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Assessment of Soil Heavy Metal Pollution and the Ecological Risk in an Agricultural Area from Sánchez Ramírez Province, Dominican Republic

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Abstract: Heavy metal pollution in agricultural soils is one of the main problems in agricultural production worldwide, which threatens human health and the environment. To evaluate the pollution levels of heavy metals and the ecological risks in an agricultural area from Sánchez Ramírez Province, Dominican Republic, the concentration levels of heavy metals (Fe, Mn, Cr, Ni, Cu, Zn, Pb, and As) were measured using energy-dispersive X-ray fluorescence spectroscopy (EDXRF). Several pollution indices, including the geo-accumulation index (I_{geo}), enrichment factor (EF), and single pollution index (PI), were used to investigate the pollution status. The spatial distribution of different heavy metals in the studied soils was also determined. The mean concentrations of Fe, Mn, Cr, Ni, Cu, Zn, Pb, and As were 73735, 1616, 426; 34; 20; 200; 43; and 5 mg kg^{-1}, respectively. These results indicated that the mean concentration of Cr, Cu, Zn, and Pb exceeded FAO-recommended levels for healthy agricultural soils. However, the potential ecological risks assessment indicated a low-risk status. The results obtained could help improve soil–rice–environment management practices and prevent heavy metal pollution in this type of production system, protecting the health of the local population and the environment.

Keywords: paddy soil; EDXRF; pollution index; spatial distribution

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Citation: Alberto Then, N.M.; Delanoy, R.; Nuñez-Ramos, P.A.; Díaz Rizo, O.; Bello, L. Assessment of Soil Heavy Metal Pollution and the Ecological Risk in an Agricultural Area from Sánchez Ramírez Province, Dominican Republic. *Land* **2024**, *13*, 684. https://doi.org/10.3390/land13050684

Academic Editor: Alexei Konoplev

Received: 15 March 2024
Revised: 25 April 2024
Accepted: 6 May 2024
Published: 14 May 2024

1. Introduction

Heavy metals represent a prevalent form of soil contaminant that can induce negative impacts on human and environmental health [1–3]. Two types of sources of heavy metals might end up in soil: natural and anthropogenic. Natural sources are mainly heavy metals present in parent materials, in forms that are not readily bioavailable for plant uptake and are generally found in low concentrations [4–8]. On the other hand, anthropogenic sources such as agricultural activities, mining, and wastewater can significantly enrich soils with heavy metals [9–11]. In the agricultural ecosystem, lime and super-phosphate fertilizers, along with containing elements necessary for plant nutrition and growth, may also introduce heavy metals like As, Cd, and Pb into the agricultural land [12]. In addition, the long-term repeated application of fertilizers, metal-containing pesticides, and fungicides may increase heavy metal concentrations in soils and plants [13,14], and these elements then become present in the food chain. Consuming crops grown in heavy-metal-contaminated soils can directly affect human health [2,15,16]. Thus, the heavy metal contamination in paddy soil–rice systems represents a potential threat to humans and ecosystems [9,17–19]. Several researchers have reported heavy metal levels in rice–paddy soil systems and rice grains (*Oryza sativa* L.), that are above the permissible limits recommended by the World Health Organization (WHO): Guo et al. [20] report high levels of Cd and Pb in soil cultivated with rice in the Jin-Qu basin of China. Khan et al. [21] found that the average levels of Fe, Cu, Mn, and Al in rice grains from crops of District Malakand, Pakistan, were higher than the permissible limits recommended by the Food and Agriculture Organization (FAO) of the United Nations and the WHO. Hasan et al. [22] observed that the concentrations of Zn, Cd, Cr, and Co in soil and rice samples from three industrial areas of Bangladesh exceeded the recommended maximum tolerance values by FAO/WHO.

The Dominican Republic is considered a self-sufficient rice-producing country, a basic crop in the local population's diet. According to the Dominican Ministry of Agriculture, the country maintains an average production of about 474,000 tons year^{-1}, in 187,000 ha. Rice production is mainly carried out under irrigation systems (98%). In 2023, the Dominican Republic cultivated an average rice area of 200,362 ha, harvested 186,355 ha, with an average yield of 3309.09 kg ha^{-1} and a total average production of 13,570,490 tons year^{-1} of white rice [23].

Despite the importance of agricultural soils for sustaining farming in the Dominican Republic, there is a significant lack of information about the heavy metal pollution in these soils. There has been limited research aimed at assessing the extent of heavy metal contamination in these areas. Consequently, there is scarce information to guide the country's regulatory or pollution control efforts. This lack of data is particularly concerning given the potential health risks associated with heavy metal exposure. Delanoy et al. [24] determined the concentration of heavy metals in the northwest region of the Dominican Republic dedicated to banana and rice cultivation, where the levels of Cr exceeded the Probable Effects Levels (PEL) provided by the National Oceanic and Atmospheric Administration (NOAA) and the Environmental Protection Agency of the United States (US EPA). In another study, Delanoy et al. [25] assessed the contents of several heavy metals in rice cultivation areas of La Vega and San Francisco de Macoris, and they reported levels of Cr and Ni higher than the maximum values recommended by NOAA-USEPA Canadian Agricultural Soil and Sediment Guide. Alberto Then et al. [26] reported that the average levels of Mn, Ni, Cu, and As in an agricultural area located in Bonao, Monseñor Noel Province, dedicated to rice production exceeded the permissible limits recommended by the FAO. Recently, Delanoy et al. [27] determined that the levels of Ni, Cu, and Cr in soils dedicated to different crops across various locations in the Cordillera Central exceeded the PEL recommended by NOAA-USEPA for agricultural soils. Therefore, it is important to establish baseline information on the current state of soil pollution in agricultural areas to protect human and environmental health. Based on this, the present study aims to evaluate heavy metal pollution, the spatial distribution, and the potential ecological risks in soils used for rice production in La Mata, Sánchez Ramírez Province of the Dominican Republic.

2. Materials and Methods

2.1. Study Area

The study area is located in La Mata, Sánchez Ramírez Province, Dominican Republic, between latitude 18°53′ North and longitude 70°22′ West, with a total geographical area of 5.8 km^2. The climate is tropical humid, with an average annual temperature of 25 °C, and an average annual rainfall of 1787 mm [28]. The economy of the Sánchez Ramírez province is based on agriculture and mining. There are two active gold mines, one 16.5 km and the other 24.3 km upstream of the Yuna River, which is the main source of irrigation of the soils in the area of study. The dominant soils in this region are, Inceptisol and to a lesser extent, Vertisol [29]. Figure 1 shows the soil sampling points and background sample location.

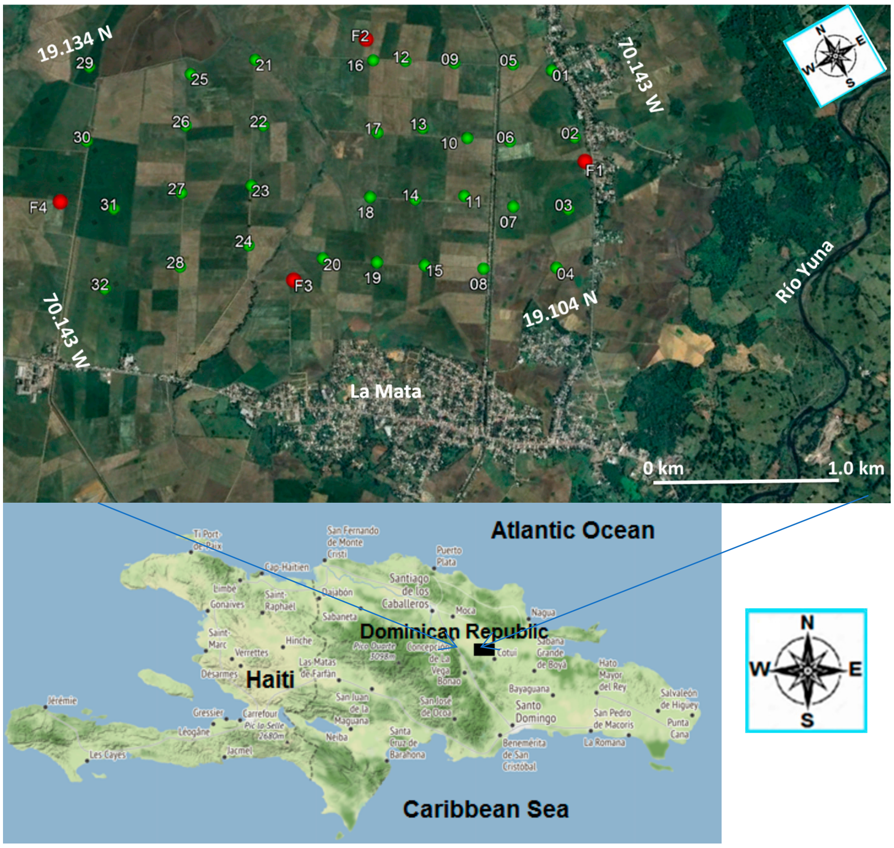

Figure 1. The study area and sampling points are located in La Mata, Sánchez Ramírez Province Dominican Republic.

2.2. Soil Sample Collection

A total of 32 surface soil samples at a depth of 0–30 cm were collected from rice-cultivated land, maintaining a distance of approximately 300 m from one sampling point to another [30]. Four subsoil samples were collected at a depth of 40–50 cm, in positions F1 to F4 (Figure 1), to estimate the local background [31]. Around 1 kg of soil was collected from each sampling point. The localization of the study points was determined using a global positioning system (GPS Differential) (Garmin GPS Map 76S, Garmin Ltd., Olathe, KS, USA).

2.3. Sample Preparation

After collection, soil samples were air-dried and cleaned to remove stones, gravel, and plant roots. Before chemical analysis, the soil samples were screened through a 2 mm sieve. For heavy metal analysis, the soil samples were further dried at 105 °C for 24 h, crushed in an agate mortar, passed through 0.2 mm nylon sieves, and stored in polyethylene jars at room temperature. For testing, 3 g of each soil sample was pressed at 15 tons into a pellet of 2.0 cm diameter and 2.0–3.0 mm height. The elemental concentrations in five replicas of the certified Standard Reference Material SRM 2711a were measured to check the quality of the analysis. Detection and quantification limits are presented in Table S1.

2.4. Soil Physicochemical Analysis

Soil particle size distribution was determined using the hydrometer method [32]. The pH of the soil samples was measured using a portable meter in a soil-to-water suspension of 1:2 (Apera Instrument, LLC, Columbus, OH, USA, model MP511, CHN). The electrical conductivity (EC) was measured with a portable meter (Apera Instrument, LLC, model EC700, CHN) in a soil-to-water suspension of 1:2 [33]. The organic matter (OM) content was determined following the Walkley and Black method [34]. The cation exchange capacity

(CEC) of the mineral soils was measured in an extractable solution with 1 M of NH_4-acetate [35].

The total concentrations of Fe, Mn, Cr, Ni, Cu, Zn, Pb, and As were determined using the EDXRF technique. The instrument used was a Skyray model EDXR-3600B X-ray spectrophotometer with a silicon detector placed at 45 degrees from the X-ray source (Ag). The excitation voltage of the X-ray-emitting source was 40 kV and 600 µA. A calibration curve was prepared for each element using Standard Reference Materials (SRM). The calibration curves obtained were evaluated using the determination coefficient (R^2), which was between 0.990 and 0.999. The intensity of the characteristic radiation of each element in the samples was obtained from the processing of the spectra with the Skyray program (Version RoHS4_1.1.47_110524_R, 2009, 20110524_R, Kunshan, China) supplied by the manufacturer (Dallas, TX, 75,238 USA).

2.5. Statistical Analysis and Spatial Distribution

The data set was analyzed using the Excel 2023 software package (Microsoft, Redmond, WA, USA) to compute descriptive statistics, which included minimum, maximum, mean, and standard deviation. A Pearson's correlation analysis was conducted to investigate the relationship between heavy metals and the examined soil properties. To create a map of the spatial distribution of heavy metals in paddy soils, Ordinary Kriging (OK) was used as the interpolation method. The mapping was carried out using SURFER 10 (Golden Software, LLC, Golden, CO, USA).

2.6. Soil Pollution Indices

Different pollution indices were estimated to evaluate the heavy metal contamination using local background (LB) and the World Average Shale (WAS), according to Turekian and Wedepohl [36], as reference values. The following indices are described below.

2.6.1. Geo-Accumulation Index (I_{geo})

The I_{geo} was calculated according to Müller [37] as follows:

$$I_{geo} = \log_2 \frac{Ci}{kxS_i} \quad (1)$$

where Ci is the concentration value of metal i in the soil, Si is the background reference value and k is the correction coefficient, which is 1.5. The classification for I_{geo} values is presented in Table S2.

2.6.2. Enrichment Factor (EF)

The enrichment factor (EF) was calculated according to Jiang et al. [38]. Elements such as Sc, Mn, Ti, Al, and Fe are usually chosen as references [39–41]. In this study, Fe was used as the reference element because of the relatively high concentration and stability in the earth's crust [13,42]. It was calculated as follows:

$$EF = \frac{(M/Fe)_{sample}}{(M/Fe)_{background}} \quad (2)$$

where (M/Fe)sample is the ratio of metal and Fe concentrations in the sample, and (M/Fe)background in the background sample, respectively. According to Sutherland (2000), the EF values must be classified into six levels as presented in Table S2.

2.6.3. Single Pollution Index (PI)

The single pollution index was used to assess the pollution levels for individual heavy metals. The PI was calculated according to Hakanson [43] as follows:

$$PI = Ci/Cb \quad (3)$$

where Ci is the concentration of the metal element i in topsoil samples, while Cb is the background value of the target element. PI values can be classified into three levels as presented in Table S2.

2.7. Potential Ecological Risk Index (RI)

The potential ecological risk index (RI) is a tool used to evaluate soil pollution based on the characteristics and environmental behavior of heavy metals [42]. It integrates the content and toxicity level of heavy metal and the sensitivity of the environment to this heavy metal. Also, it is widely used in soil ecological risk assessment [19,44]. RI was calculated as follows:

$$\mathrm{RI} = \sum_{i}^{n} \mathrm{E}_{r}^{i} = \sum_{i}^{n} \mathrm{T}_{r}^{i} \times \frac{\mathrm{C}_{s}^{i}}{\mathrm{C}_{n}^{i}} \quad (4)$$

where E_r^i is the potential ecological risk factor of an individual heavy metal, T_r^i is the toxic response factor, C_s^i is the measured concentration of the heavy metal, and C_n^i is the soil background values of the heavy metal. The standard toxic response factors for Cr, Cu, Ni, Pb, Zn, and As are 2, 5, 5, 5, 1, and 10, respectively [45]. The values of RI are presented in Table S2.

3. Results and Discussion

3.1. Physicochemical Properties

Table 1 presents the physicochemical properties evaluated in the surface soil samples of La Mata, Dominican Republic. The soil pH ranged between 5.08 and 6.96 with a mean value of 5.8, and its coefficient of variation in the total samples was relatively low. Salinity (EC) was less than 0.75 (mS cm^{-1}) in all samples. The OM content ranged between 1.6% and 6.5% with a mean value of 4.4%. The cation exchange capacity (CEC) varied between 11 and 30 meq 100 g^{-1} with a mean value of 15 meq 100 g^{-1}. The soil texture has a content of silt, clay, and sand in the ranges of 22–51%, 16–54%, and 13–55%, respectively. These results are similar to those reported by Alberto Then et al. [26] in Bonao, Dominican Republic.

Table 1. Selected physicochemical properties in agricultural soils of La Mata, Dominican Republic.

Soil Parameter	Min	Max	Mean ± STD	CV (%)
pH (1:2)	5.08	6.96	5.8 ± 0.4	7
EC (mS cm^{-1})	0.06	0.60	0.16 ± 0.09	43
OM (%)	1.6	6.5	4.4 ± 1.2	27
CEC (meq 100 g^{-1})	11	30	15 ± 3	21
Silt (%)	22	51	32 ± 6	18
Sand (%)	13	55	23 ± 7	32
Clay (%)	16	54	45 ± 9	20

Min, minimum; Max, maximum; STD, standard deviation; CV, coefficient of variation.

3.2. Heavy Metal Concentration in Soils

The mean concentration of heavy metals in the surface soil was 73735, 1616, 426, 34, 20, 200, 43, and 5 mg kg^{-1} for Fe, Mn, Cr, Cu, Ni, Zn, Pb, and As, respectively (Table 2). Generally, average metal concentrations were found in the order of Fe > Mn > Cr > Zn > Pb > Cu > Ni > As. The trend for Fe, Mn, and Cr is similar to the results of Alberto Then et al. [26] in Bonao rice soils. The mean concentration of Fe, Mn, Cr, and Pb in the surface soil exceeded the local background values (LB), and the World Average Shale (WAS). In the case of Cu, Ni, and As, the mean concentrations are higher than LB but lower than WAS. It is important to note that the concentrations of Fe, Mn, Cr, and Pb in the local background were higher than those in WAS, suggesting elevated natural levels and likely a geogenic origin for these metals in the soils of the study area. Cr, Cu, Zn, and Pb concentrations are higher than the maximum values for healthy agricultural soils adopted by the FAO [46]. The mean concentrations of Cr, Zn, and Pb are higher than those reported by Alberto Then et al. [26] in Bonao. However, the Ni concentration is lower than the maximum values given by

FAO and those reported by Alberto Then et al. [26] in Bonao, and by Delanoy et al. [25] in La Vega and San Francisco de Macoris. These are three different agricultural areas of the Dominican Republic where rice is cultivated under similar climatic and rice management conditions. Despite this, there was no significant difference in the As concentration reported in this study and these areas. The total heavy metal concentration for all sampling points can be found in Tables S4 and S5.

Table 2. Descriptive statistics of the total heavy metal concentrations, and local background values in surface soils of La Mata (mg kg^{-1}).

Heavy Metal	Fe	Mn	Cr	Cu	Ni	Zn	Pb	As
Min	48,521	170	285	2	<0.1	23	24	3
Max	100,708	7340	709	73	179	578	72	6
Mean ± STD *	73735 ± 13109	1616 ± 1691	426 ± 130	34 ± 19	20 ± 39	200 ± 167	43 ± 12	4.8 ± 0.7
CV (%)	18	105	30	55	198	83	27	14
Local Background ± STD *	56,916 ± 16,481	1387 ± 1549	417 ± 117	27 ± 20	9.5 ± 2.1	215 ± 109	38 ± 15	4.0 ± 1.4
WAS [a]	47200	850	90	45	68	95	20	13
FAO [b]	-	<0.01	70	30	50	90	35	-

* STD: Standard deviation; CV: coefficient variation; [a] WAS: World Average Shale [37]; [b] FAO: Maximum heavy metal concentration values for healthy agricultural soil, according to the Food and Agriculture Organization (FAO) [46].

The coefficient of variation (CV) values of the seven heavy metals and As in the study area ranged from 14% to 198%. Table 2 shows that the CV of Mn, Ni, and Zn was 105%, 198, and 83%, respectively, with high variation (CV > 75%), and the CV of Cr, Cu, and Pb was 30%, 55%, 27%, respectively, with medium variation (25% < CV < 75%). Therefore, the main inputs of the contents of Mn, Ni, Zn, Cr, Cu, and Pb may be influenced by anthropogenic sources [13,47]. The CV of Fe and As was 18% and 14%, respectively, with low variability (CV < 25%), indicating that the inputs of these metals in the studied area may be influenced by the parent material of the soil.

3.3. Pearson's Correlation of Soil Heavy Metals and Physicochemical Properties

Cr had a significant correlation at the 0.05 level with Ni and Zn, and Zn with Ni and As (Table 3). However, Pb had a moderate negative correlation with As, indicating that the mentioned heavy metals must be associated with the different pollution sources. No significant correlation was found between pH and OM with the presence of heavy metals.

Table 3. Pearson's correlation coefficients between heavy metals and physicochemical properties.

	Fe	Mn	Cr	Cu	Ni	Zn	Pb	As	pH	OM
Fe	1									
Mn	0.27	1								
Cr	−0.24	0.43	1							
Cu	−0.22	−0.21	−0.24	1						
Ni	−0.24	0.47	**0.53 ***	0.13	1					
Zn	−0.10	0.41	**0.61 ***	0.10	**0.59 ***	1				
Pb	0.47	−0.08	−0.13	−0.38	−0.22	−0.18	1			
As	−0.24	0.14	0.24	0.31	0.32	**0.51 ***	**−0.56 ***	1		
pH	−0.18	0.07	0.31	−0.04	0.27	0.25	−0.03	−0.09	1	
OM	0.07	0.07	−0.11	−0.16	−0.11	0.04	−0.21	0.04	**−0.50 ***	1

* Correlation is significant at the 0.05 level.

3.4. Spatial Distribution of Heavy Metals

Figure 2 presents the spatial distribution of the concentrations of seven heavy metals and As in the study area. The study area was found to have high concentrations of Fe in its central part, while high concentrations of Mn were observed in the northwest and southeast areas. Additionally, Cr was found to have high concentrations in almost all parts of the study area. Ni had high concentrations in the eastern and southwest parts, whereas

Zn was observed to have high concentrations in the northwest and southeast areas. Cu, Pb, and As had similar distribution patterns, with low concentrations observed in almost all parts of the study area. Therefore, the distribution pattern of each metal is different, indicating they have different sources of origin.

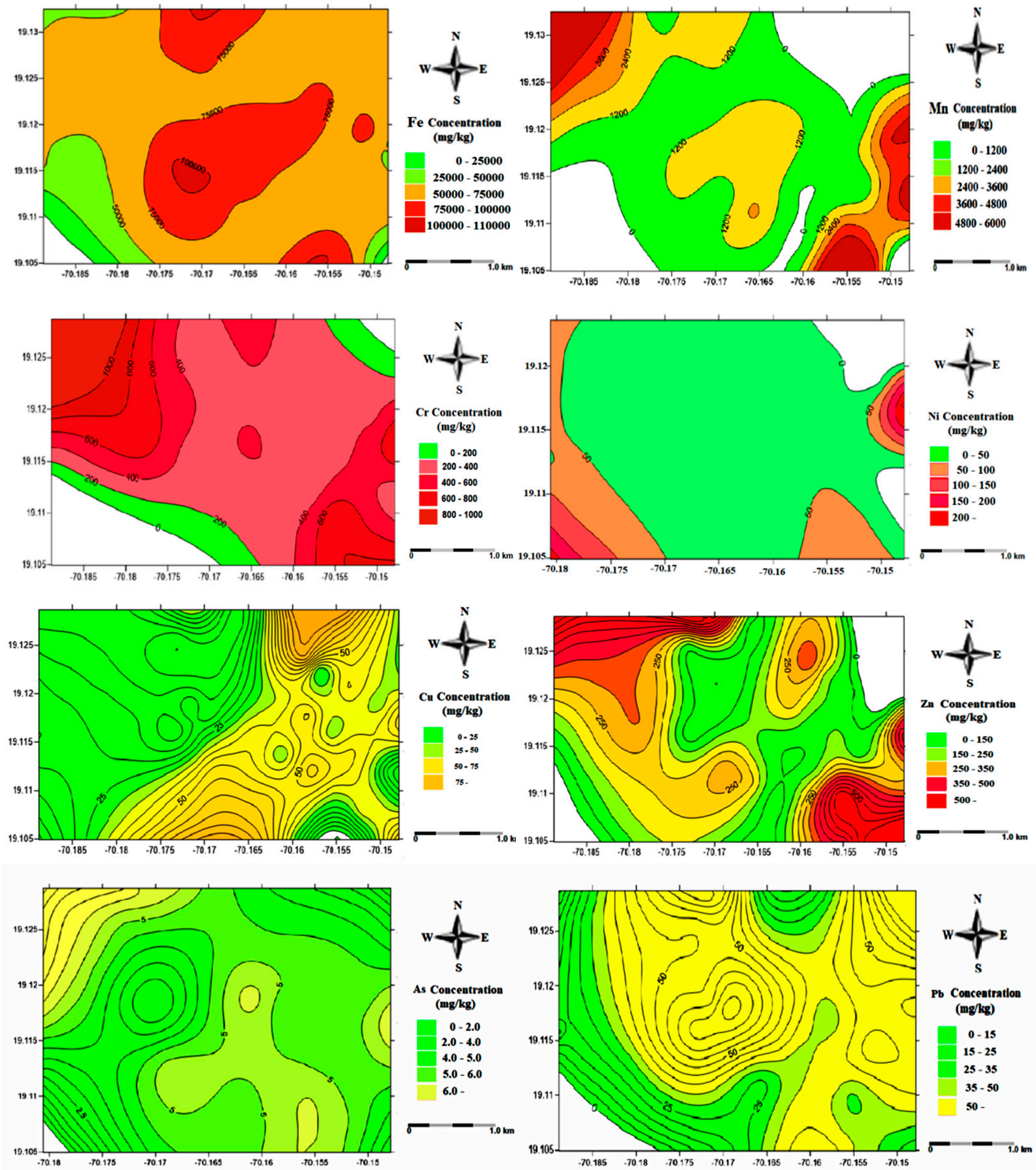

Figure 2. Spatial distribution maps of heavy metals in soils.

3.5. Soil Pollution Indices

3.5.1. Geo-accumulation Index (I_{geo})

The average I_{geo} value is lower than 0 for Mn, Cu, Zn and As using both LB and WAS (Figure 3), indicating that the soils are not polluted by these elements. However, the mean value for Cr is lower than 0 using LB but exceeds 1 using WAS, which classifies the soils as partially moderately polluted. For Pb, the mean value exceeds 0 using WAS, which classes the soils as slightly polluted, while using LB, the mean value is lower than 0, which classes them as not polluted. For Ni, the mean value exceeds 0 using LB and is lower than 0 using WAS.

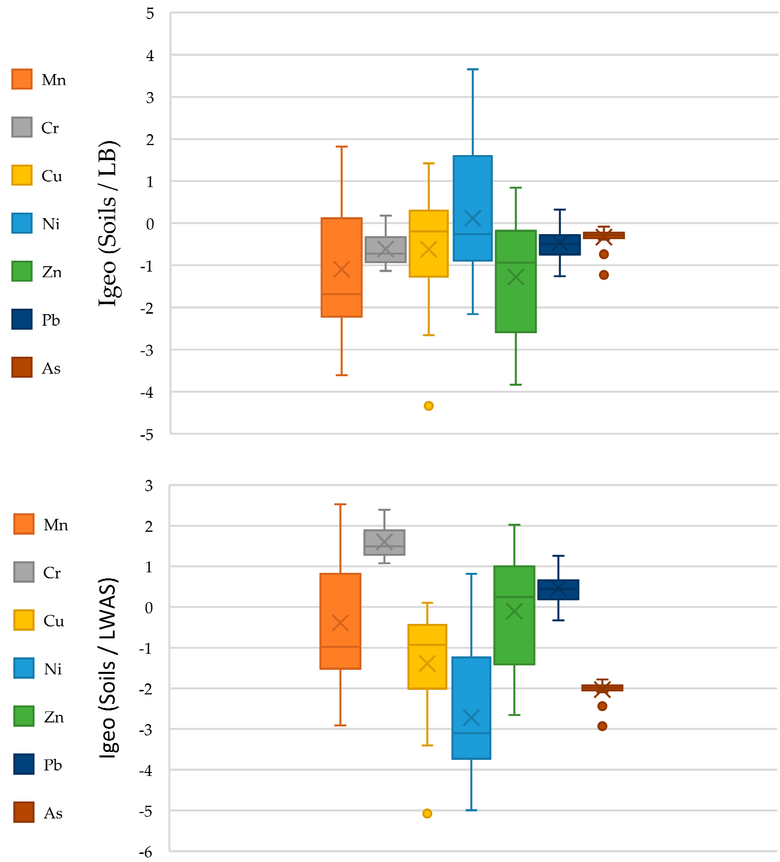

Figure 3. I_{geo} values of soils using LB and WAS as reference values.

3.5.2. Enrichment Factor (EF)

The mean EF values suggest no enrichment for Mn, Zn, and Pb using LB and minimal enrichment using WAS, unlike Cu and Ni, which indicate minimal enrichment using LB and no enrichment using WAS. For Cr, the mean EF value shows no enrichment using LB and moderate enrichment using WAS, and finally, As falls into the no-enrichment category using LB and WAS as reference values (Table 4). The average EF value ranks heavy metals in the following order using WAS as the reference value: Cr > Zn > Pb > Mn > Cu > As > Ni. Using LB as the reference value, the order is as follows: Ni > Cu > As > Mn > Pb > Cr > Zn.

Table 4. Average enrichment factor (EF) using LB and WAS as reference values.

Heavy Metal	FE (Soils/LB)	Enrichment	FE (Soils/WAS)	Enrichment
Mn	0.89	No enrichment	1.20	Minimal
Cr	0.82	No enrichment	3.17	Moderate
Cu	1.02	Minimal	0.51	No enrichment
Ni	1.81	Minimal	0.21	No enrichment
Zn	0.75	No enrichment	1.41	Minimal
Pb	0.87	No enrichment	1.38	Minimal
As	0.97	No enrichment	0.25	No enrichment

LB: Local background; WAS: World Average Shale [36].

3.5.3. Single Pollution Index (PI)

Table 5 shows the average of the single pollution index (PI) for the studied elements using LB and WAS as background. For Cu, Ni, and As, a low pollution level was recorded using LB and WAS as background. However, Mn, Zn, and Pb were classified as having low pollution levels using LB, and moderate pollution levels using WAS. Cr is classified with a low pollution level using LB, and with a strong pollution level using WAS as a reference value.

Table 5. Average Pollution Index (PI) using LB and WAS as reference values.

Heavy Metal	PI (Soils/LB)	Pollution Status	PI (Soils/WAS)	Pollution Status
Mn	0.94	Low	1.9	Moderate
Cr	0.95	Low	4.73	Strong
Cu	0.95	Low	0.75	Low
Ni	0.72	Low	0.29	Low
Zn	0.94	Low	2.11	Moderate
Pb	0.97	Low	2.14	Moderate
As	0.96	Low	0.37	Low

LB: Local background; WAS: World Average Shale [36].

3.6. Potential Ecological Risk (RI)

The potential ecological risk index (RI) of the six heavy metals and As in the study area is presented in Table 6. The findings of this study indicate that the level of pollution from heavy metals, using both LB and WAS as reference values, was low. Therefore, there is no significant risk to human or environmental health.

Table 6. The potential ecological risk using LB and WAS reference values.

Heavy Metal	Ei (Soils/LB)	Risk Degree	Ei (Soils/WAS)	Risk Degree
Mn	2.33	Low	1.90	Low
Cr	2.04	Low	4.73	Low
Cu	1.25	Low	0.75	Low
Ni	2.07	Low	0.29	Low
Zn	4.65	Low	2.11	Low
Pb	5.59	Low	2.14	Low
As	12.08	Low	0.37	Low
RI	30.02	Low	12.29	Low

LB: Local background; WAS: World Average Shale [36].

4. Conclusions

The average concentrations of elements present in rice-cultivated soils in La Mata, Dominican Republic, are in the following order: Fe > Mn > Cr > Zn > Pb> Cu > Ni > As. The evaluation of contamination levels in soils through various indices, I_{geo}, EF, and PI, shows that the results obtained using WAS as a reference value indicate more pollution than those obtained using LB. Although the potential ecological risk assessment indicated a low-risk status, the mean concentrations of Mn, Cr, Cu, Zn, and Pb are higher than the maximum levels of heavy metals for healthy agricultural soils adopted by the FAO. The slight pollution of these heavy metals was probably caused by mining activities and the excessive use of fertilizers and pesticides, which are the primary sources of pollution in the study area. This study provides valuable information about the current soil quality status that can be used as a baseline for future comparisons with other agricultural areas in the Dominican Republic.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/land13050684/s1, Table S1a. XRF analysis of the standard reference material (SRM 2711a), %SR values (n = 5), and detection limits; Table S1b. Determination of detection limit (DL) and quantification limit (LOQ) of the standard reference material (SRM 2710a); Table S2. Pollution/ecological Indices, classification and interpretation; Table S3. Geographic location and physicochemical properties of La Mata, Dominican Republic; Table S4. Total heavy metal concentrations in surface soils of La Mata, Dominican Republic; Table S5. Descriptive statistics of pH, % Organic Matter (OM), and the heavy metal concentrations of local background samples in soils La Mata, Dominican Republic (n = 4); Table S6. Geo-accumulation Index (Igeo) in surface soils of La Mata, Dominican Republic, using LB as background; Table S7. Geo-accumulation Index (Igeo) in surface soils of La Mata, Dominican Republic, using World average shale (WAS) as background; Table S8. Enrichment Factor (EF) in surface soils of La Mata, Dominican Republic, using LB as background; Table S9. Enrichment Factor (EF) in surface soils of La Mata, Dominican Republic, using World average shale (WAS) as background; Table S10. Single Pollution Index (PI) in surface soils of La Mata, Dominican Republic, using LB as background; Table S11. Single Pollution Index (PI) in surface soils of La Mata, Dominican Republic, using WAS as background; Table S12. The potential Ecological risk factor (E_r^i) in surface soils of La Mata, Dominican Republic, using LB as background; Table S13. The potential Ecological risk factor (E_r^i) in surface soils of La Mata, Dominican Republic, using WAS as background. Refs. [48–50] have been cited in the Supplementary Materials.

Author Contributions: N.M.A.T.: design of this study and writing of this manuscript; R.D.: validation and formal analysis; O.D.R.: analysis and interpretation of the data; P.A.N.-R.: review; L.B., writing—review and editing; L.B.: project administration. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the government of the Dominican Republic through the Ministry of Higher Education, Science, and Technology (project No. 2020-2B6-037) and by the Instituto Tecnológico de Santo Domingo (INTEC) (project No. CBA-330811-2020-p-1).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within this article and Supplementary Materials.

Acknowledgments: The authors thank the Environmental Science Doctoral program of INTEC for their support in this work. We also thank María Cristina Suárez for her contribution to the soil samples analysis, and Diana Rodríguez Alberto for her support in writing this manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

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