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Determination of the Presence of Heavy Metals in Raw Bovine Milk in the Parish Bilován, Province of Bolívar (Ecuador)

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ABSTRACT
The objective of the research was to determine the presence of heavy metals in raw bovine milk, which was carried out in the parish of Bilován in the canton of San Miguel (Ecuador) considering that the presence of said chemical elements in milk leads to a damage to the health of the animal and subsequently to the consumer. The research was carried out with a population of 15 female cattle obtaining 100 mL of sample. A parameter to consider was how the consumption of offal from the corn harvest influences when estimating the influence of the various agrochemical products used in the crops. After the analysis, with respect to the number of APU they are in a range between 1 to 5 AUP, in addition, that 1 to 5 cows are destined for the production of milk, where 66.67 of milk is destined for the production of cheese. On the other hand, in the type of forage used, there is a certain similarity between kikuyo and corn crop waste, 66.66 of those consulted say that they apply pesticides only once during cultivation, however, the rest of those consulted say that they apply three times. Through the analysis of metal residues such as Pb and Cu; in Pb was found in a concentration of <0.02 mg/Kg; and Copper of 0.38 mg/Kg. In conclusion, the concentrations of lead and copper do not exceed the maximum permissible limits in the milk samples analyzed.

Keywords: Metabolizable, pesticide, residue, heavy metals, milk

INTRODUCTION
Today's cattle are divided into two species *Bovidae taurus,* which originated in Europe and includes most modern varieties of dairy and beef cattle, and *Bovidae Indicus* which originated in India is characterized by a hump on the withers, at present the bovine plays an important role contributing milk and meat (**Winer, 2019).** Generally, its origin dates back to the Aurochs *Bos primigenius*, extinct in 1627, first domesticated in Europe and Asia during the Neolithic period, there are two main types of domestic cattle, the zebu (*Bos Indicus*) that has a marked jibá at the level of the back and the Taurinos (*Bos Taurus*) that do not have cuttlefish (**Inatec, 2017**).

The production systems are determined according to the production to which the breed is destined, its adaptability in the environmental conditions of the area and the availability of food that the producer has.

In Ecuador, the main dairy breeds are: Holstein, Jersey, Guerrsey, Pardo Suizo, Ayrshire (**Bazurto, 2014; EcuRe, 2018; Productor, 2018**).

Dairy production is of vital importance in family farming systems, fundamentally because it improves the nutrition of the producers themselves and their families. In this sense, the growing interest in the content of mineral elements in meat and milk is given by its use as a quality indicator, whose final objective is to ensure and offer a safe and nutritious food. The intense and constant increase in anthropogenic and industrial activities has favored the emission of polluting substances into ecosystems, which are intimately related to particularly domestic animal species and man. (**Martínez-Vasallo et al., 2017; García Romero et al., 2020**).

Highlighting the conditions caused by the heavy metals, copper and lead in bovines, so it is not surprising the prevalence of diseases associated with these elements, where the main routes of entry are dermal routes, by ingestion and by inhalation. Heavy metals, including copper, are very harmful as they are not easily degraded in a biological way (**Heredia, 2017**).

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Table 1. Maximum permissible concentration limits for heavy metals (Hg, As, Cd and Pb) in water, soil and food for human consumption.

Food	Unit	Hg	As	Cd	Pb
Water for human consumption	mg /L	0.001	0.05	0.01	0.05
Sea water and estuaries		0.0001	0.05	0.05	0.01
Agricultural water		0.001	0.1	0.01	0.05
Water for livestock use		0.01	0.2	0.05	0.05

Sourse: *(Codex, 1995; UE, 2016)*

What facilitates the entry of these toxic substances into the food chain, the residual content of some elements of milk and meat is a direct indicator of the degree of contamination and an indirect indicator of local or peripheral environmental conditions, mainly of soil, water, air and vegetation (pastures for consumption) (**González, 2009**).

According to studies, the application of fertilizers, Agrochemicals and irrigation with urban and industrial effluent waters are some of the activities that pollute the environment and specifically agricultural and grazing soils. The concentration of these elements can also derive from other factors directly related to the production chain, both in the milking and transport of meat and milk (**Wlostowski, 2005).**

Both nationally and internationally, a series of standards have been established in order to guarantee, throughout the production chain, the safety of meat and dairy products. Within this regulation, the control of heavy metals in bovine productions, plays a prominent role, in order to mitigate environmental impact and encourage cleaner production (**Alcocer, 2007).** Therefore, it is of great importance, the use of techniques and methods for the detection of heavy metals, among these this emission spectroscopy, atomic absorption, which is a sensitive and specific method for the quantitative determination of more than 60 metals or metalloids. This analytical technique has a very wide field of application, including food (determination of contaminants such as Pb, As, Cu, Hg, and Cd) and medicine (analysis of Pb, Na, K, Ca in the blood) (**Armenta et al., 2005**).

Considering the previously described, the objective of this research was: to determine the presence of heavy metals (Cu and Pb) in raw bovine milk in the Bilován parish, Bolívar province.

MATERIALS AND METHODS

The research was carried out in the province of Bolívar, canton San Miguel, parish of Bilován, (Ecuador), where surveys, samples and subsequent analysis were carried out in the research laboratory of the Universidad Estatal de Bolívar. Experimental material, for the research work 6 months were used.

The sample consisted of 15 cattle of different age, breed and type of diet.

The present research work was carried out with the investigative and experimental method.

Study Factors

Fifteen samples of raw milk from bovines of different races and ages from the Bilován parish were used, which were used for the analysis of milk in relation to the type of food they consume.

Design Type

For the present research work the descriptive statistical design was used. The samples were taken in the first hours of the day after the first milking.

Methodology

The samples were digested according to a two-stage protocol and then quantified. For digestion, in a first stage 0.5 g of homogenized whole milk was weighed, 2 mL of nitric acid, 1 mL of hydrogen peroxide (30%) and 2.5 mL of ultra-pure water was added. The samples were heated for 10 min at 200 ºC, then they will be allowed to cool to <50 ºC, where 1.4 mL of hydrochloric acid will be added. Subsequently, the samples were subjected to a program of 5 min of 130 ºC

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and maintenance for 10 min 130. Then the samples will be cooled <50 ºC and 0.4 mL of urea (50% m/v) was added and they were volumetric at 20 mL.

RESULTS AND DISCUSSION

APU Number.

When analyzing the results of the research with respect to the number of APU (agricultural production unit) it can be seen that a larger group of producers consulted based on their response are in a range between 1 to 5 APU and in relation to 6 to 10 there was a lower percentage as shown evidence in the table 2; Values obtained in relation to the survey carried out by the different producers. In this way, it can be said that in the study there are larger producers who have a number of APU`s that are between the ranges 1 to 5.

Table 2. APU number

Alternatives	Frequencies	Percentages
1 to 5	4	66.66
6 to10	2	33.34

Number Of Cows Destined For Milk Production

Regarding the number of cows destined for milk production, the surveyed producers affirm that they have between 1 to 5 cows destined for milk production.

Destination Of Milk Production

After the survey, a larger group of producers responded that they use milk to make cheese and in relation to the sale and consumption there was a lower percentage (Table 3).

Table 3. Destination of milk production

Alternatives	Frequencies	Percentages
Sale	1	16.66
Consumption	1	16.67
Cheese making	4	66.67

Type Of Feeding.

Table 4. Type of feeding

Alternatives	Frequencies	Percentages
Corn crop forage (*Zea mays*)	6	100
Kikuyo (*Pennisetum clandestinum*)	6	100

In the table 4, it can be seen that the type of food supplies their animals, forage from the harvest of the corn crop (Zea mays) and Kikuyo, these results allow us to interpret that there is some similarity between these alternatives; the Kikuyo (*Pennisetum clandestinum*) is the predominant pasture in this area of the country and also estimating that at this time due to the harvest of the corn crop, the spoils of the corn crop predominate, considering that in the parish of Bilován corn crops prevail.

Supply Frequency And Type Of Food.

From the information collected, 100% of the producers frequently supply their animals with the forage from the corn crop harvest every day, stating that they have to take advantage of the residues that remain from the corn harvest activity.

Regarding the supply of Kikuyo, half of the respondents assured that they do it every three days (Figure 1)

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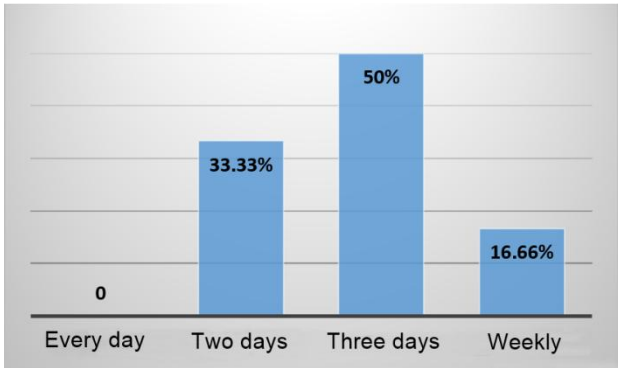

Figure 1. Frequency that supplies the Kikuyo

Type Of Water You Supply To Your Animals

It can be seen that a larger group of producers consulted responded that they supply water from ditches or natural springs and in relation to supplying piped water, there was a lower percentage (Table 5).

Table 5. Type of water supplied to cattle

Alternatives	Frequencies	Percentages
Tubing water	1	16.66
Ditch water	5	83.33

Waste Management.

According to the respondents, they fully affirm that if they handle the waste considering it as the animals that die, the process they follow to eliminate the bodies, if incinerate and disinfect.

Use Of Pesticides On Your Crops

According to the information obtained and represented in Table 6, it can be seen that the surveyed producers affirm in their entirety that they do use pesticides in their corn crops.

Table 6. Pesticide use and frequency

Alternatives	Frequencies	Percentages
One time	4	66.66
Three times	2	33.3
Total	6	100

Analyzing the results, it can be seen that a larger group of producers use these products twice in the entire corn production cycle, in relation to those who uses three times.

Analysis Of The Presence Of Lead In Raw Milk

After this analysis of atomic emission, the presence of Pb in a concentration lower than the reference value was determined (Table 7).

Table 7. Pb analysis results in raw milk

Physical-chemical analysis report				
Parametet	**Unit**	**Results**	**Reference value**	**Testing method**
Lead (Pb)	Mg/kg	<0,02	Max.o,o2	PEE.LASA.BR.37 UNE-EN 140884:2003

In a study developed by **Pernia,** (**2015**), for the determination of cadmium and lead in cow's milk commercialized in the city of Guayaquil, Ecuador in relation to the varying percentages of heavy metals in raw bovine milk from the Bilován parish, points out that. The percentage of processed samples were (<0.002 ppm) of cadmium and (<0.045 ppm) of lead, determining the absence of heavy metals in the liquid milk, having some similarity with the results obtained in said investigation.

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Analysis Of The Presence Of Copper In Raw Milk

Rodríguez Fuentes (**2005**), from the University of Mexico, in his research on heavy metals in raw bovine milk in relation to copper, is an average of 0.4125 mg/kg, presenting an analogy with the research since copper is found in a 0.038 mg/100 g range.

Table 8. Result of analysis of copper (Cu) in raw milk

Sample	Concentration [ppb]	Concentration [ppm]	sample weight (g)	Concentration [mgCu/100g of sample]
1	19.5967	0.0196	0.5106	0.0384
2	21.4095	0.0214	0.534	0.0401
3	22.9279	0.0229	0.5199	0.0441
4	19.4001	0.0194	0.5245	0.0370
5	18.4292	0.0184	0.5263	0.0350
6	22.1295	0.0221	0.5223	0.0424
7	19.9458	0.0199	0.5237	0.0381
8	21.8794	0.0219	0.491	0.0446
9	19.3088	0.0193	0.5387	0.0358
10	17.6977	0.0177	0.5098	0.0347
11	21.1963	0.0212	0.5245	0.0404
12	20.0885	0.0201	0.5269	0.0381
13	19.0120	0.0190	0.5308	0.0358
14	17.9186	0.0179	0.5235	0.0342
15	16.3981	0.0164	0.5292	0.0310

CONCLUSION

According to the Ecuadorian Technical Standard NTE INEN 1108 (2011) for drinking water, lead and copper concentrations do not exceed the maximum permissible limits for all raw milk samples analyzed.

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