Article

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Abstract: The Santiago-Guadalajara River Basin has an area of 10,016.46 km². The Metropolitan Area of Guadalajara, within the basin, is the second largest city in the country, with more than 5 million inhabitants. The growths of the urban population, as well as industrial and agricultural activities with insufficient infrastructure for the sanitation of wastewater and its reuse, have caused environmental deterioration of surface waters and gradual depletion of groundwater resources. To assess the level of contamination in surface waters from the presence of heavy metals in the basin, a monthly monitoring campaign was carried out at 25 sampling stations located in the main and tributary streams from July 2021 to April 2022. The following decreasing sequence was found according to the mean concentration values: Fe>Al>Mn>B >Ba>Zn>As>Cu>Cr>Ni>Cd. The Heavy Metal Pollution Index (*HPI*) method was applied to assess the level of risk to aquatic life, finding an average global *HPI* value of = 314.436 for the basin, which classifies it as in the critical contamination range. Regarding human health, results reflect concerning health risks due to presence of As, Cd, and Ni in some stations and require increased monitoring measures, as well as pollution control, to protect aquatic life and human health from heavy metal contamination.

Keywords: water quality; environmental monitoring; arsenic; heavy metal pollution index; hazard index; total carcinogenic risk

1. Introduction

Human activities, driven by population, industrial and agricultural growth have significantly impacted water supplies, leading to overexploitation and contamination [1,2]. Global climate change has further affected the availability and quality of water resources [3]. Cities worldwide face water scarcity issues, such as potable water availability, lack of adequate wastewater treatment, and depletion of ground water [4]. On the other hand, the severity of urban floods has been increasing

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since at least the 1980s due to climate change, increase of population, poor management of stormwater, land use changes, and inadequate infrastructure [5].

Approximately one-third of the world faces water pollution issues [6,7], with one billion people lacking access to clean drinking water [8] and over 2.2 million people in underdeveloped countries like Bangladesh, Pakistan and India facing life-threatening risks [9]. According to WHO and UNICEF, around 60% of infants are at risk of diseases transmitted through contaminated water [10]. Urban and peri-urban areas frequently pollute water bodies by altering natural flows and by discharging industrial waste, leading to high levels not only of heavy metals and other emerging contaminants which pose significant threats to aquatic life and human populations [11,12].

With regard to heavy metals such as arsenic, chromium, mercury, lead, cadmium, and copper, they naturally occur in soil and water due to weathering and erosion, [13–15]. However, industrial emissions of heavy metals have increased in recent decades, threatening aquatic ecosystems and human health [16,17]. As with some organic pollutants, heavy metals persist in the environment, accumulating to toxic levels and disrupting physiological and biochemical functions in organisms [18–20].

Pollutants dissolve because of the solvent properties of water [21–23]. Heavy metals migrate to water bodies and to sediments via industrial and municipal discharges and by runoff [23]. A primary pollution source is the improper or untreated discharge of industrial wastewater into rivers [24–26].

In Mexico over 60% of river systems show signs of contamination, mainly from agricultural runoff and industrial and urban wastewater discharge [27,28]. This issue is evident in the Lerma-Chapala and upper Santiago Hydrological System, including the Santiago-Guadalajara River Basin (SGRB) [26]. In developing countries, untreated or poorly treated industrial wastewater discharges and mining are significant metal pollution sources to freshwater [27]. Heavy metals may also enter water through atmospheric deposition [28–30]. Risks associated with dermal contact, inhalation, and ingestion have been evaluated in soils contaminated with heavy metals [31–33]. The risks associated to human health could increase if people consumed animal and plant products contaminated with heavy metals [32–34].

The Heavy Metal Pollution Index (HPI) plays a crucial role in evaluating the level of metal contamination in river systems, allowing researchers and environmental managers to assess the impact on ecosystems [35,36]. The HPI provides a valuable tool for monitoring the concentration of metals in rivers over time, enabling early identification of pollution issues and allowing for timely corrective actions [37,38].

On the other hand, recent studies have reported the Hazard Index (*HI*) and the Total Carcinogenic Risk (*TCR*) as widely used indicators to measure the carcinogenic and non-carcinogenic risks to human health associated with heavy metals in river waters [33,35,36]. The *HI* is typically used to assess the potential non-carcinogenic health risks, while TCR is used to estimate the cancer risk associated with long-term exposure to these pollutants. Together, these indices offer a thorough evaluation of the health risks posed by heavy metal contamination in aquatic environments, helping to pinpoint areas where public health may be at significant risk [37,38]. The objective of this study was to evaluate spatially the potential toxicity of heavy metals to aquatic life and their risks to human health throughout the SGRB, using the Heavy Metal Pollution Index, the Hazard Index and the Total Carcinogenic Risk.

2. Materials and Methods

2.1. Study Area

The Santiago-Guadalajara River Basin (SGRB) is in the Central Western Meso-Hydrological Region, in the R12 "Lerma-Santiago" Hydrological Region (Figure 1). It belongs to the Santiago River Basin Council, which is part of the Hydrological-Administrative Region VIII named Lerma-Santiago-Pacific, and accounts for 97% of the state of Jalisco. This coincides with the Groundwater Technical Committee of the VIII Hydrological-Administrative Region, named Irapuato-Valle de Santiago. It is

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made up of 10 tributary sub-basins of the Río Grande de Santiago whose main currents are the Zula (1), Calderón (4), La Laja (Arroyo Grande) (3), Corona (La Cañada) (2), Los Sabinos, El Ahogado, Verde, Blanco, La Soledad, Cuixtla and Chico rivers.; and important natural bodies of water (Lake Chapala, Lake Cajititlán) and dams (Calderón, El Ahogado, La Colonia, El Tule, Dos Cauces, Santa Rosa, La Yesca, El Cajón, Aguamilpa, among others). In the basin there are 41 municipalities, of which four belong to Zacatecas, one to Nayarit and 36 to Jalisco [39].

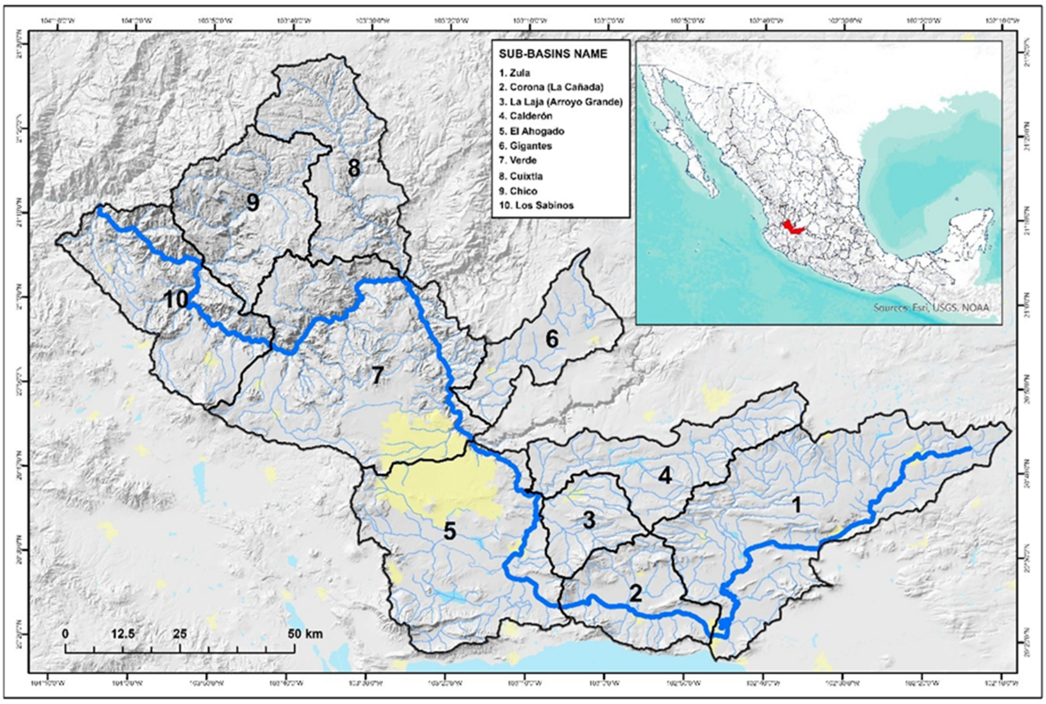

Figure 1. Geographic location of the Santiago-Guadalajara River Basin.

The total surface area of the SGRB was approximately 10,016.46 km^2, with an altitude range from 1,423.8 m to 2,960 m above sea level [39]. The highest elevations in the basin are located at the Tequila Volcano and the mountainous formation known as Cerro Viejo, at 2,920 m and 2,960 m above sea level, respectively [39]. The lowest areas are found at the bottom of the Río Grande de Santiago canyon [39]. The density of the main streams is medium to high, between 2 km and 8 km per square kilometer, covering 75% of the surface [39]. In 2018, the predominant land use type in the basin was agriculture (45.05%), followed by forests (20.9%), deciduous forests (14.8%), grasslands (10.5%), and urban areas (7.5%) [39] (Figure 2).

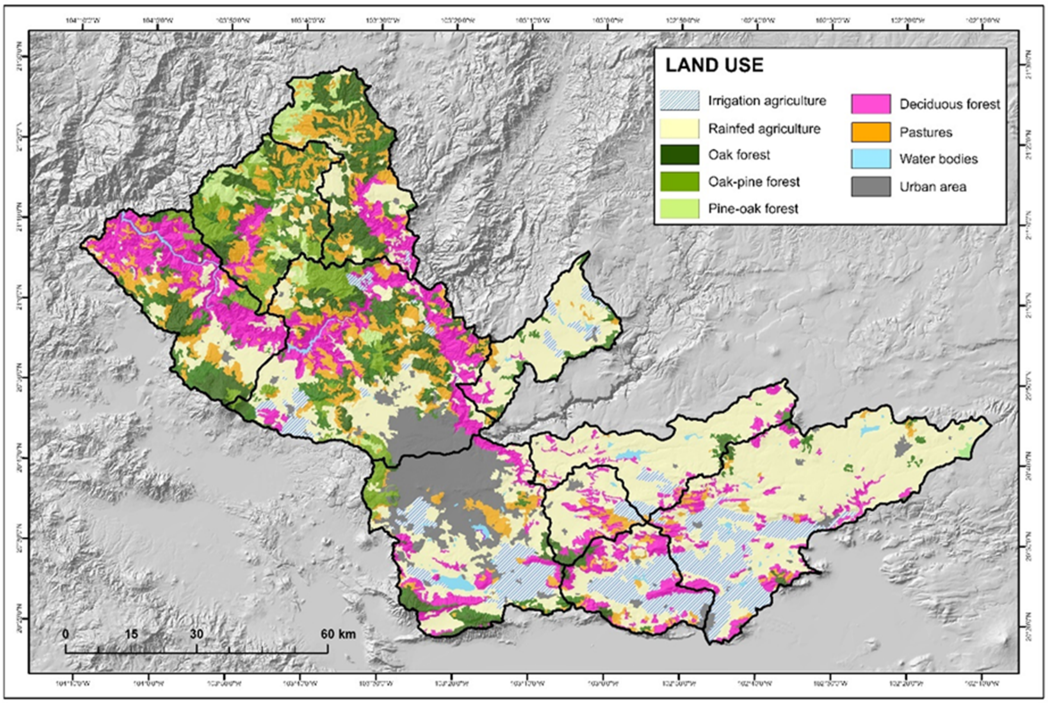

Figure 2. Land use and vegetation type in the study area.

Owing to increasing pollution in Mexico's rivers, in recent years it has become imperative to implement policies aimed at cleaning and restoring these waterbodies to assure the sustainability of water resources [26,40]. Among the rivers in the country identified as the most contaminated are the Tula River in the state of Hidalgo [41,42], the Atoyac River [43] and the Lerma-Santiago River System [26,44]. The Metropolitan Area of Guadalajara (MAG) has experienced a rapid population growth, and according to the INEGI in 2020, it has a population of 5,268,642 inhabitants, making it the second largest metropolitan area in the country [26,45]. The main river running near the city is the Santiago River, which originates in Lake Chapala and flows approximately 562 km into the Pacific Ocean [46]. The Grande de Santiago River Basin covers approximately 76,400 km2 and has an average flow rate of 320 m3/s [46]. The upper basin of the Santiago river, known as the SGRB extends from the city of Ocotlán, near Lake Chapala, to the Santa Rosa Dam, crossing to the east through the Metropolitan Area of Guadalajara (MAG) [26,39] (Figure 1).

The Zula River stream (sub-basin 1) and the El Ahogado stream (sub-basin 5) are significant tributaries of the SGRB, traversing areas with intense agricultural and urban-industrial activity, respectively [46]. Studies have shown that the El Ahogado stream receives untreated urban wastewater with a significant load of pollutants [46,47]. These are partially collected and treated in the local Wastewater Treatment Plant (WWTP), with a capacity of approximately 2,250 L/s. In the northern area of Guadalajara, the Agua Prieta WWTP with an estimated treatment capacity of 8,500 L/s also discharges into the Santiago River. It is operated below its design capacity because the San Gaspar-Agua Prieta wastewater collection works from the East part of the MAG have not yet been completed [47]. The El Ahogado and Agua Prieta WWTPs are planned to receive about 20% and 80% of the wastewater from the MAG, respectively [47]. Despite the heavy reliance of the more than five million inhabitants of the MAG on surface water from the SGRB to meet their water needs [48], a significant portion of the industrial and urban wastewater generated within the basin is still discharged untreated directly into the Santiago River or indirectly through its tributaries [48].

For the National Statistical Directory of Economic Units (NSDEI, DENUE by its acronym in Spanish), an economic unit is an establishment that combines resources and actions to carry out production activities, purchase and sale of goods or provision of services [49]. The last update carried out in 2021 by the NSDEI reported a total of 27,858 economic units in the SGRB, equivalent to 13.1%

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of all business activities in the state of Jalisco. Of these, more than 27,000 economic units (85.8%) are located within the MAG (see Figure 3) [49].

Of the total economic units found in the basin, 2.6% are considered large, with more than 250 people working in the establishment, 23.3% corresponded to medium-sized businesses, and 74% were small businesses, that is, with fewer than 50 people working in the firms. The largest number of economic units in the manufacturing industry was related to the food industry (22.9% of the total economic units in the basin), where animal feed, grain and seed milling, slaughter, and processing of livestock and poultry are processed and produced. The textile industry represents 5.2% of the total economic units in the study basin, whereas the industry that produces plastics and rubber is equivalent to 3.0%. The chemical industry represents only 2.1% of the economic units in the basin, but most of them are large economic units, where resins, synthetic rubbers, fertilizers, pesticides, paints, adhesives, among other chemical products, are produced [49]. Figure 3 shows the concentration of the main industrial activities in the basin.

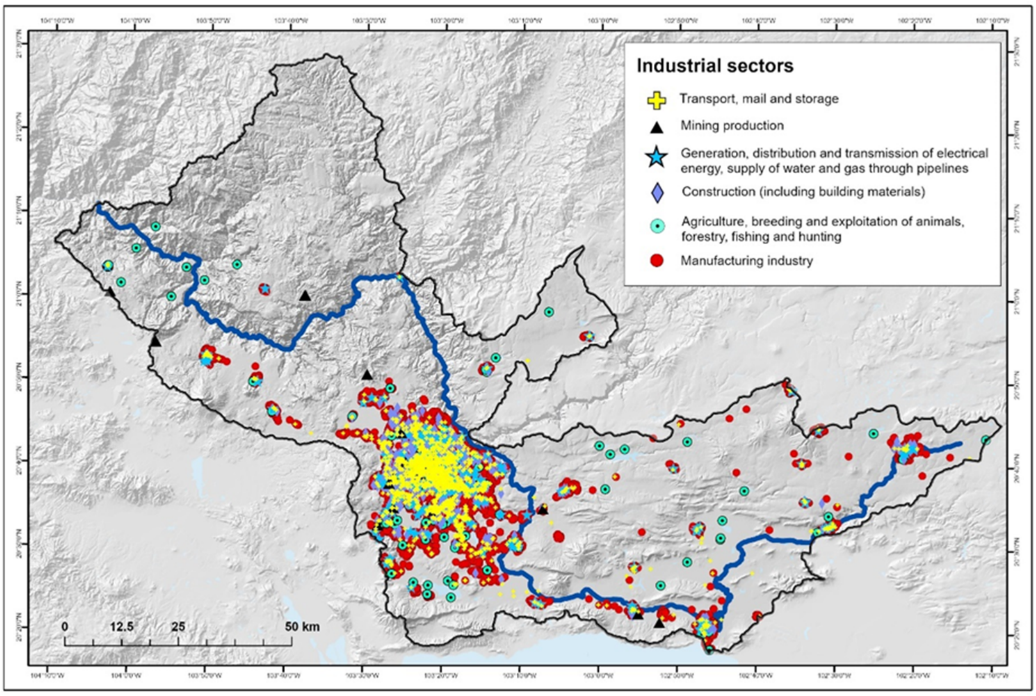

Figure 3. Location of the main industrial sectors in the Santiago-Guadalajara River Basin.

The importance of ensuring the sustainability of watersheds has become increasingly recognized, as it is acknowledged that maintaining their functions is essential for a sustainable future and human security [50,51]. The SGRB is vital for the inhabitants of the MAG and its conservation is a priority today, hence the concern that exists about emerging pollutants that are present in the river and their potential effects on aquatic life and human health [26,46].

2.2. Monitoring Stations

To study the presence of heavy metals in the surface waters of the SGRB, the selection of the monitoring stations was carried out considering the following criteria: accessibility, hydrological regime of the watercourse throughout the year, population in the local watershed, and socioeconomic level of the population. Table A1 (in Appendix A) describes the names and geographical coordinates of each of the selected monitoring stations, and Figure 4 shows their geographic locations.

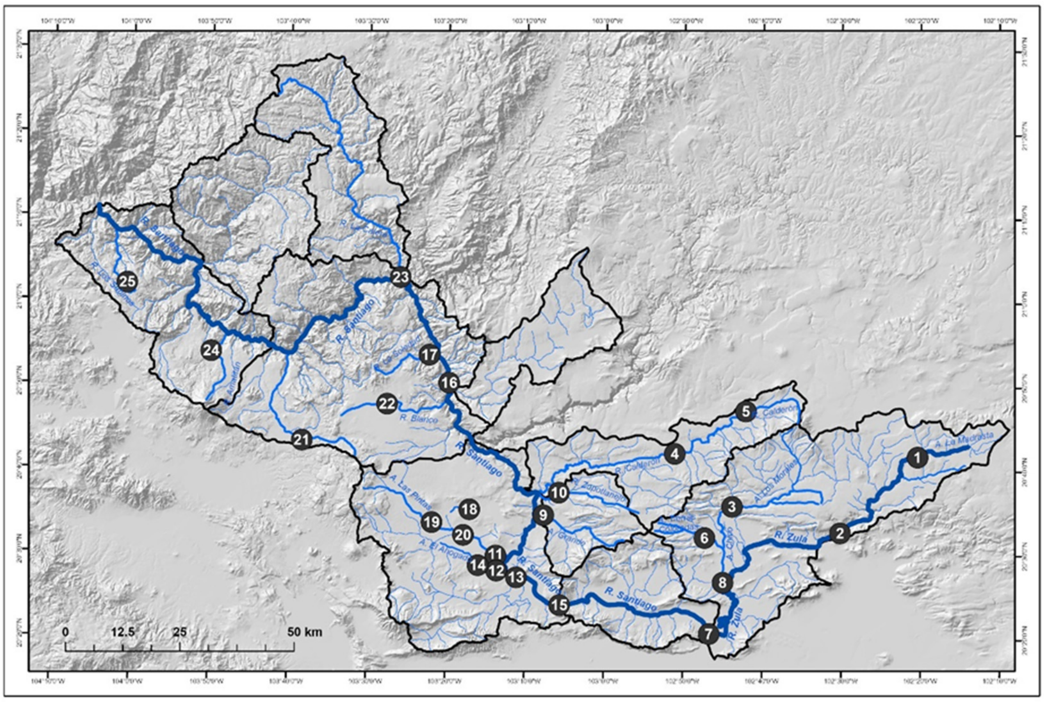

Figure 4. Location of the 25 monitoring stations located in the main and secondary streams of the Santiago-Guadalajara River Basin.

2.3. Analytical Methods

The water samples were digested using the US EPA 3005A method with some modifications, which involved acid digestion of the water to recover heavy metals and subsequent analysis by inductively coupled argon plasma spectroscopy (ICP) [52]. All samples were acidified with nitric acid (5 mL/L), thoroughly homogenized, and a 100 mL aliquot was taken for digestion. Then, 0.2 mL of concentrated nitric acid and 0.5 mL of concentrated hydrochloric acid were added. The sample was covered with a watch glass and placed on a heating plate at a temperature of 90 to 95°C until the volume was reduced to 3 or 5 mL, avoiding boiling. The sample was removed from the plate and brought to a total volume of 10 mL using deionized water. The determination of total heavy metals (Al, As, B, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Se, Zn) was performed using a modification of the standard NMX-AA-051-SCFI-2001 "Water analysis - Determination of metals by atomic absorption in natural, potable, residual, and treated residual waters" [53]. The technique used for the determination of heavy metals was atomic emission (ICP-OES) using an inductively coupled plasma emission spectrometer, model Optima 8300 DV (Dual View) (Perkin Elmer). The standards used for the preparation of the calibration curves are certified and traceable in accordance to NIST [54]. For each group of processed samples, a reagent blank must be run from sample preparation to the analytical process. The coefficient of variation percentage for the results of these samples must not exceed 3%. The detection limits for the determination of heavy metals are shown in Table 1.

Table 1. Detection limits of heavy metals that were analyzed.

Heavy metal	Symbol	Detection limit (mg/L)
Aluminum	Al	< 0.05
Arsenic	As	< 0.01
Boron	B	< 0.001
Barium	Ba	< 0.01

Cadmium	Cd	< 0.002
Total Chromium	Cr	< 0.01
Copper	Cu	< 0.01
Iron	Fe	< 0.01
Mercury	Hg	< 0.001
Manganese	Mn	< 0.01
Nickel	Ni	< 0.01
Lead	Pb	< 0.01
Antimony	Sb	< 0.005
Selenium	Se	< 0.002
Zinc	Zn	< 0.01

2.4. Assessment Criteria

Since 2009 the Federal Rights Act (FRA, LFD by its acronym in Spanish) considers the Santiago River and its direct and indirect tributaries as type "C" waterbody [55]. A type "C" waterbody means that it must comply with the permissible limits of contaminants for fresh waterbodies where the protection of aquatic life is set as a priority [55]. The strategy to assess the toxicity risk of heavy metals and metalloids measured in the SGRB considers 1) the FRA [55], 2) the maximum permissible thresholds of acute and chronic toxicity for the protection of freshwater vertebrate and invertebrate organisms established by the U.S. Environmental Protection Agency [56], 3) Water Quality Guidelines for the Protection of Aquatic Life established by the Canadian Council of Ministers of the Environment (CCME) [57] and 4) Australian and New Zealand Guidelines for Freshwater and Marine Water Quality (ANZECC & ARMCANZ) [58] (see Table 2).

Table 2. Permissible limits of heavy metals and metalloids for the protection of aquatic life according to four different guidelines.

Heavy metal	**Symbol**	**Federal Rights Act**	**Fresh-water CMC[1]** (acute)	**Fresh-water CCC[2]** (chronic)	**Long-term exposure**	**Short-term exposure**	**Guidelines for fresh and marine water quality**
		FRA [55]	US EPA [56]	US EPA [56]	CCME [57]	CCME [57]	ANZECC & ARMCANZ [58]
		µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Aluminum	Al	50.000	N.D.	N.D.	N.D.	N.D.	55.000
Antimony	Sb	90.000	N.D.	N.D.	N.D.	N.D.	9.000
Arsenic	As	200.000	340.000	150.000	5.000	5.000	24.000
Barium	Ba	10.000	N.D.	N.D.	N.D.	N.D.	N.D.
Boron	B	N.D.	N.D.	N.D.	1,500.000	29,000.000	940.000
Cadmium	Cd	4.000	1.800	N.D.	0.090	1.000	0.200
Copper	Cu	50.000	N.D.	N.D.	N.D.	N.D.	0.470
Total Chrome	Cr	50.000	N.D.	N.D.	N.D.	N.D.	N.D.

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Cr (III)	Cr^{+3}	N.D.	570.000	74.000	8.900	8.900	3.300
Cr (VI)	Cr^{+6}	N.D.	16.000	11.000	1.000	1.000	1.000
Iron	Fe	1,000.000	N.D.	1,000.000	N.D.	N.D.	300.000
Manganese	Mn	N.D.	N.D.	N.D.	430.000	3,600.000	1,900.000
Mercury	Hg	0.500	1.400	0.770	0.026	0.026	0.600
Nickel	Ni	600.000	470.000	52.000	87.000	87.000	11.000
Lead	Pb	30.000	65.000	2.500	N.D.	N.D.	3.400
Selenium	Se	8.000	N.D.	N.D.	N.D.	N.D.	11.000
Zinc	Zn	20.000	120.000	120.000	7.000	37.000	8.000

1/ CMC: Criterion Maximum Concentration; 2/ CCC: Criterion Continuous Concentration; N.D. No limits available.

2.5. The Heavy Metal Pollution Index (HPI)

The Heavy Metal Pollution Index (*HPI*) is a method originally developed by Mohan and Nithila in 1996 [59]. *HPI* is a numerical value that measures the overall influence of each heavy metal contributing to contamination in water bodies. It helps to assess and evaluate the combined influence of all heavy metals on overall water quality and uses a weighted arithmetic mean method in the assessment of overall water quality with respect to heavy metal pollution (equation 1) [60].

$$HPI = \frac{\sum_{i=1}^{n} W_i Q_i}{\sum_{i=1}^{n} W_i} \quad (1)$$

where Q_i is the subindex of the i-th parameter, and the W_i is the unit weight of the i-th parameter and n is the number of parameters considered. The subindex Q_i of the *HPI* described in Equation 1 is defined as:

$$Q_i = \sum_{i=1}^{n} \frac{|M_i - I_i|}{S_i - I_i}, \quad (2)$$

where M_i (μ/L) is the monitored value of the i-th parameter. I_i is the ideal value of the i-th parameter and S_i is the standard value reported by the environment or health agencies. In this study, the standard values are those reported by federal agencies to protect aquatic life, as shown in Table 2.

Equation 2 was adapted from the original equation to follow a criterion similar to that proposed by Caerio et al. in 2005 [61]. The critical value of the *HPI*, which establishes the acceptable limit for drinking water, is 100 [59]. Therefore, *HPI* was used to classify the values that we obtained into three categories: "low" when the index value is below 100, "near threshold" at 100, and "high" or "critical" when exceeding 100 [60,62].

2.6. Human Health Risk Assessment

Human Health Risk Assessment (*HHRA*) is directly related to the nature and severity of the negative health effects that individuals may experience when exposed to environmental contaminants [63]. In the case of heavy metals, consuming drinking water contaminated with them increases the risk of developing both cancerous and non-cancerous diseases in humans [64]. In this study, we considered the carcinogenic and non-carcinogenic risks of the metals identified at the sampling stations using the methods specified by the US EPA [65]. This represents the first study reported in the literature considering these risks from heavy metals in the basin. The health risk assessment was calculated through oral and dermal exposures: non-cancerous (Fe, Zn, As, Cu, Cr, Cd) and cancerous risks from the dissolved metals (As, Cr, Cd) according to [66,67].

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The hazard indices for non-cancerous health risks were calculated using Hazard quotients (HQ) from the ingestion and dermal absorption pathways. These indices reflected the overall potential health risks to both children and adults posed by various heavy metals.

2.7. Exposure Assessment

A technique for measuring the health risks posed by heavy metals, analyzing both non-cancerous and cancerous effects, involves considering the direct ingestion and absorption of contaminated water or skin contact during certain activities [65–67].

2.8. Non-Cancerous Health Risk

According to Selvam et al. [64], the dose of the contaminant consumed by humans is calculated using the chronic daily intake (*CDI*) in mg/kg/day, obtained through the ingestion pathway ($CDI_{ingestion}$) and dermal absorption (CDI_{dermal}) using the equations 3 and 4.

$$CDI_{ingestion} = C_{water}\ X\ \frac{(IR\ x\ EF\ x\ ED)}{(BW\ x\ AT)}, \quad (3)$$

$$CDI_{dermal} = C_{water}\ X\ \frac{(SA\ x\ Kp\ x\ ET\ x\ EF\ x\ ED\ x\ CF)}{(BW\ x\ AT)}, \quad (4)$$

where C_{water} is the average concentration of heavy metals in the water (mg/L), *IR* is the ingestion rate per unit time (0.64 L/day for a child and 2 L/day for an adult); *EF* is the exposure frequency (365 days/year); *ED* is the exposure duration (6 years for child and 70 years for adult); *SA* is the skin area (6600 cm^2 for children and 18 000 cm^2 for adults): K_p is the permeability coefficient (0.001 cm/h for Cr and 0.001 cm/h for other metals); *ET* is the exposure time (1 h/day for children and 0.58 h/day for adults); *CF* is the conversion factor (0.001 L/cm^3); *BW* is body weight (20 kg for child and 70 kg for adult); *AT* is the averaging exposure time (for cancerous, *AT* (70×365) is 25 550 days for both children and adults; for non-cancerous, *AT* (*ED*×365) is 2 190 days for children and 10 950 days for adults [64,66,67].

The *HQ* was calculated from *CDI* ($CDI_{ingestion}$ and CDI_{dermal}) and *RfD* ($RfD_{ingestion}$ and RfD_{dermal}) using the equation 5:

$$\frac{HQ_{ingestion}}{HQ_{dermal}} = \frac{CDI_{ingestion}/\ CDI_{dermal}}{RfD_{ingestion}/RfD_{dermal}}, \quad (5)$$

where *RfD* is the reference dose in mg/kg/day for ingestion is: 0.3 for arsenic (As), 1.4 for Lead, 0.5 for cadmium (Cd), 40 for copper (Cu), 300 for zinc (Zn), 300 for iron (Fe), 3.0 for chrome (Cr), 2.00 E-02 for nickel (Ni); and for dermal absorption: 0.123 for arsenic (As), 0.42 for lead (Pb), 0.005 for cadmium (Cd), 12 for copper (Cu), 60 for zinc (Zn), 45 for iron (Fe), 0.015 for chrome (Cr) and 5.60 E-03 for nickel (Ni) [70]. Finally, the total potential non-cancerous risks were evaluated by calculating the hazard index (*HI*) using the equation 6:

$$HI = HQ_{ingestion} + HQ_{dermal} = \frac{CDI}{RfD}, \quad (6)$$

Toxic metals with an *HI* and *HQ* greater than 1.0 may cause negative effects, while those with values less than 1 are unlikely to have adverse effects on human health [64].

2.9. Cancerous Health Risk

The cancerous risks (CR) were evaluated using the following equations:

$$CR = CDI\ x\ CSF, \quad (7)$$

$$TCR = CR_{ingestion} + CR_{dermal}, \quad (8)$$

where *TCR* means Total Carcinogenic Risk. The standard values of the cancer slope factor (*CSF*) oral for assessing risks are: 0.5, 1.5, 6.1, 0.0085 and 1.7 ppb/day for Cr, As, Cd, Pb and Ni respectively [68].

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The *CSF* dermal for assessing risks is: 41, 1.5, 6.3, 0.043 and 2 ppb/day for Cr, As, Cd, Pb and Ni respectively. The acceptable or tolerable range for carcinogenic risk is from 0.000001 to 0.0001. If the *CR* or *TCR* of an element exceeds 0.0001 , it could have harmful effects on human health [64].

2.7. Statistical Analyses

All data were processed and analyzed using Excel 2021. Correlation analysis was performed by Pearson's correlation method using Minitab statistical software version 21.4.2 (Minitab Ltd., Coventry, UK). Additionally, a scatter plot matrix was created to analyze the patterns and relationships between heavy metals, considering the concentrations found in the samples.

3. Results and Discussion

3.1. Heavy Metals in the Monitoring Stations

The concentrations, means and standard deviations of the heavy metals in this study are listed in Table 3. The following sequence (highest to lowest) was found according to the mean concentration values calculated: Fe>Al>Mn >B>Ba>Zn>As>Cu>Cr>Ni>Cd. It was found that Fe, Mn, As, Cu, Cr, Ni, and Cd are below the permissible limits according to the Federal Rights Act in Mexico for the protection of aquatic life in freshwater bodies [55], whereas Al, B, Ba, and Zn exceed the established limits.

The metal with the highest mean concentration was Fe (0.421 mg/L), followed by Al (0.372 mg/L), Mn (0.165 mg/L), and B (0.153 mg/L). Iron concentrations do not represent a risk in the SGRB, as their presence is associated with the natural conditions of rocks and in sediments and the mineralization process [69]. This metal, when found with manganese (Mn), gives rise to the formation of reactive minerals that influence the behavior of organic matter in soil or water. Iron (Fe) forms minerals in different chemical forms, such as oxides (formed by the reaction of iron with oxygen), hydroxides (when combined with water) and oxyhydroxides (when combined with oxygen and water) [70]. Some of these minerals include ferrihydrite, goethite, and lepidocrocite, which are common forms of reactive iron minerals [70]. These minerals do not remain where they were formed but can be transported to other locations through natural processes such as hydric or wind erosion, these minerals then reach soils and sediments in rivers and streams, where they are deposited [71].

Aluminum (Al) was detected in all sampling stations above the maximum permissible limit (0.05 mg/L) (see Table 2). Boron (B) was detected in all stations; however, concentrations of this metal are not regulated in Mexico (see Table 2). Its presence in river systems is associated with mineralization processes but also with wastewater discharges resulting mainly from agriculture activities [72,73]. Barium (Ba) exhibited a similar behavior, with a permissible limit of 0.01 mg/L (see Table 2). However, its presence may be primarily associated with river sediments containing minerals composed of aluminum oxide and silicon dioxide, which are typically derived from igneous and metamorphic rocks [74]. Therefore, the presence of Fe, Al, Mn, B, and Ba metals in all monitored stations is mainly due to the dissolution of minerals that are specific to the regional geology and to various geochemical processes rather than to anthropogenic activities [69].

In the case of Zn, only six of the 25 sampling stations (S1, S10, S16, S18, S19, and S20) showed concentrations above the established limit (0.02 mg/L). These higher values may be associated with an increase in natural processes such as soil/rock leaching and forest fires, as well as anthropogenic activities such as steel production, domestic sanitation systems, disposal of industrial and urban wastewater, mining, leachate from municipal landfills, application of fertilizers, insecticides, fungicides, cosmetics, and/or paints [75].

For As, Cu, Cr, Ni, and Cd a standard deviation slightly higher than the total mean obtained at all sampling stations was observed (Table 3). Natural processes, such as precipitation, erosion, water flow, and biological activity can cause significant fluctuations in the concentrations of heavy metals, contributing to a high standard deviation [76,77]. It could also be associated with the polluted

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environment of the river basin itself, as heavy metal concentrations can vary widely from one point to another due to water flows and sedimentation processes [78].

On the other hand, the existence of sampling points with extremely high concentrations, as often happens with outliers, can increase the standard deviation. Studies have shown that a significant factor explaining these increases is associated with a small sample size that does not adequately capture the spatial and temporal variability [79].

Conversely, the metal with the lowest average concentrations detected was cadmium (Cd) with a value of 0 mg/L, which had a very small sample size, ranging from 0 to 2 values per station, which could be associated with the higher standard deviation value obtained for the mean. Despite the few samples detected during the study at the different sampling stations, the highest average value for this metal was at station S16 (0.003 mg/L), which is below the permissible limits established for river water according to the Federal Rights Act in Mexico [55]. Therefore, this metal does not pose any danger to aquatic life in the Santiago-Guadalajara River Basin. The few concentrations of cadmium (Cd) detected could be associated with certain phosphate fertilizers that contain it and that infiltrate the soil during their application in crops [80].

Table 3. Mean concentrations of heavy metals at 25 sampling stations in the SGRB from July 2021 to April 2022.

Monitoring Stations	Al	As	Ba	B	Cd	Cu	Cr	Fe	Mn	Ni	Zn
S1	0.208	0.000	0.064	0.023	0.000	0.000	0.000	0.885	0.452	0.000	0.026
S2	0.094	0.000	0.006	0.039	0.000	0.002	0.001	0.232	0.041	0.000	0.012
S3	0.205	0.000	0.048	0.041	0.000	0.000	0.000	0.591	0.069	0.002	0.007
S4	0.178	0.000	0.049	0.050	0.000	0.000	0.001	0.285	0.063	0.000	0.005
S5	0.138	0.008	0.037	0.074	0.000	0.001	0.000	0.405	0.093	0.000	0.006
S6	0.260	0.000	0.041	0.078	0.000	0.000	0.000	0.263	0.044	0.000	0.004
S7	0.558	0.003	0.076	0.240	0.000	0.001	0.000	0.611	0.109	0.000	0.004
S8	0.687	0.000	0.041	0.125	0.000	0.000	0.000	0.545	0.132	0.000	0.005
S9	0.328	0.001	0.078	0.228	0.000	0.005	0.000	0.658	0.300	0.000	0.009
S10	0.474	0.000	0.023	0.049	0.000	0.005	0.006	0.401	0.195	0.003	0.067
S11	0.235	0.002	0.041	0.415	0.000	0.001	0.000	0.193	0.155	0.000	0.016
S12	0.282	0.002	0.077	0.215	0.000	0.000	0.002	0.261	0.116	0.000	0.011
S13	0.218	0.002	0.072	0.234	0.000	0.000	0.000	0.222	0.104	0.000	0.003

S14	0.281	0.002	0.076	0.214	0.000	0.000	0.002	0.254	0.125	0.000	0.010
S15	0.099	0.002	0.064	0.217	0.000	0.000	0.000	0.158	0.126	0.000	0.006
S16	0.697	0.001	0.047	0.177	0.003	0.008	0.000	0.434	0.128	0.003	0.026
S17	0.522	0.003	0.016	0.050	0.000	0.001	0.002	0.387	0.002	0.000	0.007
S18	1.007	0.001	0.070	0.193	0.000	0.002	0.000	1.174	0.320	0.000	0.048
S19	0.146	0.000	0.023	0.178	0.000	0.000	0.000	0.173	0.153	0.000	0.025
S20	0.966	0.001	0.053	0.343	0.000	0.002	0.000	0.420	0.187	0.022	0.043
S21	0.222	0.001	0.031	0.110	0.000	0.000	0.001	0.553	0.544	0.000	0.004
S22	0.200	0.000	0.031	0.035	0.000	0.009	0.000	0.331	0.148	0.000	0.128
S23	0.822	0.011	0.053	0.191	0.000	0.001	0.001	0.347	0.228	0.000	0.002
S24	0.253	0.000	0.053	0.082	0.000	0.001	0.001	0.464	0.112	0.003	0.016
S25	0.233	0.002	0.085	0.236	0.000	0.002	0.000	0.272	0.168	0.000	0.010
Min	0.094	0.000	0.006	0.023	0.000	0.000	0.000	0.158	0.002	0.000	0.002
Max	1.007	0.011	0.085	0.415	0.003	0.009	0.006	1.174	0.544	0.022	0.128
Mean	0.372	0.002	0.050	0.153	0.000	0.002	0.001	0.421	0.165	0.001	0.020
SD	0.269	0.003	0.022	0.103	0.001	0.003	0.001	0.236	0.125	0.004	0.028

3.2. Heavy Metal Pollution Index

The mean concentrations of the 11 heavy metals were considered in the *HPI* calculation. Table 4 shows the values of the assigned unit weights (W_i), calculated sub-index values (Qi), and HPI. The critical value for HPI is 100, and any value above 100 indicates that the level of heavy metal contamination in the river is significantly high [60]. The heavy metal pollution index was calculated for each sampling site (Table 4). In the study area of the SGRB, the average *HPI* value obtained was = 314.436, which is an indication of highly polluted water (HPI > 100) and therefore not suitable for drinking.

Table 4. Values of *HPI* based on mean concentrations of heavy metals for all sampling stations in the SGRB.

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Heavy metal	Mean Concentration (mg/L)	(S_i)	(I_i)	(W_i)	(Q_i)	W_iQ_i	*HPI*
Al	0.372	0.055	0.050	18.182	6,449.096	117,256.283	277.572
As	0.002	0.340	0.005	2.941	0.896	2.634	0.006
Ba	0.050	0.010	0.000	100.000	501.360	50,136.000	118.683
B	0.153	29.000	0.940	0.034	2.794	0.096	0.000
Cd	0.000	0.004	0.000	250.000	0.870	217.391	0.515
Cu	0.002	0.050	0.000	20.000	2.532	50.636	0.120
Cr	0.001	0.050	0.000	20.000	1.378	27.556	0.065
Fe	0.421	1.000	0.300	1.000	17.255	17.255	0.041
Mn	0.165	3.600	0.430	0.278	8.360	2.322	0.005
Ni	0.001	0.600	0.011	1.667	1.698	2.830	0.007
Zn	0.020	0.120	0.007	8.333	11.504	95.870	0.227

Critical values above 100 were obtained for the 25 sampling stations, except for S2, corresponding to El Taretán stream (72.238) and S17 in La Soledad River (80.952). The highest values of this index were recorded for three stations: S7 of the Zula River (618.045), S20 corresponding to El Ahogado stream (914.455), and S23 in the La Calera River (790.226). These points indicate high heavy metal contamination and high risk for aquatic life. In the El Ahogado basin, a high degree of environmental degradation has been documented [81,82]. This has caused severe socio-environmental conflicts, leading to collective action by organizations at local, national, and international levels to combat the problem affecting the entire basin [26,83]. Along with the three stations, S1 (Arroyo La Madrastra) = 331.960, S4 (Arroyo Los Morales) = 449.830, S9 (Arroyo Grande) = 430.390, S10 (Río Zapotlanejo) = 419.711, S12 (Río Santiago) = 382.528, S14 (Arroyo Las Pintas) = 378.107 and S25 (Río Los Sabinos) = 358.711 showed values above the average HPI, while the rest were below but still represented highly contaminated sites with heavy metals, with HPI values > 100.

Table 5. Heavy metal pollution index estimated for each sampling station in the Santiago-Guadalajara River Basin.

Sampling station	*HPI*	STD	Sampling station	*HPI*	STD
S1	331.960	± 67.307	**S14**	378.107	± 76.531
S2	72.238	± 17.685	**S15**	193.424	± 46.154
S3	156.798	± 35.396	**S16**	195.640	± 35.315
S4	449.830	± 73.230	**S17**	80.952	± 16.351
S5	163.180	± 33.064	**S18**	209.048	± 50.104
S6	140.513	± 30.860	**S19**	137.450	± 28.418
S7	618.045	± 137.569	**S20**	914.455	± 236.881
S8	139.547	± 30.602	**S21**	221.258	± 47.736
S9	430.390	± 86.589	**S22**	205.968	± 42.750
S10	419.711	± 109.551	**S23**	790.226	± 200.020
S11	256.725	± 53.726	**S24**	299.991	± 61.602
S12	382.528	± 77.401	**S25**	358.711	± 73.150
S13	314.205	± 63.775	**Mean**	**314.436**	

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3.3. Correlation Analysis

The correlation index is used to understand the linear relationship between two variables [84], such that high correlation coefficient values show good correspondence between them and vice versa. If the correlation coefficient (r) value is close to zero, there is no correspondence between the variables [85]. However, a value close to one means there is a good relationship, but if it is negative, then it is an inverse correlation. To understand the relationship between the heavy metals analyzed in this study, Pearson correlation coefficients were calculated for the water samples from the SGRB, and the results are summarized in Table 6.

Table 6. Pearson´s correlation matrix of heavy metals in water samples of SGRB.

Heavy metal	Al	As	Ba	B	Cd	Cu	Cr	Fe	Mn	Ni	Zn
Al	1.000										
As	0.275	1.000									
Ba	0.133	0.095	1.000								
B	0.302	0.167	**0.492***	1.000							
Cd	0.245	-0.047	0.013	0.069	1.000						
Cu	0.211	-0.075	-0.151	-0.081	**0.564***	1.000					
Total Cr	0.003	-0.046	-0.3	-0.296	-0.128	0.072	1.000				
Fe	**0.481***	-0.062	0.243	-0.165	0.043	0.092	-0.144	1.000			
Mn	0.145	-0.005	0.186	0.059	-0.027	0.026	-0.013	**0.566***	1.000		
Ni	**0.474***	-0.084	-0.011	0.32	0.052	0.095	-0.008	0.016	0.012	1.000	
Zn	0.145	-0.268	-0.228	-0.174	0.033	**0.709***	0.177	0.135	0.126	0.214	1.000

*Correlation is significant at the 0.05 level (2-tailed).

A correlation analysis was performed between the average concentrations of heavy metals in water samples from the basin to evaluate possible similar sources of origin. It was observed that there is an inverse relationship between the metals Cd, Cu, Cr, Fe, Mn, Ni, and Zn with Arsenic, between Cu, Cr, Ni, and Zn with Ba, between Cu, Cr, Fe, and Zn with B, between Cr and Mn with Cd, as well as between Fe, Mn, and Ni with Cr. This means that the appearance of metals in the basin is not related to each other and that they have different sources of origin. In Figure 5, it can be observed that there is a positive correlation between B-Ba (r = 0.492), Fe-Al (r = 0.481), Ni-Al (r = 0.474), Cu-Cd (r = 0.564), Zn-Cu (r = 0.709), and Mn-Fe (r = 0.566). The strong correlations found between B-Ba, Fe-Al, and Mn-Fe demonstrates that these metals are indeed strongly associated with the composition of the lithological structure of the region [69].

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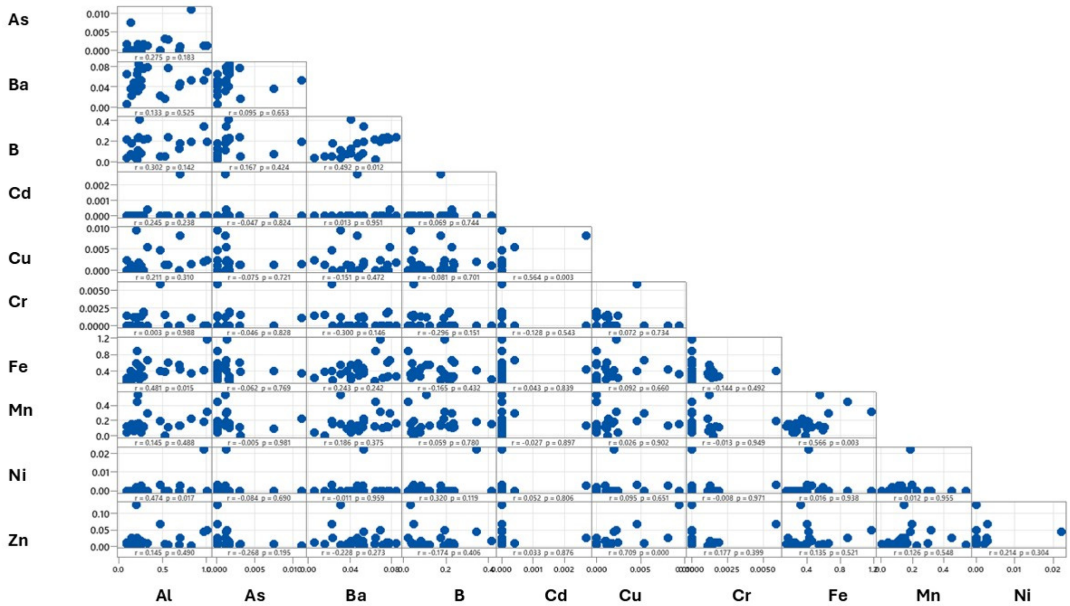

Figure 5. Plot matrix showing relationships of heavy metals in water samples from the SGRB.

The correlations found between Ni-Al, Cu-Cd, and Zn-Cu could be associated with anthropogenic sources of contamination. These correlations suggest that industrial effluents, toxic waste from processing units near the sampling stations in the SGRB, and untreated domestic wastewater discharges are the main sources of contamination, as has already been demonstrated in other river basins by other authors [60]. These results may be relevant for understanding common sources of contamination and indicate the interactions between these pollutants in the environment and their potential effects on health and the ecosystem.

3.4. Human Health Risk Assessment

3.4.1. Non-Cancerous Health Risks

The results presented in Table 7 show the non- cancerous hazard index (*HI*) for various heavy metals in water samples from different stations in the SGRB. The Hazard Quotient (*HQ*) and Hazard Index (*HI*) were used to assess the risk, where an HI greater than 1.000 indicates a potential risk for adverse health effects, and an *HI* less than 1.000 suggests that the exposure is unlikely to cause harmful effects. These values are calculated for both children and adults, and the metals assessed were As, Cd, Cu, Cr, Fe, Pb and Zn. The average values of the *HQ* for both children and adults are generally low, with the lowest average value found being 0.001 for copper (Cu) in children and adults, while the highest was 0.176 for Arsenic (As) in children. These values indicate that, in general, the water in the SGRB does not pose an immediate non-carcinogenic health risk related to the metals studied, as values below 1.000 are unlikely to have adverse effects on human health, as reported [86,87]. However, some sampling stations show higher values, suggesting the possibility of localized contamination in these areas.

Table 7. The hazard index (*HI*) of non-carcinogenic risk (*HQ*) among children and adults in the water of the SGRB.

	***HQ* Children**	***HQ* Adults**

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Monitoring Stations	**As**	**Cd**	**Cu**	**Cr**	**Fe**	**Ni**	**Pb**	**Zn**	***HI***	**As**	**Cd**	**Cu**	**Cr**	**Fe**	**Ni**	**Pb**	**Zn**	***HI***
S1	0.000	0.000	0.000	0.000	0.101	0.000	0.000	0.003	0.104	0.000	0.000	0.000	0.000	0.087	0.000	0.000	0.003	0.090
S2	0.000	0.000	0.002	0.077	0.026	0.000	0.000	0.001	0.106	0.000	0.000	0.002	0.041	0.023	0.000	0.000	0.001	0.067
S3	0.000	0.000	0.000	0.000	0.067	0.003	0.000	0.001	0.072	0.000	0.000	0.000	0.000	0.058	0.003	0.000	0.001	0.062
S4	0.000	0.000	0.000	0.066	0.032	0.000	0.000	0.001	0.099	0.000	0.000	0.000	0.035	0.028	0.000	0.000	0.000	0.064
S5	**0.820**	0.000	0.001	0.000	0.046	0.000	0.000	0.001	**0.868**	**0.723**	0.000	0.001	0.000	0.040	0.000	0.000	0.001	**0.765**
S6	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.026
S7	0.328	0.000	0.001	0.000	0.070	0.000	0.000	0.000	0.399	0.289	0.000	0.001	0.000	0.060	0.000	0.000	0.000	0.351
S8	0.000	0.000	0.000	0.000	0.062	0.000	0.000	0.001	0.063	0.000	0.000	0.000	0.000	0.054	0.000	0.000	0.000	0.054
S9	0.131	0.052	0.004	0.000	0.075	0.000	0.000	0.001	0.264	0.116	0.035	0.004	0.000	0.065	0.000	0.000	0.001	0.220
S10	0.000	0.000	0.004	0.323	0.046	0.005	0.000	0.007	0.385	0.000	0.000	0.003	0.174	0.040	0.004	0.000	0.007	0.227
S11	0.164	0.000	0.001	0.000	0.022	0.000	0.000	0.002	0.189	0.145	0.000	0.001	0.000	0.019	0.000	0.000	0.002	0.166
S12	0.175	0.000	0.000	0.104	0.030	0.000	0.000	0.001	0.310	0.154	0.000	0.000	0.056	0.026	0.000	0.000	0.001	0.237
S13	0.175	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.201	0.154	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.177

S14	0.175	0.000	0.000	0.093	0.029	0.000	0.000	0.001	0.298	0.154	0.000	0.000	0.050	0.025	0.000	0.000	0.001	0.230
S15	0.175	0.000	0.000	0.000	0.018	0.000	0.000	0.001	0.194	0.154	0.000	0.000	0.000	0.016	0.000	0.000	0.001	0.170
S16	0.119	0.351	0.007	0.000	0.050	0.004	0.000	0.003	0.534	0.105	0.235	0.006	0.000	0.043	0.004	0.000	0.003	0.395
S17	0.339	0.000	0.001	0.082	0.044	0.000	0.000	0.001	0.467	0.299	0.000	0.001	0.044	0.038	0.000	0.000	0.001	0.383
S18	0.142	0.000	0.002	0.000	0.134	0.000	0.544	0.005	**0.827**	0.125	0.000	0.002	0.000	0.116	0.000	0.478	0.005	**0.725**
S19	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.003	0.022	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.002	0.019
S20	0.131	0.000	0.002	0.000	0.048	0.037	0.000	0.005	0.222	0.116	0.000	0.001	0.000	0.041	0.032	0.000	0.004	0.195
S21	0.121	0.000	0.000	0.067	0.063	0.000	0.000	0.000	0.252	0.107	0.000	0.000	0.036	0.055	0.000	0.000	0.000	0.198
S22	0.000	0.000	0.008	0.000	0.038	0.000	0.000	0.014	0.060	0.000	0.000	0.007	0.000	0.033	0.000	0.000	0.012	0.052
S23	**1.225**	0.000	0.001	0.060	0.040	0.000	0.000	0.000	**1.326**	**1.080**	0.000	0.001	0.032	0.034	0.000	0.000	0.000	**1.148**
S24	0.000	0.000	0.001	0.071	0.053	0.005	0.000	0.002	0.131	0.000	0.000	0.001	0.038	0.046	0.004	0.000	0.002	0.090
S25	0.175	0.000	0.001	0.000	0.031	0.000	0.000	0.001	0.209	0.154	0.000	0.001	0.000	0.027	0.000	0.000	0.001	0.183
Min	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.019
Max	1.225	0.351	0.008	0.323	0.134	0.037	0.544	0.014	1.326	1.080	0.235	0.007	0.174	0.116	0.032	0.478	0.012	1.148

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Mean	0.176	0.016	0.001	0.038	0.048	0.002	0.022	0.002	0.305	0.155	0.011	0.001	0.020	0.041	0.002	0.019	0.002	0.252
Samples exceeding the limit	**1.000**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**1.000**	**1.000**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**1.000**
% of samples exceeding the limit	**4.0**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**4.0**	**4.0**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**4.0**

There is considerable variation in the *HI* values between the different stations. Stations S5 (Río Calderón), S18 (Arroyo El Ahogado), and S23 (Río La Calera) showed the highest levels, while station S19 displayed the lowest values. Although the *HI* values obtained at S5 were below 1.000 (0.868 for children and 0.765 for adults) and at station S18 as well (0.827 for children and 0.725 for adults), continuous monitoring should be implemented to study the behavior of these sites over time and prevent potential future impacts on human health. These locations may represent critical points of pollution that require further investigation and action. The highest *HI* value for children was obtained at station S23, with a value of 1.326, while for adults it was 1.148 (*HI* >1). Station S23 showed elevated *HQ* values for both children and adults for arsenic, indicating that heavy metal concentrations here could pose health risks. The metal contributing to this high *HI* is arsenic (As). The results suggest that long-term exposure at this station could lead to harmful effects such as neurological damage or carcinogenic effects (associated with As), emphasizing the importance of closely monitoring this area.

Similar results have been reported in other recent studies for this heavy metal, indicating a potentially significant risk [86,88]. Since the communities in this basin are primarily engaged in agriculture, small-scale manufacturing, and trade, the sources of arsenic in this basin are likely associated with the presence of this metalloid in groundwater, which may surface during rainfall and flow into the tributary channels of this river.

The values obtained in S5 for arsenic (As) are the highest values for this metal, after those found at S23. Low levels of arsenic exposure can have serious health consequences, particularly over the long term [86,88]. Unlike station S5, station S23 is located at a point where treated and untreated wastewater from the basin converge, making it more representative of the urban, industrial and agricultural pollution generated in the basin.

In general, HI values for children are higher than those for adults, which is expected due to children's increased vulnerability to toxic pollutants. This is because children have a lower body weight and might consume or use more water relative to their size, making them more susceptible to contaminants [87]. Since children are in a critical stage of development and are more likely to experience adverse health effects from waterborne pollutants, their exposure levels must be a key focus in water quality assessments [89].

Arsenic is the primary metal contributing to elevated HI values at several stations. Long-term exposure to arsenic is known to cause skin issues, lung and organ damage, and increase the risk of cancer [90–92]. While this study focuses on non-cancer risks, it is still crucial to address arsenic levels. Lead (Pb), another key concern, showed high HI values in places such as S18. Lead (Pb) is a well-known neurotoxin, and its presence in drinking water is particularly dangerous for children, affecting brain development and overall health [93–95]. Most of the stations exhibit HI values below critical limits, but the higher readings at stations like S5, S18, and S23 highlight areas that may present more significant risks, particularly for vulnerable groups such as children.

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While the overall water quality in the SGRB appears to meet safety standards for non-cancer risks, there are still concerns about point source contamination, particularly from arsenic and lead. Arsenic (As) requires closer monitoring at S23 because, in this study, the concentrations exceeded recommended safety levels. The higher HI values for children emphasize the importance of prioritizing their health in water quality regulations, and child-specific safety standards should be implemented. Ongoing monitoring and public awareness campaigns can help minimize exposure to these harmful pollutants. Additionally, further research into the sources of contamination in these high-risk areas is essential to prevent future problems. SGRB is generally acceptable in terms of non-cancer risks, but certain areas, especially those with high arsenic (As) and lead (Pb) concentrations, require more research, monitoring, regulation focused attention, particularly due to the heightened risks for children.

3.4.2. Cancerous Health Risk

The cancer risk (*CR*) was calculated for As, Cd, Cr, Ni, and Pb. Table 8 shows the overall carcinogenic risk (*TCR*), which is the sum of *CR* from both ingestion and dermal contact exposure, for both children and adults. Our results for the *TCR* associated with these metals showed a minimum mean value of 0.0E+00 for both children and adults, and a maximum mean value of 1.3E-03 for children only. In some stations such as S3, S5, S7, S16, S17, S23, and S24, the obtained values for *CR* and *TCR* were higher than 0.0001, which could indicate potential health problems.

The lowest *TCR* values at most sampling sites were 0.0E+00, indicating that no detectable carcinogenic risk from metals was found in these areas. This implies that the water in those locations is free of carcinogenic metals or minimally contaminated by them, which is a positive finding for public health [96,97]. At station S5, high concentrations of arsenic (As) for both children and adults with values of 3.6E-04 and 3.2E-04 respectively exceed the critical limit of 0.0001 for cancer risk. This suggests that the arsenic (As) risk at this station may be significant, particularly for children who are more vulnerable to environmental pollutants. Cadmium (Cd) and nickel (Ni) at station S16 showed values exceeding the threshold. For cadmium, a value of 5.3E-04 was obtained, while for nickel it was 1.4E-04 for children. For adults, the obtained value for cadmium was 4.7E-04, and for nickel it was 1.3E-04. This highlights the importance of closely monitoring these metals in areas where industrial pollution may contribute to environmental contamination. Meanwhile, nickel (Ni) at station S20 also shows concerning levels above the established limit, with values of 1.2E-03 for children and 1.1E-03 for adults. This indicates a significant cancer risk, especially from exposure to this metal.

Table 8. The overall carcinogenic risk (*TCR*) for both children and adults in water in the SGRB.

Monitoring Stations	CR in Children					TCR	CR in Adults					TCR
	As	Cd	Cr	Ni	Pb		As	Cd	Cr	Ni	Pb	
S1	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
S2	0.0E+00	0.0E+00	6.0E-05	0.0E+00	0.0E+00	6.0E-05	0.0E+00	0.0E+00	3.7E-05	0.0E+00	0.0E+00	3.7E-05
S3	0.0E+00	0.0E+00	0.0E+00	1.1E-04	0.0E+00	1.1E-04	0.0E+00	0.0E+00	0.0E+00	9.8E-05	0.0E+00	9.8E-05
S4	0.0E+00	0.0E+00	5.2E-05	0.0E+00	0.0E+00	5.2E-05	0.0E+00	0.0E+00	3.2E-05	0.0E+00	0.0E+00	3.2E-05
S5	3.6E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	3.6E-04	3.2E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	3.2E-04

S6	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
S7	1.5E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	1.5E-04	1.3E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	1.3E-04
S8	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
S9	5.8E-05	7.9E-05	0.0E+00	0.0E+00	0.0E+00	1.4E-04	5.2E-05	7.0E-05	0.0E+00	0.0E+00	0.0E+00	1.2E-04
S10	0.0E+00	0.0E+00	2.5E-04	1.7E-04	0.0E+00	4.2E-04	0.0E+00	0.0E+00	1.6E-04	1.5E-04	0.0E+00	3.0E-04
S11	7.3E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	7.3E-05	6.5E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	6.5E-05
S12	7.8E-05	0.0E+00	8.2E-05	0.0E+00	0.0E+00	1.6E-04	6.9E-05	0.0E+00	5.0E-05	0.0E+00	0.0E+00	1.2E-04
S13	7.8E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	7.8E-05	6.9E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	6.9E-05
S14	7.8E-05	0.0E+00	7.3E-05	0.0E+00	0.0E+00	1.5E-04	6.9E-05	0.0E+00	4.5E-05	0.0E+00	0.0E+00	1.1E-04
S15	7.8E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	7.8E-05	6.9E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	6.9E-05
S16	5.3E-05	5.3E-04	0.0E+00	1.4E-04	0.0E+00	7.3E-04	4.7E-05	4.7E-04	0.0E+00	1.3E-04	0.0E+00	6.5E-04
S17	1.5E-04	0.0E+00	6.5E-05	0.0E+00	0.0E+00	2.1E-04	1.3E-04	0.0E+00	4.0E-05	0.0E+00	0.0E+00	1.7E-04
S18	6.3E-05	0.0E+00	0.0E+00	0.0E+00	6.6E-06	7.0E-05	5.6E-05	0.0E+00	0.0E+00	0.0E+00	5.7E-06	6.2E-05
S19	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
S20	5.8E-05	0.0E+00	0.0E+00	1.2E-03	0.0E+00	1.3E-03	5.2E-05	0.0E+00	0.0E+00	1.1E-03	0.0E+00	1.1E-03
S21	5.4E-05	0.0E+00	5.3E-05	0.0E+00	0.0E+00	1.1E-04	4.8E-05	0.0E+00	3.2E-05	0.0E+00	0.0E+00	8.0E-05
S22	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
S23	5.4E-04	0.0E+00	4.7E-05	0.0E+00	0.0E+00	5.9E-04	4.8E-04	0.0E+00	2.9E-05	0.0E+00	0.0E+00	5.1E-04
S24	0.0E+00	0.0E+00	5.6E-05	1.5E-04	0.0E+00	2.1E-04	0.0E+00	0.0E+00	3.4E-05	1.4E-04	0.0E+00	1.7E-04
S25	7.8E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	7.8E-05	6.9E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	6.9E-05
Min	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00

Max	5.4E-04	5.3E-04	2.5E-04	1.2E-03	6.6E-06	1.3E-03	4.8E-04	4.7E-04	1.6E-04	1.1E-03	5.7E-06	1.1E-03
Mean	7.8E-05	2.4E-05	3.0E-05	7.2E-05	2.6E-07	2.0E-04	6.9E-05	2.2E-05	1.8E-05	6.4E-05	2.3E-07	1.7E-04

The presence of these metals at low concentrations at some sampling stations in the SGRB could be attributed to agrochemical industries, for example, as their wastewater often contains components of pesticides and fertilizers. Even low levels of heavy metals have been shown to accumulate over time, leading to chronic exposure and long-term health risks, including cancer [98]. Despite the low concentrations observed at some monitoring stations, protecting children from exposure to heavy metals should remain a key focus for future studies and environmental monitoring, as chronic exposure to low levels of these contaminants can have cumulative impacts on children's development, cognitive health, and immune system function [96–99]. When the *CR* or *TCR* exceeds the critical value, there is a heightened concern for carcinogenic health risks. Long-term exposure to these elevated levels of metals like arsenic, cadmium, chromium and nickel can increase the likelihood of cancer, particularly among vulnerable populations such as children [64].

The percentage of samples exceeding the permissible limit varies for different metals. For example, arsenic (As) and nickel (Ni) exceed the threshold in more than 16% and 20% of the samples, respectively, at some stations, indicating that environmental interventions may be necessary to reduce exposure. Nickel (Ni) showed the most concerning pattern, with 20% of the samples exceeding the threshold at some stations (S3, S10, S16, S20, and S24), which represents a serious health issue and is consistent with results obtained by other authors [100–103]. Some metals fall within an acceptable or tolerable risk category, such as arsenic (As) and total chrome (Cr), with percentages of 44% and 32%, respectively. This suggests that, for the most part, these metals do not pose an immediate health hazard. However, long-term exposure within the tolerable range still needs to be monitored to prevent reaching harmful levels. The results indicate that while many stations have concentrations of heavy metals below the threshold established for *TCR*, there are several areas where the cancer risk is high due to metals such as As, Cd, Cr, and Ni. The highest risks were observed at stations S5, S16, and S20, particularly for children. Tighter environmental monitoring and public health measures are recommended in these areas to reduce exposure. Continuous monitoring and efforts to reduce industrial emissions and vehicular pollution are essential to ensure public health, especially in sensitive populations like children.

4. Conclusion

This work carried out a comprehensive study of the presence of heavy metals in the main and secondary streams located in the SGRB. Data collected in a monitoring campaign carried out at 25 sampling stations monthly from July 2021 to April 2022 were used. Analysis of the values obtained for heavy metals revealed critical results for the contamination index of these elements. Of the 25 sampling stations, 23 showed *HPI* results above 100 (critical values), representing 92% of the sampled locations, except for stations S2, stream El Taretán (72.238) and S17, La Soledad River (80.952), whose *HPI* were below 100. The water quality in the Santiago River basin in Guadalajara did not present immediate non-carcinogenic health risks at most monitoring stations, as the Hazard Index (*HI*) values remained low, below the established threshold (<1.000). The highest HI value was recorded at Río La Calera, at station S23 (children: 1.326, adults: 1.148) for arsenic (As). These *HI* values exceeded the established limit, suggesting that heavy metal levels at this location may pose potential health risks. This indicates localized contamination, and frequent periodic monitoring is required as the levels exceed the recommended safety limits.

Cancer risk (*CR*) and total carcinogenic risk (*TCR*) were assessed for arsenic, cadmium, total chrome, nickel, and lead. Most sampling sites did not show detectable cancer risk, but certain stations

(S3, S5, S7, S16, S17, S23, S24) had values exceeding the established limit (0.0001), indicating potential health risks, particularly from arsenic (As), cadmium (Cd), and nickel (Ni). According to national and international criteria, the surface waters of the river are not suitable to sustain aquatic life and may require advanced treatment processes if the water is to be used for other purposes such as agriculture irrigation or as a drinking water supply. The findings of this research may be valuable to government authorities and stakeholders, enabling them to take necessary actions owing to the considerable risk to human health and the river ecosystem posed by the surface waters of the basin. The application of environmental regulations, as well as the implementation of monitoring and control measures, is necessary to reduce the risks of toxicity to aquatic life and the population exposed to contact with river waters.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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Abbreviations

The following abbreviations are used in this manuscript:

ANZECC & ARMCANZ	Australian and New Zealand Guidelines for Freshwater and Marine Water Quality
CCME	Canadian Council of Ministers of the Environment
CDI	Chronic Daily Intake
CSF	Cancer Slope Factor
FRA	Federal Rights Act
HI	Hazard Index
HHRA	Human Health Risk Assessment
HPI	Heavy Metal Pollution Index
HQ	Hazard quotients
ICO-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
ICP	Inductively Coupled Plasma Spectroscopy
MAG	Metropolitan Area of Guadalajara
NIST	National Institute of Standards and Technology

NSDEU	National Statistical Directory of Economic Units
RfD	Reference Dose
SGRB	Santiago-Guadalajara River Basin
STD	Standard Deviation
TCR	Total Carcinogenic Risk
UNICEF	United Nations International Children's Emergency Fund
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
WWTP	Wastewater Treatment Plant
SGRB	Linear dichroism

Appendix A

Appendix A.1

Table A1. Description of the geographical coordinates of the sampling points in the Santiago-Guadalajara River Basin.

Sampling site	Official Name of the sampling site	Name of the sampled tributary	West Longitude	North Latitude
1	Arandas	Arroyo La Madrastra	102° 20' 23''	20° 41' 46''
2	Atotonilco el Alto	Arroyo El Taretán	102° 30' 09''	20° 32' 43''
3	La Ladera	Arroyo Los Morales	102° 43' 50''	20° 35' 50''
4	Gaviotas	Río Calderón	102° 51' 06''	20° 42' 06''
5	San José de Gracia	Río Calderón	102° 42' 07''	20° 47' 10''
6	San Miguel	Arroyo Tierras Coloradas	102° 47' 18''	20° 32' 06''
7	Ocotlán Centro	Río Zula	102° 46' 41''	20° 20' 41''
8	Los Cerritos	Arroyo Chico	102° 44' 58''	20° 26' 47''
9	La Laja	Arroyo Grande	103° 07' 40''	20° 34' 41''
10	Río Zapotlanejo	Río Zapotlanejo	103° 05' 44''	20° 37' 23''
11	La Azucena	Arroyo El Ahogado	103° 13' 40''	20° 29' 51''
12	La Noria	Río Santiago	103° 13' 35''	20° 28' 03''
13	Río Santiago[1]	Río Santiago	103° 11' 04''	20° 27' 17''
14	Carretera Guadalajara – Chapala	Arroyo Las Pintas	103° 15' 55''	20° 28' 41''

15	Presa Corona	Río Santiago	103° 05' 35''	20° 24' 01''
16	Paso a Guadalupe	Río Santiago	103° 19' 44''	20° 50' 20''
17	Rancho La Soledad	Río La Soledad	103° 22' 15''	20° 53' 40''
18	Plan de Oriente	Arroyo El Ahogado	103° 17' 03''	20° 35' 19''
19	Villa Fontana	Arroyo Las Pintas	103° 21' 48''	20° 33' 46''
20	San José del Quince	Arroyo El Ahogado	103° 17' 48''	20° 32' 16''
21	El Arenal	Río Arenal	103° 38' 19''	20° 43' 24''
22	San Isidro	Río Blanco	103° 27' 34''	20° 47' 47''
23	San Cristóbal de la Barranca	Río La Calera	103° 25' 59''	21° 02' 51''
24	Tequila	Río Amatitán	103° 49' 54''	20° 53' 54''
25	Hostotipaquillo	Río Los Sabinos	104° 00' 40''	21° 01' 56''

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