


Article

Generation of Tequila Vinasses, Characterization, Current Disposal Practices and Study Cases of Disposal Methods

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Abstract: Tequila vinasse is a liquid waste generated during the production of tequila, an emblematic alcoholic beverage in Mexico. The objective of this study was to carry out an investigation on the tequila factories located in the state of Jalisco in order to know the location of the factories in the state, the characterization of the vinasses including factories of different sizes, the current treatment methods, and disposal practices as well as the impacts of common practices of vinasse disposal. Part of the information was collected by applying a questionnaire to the tequila factories previously contacted (and physically located). For the vinasse characterization, 24 tequila factories provided a composite sample of vinasse. To assess the impact of common vinasse disposal practices, a stream running through tequila factories, soil that has been used for vinasse discharge for 14 years, and a well located near the soil were evaluated. In two main regions (Valle and Altos Sur), 110 tequila factories distributed in 10 municipalities, were identified. Vinasse disposal and treatment problems are mainly related to micro-factories that do not treat their vinasse at all. The most common method of disposal is discharging on soils. Only in the Valle region is disposal in surface waters a common practice, as well as discharges into sewage systems. The monitored stream is totally degraded with low pH, high concentrations of organic matter, suspended solids, etc. Soil fertility has not been affected due to a method of vinasse discharge-soil rest. The texture of the soils (high content of clay and silt) has been decisive in protecting groundwater from the infiltration of vinasse. The results obtained in this study could help the authorities to develop adequate strategies for the management of vinasses (treatment and disposal), mainly in micro and small tequila factories.

Keywords: groundwater quality; distillery stillage; wastewater characterization; disposal sites; soil fertility



Citation: Zurita, F.; Tejada, A.; Montoya, A.; Carrillo, I.; Sulbarán-Rangel, B.; Carreón-Álvarez, A. Generation of Tequila Vinasses, Characterization, Current Disposal Practices and Study Cases of Disposal Methods. *Water* **2022**, *14*, 1395. <https://doi.org/10.3390/w14091395>

Academic Editors:
Mariusz Adynkiewicz-Piragas and
Daniel Gebler

Received: 4 April 2022

Accepted: 24 April 2022

Published: 26 April 2022

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1. Introduction

Tequila is a Mexican alcoholic beverage produced from the blue variety of the agave species *Agave tequilana* Weber. The tequila production process involves the extraction of sugars from the cooked agave, the fermentation of the sugars, and the distillation of the fermented juice. The tequila Designation of Origin establishes that the blue variety of *Agave tequilana* Weber can only be produced in a specific geographical region that includes all the territory of the State of Jalisco and some municipalities of other states (Michoacán, Nayarit, Tamaulipas, and Guanajuato). Currently, 91% of tequila factories are located in Jalisco [1], but there is no available information regarding the factory distribution in the state which is divided into 12 regions, i.e., Norte, Altos Norte, Altos Sur, Ciénega, Sureste, Sur, Sierra de Amula, Costa Sur, Costa Norte, Sierra Occidental, and Valles y Centro. The production

of this emblematic alcoholic beverage is one of the most important economic activities in Mexico. In 2019, 245.8 million liters of tequila were for export from the 351.7 million liters of tequila produced [2]. In the process of tequila production, there is a generation of a residual liquid (vinasse), which is produced mainly during the distillation process of the fermented agave juice, but also during the cooking of agave and the extraction of sugars. In general, it is considered that around 10–15 L of vinasse are generated per liter of tequila produced [3] depending on the production practices of each factory.

The Mexican Tequila Regulatory Council (CRT by its acronym in Spanish) determines the size of a tequila factory based on the volume of tequila produced per year. In this way, the factories are classified into micro (from 1 to 300 m³/year), small (from 300 to 1000 m³/year), medium (1000 to 3000 m³/year), and large (more than 3000 m³/year) factories [4]. Based on an estimate by [4], around 72 and 75% of tequila factories are micro in size and only between 10.3 and 12% are large. However, the actual total number of tequila factories is unknown, mainly because not all factories are registered in CRT, especially some of the micro and small size. Additionally, it is common for some tequila factories to stop operating without notifying CRT.

On the other hand, according to [5] based on the analysis of other authors, vinasse without treatment is commonly disposed of by direct irrigation in the field or direct discharge into aquatic bodies; while the technologies used for the vinasse treatment include activated sludge and anaerobic digestion with methane recovery. However, the extent of these practices was not analyzed by the authors nor was the influence of the size of the tequila factories on these practices. Vinasse treatment and disposal practices are likely to be determined by the size of the factory and knowing this information is relevant to developing strategies for managing them. Vinasse is considered among the most difficult industrial effluents (similar to other distillery effluents) to treat due to its physicochemical characteristics such as high concentrations of organic matter measured as chemical oxygen demand (COD) and biochemical oxygen demand (BOD₅), high concentrations of total suspended solids (TSS) and total dissolved solids (TDS), acidic pH values, high conductivity values, high concentrations of fats and oils, Ca⁺², Mg⁺² and K. The high COD and BOD₅ values are due to the presence of different organic compounds, such as polysaccharides, reduced sugars, lignin, proteins, melanoidin, waxes, etc. [6]. For the treatment of vinasse to be adequate and comply with Mexican regulations, a combination of different processes in many stages is required, which makes it costly in general.

Despite the relevance of tequila production to Mexico, there is limited information on the generation of vinasse that includes its characterization, treatment, and current disposal practices in factories of different sizes, nor are there studies that evaluate the impact of common disposal practices. A recent study by [5] evaluates the characterization of vinasses from grab samples which are not adequate due to the impact of the different stages of the production process on the concentration of contaminants, that are not necessarily carried out simultaneously. Additionally, the authors used official information (for some analysis) that might be erroneous mainly because it is frequently out of date. Therefore, the aim of this study supported by the National Water Commission (CONAGUA) of Mexico was (1) to carry out an investigation of the tequila factories located in the state of Jalisco (since it is the state that concentrates the largest number of factories) to identify their location in the state, the characterization of vinasses including factories of the four sizes and the current disposal practices, as well as (2) to evaluate some sites where vinasses are currently discharged. The information obtained from the study could be useful to the authorities to generate solutions for the current environmental problems in this industrial sector in the state of Jalisco (one of the richest in the country).

2. Materials and Methods

2.1. Collecting Information on Tequila Factories

To identify the tequila factories (name, addresses, contact details) located in the 12 regions of the state of Jalisco, an exhaustive search was carried out. This was done through different means such as internet pages, databases of the Tequila Regulatory Council

(CRT), Secretariat of the Environment and Territorial Development (SEMADET), National Chamber of the tequila industry (CNIT), State Water and Sanitation Commission of Jalisco (CEAS) and different offices of the municipalities where the tequila companies are located.

Subsequently, contact was established by telephone and by email with each identified tequila factory. In some cases, physical visits were necessary. The contact with the factories was done to ask them to answer a confidential questionnaire developed through Google Forms. The questionnaire was elaborated to gather information regarding general information about the factories in order to know its size, the volume of produced tequila and vinasses, its current method of final disposal of the vinasse, etc., and above all, the willingness to provide us with a composite sample of vinasse for characterization.

Once we had the addresses of the tequila companies, we proceeded to field visits to verify their existence and geolocate them with a Garmi Oregon 650 model GPS. With this information, geographic maps were generated with QGIS software.

2.2. Vinasse Characterizations

A total of 42 companies responded to the questionnaire; 38 responded that they would provide vinasse for characterization, but finally, only 24 did. Of those who responded, based on tequila production, 3 were large, 6 were medium, 1 was small and 15 were micro. Therefore, tequila factories of all 4 sizes were represented in the study. The characterization of the vinasses was carried out from 24-h composite samples provided by the tequila factories (4 samples taken every 8 h). The following parameters commonly used to characterize vinasses were measured: COD, BOD₅, Organic nitrogen (org-N), ammonia (NH₄⁺-N), nitrate (NO₃-N), total phosphorus (TP), TSS, VSS, TDS, settleable solids (SetS), pH, conductivity, fats, and oils (FO), total phenols (TPh). Additionally, some minerals that might be found in vinasses were also quantified Ca⁺², Mg⁺², K, Na, Fe, Cu, Zn, and Ni. The analysis was performed in a certified laboratory by the Water National Commission and the Mexican Accreditation Entity according to Mexican Standards which are based on the Standard Methods for the analysis of water and wastewater [7].

2.3. Analysis of Current Disposal Practices for Treated and Untreated Vinasses

The information provided by the 42 companies that responded to the questionnaire was analyzed in order to classify the tequila companies by size, calculate the amount of vinasse generated by the size of the tequila factory and detect the common treatments and disposal methods. In addition, an estimate of the total volume of vinasse was made for the state of Jalisco and for the methods of treatment and disposal for the entire state of Jalisco. The information was organized in tables and graphs.

2.4. Case Studies to Assess the Impact of Current Vinasse Disposal Practices

The information gathered by the procedure described in Sections 2.2 and 2.3, complemented with field visits in the three main tequila production regions, allowed us to select three case studies to evaluate the impact of the current vinasse disposal practices. As a result, the following three cases studies were selected:

- (a) Monitoring of surface water in Tequila, Jalisco;
- (b) Characterization of a soil irrigated with vinasse for 14 years;
- (c) Monitoring of groundwater in a well located near soils used for vinasse disposal

For the first case, the Atizcoa stream was selected. This stream is located in Tequila, Jalisco and it is the main surface water body in the locality (Figure 1). It runs about 16.5 km from its source in the Tequila volcano to its inflow into the Santiago River. The flow rate along the stream was estimated by the well-known velocity/area method which consisted of measuring the average velocity of flow and the cross-sectional area of the stream (Flow rate (Q) = A (cross-sectional area) × V (water velocity at the surface)). The monitored section was around 5 km and covered the portion of the stream that runs in the middle of tequila factories (Figure 1). Three sampling campaigns were carried out every 15 days, between 2 February and 1 March 2021, in the dry season at 4 different points (P1, P2, P3,

P4). The samples were kept at 4 °C until their processing in the Environmental Quality Laboratory of the University of Guadalajara campus (Centro Universitario de la Ciénega), within 24 h after sampling. Conductivity, pH, temperature, and dissolved oxygen analyzes were measured in situ, with an HQ40d meter and heavy-duty Intellical probes. The other parameters were performed in accordance with the official Mexican standards based on the Standard Methods for Analysis of water and wastewater [7].

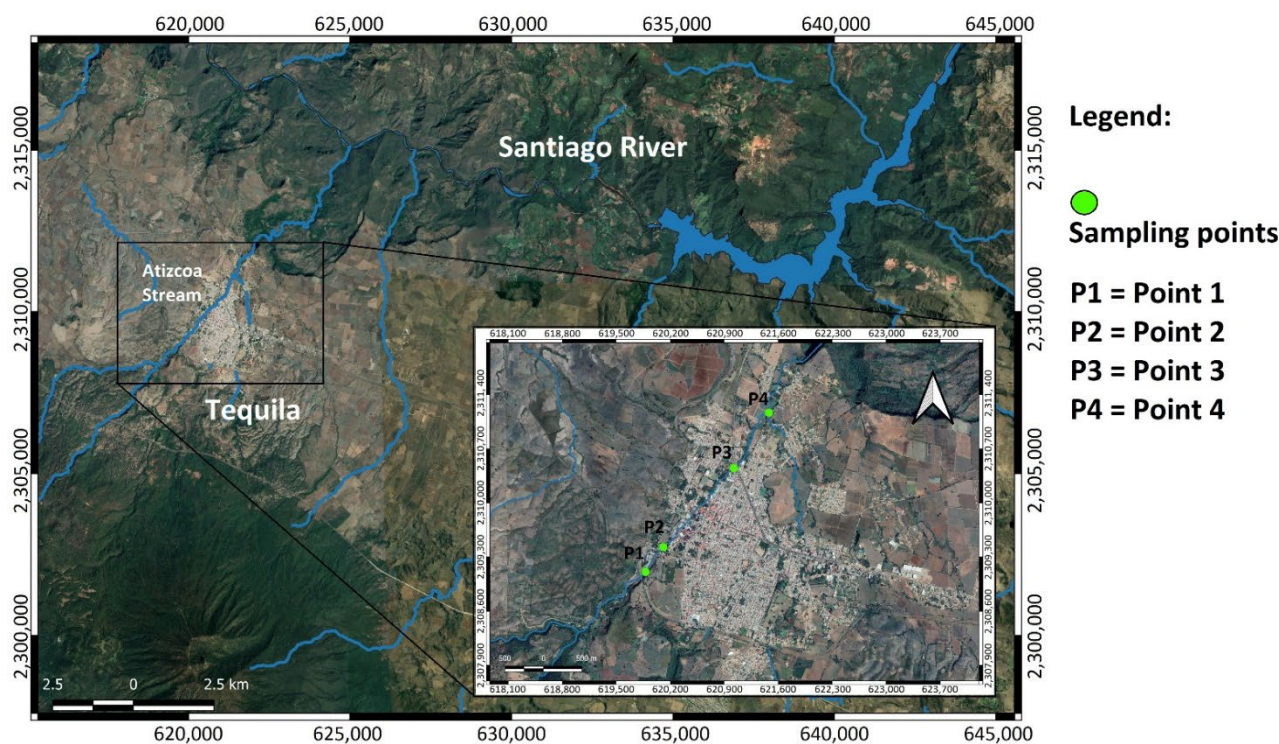


Figure 1. Sampling points along the Atizcoa stream.

Regarding the second case, the soil irrigated with vinasse is located in the municipality of Atotonilco, Jalisco, in the Ciénega region and is within a tequila industry classified as micro-size. This soil has been used for vinasse disposal for the last 14 years. The vinasse is discharged continuously only for 3 or 4 months each year, with a total dose of 1200 m³/ha during these months. The vinasse that is discharged into the soil is only cooled in a retention pond and its pH is adjusted to a neutral value. In this soil grows wild grass that is used mainly as food for cattle that were observed grazing. The total extension of the soil area was 2065 m² with a trapezoidal shape and it was determined with a Garmin GPS model etrex10. A total of 36 sampling points distributed in an “N” or zigzag shape were determined (Figure 2). For each sampling point, a 25 to 30 cm excavation was made [8,9] and approximately 1 kg of soil was taken. The different samples were mixed to form a composite sample. The physicochemical characterization of the soils was carried out in the Research Center in Environmental Quality of the Centro Universitario de la Ciénega of the University of Guadalajara. Soil samples were immediately processed for analysis requiring fresh soil. The rest of the samples were stored at 4 °C to later be used in the determination of several physicochemical parameters according to different references in the literature. Total nitrogen and all the different forms that compose it was determined according to what was reported in [10–12]; total and available phosphorous, according to the analytical methods in [8,13,14]. On the other hand, refs. [15,16] were used to determine texture, as well as, fats and oils, respectively. The rest of the parameters were determined based on the procedures described in [9,17–29].

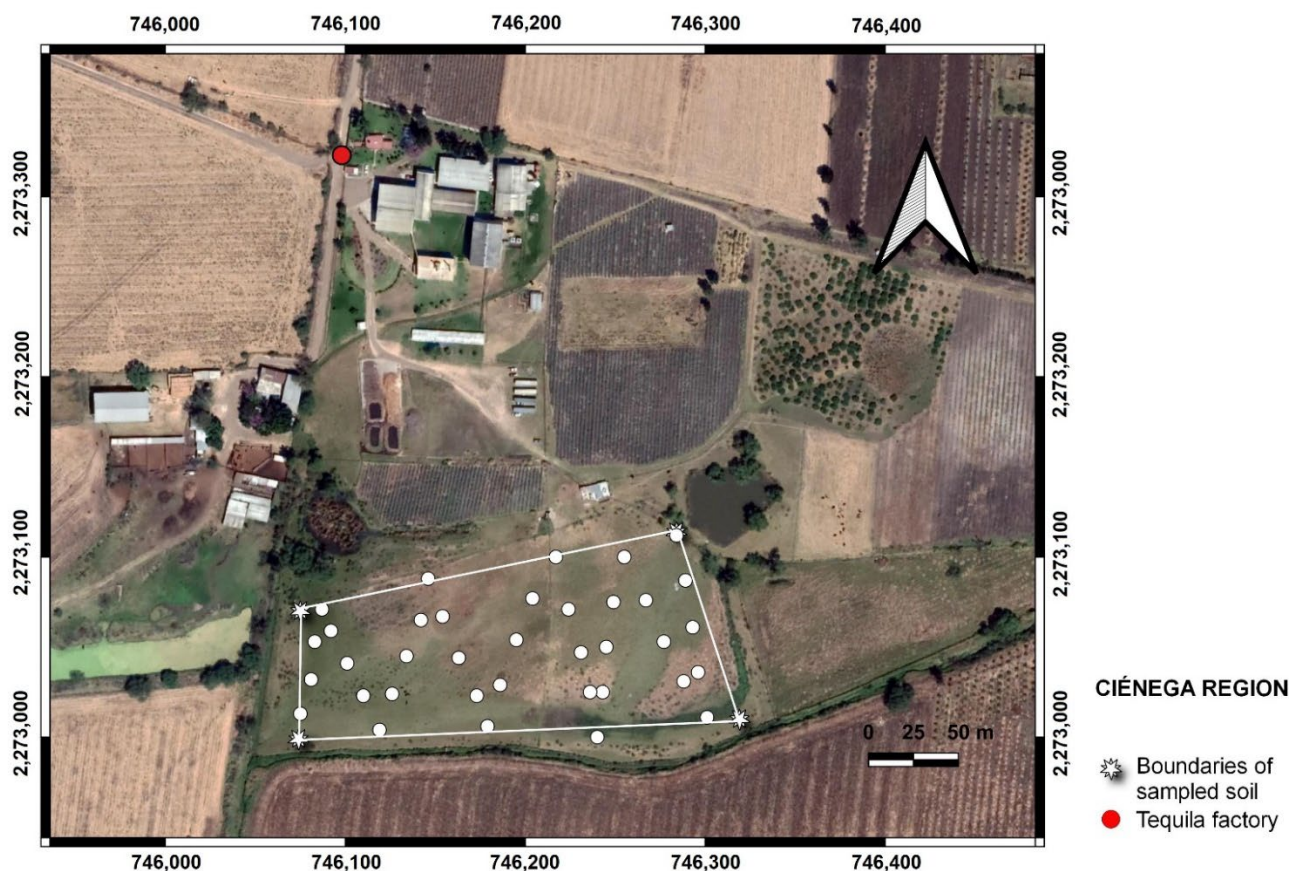


Figure 2. Location of the soil monitored in the Ciénega Region.

For the last case, the monitoring of groundwater, a well located in the municipality of Atotonilco, Jalisco in the Ciénega region was selected (Figure 2). The well has a depth of 120 m and is located 100–150 m from the soil that is irrigated with vinasses. Three sampling weekly campaigns were carried out in the dry season between 24 May–7 June 2021. Approximately 1 liter of the sample was taken at each point and the samples were kept at 4 °C until their analysis within the next 24 h.

The characterization of the samples was carried out both in situ and in the Research Center on Environmental Quality of the Centro Universitario de la Ciénega of the University of Guadalajara. Regarding the in situ parameters, electrical conductivity, pH, and temperature were measured with a HACH model HQ40d portable meter with INTELLICAL probes. On the other hand, the laboratory techniques used for the analysis of the water samples include parameters such as nitrate, nitrite, total Fe, Mn, Cl^- , F^- , hardness, alkalinity, N-NH_4^+ , turbidity, SO_4^{2-} , color, reactive phosphorus, total phenols, fats and oils, Ca^{2+} , copper, K^+ , Mg^{2+} , Na^+ , nickel, and zinc, were carried out according to standard methods [7]. It is important to clarify that the measured parameters were selected based on those pollutants that might be present in the tequila vinasse and that may infiltrate from the discharges into soils.

3. Results and Discussion

3.1. Distribution of Tequila Factories in the State of Jalisco

In total, 110 tequila factories, whose existence and operation was verified in the field, were identified. This number differs from the 131 tequila companies in the state of Jalisco, reported by the CRT in January 2021 [1] and also, in comparison to the 158 tequila factories reported by [5] with the information of [30]. These differences between the official information and the results from our field study are mainly due to the fact that many companies

registered in CRT are only bottling companies, not distilleries. Additionally, many factories operate for a time and then cease to exist; this was a repeated situation during the pandemia. On the other hand, many micro-size tequila factories are not registered in CRT.

Tequila factories are located mainly in three regions: Valles (45 factories), Altos Sur (31 factories), Ciénega (20 factories), and to a lesser extent in the Centro region (9 factories); while in the Laguna region there are 3 factories and in the Sureste and Costa Sur regions there is only 1 factory (Figure 3, Table 1). Furthermore, in these regions, the factories are concentrated in some municipalities. In the Valles Region, the municipalities of Tequila, Amatitán, and El Arenal stand out. In the Ciénega region, the main municipality is Atotonilco El Alto, and in the Altos Sur region, the municipality that has a concentration of tequila factories is Arandas (Table 1). Other municipalities such as Guadalajara, Tlaquepaque, and Tlajomulco de Zúñiga in the Centro region have two tequila factories each. This information differs from that reported by [5] because the authors only focused on the large tequila factories, which according to them, produce 80% of the tequila in the State of Jalisco. However, as will be seen later, the problem of managing vinasses is mainly a problem for tequila factories of other sizes rather than for large ones.

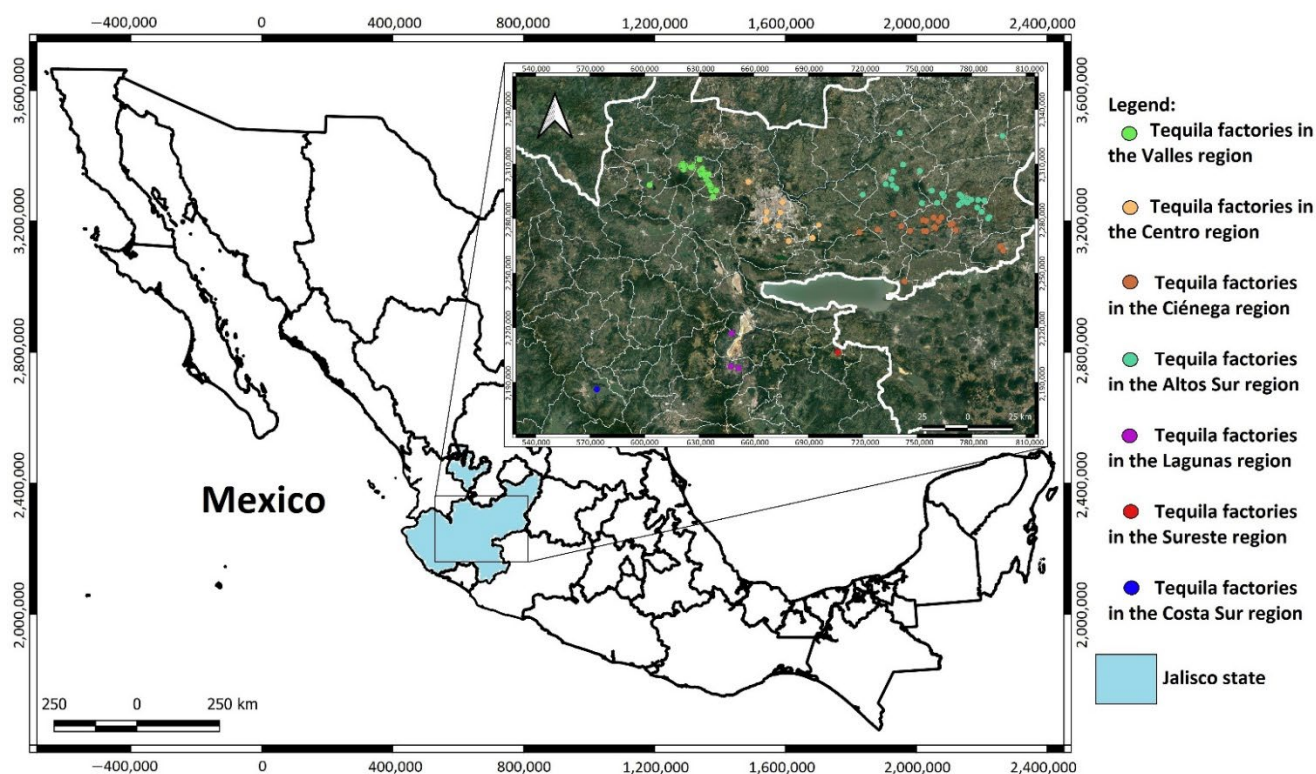


Figure 3. Tequila factories located throughout the state of Jalisco (a total of 110 facilities).

Table 1. Municipalities with the largest numbers of tequila companies.

Municipality	Region	Tequila Factories
Tequila	Valles	22
Arandas	Altos Sur	15
Amatitán	Valles	13
Atotonilco el Alto	Ciénega	12
El Arenal	Valles	9
Tepatitlán	Altos Sur	7
Jesús María	Altos Sur	3
Tototlán	Ciénega	3

3.2. Distribution of Tequila Factories in the State of Jalisco

The average values of the measured parameters are shown in Table 2. Additionally, in Table 3, the results are shown by the size of the tequila factory. As can be seen, no clear differences are observed when comparing the characteristics of the vinasse generated, depending on the size of the factory. However, it seems that the concentration of COD, TSS, VSS, TDS, conductivity, and org-N tend to be lower in the vinasse of micro tequila factories. This could be related to a dilution effect due to less efficient water management in these companies, for example, during the incorporation of hot water for agave grinding. Nor were clear differences found when the company does not produce 100% agave tequila.

Table 2. Characterization of Tequila vinasse (Average \pm Standard Dev. $n = 24$) and values of the characterization performed by López-López et al. (2010).

Parameter	This Study	Lopez-Lopez et al., 2010	Parameter	This Study	Lopez-Lopez et al., 2010
Total BOD ₅ (mg/L)	23,254 \pm 7924 (3718–35,516)	35,000–60,000	Ammonia	(<0.40–15.8)	15–40
Total COD (mg/L)	45,381 \pm 17,369 (7565–73,690)	60,000–100,000	Nitrite	<0.00199	—
TSS (mg/L)	7127 \pm 7294 (190–24,800)	2000–8000	Nitrate	<0.100	—
VSS (mg/L)	6418 \pm 6451 (185–23,000)	1990–7500	Ca ²⁺	516.4 \pm 292.1 (123–1287)	200–1100
SetS (ml/L)	235.6 \pm 306.1 (0.1–900)	10–900	Cu	1.22 \pm 2.5 (<0.05–12.5)	<3.0
TP (mg/L)	<0.3	32–228	Fe	18.5 \pm 15.8 (0.24–61.5)	<45
Fats and Oils (mg/L)	119 \pm 109 (6–423)	10–100	Mg ²⁺	168 \pm 92 (10.4–382.0)	100–300
Total Phenols	<0.0103	—	K	297.5 \pm 212.5 (3.68–961.8)	150–650
TDS (mg/L)	19816 \pm 8550 (2055–33,970)	23,000–42,000	Na	45.1 \pm 37.7 (9.2–161.9)	—
Conductivity	3044 \pm 1007 (523–5385)	—	Ni	<0.1–0.54	<0.02
pH	3.6 \pm 0.2 (3.4–3.9)	3.4–4.5	Zn	1.03 \pm 0.76 (<0.1–3.0)	<1.0
Org-N	254 \pm 127 (24–574)	5.0–10			

Table 3. Characterization of Tequila vinasse by factory size (3 L, 6 M, 15 Micro).

Parameter	Average Value		
	Large	Medium	Micro
Total BOD ₅ (mg/L)	19,587	21,084	24,856
Total COD (mg/L)	47,776	49151	43,394
TSS (mg/L)	9472	8242	6211
VSS (mg/L)	8675	7524	5524
TDS (mg/L)	22,624	22,536	18,168
Conductivity	3321	3158	2943
pH	3.5	3.7	3.6
Fats and Oils (mg/L)	130	87	130
Org-N	262	335	219

It is important to emphasize that the ranges of values found for the different parameters differ from that reported by [3], which has been taken as a reference in the literature and was obtained from the characterization of only four tequila factories. The results are also different from those recently reported by [5] who obtained higher values in grab samples for seven parameters. Some of the most important parameters, total COD and BOD₅, and TSS are reported between 60,000–100,000 mg/L, 35,000–60,000, and 2000–8000 mg/L respectively by [3] and we found a much lower range (Table 2). Furthermore, [5] who did not measure COD; reported an extremely high average concentration of BOD₅ which is 119,011.77 mg/L. In comparison to these authors, the results we obtained by including a greater number of effluents from companies of different sizes and using composite samples, allow us to assume that they are closer to reality and reflect the high variability in the characteristics of tequila vinasse. This is due to multiple factors that range from the characteristics of the agave used (origin, age, etc.), the incorporation of other sugars, the processing and practices in the use of water during cooking and juice extraction, among others. One of the main differences between tequila plants is found in the method of extracting sugars from the agave. Most large and medium-sized companies carry out extraction by diffusion, which consists of tearing the agave to the smallest size possible and the subsequent extraction of sugars with hot water against the current. In contrast, in general, most tequila companies first cook the agave in autoclaves and the extraction of sugars is carried out in mills.

3.3. Current Disposal Practices and Treatments of Tequila Vinasse

As previously mentioned, a total of 42 tequila factories of the four sizes responded to the questionnaire (4 anonymously): micro-size (29) 69%, small (3) 7.1%, medium (6) 14.3%, and large (4) (9.5%). This distribution in size is very similar to the numbers reported by [4]: micro, 72–75%; small, 6.5–9.6%, medium 6.8–7.7%, and large, 10.3–12%, respectively. Due to the fact that all sizes of tequila factories were represented in the collected information, an estimate of the total volume of vinasses generated in the state of Jalisco was made. The estimate was made considering that the total number of tequila factories we found is 2.6 times the number of factories that participated in the study (110/42). Therefore, being conservative we considered that the total volume of tequila vinasse generated in Jalisco is between 2–3 times the volume calculated from the field study (Table 4).

Table 4. Tequila vinasses generated in Jalisco state (collected and estimated data).

Size	Micro	Small	Medium	Large	Total
Data collected in a survey (m ³ /year)	27,278 (3.8%)	13,250 (1.8%)	127,164 (17.6%)	556,000 (76.8%)	723,692
Estimate for Jalisco State (m ³ /year)	55,000–82,501	26,053–39,079	254,740–382,109	1,111,591–1,667,386	1,447,384–2,171,076

The estimated maximum value is close to the 2,645,655.69 m³/year of vinasses produced in the state of Jalisco which was estimated from official information by [5].

On the other hand, the common treatments given to vinasses are presented in Table 5. It was found that 28.6% of the companies recognized that they do not provide any treatment to the vinasses, while 52.4% only cool and adjust the pH to a value around 7, and only 27.3% of companies give a complete treatment to vinasses (4 large and 2 medium sizes).

Table 5. Treatments given to vinasses in tequila factories.

Type of Treatment	Number of Tequila Factories	Size of Tequila Factories
Retention lagoon and pH adjustment	20	Micro (16), Small (1) Medium (2) Large(1)
Sedimentation pond	2	Medium (2)
Full biological treatment	6	Micro (3), Medium (2) Large (1)
Full physicochemical treatment (coagulation-floculation)	2	Large (2)
No treatment	12	Micro (8), Small (2) Medium (2)

Regarding the volume of vinasse disposed of by the different methods, Table 6 shows the information collected in the survey, and Table 7 shows the estimate for the state of Jalisco.

Table 6. Disposal methods according to the information collected in the field study.

	Disposal in Surface Waters (m ³ /year)	Disposal in Soils (m ³ /year)	Use of Vinasse for Irrigation (m ³ /year)	Treated by an External Company (m ³ /year)	Disposal into Municipal Sewer Systems (m ³ /year)	Total (m ³ /year)
No treatment	16	2500	3000	27,395	-	32,911
Incomplete treatment	200	90,050	90,159	4000	150	184,559
Full treatment	297,200	7000	202,022	-	-	506,222

As can be seen, according to the applied questionnaire, the largest amount of vinasse generated receives a complete treatment (Table 6). This is due to the fact that large and some medium-sized companies are the ones that have complete treatment systems. Even so, there is an enormous amount of vinasse that does not receive any treatment (32,911 m³/year) or that is only cooled and neutralized (184,560 m³/year).

Table 7. Estimate of the vinasses disposed of annually in the state of Jalisco.

	Disposal in Surface Waters (m ³ /año)	Disposal in Soils (m ³ /año)	Use of Vinasse for Irrigation m ³ /año)	Treated by an External Company (m ³ /año)	Disposal into Municipal Sewer Systems (m ³ /año)
No treatment	32–48	5000–7500	6000–9000	27,395	-
Incomplete treatment	400–600	180,100–270,150	180,319–270,748	8000–12,000	300–450
Full treatment	594,400–981,600,	14,000–21,000	404,044–606,067	-	-

3.4. Evaluation of the Impact of Vinasse Disposal in Atizcoa Stream

The flow rate in the Atizcoa stream was around 23 L/s and 96 L/s in P1 and P2, respectively, suggesting an increase in the volume of water due to wastewater discharges, mainly vinasses. Table 8 shows the average values of the parameters measured in situ during the sampling campaigns. A clear reduction in DO concentrations from P1 to P4 is observed, reaching anoxic-anaerobic conditions in P3 and P4. Usually, a reduction in DO concentrations in surface waters is due to the discharges of high concentrations of biodegradable organic matter. Two parameters that confirm that vinasses are responsible for these conditions in the Atizcoa stream are pH and temperature. The values of pH changed from 6.9 (P1) to 4.7 (P4) and such reduction could only be from the inflow of acidic effluents as vinasses. When the inflows are from municipal wastewater, the changes in pH are not of that magnitude as reported by Toure, et al. [31] who found variations of pH between 6.30 and 6.54 in the Comatex stream in Mali which receives discharges of municipal wastewater. Additionally, Thai-Hoang, et al. [32] found that in the Saigon River

in Vietnam, the pH values varied from 6.46 to 6.75 during the rainy season and from 6.13 to 7.06 during the dry season. Moreover, the increase in temperature in P2 is due to the direct disposal of a tequila factory located nearby the stream; the vinasses are generated at around 90 °C [33]. With respect to the conductivity, the increase registered from P1 to P4 is unusual too. In a river sampling point with municipal wastewater discharges, a value of 647.27 $\mu\text{S}/\text{cm}$ was found [31].

Table 8. Parameter measured in situ along the four sampling points. Mean \pm Standard deviation.

Parameter	Point 1	Point 2	Point 3	Point 4
Temperature	24 \pm 1.8	35.8 \pm 1.6	27.1 \pm 1.0	26.0 \pm 1.7
pH	6.9 \pm 0.5	5.4 \pm 1.0	5.7 \pm 1.1	4.7 \pm 0.4
DO	6.3 \pm 0.2	3.2 \pm 0.3	0.5 \pm 0.3	0.4 \pm 0.0
Conductivity	82.1 \pm 0.9	155.7 \pm 56.2	712.0 \pm 145.9	1384.7 \pm 63.5

In addition, the discharge of tequila vinasses in the Atizcoa stream was also evident in the concentrations found for COD, BOD₅, TSS, fats and oils, TP, and TKN (Figure 4) in the three sampling campaigns. In general, an increase in pollutant concentrations was observed as the stream run from sampling point 1 (almost zero contaminant concentration) to number 4. For example, COD and BOD₅ concentrations reached values of more than 5000 mg/L and 3000 mg/L, respectively at sampling P4, after the stream passed through the numerous tequila factories. TSS concentrations at the last sampling point reached concentrations between 1000 and almost 2500 mg/L. The values observed for these parameters are many times higher than the limit value from which surface water is considered highly polluted in Mexico according to the Water National Commission (Table 9) [34]. The values are also very superior to those in a river in Veracruz, Mexico which receives mainly municipal wastewater (COD, 318 mg/L; TSS, 696 mg/L, etc.) [35]. Fats and oils reached a maximum value of almost 60 mg/L and the concentration of this pollutant in tequila vinasses vary between 10–100 mg/L [3].

Table 9. Indicator of surface water quality in Mexico.

Parameter	Excellent Quality	Good Quality	Acceptable Quality	Polluted	Highly Polluted
BOD ₅ (mg/L)	≤ 3	>3 ≤ 6	>6 ≤ 30	>30 ≤ 120	>120
COD (mg/L)	≤ 10 mg/L	>10 ≤ 20	>20 ≤ 40	>40 ≤ 200	>200
TSS (mg/L)	≤ 25 mg/L	>25 ≤ 75	>75 ≤ 150	>150 ≤ 400	>400
Fecal Colifoms (MPN/100 mL)	≤ 100	>100 ≤ 200	>200 ≤ 1000	>1000 $\leq 10,000$	>10,000

Regarding nutrients, TP and TKN reached maximum concentrations of almost 60 mg/L and 100 mg/L, respectively. It is well-known that nitrogen and mainly phosphorus are responsible for eutrophication in aquatic bodies. As aforementioned, this stream is a tributary of Santiago River which is one of the most contaminated rivers in the country [36,37], and in some parts, the river is fully covered by *Eichornia crassipes* [38].

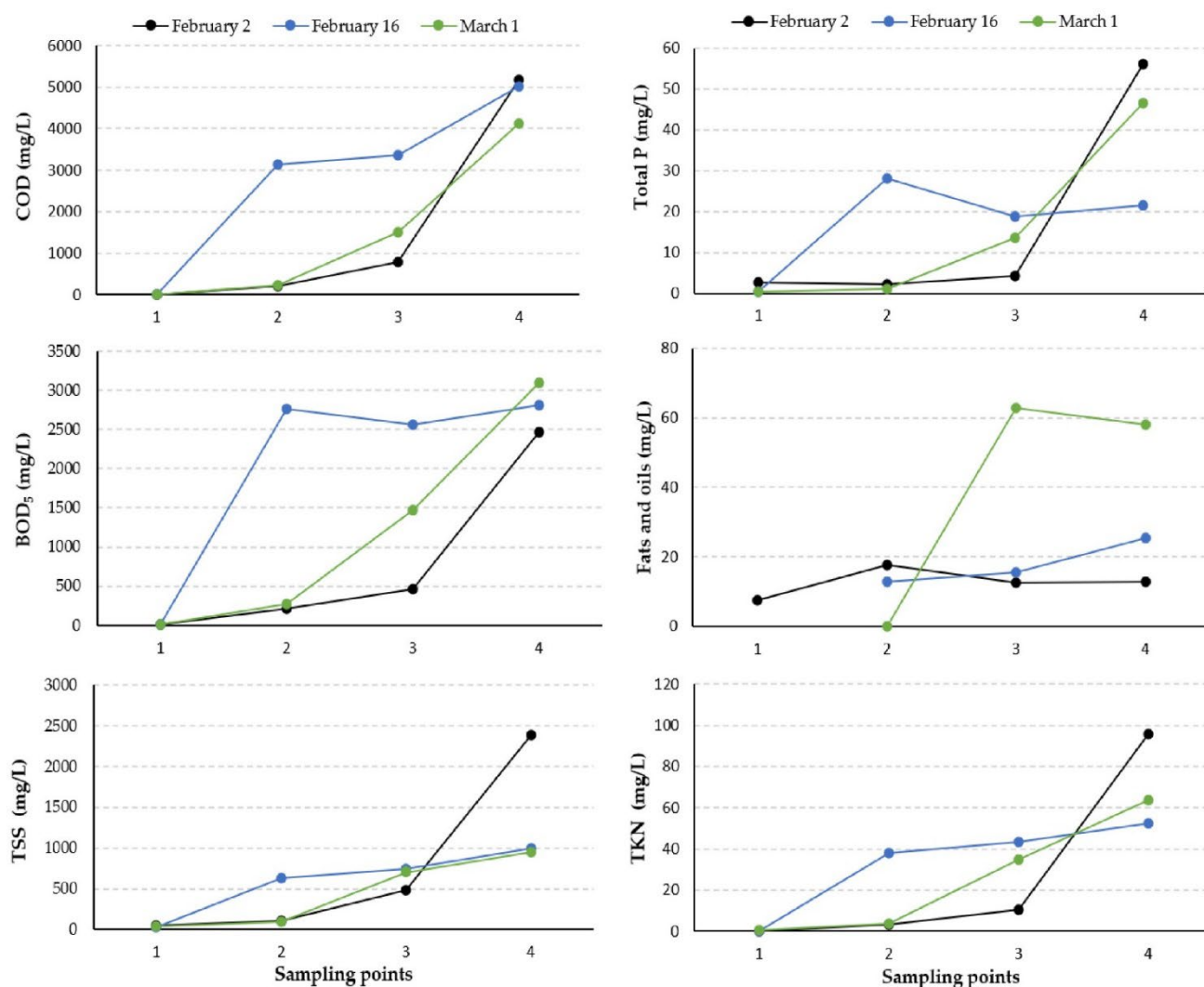


Figure 4. Concentrations of COD, BOD₅, TSS, TKN, fats and oils, and phosphorus found in the Atizcoa stream during the three sampling campaigns.

3.5. Evaluation of a Soil Irrigated with Vinasse for 14 Years

The textural classification of the soil that was evaluated is clay due to its high clay content, that is, 45.13% (Table 10). Soil texture is the relative percentage of sand, silt, and clay particles in the inorganic fraction of the soil. Clay particles are the smallest, measuring less than 0.002 mm, while silt particles range from 0.002 to 0.05 mm and sand particles from 0.05 to 2.0 mm [39]. Soil texture influences soil biophysical properties because it is associated with soil porosity, which regulates water holding capacity and water movement, as well as gaseous diffusion, which together determine soil health [40].

As a result of the high content of clay and silt, this soil has very low permeability and high water holding capacity [39]. In general, the higher the clay content, the lower the hydraulic conductivity [41]. Hydraulic conductivity determines the relative ease of water flow through soils. This implies that the risk of vinasse infiltration in nearby groundwater is low.

On the other hand, the pH value in this soil was found to be moderately alkaline due to the use of calcium hydroxide (hydrated lime) for the neutralization of the vinasse before its disposal; this practice was also manifested in the very high concentrations of Ca²⁺ in the soil. In addition, concentrations of Ca²⁺, K⁺, and Mg²⁺ were much higher than the concentration of Na⁺ which minimize the risk of soil sodification [42]. In addition, the estimated soil organic content (SOC) was 0.097 kg/m² and it is similar to the minimum value of 0.1 kg of C/m² in the 0.30 cm topsoil reported by [43] in a review of degraded and

non-degraded grasslands worldwide. Probably, this low accumulation of SOC is due to the short time of vinasse discharge per year in this soil.

Table 10. Physicochemical characteristics of the evaluated soil.

Parameter	Value
Textural classification	Clay
Sand (%)	23.51 ± 3.12
Clay (%)	45.13 ± 5.42
Silt (%)	31.36 ± 5.47
pH	7.90 ± 0.20
Electrical conductivity (µs/cm)	425.08 ± 52.34
Moisture (%)	19.81 ± 0.13
Water retention capacity (%)	64.94 ± 1.98
Cation exchange capacity (meq/100 g)	46.80 ± 2.26
Density (g/cm ³)	1.29 ± 0.014
Organic carbon (%)	9.38 ± 0.626
Chloride (ppm Cl ⁻)	93.33 ± 7.64
Total phosphorus (ppm P)	44.66 ± 11.01
Phosphorus available to plants (ppm P)	14.89 ± 1.95
Total Nitrogen (ppm)	2688.06 ± 195.08
Total Kjeldahl Nitrogen: N-org. y NH ₃ (ppm)	2674.56 ± 200.90
Nitrate (NO ₃ ⁻ ppm)	18.50 ± 5.89
Potassium (ppm K)	690.50 ± 12.02
Ca ²⁺ (ppm Ca)	6247.50 ± 212.84
Magnesium (ppm Mg)	1593.00 ± 135.76
Sulfur (SO ₄ ⁻ ppm)	28.00 ± 0.00
Boron (B ppm)	1.20 ± 0.141
Copper (Cu ppm)	5.60 ± 0.424
Iron (Fe ppm)	100.00 ± 11.31
Manganese (Mn ppm)	304.00 ± 21.21
Zinc (Zn ppm)	2.95 ± 0.495
Sodium (Na ppm)	120.50 ± 6.36
Fats and oils (g/kg soil)	21.71 ± 3.55

Regarding the available nutrients, low concentrations of nitrate were found although the organic nitrogen values found were very high. Probably, the vinasse addition to the soil has changed the microbial population and altered or reduced the biochemical cycles by which organic nitrogen is transformed into available nitrogen for plants (NH₄⁺ and NO₃⁻) [44]. Soil health is highly dependent on the activity of aerobic microbial populations that not only decompose organic matter [42] but also transform nitrogen compounds into oxidized forms usable by plants; the discharges of vinasse might create anaerobic microsites when flooding the pores [44].

However, in general, after 14 years of being used for tequila vinasse disposal, the impact on soil fertility is null. This means that this vinasse disposal-soil rest method appears to be suitable to produce crops during the soil rest period, rather than direct fertigation. This, although the dose of vinasse applied to the soil is 4 times the maximum doses of 300 m³/ha used to irrigate sugar cane plantations with alcohol vinasses without a negative effect on growth [44–46].

3.6. Physicochemical Characterization of Groundwater near to Soils Used for Vinasse Disposal

A major concern when stillage is disposed of on soil or used for fertigation is groundwater contamination [44]. However, in this case, in general, the results obtained from the characterization of the water from the well located near the soil that is irrigated with neutralized vinasse (Table 11) showed that the quality of the groundwater has not been affected. The physicochemical characteristics of the water comply with the provisions of the modification of NOM-127-SSA1-1994 [47], which establishes the maximum permissible limits of the different components of water to make it suitable for human consumption in

Mexico. This is probably related to the type of soil in the area where the well is located, as well as the way in which the vinasse is being applied (only three or four months per year). As mentioned above, high content of clay (45%) and silt (31%) allows the soil high retention of water and nutrients [48] and reduces permeability, which in turn limits the rate of infiltration [41,49] of vinasse and its possible impact on groundwater. The texture and chemical composition of the soil, as well as the dose of vinasse deposited in the soils, largely determine the rate of infiltration and the impact on nearby groundwater [50].

Table 11. Physicochemical characteristics of the evaluated well in comparison with what is established in the modification of NOM-127-SSA1-1994.

Parameter	Value on This Study	Mexican Standards
Nitrate (mg/L)	9.366 ± 2.484	10
Nitrite (mg/L)	0.058 ± 0.041	1
Total Fe (mg/L)	0.026 ± 0.0115	0.30
Mn (mg/L)	0.9 ± 0.1	0.15
Cl ⁻ (mg/L)	17.0 ± 4.334	250
F ⁻ (mg/L)	0.306 ± 0.4	1.5
Hardness (mg/L CaCO ₃)	376.66 ± 2.88	500
Alkalinity (mg/L CaCO ₃)	327.10 ± 16.07	N.R.
N-NH ₄ ⁺ (mg/L)	0.0 ± 0.0	0.5
Turbidity (NTU)	0.296 ± 0.513	5 NTU
Sulfate SO ₄ ²⁻ (mg/L)	60.6 ± 1.154	400
pH	8.083 ± 0.155	6.5–8.5
Conductivity (µs/cm)	722.67 ± 214.13	N.R.
Color (Units of Pt-Co)	3.0 ± 2.0	20
Reactive phosphorus (mg/L)	0.054 ± 0.0188	N.R.
Total Phenols (mg/L)	<0.0101	0.3
Fats and oils (mg/L)	<5.00	N.R.
Ca ²⁺ (mg/L)	82.40 ± 5.234	N.R.
Copper (mg/L)	<0.020	2
K ⁺ (mg/L)	3.298 ± 0.240	N.R.
Mg ²⁺ (mg/L)	41.60 ± 2.515	N.R.
Na ⁺ (mg/L)	18.50 ± 2.656	200
Nickel (mg/L)	<0.020	N.R.
Zinc (mg/L)	<0.020	5

Note: N.R. (Not Reported).

4. Conclusions

By performing this study, we identified two regions as the main producers of tequila and vinasse (Valle and Altos Sur region) and 10 municipalities with the highest number of factories. In addition, we found a very high range of variation in the concentrations of contaminants in the vinasses, e.g., COD varied between 7565–73,690 mg/L; BOD₅ from 3718 to 35,516 mg/L, TSS from 190–24,800, Org-N between 24–574 mg/L, and Ca²⁺ from 123 to 1287 mg/L. On the other hand, we corroborated that the main environmental problems are related to micro-size factories which do not treat their vinasses at all. Additionally, the most common method of disposal is the discharge on soils along with fertigation. Apparently, only in the Valle region, the disposal into surface water is a common practice as well as discharges into sewage systems. The degradation of the monitored stream (that runs in the middle of tequila factories) was evident not only by the physicochemical characterization, with the worst conditions in P4 (pH, 4.7; OD, 0.4 mg/L; COD, more than 5000 mg/L and BOD₅, more than 3000 mg/L), but also visually through the odors and the brown color of the water. Furthermore, the method of vinasse disposal-soil rest has not affected soil fertility (after 14 years), but it must be thoroughly evaluated to generate general guidelines that include the optimal application doses and rest periods. In this regard, the texture of soils (high content of clay and silt) is decisive in protecting groundwater from vinasse infiltration. Finally, the results obtained in this study could help the authorities to develop

adequate strategies for the management of vinasses (treatment and disposal), mainly in micro and small tequila factories, as well as to focus future research on the improvement and correction of disposal practices, in order to prevent environmental pollution.

Author Contributions: Conceptualization, F.Z. and A.T.; methodology, F.Z., A.T., B.S.-R. and A.C.-Á.; software, A.M., I.C. and B.S.-R.; validation, F.Z. and B.S.-R.; formal analysis, A.C.-Á., A.M. and I.C.; investigation, A.T., A.C.-Á., I.C. and A.M.; resources, F.Z.; writing—original draft preparation, F.Z.; writing—review and editing, F.Z. and A.T.; visualization, B.S.-R., A.M. and I.C.; supervision, F.Z. project administration, F.Z.; funding acquisition, F.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Water Commission and National Council of Science and Technology of Mexico through the “FONDO SECTORIAL DE INVESTIGACIÓN Y DESARROLLO SOBRE EL AGUA” to the project “TECNOLOGÍAS SUSTENTABLES PARA TRATAMIENTO DE VINAZAS Y SU REUSO EN ACTIVIDADES PRODUCTIVAS”; grant number A3-S-66470.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Graduate scholarship for Isaías Carrillo was provided by National Council for Science and Technology of México.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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