Fertilizantes como fuente de contaminación por Cd en suelos cultivados y arroz en la provincia de Guayas-Ecuador

Fertilizers as a source of Cd contamination in cultivated soils and rice in the province of Guayas, Ecuador

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Resumen

El cadmio (Cd) es un metal pesado tóxico que contamina el arroz y los fertilizantes representan una fuente significativa de esta contaminación. Este estudio se centró en detectar la presencia de Cd en fertilizantes, suelos y plantas de *Oryza sativa* en los cantones de Daule y Nobol, en la provincia de Guayas, Ecuador. Se analizaron nueve muestras de fertilizantes inorgánicos y se recolectaron muestras triplicadas de suelos y plantas en 12 estaciones. Utilizando un espectrofotómetro de absorción atómica, se encontró que la concentración máxima de Cd en los fertilizantes alcanzó 41.30±1.65 mg/kg, superando en 41 veces el límite recomendado. En el suelo, las concentraciones máximas fueron de 2.59±0.31 mg/kg, mientras que en las raíces, hojas y granos fueron de 0.50±0.07, 0.44±0.08 y 0.19±0.050 mg/kg, respectivamente. Los resultados indicaron que en Nobol, la concentración de Cd en el suelo excedió cinco veces el límite permitido por la normativa ecuatoriana, aunque los niveles en el arroz se mantuvieron dentro de los límites de la Unión Europea. Este estudio concluye que los fertilizantes son una fuente de contaminación por Cd en estas áreas, siendo Nobol el cantón más afectado.

Palabras clave: cadmio, metales pesados, *Oryza sativa*, seguridad alimentaria.

Abstract

Cadmium (Cd) is a toxic heavy metal that contaminates rice, and fertilizers represent a significant source of this contamination. This study focused on detecting the presence of Cd in fertilizers, soils, and *Oryza sativa* plants in the cantons of Daule and Nobol in the province of Guayas, Ecuador. Nine samples of inorganic fertilizers were analyzed, and triplicate samples of soils and plants were collected at 12 stations. Using an atomic absorption spectrophotometer, the maximum concentration of Cd in the fertilizers was determined to be 41.30 ± 1.65 mg/kg, over 41 times the recommended limit. In the soil, the maximum concentration was 2.59 ± 0.31 mg/kg while, in the roots, leaves, and grains, the maximum concentrations were 0.50 ± 0.07, 0.44 ± 0.08, and 0.19 ± 0.050 mg/kg, respectively. The results indicate that, in Nobol, the concentration of Cd in the soil was over five times the limit allowed by Ecuadorian regulations, although the levels in the rice remained within the limits set by the European Union. This study indicates that fertilizers are a source of Cd contamination in these areas, with Nobol being the most affected canton.

Keywords: cadmium, food safety, heavy metals, Oryza sativa

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Introduction

Cadmium (Cd) is one of the most toxic heavy metals found in agricultural soils. It has high mobility and is transferred to plants, in which it inhibits growth, generates a water deficit, and inhibits photosynthesis and germination, among other effects (He *et al.*, 2008; Pernía *et al.*, 2008).

In consequence, these effects reduce crop productivity, cause economic losses in the agricultural sector, and jeopardize food safety (Sharma and Archana, 2016). In addition, the Cd enters the trophic chain and affects human beings, with correlations between Cd consumption and diseases such as diabetes, osteoporosis, hypertension, renal damage, and cancer having been demonstrated (Clemens *et al.*, 2013). For instance, cadmium poisoning in rice paddies in Japan caused dozens of deaths from itai-itai, a degenerative bone disease (Inaba *et al.*, 2005).

In this sense, one of the routes of Cd intake is through the consumption of contaminated foods such as rice, which is an essential product in human food. Notably, studies have shown the presence of Cd in grains as a consequence of the contamination of agricultural soils (Pozo *et al.*, 2011; Zhang *et al.*, 2013). According to Liu *et al.* (2019), rice appears to bioaccumulate Cd more than other crops. In Ecuador, there is evidence of Cd contamination in agricultural soils in several provinces: Esmeraldas (0.22-0.88 mg/kg Cd), Santo Domingo (0.33-0.53 mg/kg Cd), Los Ríos (0.17-0.66 mg/kg Cd), Manabi (0.46-2.37 mg/kg Cd), and Guayas (0.25-1.65 mg/kg Cd) (Mite *et al.*, 2010). Moreover, Chávez et al. (2015) reported high values of Cd (0.66-2.59 mg/kg Cd) in Guayas Province. Moreover, in the soils of rice crops, Cd concentrations of 0.04-0.69 mg/kg have been reported (Pozo *et al.*, 2011; Ochoa *et al.*, 2020; Atiaga *et al.*, 2021).

The presence of Cd in the province of Guayas is due to natural geogenic factors, such as the weathering of parent material from the Andes Mountains, and anthropogenic factors, such as mining activity, accumulation of industrial waste, and burning of garbage (Pozo *et al.*, 2011). However, in agricultural soils, the main factors responsible for Cd contamination are the use of phosphate fertilizers and the use of irrigation water from contaminated rivers (Pozo *et al.*, 2011; Chávez *et al.*, 2015). According to Rodríquez-Serrano *et al.* (2008), in general, phosphate fertilizers are the main source of heavy metal contamination, with a contribution of 34%, followed by natural sources at 21%; fossil combustion at 21%, metallurgical production at 12%; and cement, waste incineration, and others, which represent 12%. Growing rice is one of the main agricultural activities in the Ecuadorian littoral region.

A large part of its production is established in the lower basin of the Guayas River and, according to statistical data from the National Institute of Statistics and Census (INEC, 2015), the province of Guayas is the largest producer of food in Ecuador, contributing about 71.8% of the national production. It is estimated that this province contains 87,888 hectares (ha) of rice crops, of which 17,027 ha correspond to the Daule canton and 5,458.50 ha correspond to the Nobol canton (GAD Provincia del Guayas, 2014). Both cantons are irrigated by the Daule River, which is contaminated with Cd at a rate of 0.011 ± 0.003 mg L^{-1} Cd, according to a study by Ramírez et al. (2016). In addition, large concentrations of fertilizers are used. More than 50% of the world's population consumes rice regularly, and it is a crucial source of calories and protein for people (Cheajesadagul *et al.*, 2013).

With an average daily consumption of 80 grams of rice per person, Latin America is the second most important rice-consuming area, after Asia. In terms of consumption per person, Ecuador (123 g/day) is one of the nations in South America with the highest rate of rice consumption (FAOSTAT, 2018). Ochoa et al. (2020) conducted studies in the province of Guayas in search of heavy metals in rice (Ochoa *et al.*, 2020). They focused on finding sources of contamination in irrigation water, where they found low Cd values (0.017 ± 0.020 μg/L). The present study focused on analyzing fertilizers as a source of Cd contamination in rice.

Therefore, the aim of this work was to determine the concentrations of Cd in fertilizers, soils, and plants of *O. sativa* in the Daule and Nobol cantons in the province of Guayas, in order to verify if they were within the maximum permissible national and international limits. Based on the results, the risk to the health of the Ecuadorian population was analyzed.

Materials and Methods

Study area

The study area was located in the Daule (1°52'00"S, 79°59'00"W) and Nobol (1°55'00"S, 80°00'42"W) cantons of the province of Guayas in the coastal region of Ecuador north of the city of Guayaquil. Daule is located at 22 m.s., with an average annual temperature of 24 ºC and an annual rainfall of 1500 mm (Figure 1).

The Nobol canton is located at 9 m.s., with an average annual temperature of 27 °C and an average annual rainfall of 500 to 1000 mm. It is characterized by agricultural and livestock production. Overall, six stations in the Daule canton and six stations in the Nobol canton were studied (Figure 1).

Sampling

At each study station, we randomly selected three patches. Then, we divided each patch into three sub-patches for use as replicas and geographically positioned them using a portable GPS (model: Triton Magellan®). From each sub-patch, we collected fully mature rice plants, including grains. In the laboratory, we separated each plant into roots, stem, leaves, and grains; washed them with bi-distilled water; and dried

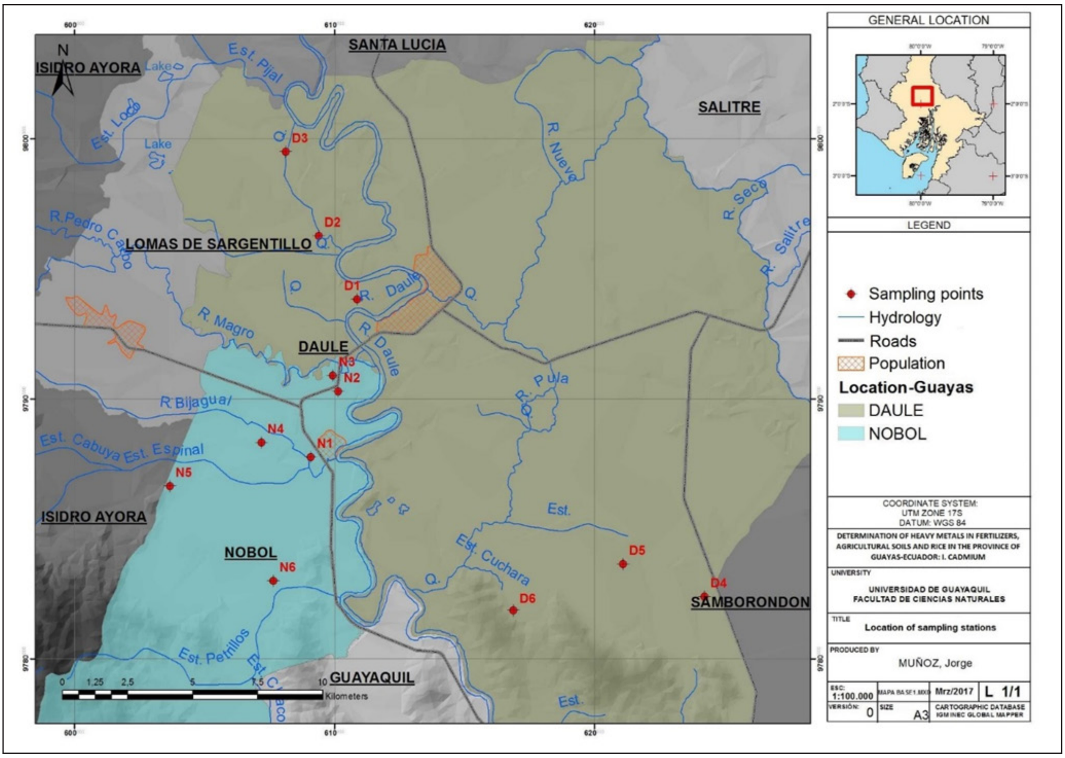

Figure 1. Location of sampling stations.

them with paper towels. Finally, the plants were dried in an oven at 80 °C for 48 hours. We took triplicate soil samples from the places where the plants were located in each sub-patch.

The Ministry of Agriculture, Livestock and Fisheries donated triplicates of samples of fertilizers used for rice cultivation: ammonium sulfate, calcium nitrate, magnesium sulfate, muriate of potash, phosphate fertilizer complex 8-20-20, phosphate fertilizer complex, potassium nitrate, and urea (Table 1).

The soil samples were collected from the top layer, between 5 and 10 cm deep, using a 1.5 m long plastic shovel. Care was taken to avoid disturbing the sediments, which were stored in airtight bags until they reached the laboratory.

Determination of cadmium in soils and plants

In the laboratory, the soils were spread out on a plastic surface and dried at room temperature for 2 weeks. Subsequently, the samples were disaggregated and sieved through a 71 µm mesh. The sieved samples were dried in an oven at 80 °C for 1 hour to remove moisture, and 1 g was weighed for further digestion in triplicate.

For detection of the total metal fraction, the procedure proposed by EPA Standard 3050B (EPA, 1996) was used. A 0.5 g sample was weighed. Then, 5 mL of 65% HNO_3 (Merck, Ensure®) was added to the sample in a hermetically sealed test tube, which was placed in a water bath at 100 °C for 1 hour. Subsequently, the solution was filtered using Whatman No. 40 paper and diluted to 100 mL with ultrapure water. Additionally, a certified reference material (CRM-016, Sigma) was used as a quality control measure. It showed a recovery percentage of 101%.

To analyze of Cd contents in the roots, stems, leaves, and grains, the dried samples were disintegrated in a mortar. Next, 1 g samples were weighed on a Sartorius analytical balance (model BL210S), digested in an open system with 5 mL of 65% HNO_3 and 3 mL of 30%

Table 1. Types of fertilizers recommended for rice cultivation.

Sample	Type	Presentation
F1	Phosphate fertilizer Complex 8-20-20	Grain
F2	Calcium nitrate	Grain
F3	Muriate of potash standard	Powder
F4	Muriate of potash	Grain
F5	Phosphate fertilizer Complex	Grain
F6	Magnesium sulfate	Grain
F7	Urea	Grain
F8	Ammonium sulfate	Grain
F9	Potassium nitrate	Powder

H_2O_2, and heated at 80 °C in a heating dish (PMC) for 1 h. Then, the resulting solutions were filtered with Whatman No. 40 paper and diluted to 50 ml with ultrapure water.

The fertilizer samples were broken down in a porcelain mortar. For their digestion, we used the methodology proposed by Borges *et al.* (2014), with certain modifications. We added 15 mL of HNO_3 to 1 g of a sample. Then, we heated the mixture at 75 ± 5 °C for 30 min, added 3 mL of 30% H_2O_2, and heated the mixture once more. The residual solution was filtered and diluted to 50 mL with ultrapure water.

The Cd present in the plants, soils, and fertilizers was analyzed in a flame atomic absorption spectrophotometer (Perkin Elmer, model: AAnalyst100) using the internal methodology of the IIRN laboratory, which is based on the methodology described in *Standard Methods* (2005).

Quality control

Quantification of the Cd concentrations in the tissues of the plants and the soils was carried out using calibration curves with a coefficient of variation of $r^2 = 0.99$. The calibration curves for Cd were generated using certified standards from Accustandard (Cd: 1000 mgL^{-1}). The assessed validation parameters included linearity, limits of detection (LOD), limits of quantification (LOQ), accuracy, and precision, and statistical tests were employed to ensure reliability.

The limit of quantification (LOQ) established for Cd was 0.028 mg kg^{-1}. Each analysis also included reagent blanks, triplicate samples, and spike samples, in order to enhance the robustness of the findings.

The samples were read at 228.8 nm for Cd, with a detection limit of 0.028 mg/L. All measurements were made in triplicate (n = 3). The certified reference material CRM-016 (trace metals/freshwater sediment) was used as quality control for the soils, and BCR-670 (duckweed trace elements) was used as quality control for the plants. The recovery percentages were 98-99%.

Bioconcentration factor

The ability to accumulate heavy metals in *O. sativa* was determined using the bioconcentration factor (BCF). This indicated the relationship between the Cd accumulated in the plant and the Cd present in the medium or substrate (Brooks, 1998) and was determined using the following formula:

$$\mathrm{BCF} = \frac{\text{Concentration of Cd in the plant}}{\text{Concentration of Cd in the substrate}}$$

Transfer factor

The Cd transfer factor (TF) indicated the ability to transfer the heavy metal from the roots to the stem (Brooks, 1998). It was determined with the following formula:

$$\mathrm{FT} = \frac{\text{Concentration of Cd in the stem}}{\text{Concentration of Cd in the root}}$$

Potential Risk Assessment

To evaluate the potential health risks associated with cadmium (Cd) exposure from rice consumption in Ecuador, we employed the Average Daily Dose (ADD) assessment method, as recommended by the United States Environmental Protection Agency (2011).

We gathered data on the cadmium concentration in rice, which was determined to be 0.19 mg/kg (the maximum value found in this study). Additionally, we obtained demographic information, including the average body weights for Ecuadorian men (74.2 kg) and women (66.9 kg), from the WorldData database (2023). The annual per capita rice consumption was 53.2 kg, equivalent to 0.145 kg/day (Zambrano *et al.*, 2018).

Average Daily Dose (ADD) Calculation: The ADD was calculated using the following formula:

$$ADD = \frac{CxIRxED}{BWxAT}$$

where C represents the heavy metal concentration in rice (mg/kg), IR is the daily rice intake (kg/day), ED is the exposure duration (days), BW is body weight (kg), and AT is the average time (days). For our analysis, we assumed a lifetime exposure duration of 70 years, which translated to 25,550 days.

We calculated the non-carcinogenic risk using the hazard quotient (HQ) approach, which was defined as follows:

$$HQ = \frac{ADD}{RFD}$$

where RfD is the reference dose for cadmium, typically estimated at 0.001 mg/kg/day. We determined HQs for both the male and female populations based on their respective ADD values.

Finally, the calculated HQ values were analyzed to assess the potential health risks associated with cadmium exposure through rice consumption. An HQ value below 1 indicates an acceptable risk level, while values above 1 suggest potential health concerns.

Statistical analysis

The results are shown as means ± standard deviations. The normality of the data was verified using the Anderson–Darling test, and homoscedasticity was verified using the Levene test. We used a one-way ANOVA to compare means to determine if there were statistically significant differences in the concentrations of metals in the different localities (taking $p<0.05$ as a significant value for an a posteriori Tukey test). For the evaluation of the cadmium contents in the grains and stems of the rice plants, the data were non-parametric, and the Kruskall–Wallis test was used.

To determine if there were correlations between the Cd present in the parts of the rice plants and the heavy metals in the soils, we calculated Spearman correlation coefficients and carried out a principal

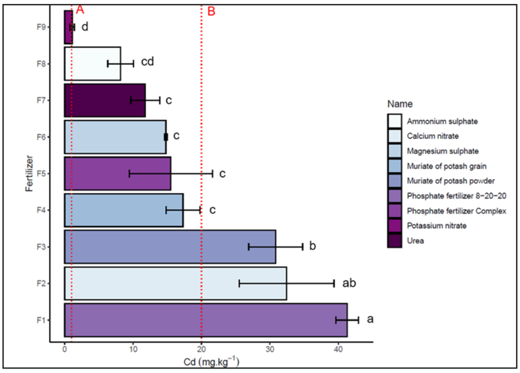

Figure 2. Concentrations of Cd in the fertilizers recommended for rice cultivation in Ecuador. A. MPL established by the Washington State Ecological Department (0.1 mg/kg Cd). B. MPL established by the Canadian regulations (20 mg/kg Cd). The bars and dots represent standard deviation (n = 3). Matching letters mean that there is no significant difference by one-way ANOVA and Tukey test (p≥0.05).

component analysis. All tests were performed and all graphics were generated with RStudio and R version 4.0.2.

Results

Cd in fertilizers used for rice crops

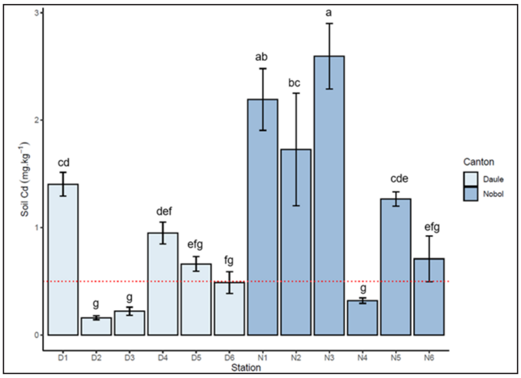

Figure 3. Cadmium total concentration in the analyzed soils in the cantons Daule and Nobol. Matching letters mean that there are no significantly difference by one-way ANOVA and Tukey test (P≥0.05) The bars mean standard deviation (n=3).

All samples studied presented concentrations of Cd, and these results are presented in Figure 2. The highest value found in the phosphorus fertilizer was observed in sample F1 (41.30 ± 1.65 mg/kg Cd), and the lowest value was observed in the F9 nitrogen (1.10 ± 0.31 mg/kg Cd). At the same time, a group with high values formed by samples F2 (32.45 ± 6.93 mg/kg Cd) and F3 (30 ± 3.94 mg/kg Cd) was detected. Another grouping of mean values was formed by F4 (17.30 ± 2.48 mg/kg Cd), F5 (15.52 ± 6.08 mg/kg Cd), F6 (14.81 ± 0.20 mg/kg Cd), F7 (11.78 ± 2.12 mg/kg Cd), and F8 (8.18 ± 1.88 mg/kg Cd). These samples were analyzed using a one-way ANOVA and a subsequent Tukey test (F = 38.42; *p* = 0.00).

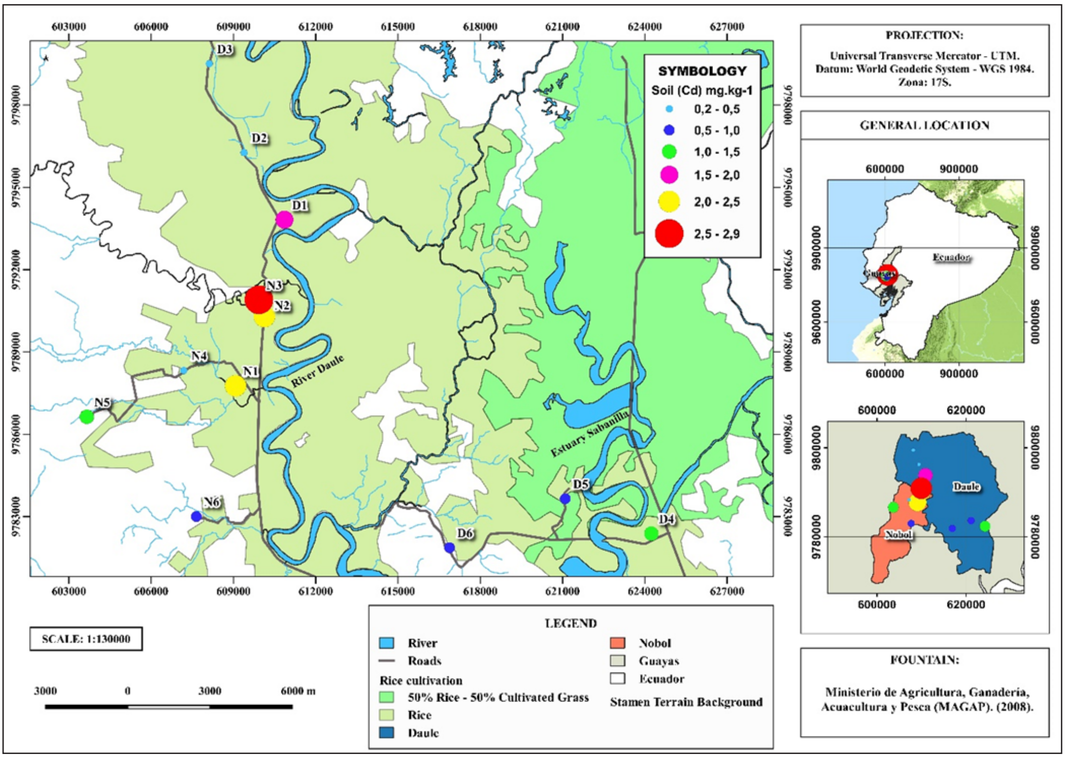

Figure 4. Geographic distribution of cadmium in agricultural soils in the cantons Daule and Nobol.

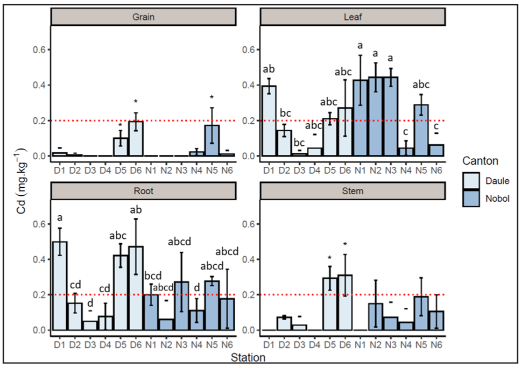

Figure 5. Cadmium concentrations detected in the plants *O. sativa* in the cantons Daule and Nobol. Red line: MPL in rice grains according to the international regulations of the European Union and the Alimentarius Codex of the WHO and the FAO. The bars mean standard deviation (n = 3). Matching letters mean that there are no significant differences by one-way ANOVA and Tukey test (P≥0.05). * statistically significant differences according to Kruskall-Wallis.

Cd in agricultural soils from Daule and Nobol cantons

Table 1 shows the types of fertilizers recommended in Ecuador for rice cultivation. We found high Cd contents in the fertilizers used in the soils from Daule and Nobol cantons that had high concentrations of this heavy metal (Figure 3). In general, the soils from the canton of Nobol (average= 1.43, min = 0.06, max = 2.86 mg/kg Cd) were more contaminated than those from Daule canton (average = 0.56, min = 0.00, Max = 1.53 mg/kg Cd). According to the results of the one-way ANOVA test (F = 13.15, *p* = 0.001), both exceeded the maximum permissible limit for agricultural soils (0.5 mg/kg).

We determined a group of soils with high concentrations of Cd (Figure 3), which was formed by N1 (2.19 ± 0.28 mg/kg Cd) and N3 (2.59 ± 0.30 mg/kg Cd), and another group with intermediate concentrations formed by the D1 stations (1.4 ± 0.37 mg/kg Cd), N5 (1.26 ± 0.06 mg/kg Cd), D4 (0.75 ± 0.42 mg/kg Cd), N6 (0.71 ± 0.21 mg/kg Cd), and D5 (0.66 ± 0.06 mg/kg Cd), which all had concentrations above the maximum permissible limits. The only stations with Cd values below the norm were D2, D3, D6, and N4.

One of the parameters that affects the bioavailability of cadmium is pH. Daule´s soils were characterized by being more acidic, with an average pH of 5.7 ± 0.2, while Nobol´s soils had an average pH of 6.5 ± 0.2.

In reference to the geographic distribution of cadmium in agricultural soils in the cantons of Daule and Nobol, Figure 4 shows that the sites most contaminated with cadmium were near the Daule River, while the sites with the lowest concentrations of this metal were far from the main roads and the Daule River.

Cd in rice plants

Figure 5 presents the Cd concentrations in the *O. sativa* plants from both cantons. In the roots of *O. sativa* belonging to station D5, we found the highest concentration of Cd (0.66 ± 0.07 mg/kg Cd). This was followed by the D1 station (0.50 ± 0.07 mg/kg Cd), D6 (0.49 ± 0.10 mg/kg Cd), and finally the D3 station, where the lowest concentration was observed (0.05 ±

Table 2. Transference Factor (TF) and Cd Bioconcentration Factor in roots (BCF roots), shoots (BCF shoot), and grains (BCF grain) of *O. sativa* plants by stations.

Stations	BCFroot	BCFshoot	FBCgrain	TF
D1	0.36±0.08^{bc}	0.28±0.02^{bc}	0.01±0.02^{d}	0.84±0.19
D2	0.93±0.22^{ab}	1.37±0.38 ^{a}	0.03±0.05^{cd}	1.59±0.63
D3	0.30±0.19^{abc}	0.18±0.22^{bc}	0.00±0.00^{d}	1.42±1.60
D4	0.12±0.03^{c}	0.04±0.07^{c}	0.00±0.00^{d}	0.44±0.63
D5	0.64±0.12^{abc}	0.77±0.18^{ab}	0.15±0.05^{b}	1.43±0.04
D6	1.02±0.52^{a}	1.19±0.51^{a}	0.39±0.02^{a}	1.71±0.77
N1	0.09±0.03^{c}	0.20±0.08^{bc}	0.00±0.00^{d}	2.43±1.48
N2	0.14±0.00^{abc}	0.37±0.13^{bc}	0.00±0.00^{d}	3.55±0.00
N3	0.11±0.06^{c}	0.20±0.03^{bc}	0.00±0.00^{d}	2.47±1.59
N4	0.36±0.24^{bc}	0.29±0.38^{bc}	0.07±0.06^{bcd}	0.84±0.46
N5	0.22±0.03^{c}	0.38±0.11^{bc}	0.13±0.07^{bc}	2.34±0.41
N6	0.32±0.12^{c}	0.20±0.02^{bc}	0.01±0.11^{d}	1.10±0.89
F	6.24	9.13	29.84	2.06
P	<0.001	<0.001	<0.001	0.07

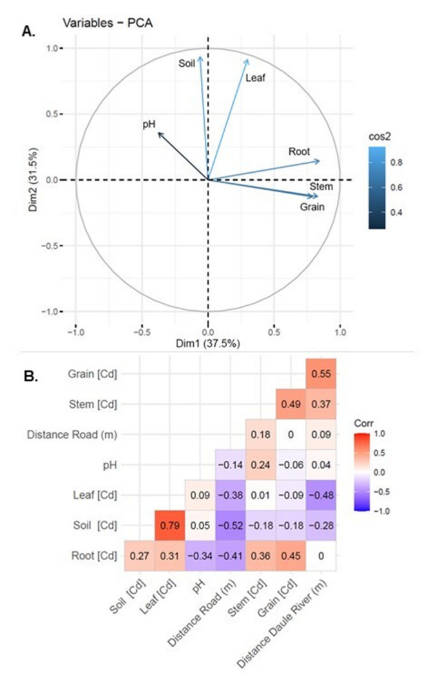

Figure 6. A. Analysis of principal components among pH, Cd concentration in roots (Root [Cd]), stems (Stem [Cd]), leaves (Leaf [Cd]), grain (Grain [Cd]) and soil (Soil [Cd]) B. Spearman Matrix between the concentration of Cd in grains, stems, roots, leaves and the concentrations in soils, and pH in Daule and Nobol.

0.06 mg/kg Cd). As for the studied stems, the metal was not detectable in the localities D1, D4, and N1. However, the D6 station had the highest concentration (0.03 ± 0.118 mg/kg Cd).

In the case of the leaves, the stations with the highest concentrations of Cd were N2 (0.44 ± 0.08 mg/kg Cd), N3 (0.44 ± 0.05 mg/kg Cd), and N1 (0.043 ± 0 mg/kg Cd), while the stations with lower concentrations were D3 (0.01 ± 0.02 mg/kg Cd), N4 (0.04 ± 0.04 mg/kg Cd), and N6 (0.06 ± 0.07 mg/kg Cd).
Concerning the grain samples, no Cd was detected at stations D3, D4, N1, N2, and N3. However, at the other stations, we found some Cd: D6 (0.19 ± 0.05 mg/kg Cd), N5 (0.17 ± 0.10 mg/kg Cd), and D5 (0.10 mg/kg Cd). Table 2 presents the transference factor and Cd bioconcentration factor of *O. sativa*. Stations D2 and D6 presented BCFs higher than one, which implies that these rice varieties are cadmium accumulators. Similarly, TFs greater than one were also observed at 9 of the 12 stations, indicating that cadmium is being transferred from the roots to the shoots efficiently, which represents a risk to food safety. Fortunately, the BCF was low in the grains.

Figure 6 shows the correlation between the concentrations of Cd found in the soils and plants. An analysis of the main components was carried out with the concentrations of Cd in the plants and soils. The test evidenced a remarkable relationship between the concentrations of Cd in the grains and stems. These values were related to the Cd in the roots, while the values of Cd in the leaves were related to the concentrations of Cd in the soils (see Figure 6A).

As shown in Figure 6B, in order to corroborate these correlations, a Spearman test was applied to the values of the correlations between the Cd concentrations in the grains and roots (r = 0,54, *p* = 0.004); stems and roots (r = 0.48, *p* = 0.001); and stems and grains (r = 0.61, *p* < 0.001). On the other hand, the Cd present in the leaves was correlated with the total concentration of Cd in the soil (r = 0.75, *p* < 0.000). Finally, we did not find correlations between pH and most of the parameters.

These results demonstrate that Cd contamination in *O. sativa* plants is a consequence of soil Cd contamination, which is caused by the use of fertilizers and proximity to the Daule River and the main roads.

Potential Risk Assessment

The assessment of potential health risks associated with Cd exposure from rice consumption revealed the Average Daily Dose (ADD) values for Ecuadorian consumers. The ADD for men was calculated to be approximately 0.00055 mg/kg/day, while for women, it was found to be around 0.00066 mg/kg/day. Subsequently, the hazard quotients (HQs) for non-carcinogenic risks were determined to be 0.55 for men and 0.66 for women, both of which are below the threshold of 1. This indicates that the risk of adverse health effects from Cd exposure through rice consumption is within acceptable limits for the average Ecuadorian consumer.

Discussion

It was observed that 33% of the analyzed fertilizers exceeded the limits established by Canadian regulations (20 mg/kg Cd), while 100% exceeded the limits allowed by the Department of Ecology of the State of Washington (1 mg/kg Cd); however, they did not exceed the limits recommended in Costa Rican regulations (80 mg/kg Cd). This finding is particularly significant, as elevated levels of Cd in fertilizers pose serious environmental and health risks. The presence of Cd can lead to bioaccumulation in plants, which may subsequently enter the food chain, affecting not only agricultural productivity but also human health and ecosystem stability (Luo *et al.*, 2024; Musa *et al.*, 2024). Given that cadmium is a toxic heavy metal, its accumulation in soil and crops can have detrimental effects on both agricultural systems and consumer safety (Suciu *et al.*, 2022; Alhaj *et al.*, 2024).

In humans, exposure to cadmium can result in toxic effects such as stunted growth, diabetes, osteoporosis, and hypertension as well as toxicity in the kidneys and liver (Clemens *et al.*, 2013). On a molecular level, Cd contributes to the generation of reactive oxygen species, causes DNA damage, and hampers DNA repair processes, which can lead to cancer (Zhang and Reynolds, 2019).

The results obtained in the present study coincide with those described by Qian et al. (2016), who presented similar Cd concentrations of up to 56 mg/kg in fertilizer samples. Similarly, they coincide with other studies that reported Cd concentrations of 1,1-3,9 mg/kg in NPK fertilizers (McBride and Spiers, 2001). Also, a Cd concentration of 38 mg/kg was observed in simple superphosphate (McBride and Spiers, 2001). This was lower than those found in di-ammonium phosphate (153 mg/kg Cd) and triple superphosphate (174 mg/kg Cd).

Furthermore, the analyzed fertilizers evidenced that phosphate had the highest values of Cd, followed by nitrogen, like those reported in Argentina by Marti et al. (2002), who found Cd concentrations of 10.43 ± 3.24 mg/kg in phosphate fertilizers and 2.03 ± 0.49 mg/kg in nitrogenous fertilizers. In summary, this suggests that the use of phosphates is the main cause of Cd contamination in agricultural soils. The origin of this Cd contamination in phosphate fertilizers has been attributed to the use of phosphate rock for its elaboration, as mentioned by Mar and Okazaki (2012), as well as apatite rock (Suciu *et al.*, 2022).

As a consequence of Cd contamination in fertilizers, and as expected, the soil exhibited high levels of Cd. In reference to the sampling stations, the station with the highest concentration of metal in the soil (N3) was found in Nobol (2.59 ± 0.30 mg/kg Cd). When comparing these results with the MPL of Cd in agricultural soils of 0.5 mg/kg, according to Annex 2 of AM097A (Ministerio del Ambiente de Ecuador, 2015), they were up to five times higher than this limit, and they exceeded the limit value of the Canadian standard of 1.4 mg/kg. These values were higher than those reported by Atiaga et al. (2021) (0.23 ± 0.14 mg/kg for rice cultivation soils in the province of Guayas), which implies that Nobol soils present more significant Cd contamination.

In our study, we found average cadmium (Cd) concentrations of 0.56 mg/kg in rice cultivation soils in Daule and 1.43 mg/kg in rice cultivation soils in Nobol, with maximum values reaching 2.86 mg/kg. These findings are concerning, especially when compared to other studies conducted in Ecuador that have documented the presence of Cd in various agricultural lands. For instance, Mite et al. (2010) reported Cd concentrations ranging from 0.46 to 2.37 mg/kg in cacao soils across different provinces, including 0.25 to 1.65 mg/kg in Guayas and 0.22 to 0.88 mg/kg in Esmeraldas. Similarly, Chávez et al. (2015) found Cd levels between 0.66 and 2.59 mg/kg in Guayas, while Arguello et al. (2019) detected maximum values of up to 8 mg/kg. These studies illustrate a worrying trend of elevated Cd levels in agricultural soils, which can pose significant risks to both environmental and human health.

Moreover, when we consider the average concentration of Cd in the Earth's crust, which was reported to be 0.15 mg/kg by Lide (2008), it becomes evident that the levels found in our study and those reported in other research are substantially higher. This discrepancy highlights the potential impact of agricultural practices, particularly the use of fertilizers and contaminated water for irrigation that may contain cadmium, leading to soil contamination.

In comparison with results from other countries, the Cd concentration in our soil samples coincides with those determined by Zhao *et al.* (2010), which ranged from 0 to 3.45 mg/kg. On the other hand, our concentration differs from the low levels reported by other authors, such as 0.77 mg/kg in Malaysia (Yap *et al.*, 2009) and 0.08 mg/kg in Finland (Singh, 1994).

In contrast, 33% of the soils presented low concentrations of Cd; the stations D2, D3, D6, and N4 registered values lower than the national and international MPLs. The soils at stations D2, D3, D6, and N4 showed lower contamination levels, likely due to their distances from main roads. In contrast, stations D1, N1, N2, and N3 exhibited higher contamination levels, primarily due to their proximity to villages and heavily trafficked roads. Additionally, the most contaminated sites were found near the Daule River, which is used for irrigation and has been documented to contain Cd (Mero et al., 2019). The contamination levels may also be influenced by the types of fertilizers used and the rice varieties cultivated in these areas.

Research conducted by Pernía *et al.* (2021) revealed that different rice varieties in Ecuador show varying levels of Cd tolerance, with 'SFL-011' and 'INIAP-Arenillas' demonstrating the highest tolerance, while 'INIAP-11' and 'INIAP-14' show the lowest tolerance to Cd.
For future research, it would be ideal to identify specific rice varieties and the types of fertilizers used in cultivation. This information will be crucial for establishing traceability and understanding the causal relationships associated with contamination. Recording these variables will enable researchers to more accurately determine how agricultural practices affect environmental health and food safety, paving the way for strategies to more effectively mitigate contamination risk.

In order to verify this result, the concentrations of Cd in the soils were compared with their proximity to the Daule River and main secondary roads using a statistical test and Spearman correlation analysis, which showed that there was a moderate correlation between the concentration of Cd in a soil and its location. These results were similar to those reported by Bedregal et al. (2012), who established critical points of contamination with Cd and other metals due to vehicular emissions.

According to the literature, the bioavailability of heavy metals depends on parameters such as pH, electrical conductivity, the content of organic matter, soil texture, and competition with other ions (McLaughlin *et al*., 1996). To verify if the bioavailability depended on the pH, we conducted a Spearman correlation analysis, which showed there was no correlation between pH and bioavailability, contrary to the results described by McLaughlin *et al*. (2000), who indicated that at a lower pH there is greater bioavailability of metals. In this case, the bioavailability may be due to other factors such as the organic matter content or competition between divalent ions in the soils of crops.

With respect to the bioconcentration factor, unlike the other stations, the rice plants at D6´s station presented BCFs higher than one. This is a criterion of hyperaccumulation, according to Brooks (1998). At the root level, the BCF was 1.02 ± 0.52, and the BCF in the stems was 1.19 ± 0.51, but this value decreased in the grains (0.39 ± 0.02). Similarly, at station D2, a stem BCF of 1.37 ± 0.38 was recorded, but the BCF decreased significantly in the grains (0.03 ± 0.05). In contrast, the stations where the plants had the highest BCFs in the grains were D5 (0.15 ± 0.05) and N5 (0.13 ± 0.07). These differences were likely due to the fact that the cultivated rice varieties were different. Unlike the present study, Song et al. (2015) compared 20 rice varieties in China and found much higher bioconcentration factors in roots (4.62-22.27) and grains (0.30-1.07). Therefore, it seems that Ecuadorian rice varieties have exclusion mechanisms for Cd.

Another variable that has been considered to estimate if a species of plant is a Cd accumulator is the transfer factor, and in this work this variable was greater than one at all stations except D1, D4, and N4. This confirmed that *O. sativa* is a Cd-accumulating species, but there are varieties with different transference and accumulation capacities for this heavy metal.

There are several factors that affect Cd accumulation in rice plants, such as the soil pH, the organic matter content, the nutrient concentration in the soil, and the rice variety (Li *et al*., 2017). With regard to the different rice varieties, it has been reported that their capacities to absorb Cd and transfer it to their stems vary widely (Ye *et al*., 2012). In the present study, the rice variety present at each sampling point was not identified, so it cannot be demonstrated if this factor influenced the differences found in the Cd in the grains. Therefore, we propose comparing the Ecuadorian varieties to detect which accumulates the least cadmium in its grains in order to propose its use in soils contaminated with this heavy metal.

We concluded that although some Daule stations (D5 and D6) had soil Cd concentrations near the PML, the plants accumulated higher concentrations of this heavy metal in their roots, stems, and grains. When we compared them with the Nobol stations, the Cd levels in the soil were very high.

In contrast, at the N1 station, where the highest soil concentrations of Cd were found, Cd was not found in rice stems or grains. These results could be explained by the differences found in the bioconcentration and transfer factors of the *O. sativa* plants in both cantons, suggesting that the variety of rice cultivated at N1 is ideal for use in soils contaminated with Cd.

The results for the rice grains did not exceed the limit values, according to the international norms that were considered, which included the European Union and the Codex Alimentary of the WHO and the FAO with a reference value of 0.2 mg/kg for Cd in grains of rice (FAO/OMS, 1995). The MPL of the national standard NTE INEN 1234 for Cd in rice grains is 0.4 mg/kg, and it also was not exceeded. These results coincide with those obtained by Atiaga et al. (2021), who found low Cd concentrations in Ecuadorian rice (0.17±0.26 mg/kg).

The low concentrations of Cd found in the grains in the present study were lower than those described by other authors in China (6 mg/kg (Wu *et al*., 2011), 0.79 mg/kg (Song *et al*., 2015), and 0.26 mg/kg (Yu *et al*., 2016)) and Colombia (0.33 mg/kg (Méndez-Fajardo *et al*., 2007)).

Although the risk of adverse health effects from cadmium (Cd) exposure through rice consumption is considered acceptable for the average Ecuadorian consumer, it is important to note that other products in Ecuador also contain cadmium. According to Benavides et al. (2022), items such as soybeans, carrots, tomatoes, lettuce, and cocoa also contribute to cadmium exposure. The cumulative effect of consuming these products could potentially increase the overall risk for consumers.

Nonetheless, it is crucial to emphasize the importance of ongoing monitoring of heavy metal concentrations in food sources, as well as the need for further research to ensure public health safety and to mitigate potential long-term health effects associated with heavy metal exposure.

It is recommended that farmers utilize fertilizers that are free from heavy metals, assess metal concentrations in their soils prior to planting, and select plant varieties that have reduced capacities to bioaccumulate these substances (Benavides *et al.,* 2022).

Conclusion

In summary, all fertilizers used for rice cultivation in Ecuador have high concentrations of Cd that exceed the limits established by Canada and the Department of Ecology of Washington State. The most-contaminated fertilizers are phosphates, followed by nitrogenated ones.

The concentrations of Cd in the agricultural soils at stations D1, D4, D5, N1, N2, N3, N5, and N6 exceeded the limits established by national and international regulations, with bioavailable concentrations occurring at all stations. Likewise, the average Cd values in each canton's soils surpassed the established maximum limits.

Comparing the concentrations of Cd in the soil and plant samples from the different cantons, it was concluded that the soils in the canton of Nobol were more contaminated, while the concentrations in the plants in Daule canton were higher.

The rice plants presented BCFs and TFs greater than one, indicating that they are Cd accumulators. However, the varieties grown at D3, D4, N1, N2, and N3 stations were able to exclude Cd from their grains. Finally, the risk of adverse health effects from cadmium (Cd) exposure through rice consumption is considered acceptable for the average Ecuadorian consumer

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