min. Quantification of pesticides was done using the internal standard method with certified standards.

### 2.5 Determination of heavy metals (Pb, Hg, Cd, Cr)

For the extraction of heavy metals from the samples, 250 mL of water was passed through columns of XAD16 resin and eluted with 1 M HCl solution in acetone [21]. The determination of heavy metals was done with atomic absorption spectrophotometry. An atomic absorption spectrophotometer Thermo electron spectrometry 711670V series S1,



26, with auto sampling FS95-/97 was used. The extracts obtained with the XA16 resin were concentrated to dryness and then diluted with 2 M HNO<sub>3</sub> prior to analysis. Quantification of heavy metals was done using the standard addition method with certified standards.

## 2.6 Experimental design and data analysis

In the experimental design used, the independent variable was the concentration of the samples of the Cauca River and the response variable was the number of spontaneous revertants of strains in each treatment, with and without enzymatic activation. To test whether differences in the results for each treatment were statistically different, we used an ANOVA and post-ANOVA Duncan and Tukey test.

## 3 Results

### 3.1 Mutagenic activities at the different sites evaluated

Preliminary studies were done in samples collected during the dry season at points P1, P3, and P4. At point P1 (river entrance to the urban area of Cali) no positive response was found to the test with the strain TA98 without S9 activator (MI=1.1). In contrast, the samples collected at points P3 and P4 showed a positive response to the Ames test, and at point P4 a cytotoxic response was found at concentrations greater than 1200 µg/plate. At point P3 the mutagenic index covered a range from 1.1 to 4.0, with an average of 2.8, and for point P4 the mutagenic index was in the range of 3.2-8.0. It was not possible to make more measurements in the dry seasons in 2008 and 2009 because of the “La Niña phenomenon”. Mutagenicity was evaluated in samples collected during the rainy seasons at the 5 sampling sites with TA98 and TA100 strains with and without S9 enzyme activator (Table 1).

The mutagenic index calculated for samples collected at the Hormiguero Bridge was between 0.60 and 1.1 with strains TA98 and TA100 respectively, which indicates no mutagenic response. At point P2 (mouth of the southern wastewater collection channel that includes lixiviates from the old sanitary landfill of Navarro) high mutagenic indices were found, MI=7.1 and 3.7 with the strain TA98 with and without S9, respectively. At this point the only positive response (MI=2.6) with strain TA100 was found. The other sites show a mild mutagenic response (MI<2.0) with the strain TA100 for the doses tested. At point P3 (intake of the drinking water treatment plant), a slight mutagenic response was detected with the two strains at the concentrations tested. In general, the TA98 strain showed greater sensitivity in the detection of potential mutagens. At point P4, the Juanchito Bridge, we found a high mutagenic index (MI=3.5) with the strain TA98 plus the S9 enzyme activator, whereas with the strain TA100 a slight mutagenic activity was found in the presence and absence of enzymatic activator. Finally, at point P5, mouth of the Cali River, a moderately high mutagenic index (MI=2.6) with the strain TA98 and S9 was found.

### **3.2 Differences between sampling sites**

The site of the mouth of the Southern Channel showed the highest mutagenic activity between the points evaluated (Table 1). To corroborate this, a post-ANOVA Duncan and Tukey test was done (Table 2). The results indicate that this site was significantly different from the Hormiguero Bridge, the Juanchito Bridge, and the mouth of the Cali River. Although the results of the test for the site of intake of the drinking water treatment plant were similar to Southern Channel, it is clear that there is not enough data for this point to get a definitive conclusion.

### **3.3 Differences in mutagenic activity in the presence and absence of enzyme activator, S9, in the Cauca River samples**

ANOVA was performed to compare the sensitivity between strains TA98 and TA100, the results are shown in Table 3. A significant difference was found in rates between the MI with the two strains. It was found that strain TA98 plus enzyme activator produced the highest values of the mutagenic index, as well as increased sensitivity to organic compounds extracted from the Cauca River.

### **3.4 Determination of pesticides by GC/MS**

Pesticides concentrations were measured on three sampling dates during the rainy seasons (September 2008, and February and June 2009) as shown in table 4. Results indicate low contamination with these compounds at all sampling points, since all concentrations are in the order of tenths of ppb.

### **3.5 Determination of heavy metals (Pb, Hg, Cr and Cd) by atomic absorption spectroscopy**

Heavy metal concentrations were determined on four sampling days in the rainy season (January and April 2008, April and June 2009). Table 5 shows the results of the analysis with atomic absorption. In general the highest concentrations found were for lead, chromium and mercury (48, 37, and 30 ppb respectively). The total concentrations of heavy metals in all the points are in general below the maximum allowed value by Colombian regulations, indicating a moderate contamination.

It is important to mention that the positive results obtained for mutagenicity cannot be adjudicated solely to the presence of the pesticides and heavy metals found (as their concentrations are relatively low), but to the presence of a large number of organic pollutants. A significant increase of the organic load was observed below the mouth of the Southern Channel. Among these substances are phenolic compounds, polycyclic aromatic compounds, and many others as evidenced in the chromatograms obtained in scan mode as shown in Fig.2.

**Table 1.** Mutagenic activity of waters collected from the Cauca River during the rainy season.

Sampling sites	Concentration (µg/plate)	TA98 without S9		TA98 With S9		TA100 without S9		TA100 with S9	
		RV	MI	RV	MI	RV	MI	RV	MI
<b>The Hormiguero Bridge</b>	0.025	20	1.0			114	1.1		
	0.05	14	0.7			85.5	0.8		
	0.10	18	0.9	ND	ND	179.5	1.7	ND	ND
	0.15	12	0.6			88.2	0.8		
<b>Mouth of Southern Channel</b>	1.25	12	0.6	39	1.1	182	1.7	78	1.0
	2.50	32	1.6	89	<b>2.5*</b>	211	<b>2.0</b>	107	1.4
	3.75	43	<b>2.1</b>	99	<b>2.7*</b>	279	<b>2.6*</b>	98	1.3
	5.00	73	<b>3.7*</b>	256	<b>7.1*</b>	ND	ND	96	1.2
<b>Intake of drinking water treatment plant</b>	0.025	14	0.7			138	1.3		
	0.05	13	0.7			137	1.3		
	0.10	14	0.7	ND	ND	133	1.2	ND	ND
	0.15	24	1.2			178	1.7		
<b>The Juanchito Bridge</b>	0.025	16	0.8	25	0.7	117	1.1	104	1.3
	0.05	16	0.8	80	<b>2.2*</b>	124	1.2	114	1.5
	0.10	17	0.9	128	<b>3.5*</b>	113	1.1	127	1.6
	0.15	15	0.8	24	0.6	116	1.1	56	0.7
<b>Mouth of Cali River</b>	1.25	23	1.2	21	0.6	64	0.6	81	1.0
	2.50	19	0.9	19	0.5	63	0.6	43	0.5
	3.75	23	1.2	19	0.5	66	0.6	121	1.6
	5.00	26	1.3	96	<b>2.6*</b>	65	0.6	76	1.0
<b>Negative control</b>	RV	20		35		103		78	
<b>Positive control</b>	RV	≤ 300		≤ 300		≤ 300		≤ 300	

RV: Average of triplicate spontaneous revertants, MI: Mutagenic Index, Positive control: 4NQNO, sodium azide and 2-aminofluoreno, Negative control: DMSO, ND: not determined,\* High MI.

**Table 2.** Post-ANOVA of the differences between the sampling sites with statistical Duncan and Tukey test.

	Sampling site	N	Subset		
			1	2	3
<b>Tukey B (a, b, c)</b>	Mouth of Cali River	53	48.85		
	The Hormiguero Bridge	29	71.38	71.38	
	The Juanchito Bridge	55	73.11	73.11	
	Intake of the water treatment plant	24		104.33	104.33
	Southern Channel	47			115.47
<b>Duncan (a, b, c)</b>	Mouth of Cali River	53	48.85		
	The Hormiguero Bridge	29	71.38		
	The Juanchito Bridge	55	73.11		
	Intake of the water treatment plant	24		104.33	
	Southern Channel	47		115.47	
	Significance		0.082	0.397	

**Table 3.** ANOVA for the difference between the MI obtained with the strains TA98 and TA100.

	Sums of squares	Degrees of freedom	Root mean square	F	Significance
<b>Contrast</b>	46.6	4	11.6	18.8	0.0
<b>Error</b>	119.9	194	0.62		

**Table 4.** Pesticides concentrations in ppb at the sampling sites during the rainy seasons.

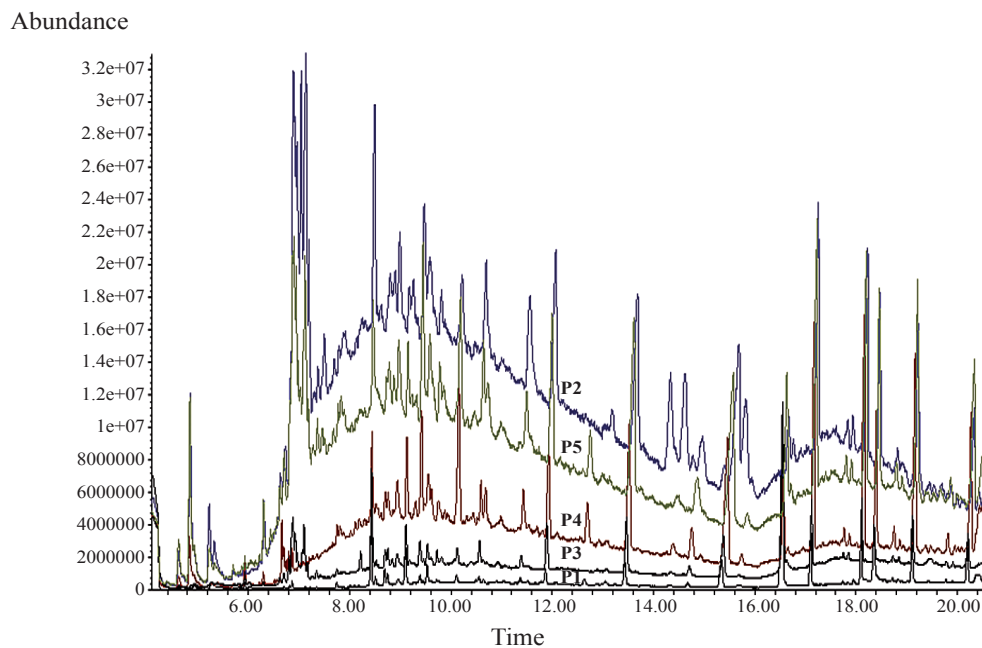
Pesticide	Sampling date	The Hormiguero Bridge	Mouth of Southern Channel	Intake of the water treatment plant	The Juanchito Bridge	Mouth of Cali River
<b>Diazinone</b>	Sept 2008	0.141	<LOD	0.111	0.097	0.061
	Feb 2009	0.224	0.012	0.187	0.009	0.009
	June 2009	0.019	<LOD	0.027	0.008	<LOD
<b>Lindane</b>	Sept 2008	0.071	<LOD	0.016	0.067	0.080
	Feb 2009	0.145	0.230	0.065	0.063	0.153
	June 2009	0.043	<LOD	0.021	0.081	<LOD
<b>Chlordane</b>	Sept 2008	0.057	0.017	0.033	0.059	0.064
	Feb 2009	0.115	0.038	0.144	0.129	0.112
	June 2009	0.035	< LOD	0.723	0.746	0.015
<b>Chlorpyrifos</b>	Sept 2008	0.034	0.021	0.049	0.056	0.029
	Feb 2009	0.095	0.065	0.132	0.239	0.081
	June 2009	0.031	< LOD	0.093	0.064	0.001
<b>Ethyl-parathion</b>	Sept 2008	< LOD	< LOD	< LOD	< LOD	< LOD
	Feb 2009	< LOD	< LOD	< LOD	< LOD	< LOD
	June 2009	< LOD	< LOD	< LOD	< LOD	< LOD
<b>Malation</b>	Sept 2008	0.011	0.019	0.008	0.032	0.007
	Feb 2009	0.271	0.039	0.129	0.093	0.099
	June 2009	< LOD	< LOD	< LOD	0.046	< LOD
<b>Heptachlor</b>	Sept 2008	< LOD	< LOD	< LOD	< LOD	< LOD
	Feb 2009	< LOD	< LOD	< LOD	< LOD	< LOD
	June 2009	< LOD	< LOD	< LOD	< LOD	< LOD
<b>Aldrin</b>	Sept 2008	< LOD	< LOD	< LOD	< LOD	< LOD
	Feb 2009	< LOD	< LOD	0.014	< LOD	< LOD
	June 2009	< LOD	< LOD	< LOD	< LOD	< LOD
<b><math>\alpha</math>-endosulfan</b>	Sept 2008	0.175	0.048	0.094	0.095	0.163
	Feb 2009	0.221	0.151	0.132	0.117	0.235
	June 2009	0.073	0.031	0.016	0.087	0.063

Limit of detection, LOD (ppb): Diazinon 0.0034, Lindane 0.0092, Chlordane 0.0004, Chlorpyrifos 0.0081, Ethyl-parathion 0.0104, Malathion 0.0315, Heptachlor 0.0003, Aldrin 0.0033,  $\alpha$ -endosulfan 0.0049.



**Table 5.** Heavy metals concentrations in ppb at the sampling sites during the rainy seasons.

Heavy Metal	Sampling date	Hormiguero Bridge	Mouth of Southern Channel	Intake of the water treatment plant	Juanchito Bridge	Mouth of Cali River
<b>Pb</b>	Jan 2008	0.16	0.04	0.01	0.03	0.04
	April 2008	32.05	37.67	47.68	3.76	27.65
	April 2009	0.91	0.77	0.38	1.3	0.29
	June 2009	1.20	0.95	0.60	1.59	0.28
<b>Hg</b>	Jan 2008	6.51	6.60	6.01	23.07	6.50
	April 2008	29.6	7.91	25.28	5.740	3.57
	June 2009	17.5	21.30	19.41	20.72	16.81
<b>Cr</b>	Jan 2008	0.18	0.21	0.17	0.27	0.31
	April 2008	9.33	12.43	5.91	7.76	36.58
	April 2009	4.75	3.11	3.26	5.90	3.80
	June 2009	5.81	4.03	4.27	6.16	3.91
<b>Cd</b>	Jan 2008	0.45	0.02	0.03	0.02	0.03
	April 2008	0.06	0.07	0.01	0.01	0.01
	April 2009	0.09	0.03	0.06	0.96	0.06
	June 2009	0.11	0.09	0.08	1.08	0.05


**Figure 2.** Chromatograms of samples in scan mode of waters collected at the sampling sites.

## 4 Discussion

Mutagenic activity was detected in the Cauca River waters at four of the five sites tested in the urban zone of the city of Cali. This shows that there is a high probability that in the Cauca River there are organic compounds that can alter DNA structure, such as those that cause mutations by base pair changes or reading frame [3]. The findings show that the current condition of the Cauca River is similar to other surface water sources that support human populations in its surroundings [6, 22].

High values of mutagenic indices with the strain TA98 plus the S9 enzyme activator found below the mouth of the Southern Channel indicate the possibility that the mutagenicity affects not only the bacteria tested, but also mammals [14, 16, 20, 23, 24]. This mutagenicity is associated with the large amount of organic compounds found at this site that include the lixiviates from the old landfill of the city of Cali. At the Juanchito Bridge, mutagenic activity was found with the strain TA98 plus S9. It is likely that the presence of mutagenic compounds at this point is influenced by the addition of pollutants from different domestic and manufacturing activities in the surroundings and by sludges coming from the water treatment plant [25].

At the junction of the Cali River with the Cauca River, the results with the strain TA98 the S9 enzyme activator indicate the presence of organic compounds that usually generate mutations in the reading frame of bacterial and mammalian DNA. Probably mutagenic compounds detected at this point come from the high pollution of the Cali River [11]. The results evaluated with statistical tests indicate that there are significant differences in mutagenicity among the Southern Channel point (site of the major mutagenic index) and the other sampling points. In general, the strain TA98 shows higher mutagenic index, in concordance with the literature [26].

It is noteworthy that there have been cases of morphologic defects at birthday in people residing within a distance of 3 km from the Cauca River in 7 of the 8 cases reported [12, 13]. Between December 2004 and May 2005, four cases of sirenornelia, and four cases of sirenornelia, and four cases of cyclopia were reported in the Hospital Universitario del Valle in Cali. The relationship between mutagenicity indices found in the Cauca River water and the occurrence of these malformations, remains a hypothesis.

## 5 Conclusions

The waters of the Cauca River showed mutagenic activity with the strains TA98 and TA100 in the four of the sites evaluated: mouth of the Southern Channel, intake of the water treatment plant, the Juanchito Bridge and the mouth of the Cali River. The samples collected at the mouth of the Southern Channel had the highest response in mutagenicity tests with TA98 and TA100 strains with and without enzymatic activator, indicating the presence of substances that potentially cause mutations in the frameshift and base pair change in DNA directly and indirectly. The TA98 strain showed better sensitivity than TA100 in detecting mutagenic organic compounds present in the samples analyzed. The positive results obtained with the two strains and enzyme activator indicate that mixtures of compounds present in the river can cause mutagenic events in mammals, and consequently the consumption of untreated water from the Cauca River is a risk for human populations and also for the biota associated with the body of water.

The chemical analysis of the collected waters shows presence of pesticides and heavy metals at low to moderate levels. In addition, a large number of organic compounds were also detected below the mouth of the Southern Channel. These compounds need to be studied to establish a correlation between the mutagenicity found and the contaminants of the river.

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