Scientia Agropecuaria 10(3): 377 – 382 (2019)

Scientia Agropecuaria

Website: http://revistas.unitru.edu.pe/index.php/scientiaagrop

Facultad de Ciencias Agropecuarias

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# Potential risk of Pb to children's health from consumption of cow's milk in areas irrigated with river water contaminated by mining activity

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Received March 31, 2019. Accepted September 8, 2019.

## Abstract

The contamination of food by heavy metals in areas irrigated with contaminated water is of great concern due to the potential risk to human health. To assess the potential risk of Pb to children's health from consumption of cow's milk in an area of the Mantaro Valley during 2018. Fifty-one children exposed to Pb and eight samples of raw cow's milk were selected. Pb concentrations were analyzed with the atomic absorption spectrometer. The mean Pb content in milk was 0.236 ± 0.057 mg.kg$^{-1}$, which exceeded the limits allowed by the Codex Alimentarius. The estimated daily intake of lead was 5.9 x 10$^{-3}$ mg.kg$^{-1}$day$^{-1}$; the estimated risk index (HQ) was below one, which represents a low risk for children. The cancer risk was below 1.0 x 10$^{-4}$. It is therefore concluded that there is no significant risk to children's health from consumption of cow's milk.

**Keywords:** estimated daily intake; risk index; health risk; contaminated milk.

## 1. Introduction

Lead (Pb) is an extremely toxic metal for all members of the food chain, is considered number two on the list of most dangerous substances in the environment by the Agency for Toxic Substances and Disease Registry (Bortey-Sam *et al.*, 2015). It accumulates in the body, mainly in bone. The International Agency for Research on Cancer classified inorganic Pb as probably carcinogenic to humans because of its long half-life in the body (WHO, 2010).

The general population is exposed to Pb through the consumption of food obtained from agricultural soils containing heavy metals (Huaranga *et al.*, 2012). According to the European Food Safety Authority there is considerable evidence showing that the developing brain is more vulnerable to Pb neurotoxicity than the mature brain and that prolonged exposure to this metal even in small amounts is dangerous to health due to bioaccumulation and biomagnification (Andrade *et al.*, 2017). Other studies refer that Pb can inhibit the biosynthesis of the hemo group, it can substitute calcium accumulating in bone tissue and teeth (Hon *et al.*, 2017).

Milk is a food of high nutritional value especially for children in many parts of the world and its consumption is suggested by FAO and UNESCO as indispensable in the diet of children for the protein contribution it represents (Castro-Gonzales *et al.*, 2017; Norouzirad *et al.*, 2018). However, in addition to being an important source of essential nutrients, milk is one of the main ways of ingesting toxic metals. Toxic metals are transferred from contaminated soil to pastures. Consumption of contaminated fodder could cause bioaccumulation in certain organs or be excreted through milk.

**How to cite this article:**
Orellana, E.; Bastos, C.; Sotelo, B.; Sarapura, V.; Custodio, M.; Cuadrado, W. 2019. Potential risk of Pb to children's health from consumption of cow's milk in areas irrigated with river water contaminated by mining activity. Scientia Agropecuaria 10(2): 377 – 382.

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DOI: 10.17268/sci.agropecu.2019.03.08

The direct ingestion of milk by people would cause health problems for consumers (Huque *et al.*, 2018).

In this context and considering that the determination of the concentration of metals in milk could be an important indicator of the degree of contamination of the environment where livestock are reared (Sobhanardakani, 2018). Several studies report the presence of heavy metals in cow's milk and other food products (Najarnezhad *et al.*, 2015; Muhib *et al.*, 2016). However, there is still a need to know about the health risk of the most vulnerable population such as children from intake of cow's milk contaminated with Pb in areas irrigated with contaminated river water from metallurgical mining activities. A potential human health risk assessment would make it possible to estimate the adverse effects of exposure to toxic substances in contaminated environments now or in the future (Sobhanardakani, 2018).

The objective of the study was to determine if the concentration of Pb in raw cow's milk exceeds the maximum per-missible limits (LMP) recommended by the Codex Alimentarius and if the daily intake of this element reaches the cancer risk index in children, in order to prevent chronic and carcinogenic diseases due to the effect of Pb.

## 2. Materials and methods

### Area of study

The town of Apata is located in the province of Jauja in the department of Junín, between the geographical coordinates: 11°53´37" latitude south and 75°21 25" longitude west, on the left bank of the Mantaro River (Figure 1). The area under study is an agricultural area with corn, potato, and cultivated grasses irrigated in the dry season with water from the Mantaro River from upstream mining and metallurgical effluents. The main economic activity of the population under study is agriculture and livestock.

### Sampling and pre-treatment

A questionnaire (Appendix) was applied to 80 families who have dairy cows previously selected intentionally in the town of Apata, Jauja to collect information on frequency, amount of cow's milk intake and body weight of children, during 2018. Previously and considering human rights and international bioethical norms, each family was informed about the reason for the study and the confidentiality of the information, agreeing to participate voluntarily and signed an informed consent form.

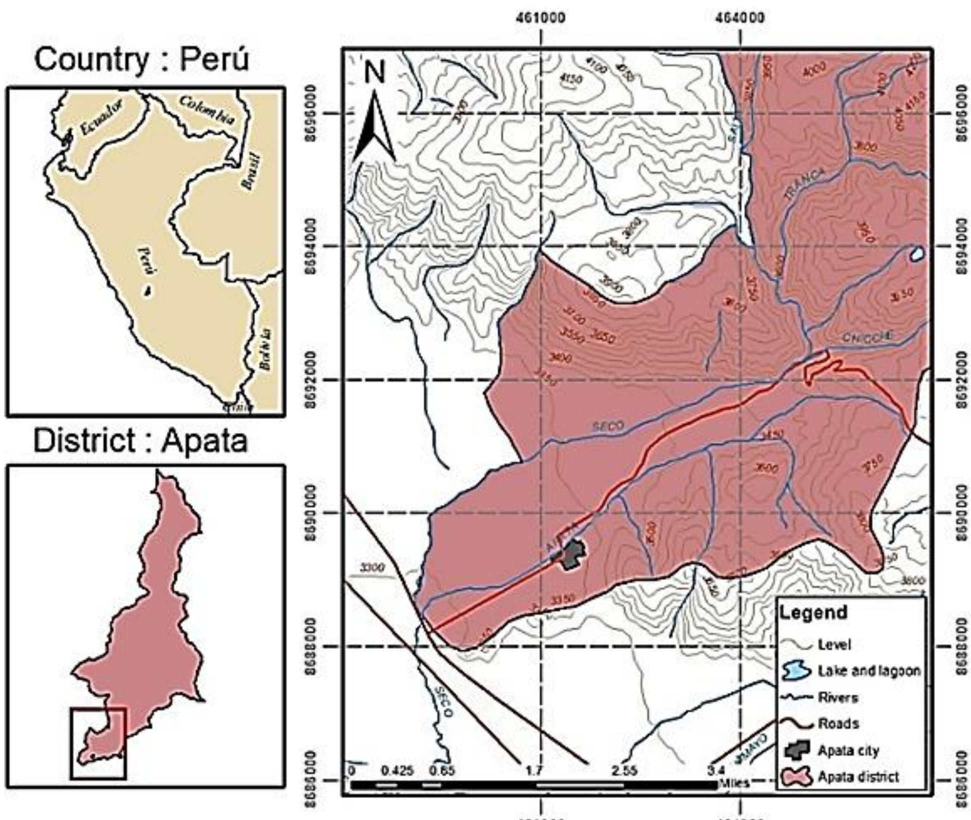


**Figure 1.** Location map of the study area in Apata, Jauja.

The questionnaire was applied to parents of children between the ages of 2 and 9 and directly to children over the age of 9. The body weight of the children studied (18 children under the age of 6, 20 children aged 6 to 9 and 13 children aged 10 to 13) was measured in the field using a pre-calibrated scale. The study subjects were children from 2 to 13 years of age with daily intake of intentionally selected cow's milk. The sample included 51 boys, of whom 63% were girls and 37% boys.

Twenty Holstein dairy cows were identified for milk sampling, then eight dairy cows were selected, fed with pastures and fodder irrigated with water from the Mantaro River, with a daily production of 15 L to 25 L. Milk samples were collected in the morning in glass bottles previously treated with 10% $HNO_3$ and rinsed three times with distilled water to remove acid residues. The samples were stored on ice and transported to the laboratory of the Faculty of Applied Sciences, Tarma headquarters of the National University of Central Peru for refrigeration at -65 °C and subsequent analysis.

**Digestion and analysis of the sample**

Milk samples were digested using the wet digestion method (Paino *et al.*, 2004). In summary, 10 mL of ultrapure nitric acid (supplied by Merck Germany) was added to 1 mL of milk in a 100 mL beaker and heated to 80 °C for 20 minutes. Immediately, 5 ml of hydrochloric acid was added to achieve complete oxidation and boiled (until water was consumed). The digested milk samples were diluted with doubly distilled water to a volume of 50 mL. Pb (mg/L) analysis was performed using the atomic absorption method using a Varian AA-240 with a graphite furnace.

**Percentage of recovery**

The standard solution for Pb was supplied by Merck with a purity level of 99.98%. The recovery percentage was calculated by adding a known amount of standard solution in milk samples. The analyses were performed with three repetitions and the average value was considered as the final concentration. The recovery rate for Pb was 95.2%.

**Determination of risk**

The estimated daily intake (EDI) of the metal was determined by the ratio: C*D/B, where C is the metal concentration found in the milk ($mg.kg^{-1}$), D is the amount of milk consumed (kg) and B is the body weight (kg).

The hazard quotient (HQ) was determined using the equation suggested by Khan *et al.* (2013): HQ = IDE/RfD; for which the oral reference dose (RfD) value for Pb was equal to $3.6 \times 10^{-2}$ $mg.kg^{-1}day^{-1}$.

Hazard Index (HI). It was determined by considering that ≥ values of 1 (HI > 1) means that it is not safe for human health according to the U.S. Environmental Protection Agency.

The cancer risk index (CRI). It was calculated using the following equation: CRI = IDE*SF, where IDE is the estimated daily intake and SF is the factor of the metals considered as carcinogenic (Cd, Cr, As) and Pb probably as carcinogenic (IARC, 2006). The SF value for oral intake of Pb was 0.0085 (USEPA, 2007).

**Statistical analysis**

Data on consumption, weight, daily intake, hazard index and cancer risk by sex and age groups were obtained using the two-way ANOVA statistic and the SPSS version 23 software.

## 3. Results and discussion

**Concentration of Pb in raw cow's milk**

The mean concentration of Pb in cow's milk was 0.236 ± 0.057 $mg.kg^{-1}$ and exceeded the permissible limits established by the European Union and the FAO/WHO standard (Codex Alimentarius Comission, 2013), which indicate a value of 0.020 mg $kg^{-1}$ for Pb. The results obtained were superior to those reported in contaminated areas by several authors (Belete *et al.*, 2014; Muhib *et al.*, 2016; Meena and Kaur, 2016; Castro-Gonzales *et al.*, 2017; Ismail *et al.*, 2017; Kabir *et al.*, 2017; Elsaim and Ali, 2018; Huque *et al.*, 2018). However, similar results were found in other studies in areas irrigated with domestic and industrial wastewater (Malhat *et al.*, 2012; Shaheen *et al.*, 2016).

The Pb concentration values in raw cow's milk were above the permitted limits. This indicates that the environment in which dairy cows are raised is contaminated and constitutes a danger to human health (Huque *et al.*, 2018; Sobhanardakani, 2018).

**Estimated Daily Intake (IDE)**

The mean daily metal intake of children overall was $5.9 \times 10^{-3} \pm 1.8 \times 10^{-3}$ $mg.kg^{-1}day^{-1}$. Significant differences ($p < 0.05$) in daily intake were detected between boys and girls with $7.1 \times 10^{-3} \pm 2.0 \times 10^{-3}$ $mg.kg^{-1}day^{-1}$ and $5.2 \times 10^{-3} \pm 1.3 \times 10^{-3}$ $mg.kg^{-1}day^{-1}$ respectively. No significant differences were observed in daily intake ($p > 0.05$) by age group. Daily intake ranges from $3.7 \times 10^{-3}$ $mg.kg^{-1}day^{-1}$ to $1.1 \times 10^{-2}$ $mg.kg^{-1}day^{-1}$ (Figure 2). Boys had higher daily intake than girls due to higher milk consumption by boys over 9 years of age. Possible factors contributing to the daily intake of Pb are the amount of milk consumed and the children's body weight.

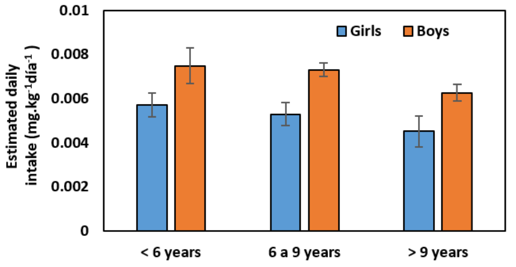


**Figure 2.** Estimated daily intake ($mg.kg^{-1}day^{-1}$) of raw cow's milk in children in Apata, Jauja.

Ismail *et al.* (2015) reported an EDI ranging from 0.051 $ug.kg^{-1}day^{-1}$ to 0.631 $ug.kg^{-1}day^{-1}$ in children and adolescents from 1 to 16 years, observing that these values were lower than what was found in the study. The daily intake of milk consumers in the Mantaro valley requires a study of other toxic and dangerous elements such as As, Cd, Cr, Hg, Zn.

### Hazard quotient (HQ)

The overall average hazard ratio for the intake of cow's milk with Pb concentrations was 0.1645 ± 0.0513, which is below 1, representing a low risk to the children participating in the study. However, significant differences were observed ($p < 0.05$) in the hazard ratio between boys and girls, with a higher hazard ratio recorded by boys with 0.1973 ± 0.0568 compared to girls with 0.1450 ± 0.0361. No significant differences were observed in the hazard coefficient ($p > 0.05$) according to age groups. The hazard coefficient ranged from 0.0728 to 0.2923 (Figure 3).

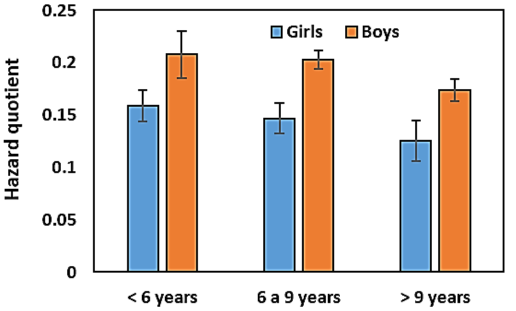


**Figure 3.** Hazard Quotient (HQ) in children for drinking Pb-contaminated cow's milk in Apata, Jauja.

The HQ was higher in boys than in girls as reported by Shaheen *et al.* (2016) in Bangladesh. However, Castro-Gonzales *et al.* (2017) detected higher HQ values in girls. The HQ values found were less than 1; therefore, they do not pose a health hazard to children ages 1 - 13. The intake of contaminated milk represents a greater risk for children due to its high degree of absorption, this population is especially vulnerable to the acute, sub-acute and chronic effects of heavy metal ingestion (Solis *et al.*, 2009).

The highest HI values are given in the first 5 years of life up to 13 years, it can be deduced that there is a certain probability of risk of non-cancerous diseases in the first years up to puberty. However, it should be noted that if children have accumulated heavy metals during this time, diseases may affect them at a later age, even if they have an HI < 1 at this time (Castro-Gonzales *et al.*, 2017).

### Risk of cancer

The risk of getting cancer from milk consumption is shown in Figure 4, there are significant differences ($p < 0.05$) in cancer risk between boys and girls. The highest cancer risk was recorded in boys with $6.0 \times 10^{-5} \pm 1.7 \times 10^{-5}$ compared to girls with $4.4 \times 10^{-5} \pm 1.1 \times 10^{-5}$. No significant differences in cancer risk index ($p > 0.05$) by age group were observed. Cancer risk ranged from $2.2 \times 10^{-5}$ to $8.9 \times 10^{-5}$ (Figure 4).

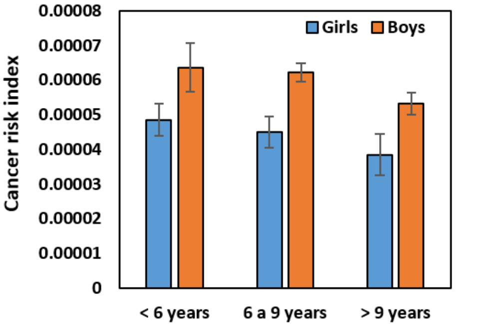


**Figure 4.** Risk of cancer in children by ingestion of raw cow's milk with Pb in the town of Apata, Jauja.

The cancer risk values found in the study are within the ranges tolerated ($10^{-6}$ a $10^{-4}$) by USEPA (2007) and lower than those reported by Hu *et al.* (2012) which at the same time indicate that values below ($1 \times 10^{-4}$) are not to be considered as potentially hazardous.

The risk of cancer obtained for Pb was lower than those reported by Islam *et al.* (2015). The carcinogenic risk value of Pb for children was $6.0 \times 10^{-5}$ slightly less than $1 \times 10^{-4}$ indicating a low potential carcinogenic risk for children under 13 years of age.

In this study, risk was calculated based on the value of milk intake per day for all ages, and the results of this risk estimate differ with the intake behavior reported by Castro-Gonzales *et al.* (2017) and Ismail *et al.* (2015) who determined a range of 0.569 $kg\ day^{-1}$ to 0.195 $kg\ day^{-1}$ in boys and girls between 1 and 16 years of age; inferring that as milk intake increases, the risk increases. Therefore, through the consumption of cow's milk, children absorb a high concentration of heavy metals and eliminate very few of them and accumulate them in the liver, kidneys, bones and mainly in the brain (Solis *et al.*, 2009) causing different health disorders.

## 4. Conclusions

The concentration of Pb in raw cow's milk produced in areas irrigated with contaminated water is a risk factor for the health of children in the medium and long term in the town of Apata, Jauja. However, from the evaluation of the HQ and the Pb cancer risk index, it is concluded that the daily intake of cow's milk does not pose a health risk to children in this study area, indicating that the consumption of small amounts of lead individually is not the cause of the risk of contracting non-cancerogenic and carcinogenic diseases.

### Acknowledgements

The authors thank the farmers and ranchers of the town of Apata, Jauja for the infor-mation provided and the samples provided for the development of this research.

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## APPENDIX

### Frequency and amount of cow's milk intake

*The purpose of this questionnaire is to know about children's exposure to lead by cow's milk intake in Apata – Jauja, Perú.*

**First and last names:** ..................................................................................................

**Locality:** ............................................................ **Date:** ............................................

**1. Overview**

**Age:** ............................................... years

Sex: Female ☐ Male ☐

**Weight:** .......................................kg

**2. Frequency and amount of cow's milk intake**

Does your child consume cow´s milk? Yes ☐ No ☐ if your answer is affirmative:

How many liters of cow´s milk does the child consume? ..................................................

How many times a day does your child, consume cow's milk?

Breakfast ☐ Lunch ☐ Dinner ☐

How often does your child consume cow's milk? (Check)

**Everyday** ☐

**Interdiary** ☐

**Twice a week** ☐

**Sometimes** ☐

**Never** ☐

**3. Milk production**

How many dairy cows do you have in your stable? ..................................................

How many liters of milk does your cows produce per day? ..........................................

*Thank you for your cooperation*