

HEAVY METALS CONCENTRATIONS IN RAW COW MILK PRODUCED IN THE DIFFERENT LIVESTOCK FARMING TYPES IN GUELMA PROVINCE (ALGERIA): CONTAMINATION AND RISK ASSESSMENT OF CONSUMPTION

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ABSTRACT

In the present study, we investigated the potential sources of environmental milk contamination; by evaluating heavy metals concentration, and the assessment of human health risks related to milk consumption in the region of Guelma, Algeria. A total of forty-eight random livestock farming (extensive, intensive and peri-urban) were selected, and a survey protocol was used to collect data on polluted environment using questionnaires, then the pollution risk indicators were calculated. From this livestock farming, 144 milk samples were collected and then mineralized; quantitative analysis of Fe, Cu, Zn, Cd, and Cr was performed using an atomic absorption spectrophotometer. Finally, for each element, the estimated daily intake (EDI) was calculated. The comparison of the synthetic pollution index according to the different types of livestock farming, revealed a significant difference between low values for extensive livestock farming and high values for peri-urban livestock farming. From the analytic analysis, the results indicated that the average milk concentrations of Fe, Cu, Zn, and Cd were higher than the suggested standards. The EDI values for Cd, Zn and Cr were higher compared to permissible values. To examine the possible effects of milk consumption on human health, further investigations of the levels of heavy metals in a greater number of milk samples from various zones are necessary.

Key words: Cow, milk, heavy metals, livestock farming, pollution, estimated daily intake (EDI).

INTRODUCTION

Cow milk is an important source of proteins, fats, vitamins and minerals, it is considered as a complete food (Enb *et al.*, 2009; Qin *et al.*, 2009; Yuzbas *et al.*, 2009; Salah and Ahmed, 2012; Seyed and Ebrahim, 2012). There are about thirty-eight micro and trace elements that have been found in raw milk from different regions around the world (Dobrzański *et al.*, 2005; Nwankwoala *et al.*, 2002). The mineral content in raw cow milk vary depending on several factors including lactation, farming practices, seasons, climatic conditions, food composition, animal health state and environmental conditions (Licata *et al.*, 2004; Yahaya *et al.*, 2010). In the same way, milk processing conditions can also affect milk mineral composition (Lante *et al.*, 2006; Salah *et al.*, 2013).

Milk micronutrients play an important and beneficial role on human health. They act as enzymatic cofactors by playing a vital role in various physiological functions of the human body; the lack of these micronutrients cause pathological problems especially in pregnant women, children, and elderly (Enb *et al.*, 2009).

Currently, increased environmental pollution has accelerated milk contamination problems and uncertainties regarding the quality of milk (Farid and Baloch, 2012). Pollution can increase milk minerals concentration; it becomes toxic when the level of concentration exceeds 40 to 200 times their recommended threshold value (Rao, 2005).

Malhat *et al.*, (2012) have shown that milk contamination is considered as one of the major hazardous aspects in recent years. Global contamination of milk with environmental pollutants and xenobiotic compounds in foodstuffs i.e. heavy metals, mycotoxins, dioxins and other pollutants, are considered as a risk factors on public health (Seyed and Ebrahim, 2012).

The main sources of heavy metal contamination are industrial or domestic effluents, combustion, decomposition of chemical fertilizers, pesticides (Degnon *et al.*, 2012). The toxicological effects of heavy metals on public health have been largely studied; chronic exposure to dietary heavy metals can cause abdominal pain, hepatotoxicity, neurotoxicity with a decreasing in intellectual quotient level, Alzheimer's disease, tissue damage, lung irritation and cancer development (Hussain *et al.*, 2010; Bushra *et al.*, 2014; Muhib *et al.*, 2016).

Moreover, heavy metals are not biodegradable in nature and accumulate in the food chain through biotransformation, bioaccumulation and biomagnification (Aslam *et al.*, 2011).

To our knowledge, in Algeria and despite the nutritional qualities of cow's milk (Boudalia *et al.*, 2016) and its wide consumption (Bousbia *et al.*, 2017), toxicological problems related to heavy metals contamination are poorly studied, only few studies have been carried out on animal-derived food products from conventional farms such as beef, sheep, camelin and white meat from chickens (Beneddouch *et al.*, 2014; Benouadah *et al.*, 2015; Yabrir *et al.*, 2016).

The aims of this study were: (1) the evaluation of the environmental risks assessment of pollution in different livestock farming types, (2) the evaluation of iron (Fe), copper (Cu), zinc (Zn), Cadmium (Cd) and Chromium (Cr) concentrations in raw milk from cows, (3) the conduction of a comparative analysis of milk qualities produced in each production system, and finally (4) the calculation of the Estimated Daily Intake (EDI) and heavy metals exposure levels related to the consumption of milk produced in Guelma region.

MATERIALS AND METHODS

Study area : The study took place in the region of Guelma (Northern East of Algeria: 36°46' N, 7°28' E) at an altitude of 279 m above sea level, covering an area of 3686.84 km² mid of the northern high plateaus and the Mediterranean sea. During the experimental study, annual average temperature is 17.3 °C. Dairy production in this region is dominated by beef production with a production close to 37 million liters in 2016 (MADR, 2017).

Protocol design and survey protocol: The study was conducted in the period from September 2016 to May 2017. A total of 48 random livestock farming (extensive, intensive and peri-urban, (Table 1) were selected, and a survey protocol was used to collect data on livestock farming characteristics and potential environmental pollution of livestock farming using questionnaires and through direct interviews with breeders at each visit during the study period.

At the end of the study period, a total of 144 survey questionnaires were validated, and data concerned:

- **Livestock structure:** general characteristics of the holdings and the occupation of the soil by the various crops, the nature of the construction of the livestock building, type of mechanization were collected.

- **Operation on livestock farming:** milk collection network, animal feed and pesticides application in livestock were recorded.

- **Livestock farming environment:** all data about sources of pollution such as proximity to road traffic, industrial activities, the presence of landfills and waste incinerators, proximity to cities, presence of intensive agricultural activities and mining sectors were analyzed.

Table 1. The various livestock types selected.

Livestock type	Abbreviation	Number	Percentage (%)
Extensive livestock	EL	17	35.41
Intensive livestock	IL	14	29.16
Peri-urban livestock	PUL	17	35.41

Pollution risk indicators: Data collected from environmental survey were transformed to pollution risk indicators according to Akhmetsadykova methodology (Akhmetsadykova, 2012), the following indicators were used:

- **Presence of waste in pasture area (PWPA):** Waste left to graze degrades and deposits very sparingly toxic elements that can be ingested by animals grazing in these areas. The scores "0", "1" and "2" were attributed respectively to "absence of waste", "presence of waste from livestock" and "presence of a landfill not far from to the livestock farming".

- **Circulation of motorized gear in pasture (CMGP):** Atmospheric pollution is the predominant soil contamination; the movement of motorized devices contributes to the deposition of toxic substances on ecosystems (Ely *et al.*, 2001). For each farm a score of "0" and "1" are attributed respectively to the absence and presence of the atmospheric pollution and soil contamination.

- **Proximity to road networks (PRN):** Proximity of farms to road networks constitutes a real risk for the sanitary quality of products. In this study and according to Miquel methodology, a score of "0" was attributed to farm located at a distance of less than 500 m and score of "1" was attributed to farm located at a distance of more than 500 m (Miquel, 2001).

- **Distance to factory, city, mining (DPCM):** The dispersion of pollutants emitted by factories is mainly due to releases to the atmosphere, but also to discharge into water (Cai *et al.*, 2009). It is known that the most polluted soils are those near the factories

According to the recommendations of Farmer and Farmer, (2000); Miquel, (2001); Cai *et al.*, (2009); Akhmetsadykova, (2012): A score of "0" was attributed to a farm located at a distance more than 400 km from a factory; A score of "1" was attributed to a farm located at

a distance between 20 km and 400 km from a factory; A score of "2" was attributed to a farm located at a distance between 5 and 20 km from a factory; A score of "3" was attributed to a farm located at a distance of less than 5 km from a factory.

- **Distance to an agricultural zone (DAZ):** Soils treatment with phyto-sanitary products is considered as a source of pollution and the risk of pollution concerns mainly the rivers close to the spreading zone. According to Akhmetsadykova, (2012) a risk zone of 1 km is considered to take into account possible contamination of streams around agricultural areas and wind dispersal. A score of "0" for a crop-free zone and a score of "1" for a growing area located at less than 1 km from a farm (Akhmetsadykova, 2012).

The values of these five indicators were aggregated in order to obtain a synthetic index (in point) representing the risk for each farm.

Samples collection: From the 48 livestock farming, a total of 144 milk samples were collected for analytic analysis, from each farm, about 500 mL were taken aseptically in sterile glass bottles following standard methods and placed immediately in a cooler, then transported to the laboratory, where they are stored at 4 °C until analysis. All bottles are previously autoclaved at a temperature of 121 °C, under a pressure of 1 bar for 15 minutes.

Table 2. Standard AAS Condition for Fe, Cu, Zn Cd, and Cr.

Metals	Wavelength (nm)	Slit (nm)	Relative Noise	Characteristic Concentration (mg/L)	Characteristic Concentration Check (mg/L)	Linear rang (mg/L)
Fe	248.33	0.20	1.0	0.11	6.0	6.0
Cu	324.75	0.70	1.0	0.077	4.0	5.0
Zn	213.86	0.70	1.0	0.018	1.0	1.0
Cd	228.80	0.70	1.0	0.028	11.5	2.0
Cr	357.87	0.70	1.0	0.078	4.0	5.0

AAS: Atomic Absorption Spectrometry

Method Validation: Direct determination of the validity of the optimized procedure towards analysis of the milk samples with respect to each of the selected metals (Fe, Cu, Zn, Cd and Cr) could not be made possible because of the absence of certified reference materials (CSRM) for these metals. As a result, three validation criteria have been selected:

Precision: The precision of an analytical procedure expresses the closeness or agreement between a replicate measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions (repeatability or reproducibility). The common terms used to measure, variability is the relative standard deviation (RSD). Which may also be expressed as a

Heavy Metals Determination: Heavy metals analysis in cow milk was achieved in two steps:

- **Step (1):** Mineralization by wet oxidation to destroy the organic matter in presence of nitric acid: After homogenization and agitation with a vortex, ten ml of milk sample was added with nitric acid reagent 68 % (CAS: 7697-37-2; Sigma Aldrich). Then the mixture was placed in a ceramic capsule on a heating plate up to 370 °C for 1 hour, and then in a muffle furnace for four hours at 500 °C. Nitric acid 68% was added again on the ashes up to the total destruction of the organic matter. When the solution was white, the solution was diluted up to 10 ml with distilled water containing 1% nitric acid.

- **Step (2):** Analysis of the solution by Flame Atomic Absorption Spectrometry: Flame Atomic Absorption Spectroscopy (FAAS) (Model: Aanalyst400, Perkin Elmer) was used for heavy metal analysis for iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and chromium (Cr), in laboratory of Industrial Analysis and Materials Engineering (University 8 Mai 1945, Guelma-Algeria).

The quantification of the elements was performed by the standard addition method, using 6 points standard curve. These solutions were prepared using appropriately diluted dilutions of the stock standard solutions. The conditions programs for determining the Fe, Cu, Zn, Cd, and Cr levels are shown in a Table 2.

percentage and it is a parameter of choice for expressing precision in analytical sciences (Mitra, 2003).

Linearity: The purpose of this test was to verify the relationship between concentration and signal (absorbance) for concentrations of different heavy metals.

Recovery Tests: The amount of spiked metal recovered after the digestion of the spiked samples was used to calculate percentage recovery as follows: % recovery= [(T-C)/T] x 100. Where T = concentration of a metal in treatment sample, and C = concentration of a metal in control sample. Procedural blanks and standard solutions were also included for analytical quality control to assure the accuracy and reproducibility of the results.

Health-risk assessment of milk consumption: The estimated daily intake (EDI) of trace metals in milk depends on metal concentrations (for dry weight basis), and daily milk consumption rate as well as the average body weight. The EDI was calculated according to Arafat *et al.*, (2014) methodology. EDI values were calculated by the formula:

$$\text{EDI} = \frac{\text{C metal} \times \text{W milk}}{\text{BW}} \text{ (mg/kg BW/day)}$$

Where C metal (mg/L) is the concentration of heavy metals in milk; W milk represents the Daily average consumption of milk and BW represents the Body Weight. This formula has been adapted in the Algerian context according to the average annual per capita consumption estimated at 100 Kg/person/year (Srairi *et al.*, 2013), i.e. the equivalent of 273.97 (g/day) is the average weight of an adult consumer that is 60 kg, is the metal concentration found in the milk (mg/L).

Statistical analysis: The results were expressed in the form of the mean \pm SD (Standard Deviation). Normality and homogeneity of the variances were checked using the Shapiro-Wilk and Levene tests before ANOVA analysis followed by LSD-test, respectively. Kruskal Wallis test was used for comparison of the mean values between the types of livestock. Dunn test was used in means analyzes when this test was found to be significant.

Significance was considered at $p < 0.05$ using SPSS® software version 20.

RESULTS AND DISCUSSION

Validation of the optimized procedure: In this study, the precision of the results were evaluated by the relative standard deviation of the results of triplicate digests. Triplicate measurements of each sample ($n = 144$) were used for the analysis of trace metals in milk samples, values of relative standard deviations (% RSD) are less than 10 % for all of the mean concentrations of metals. The results of percentage recoveries for the studied metals were all ranged between 98.29 to 101.25 %. Generally, good recoveries were obtained for all metals. The results of the recovery tests for samples were within the acceptable range for most metals 85 at 103% (Mico *et al.*, 2006). For all metals, correlation coefficient of the regression line was greater than 0.99.

Situation of different types of farms compared to pollution indicators: The environmental survey showed that the environment in which animals live is subject to multiple sources of pollution. Table 3 presents the average values of the indicators for each type of farm.

The proximity of farms to houses has allowed increasing PWPA indicator, this indicator showed a

significant difference between farms types ($p < 0.05$). Thus, the highest score was recorded in peri-urban farms, with an average score of 1.294 points (Table 3).

Analysis of variance showed no significant difference between farms types for CMGP and PRN indicators (Table 3).

Irrespective of the type of livestock farming, industrial activities threaten all livestock farms. Indeed, the mean of the DPCM indicator was very high for the three types of breeding, but no significant difference was recorded. Concerning DAZ indicator, there is no significant effect; however, extensive farms obtained the highest mean compared to the other farms types (Table 3).

Despite, the no significant effects of different pollution indicators depending on the type of farm, results from this study showed high indicators pollution risk. The comparison of pollution indicators vs farms types with results reported by many authors confirms that agricultural ecosystems are strong emitters of pollutants, particularly those related to inputs on cultivated plots (Stella *et al.*, 2013). Also, farms near high-traffic highways appear to be more polluted by heavy metals such as Cadmium (Al-Khashman, 2004), and milk produced in farms located nearby heavy industry appear more contaminated with heavy metals (Temiz and Soylu, 2012).

Average pollution index varies from 5 to 6 points depending on the farm. A significant difference was observed between extensive and peri-urban farms (Table 3).

Indeed, cows belong to the peri-urban system are more exposed to pollution and can be the vector of human's food contamination. Peri-urban areas, transition zones between urban and rural areas, are subject to several pollution types (gaseous and particulate emissions).

These pollutions come from local sources, such as residential activities, road traffic and agricultural activities (Lee and Fernando, 2013).

Assessment of raw milk contamination in heavy metals: Results from raw milk analysis from all farms combined are presented in Table 4. These results are compared to the standards values of the International Dairy Federation (IDF, 1979).

Despite the very heterogeneous metals concentrations, the order of importance of the metal contents was as follows: Zn > Fe > Cu > Cd > Cr. Indeed, heavy metal concentrations are characterized by a hierarchy of concentrations; the highest being those of Zn followed by Fe and Cu. This finding was also found by Chafaa *et al.*, (2015), which states that Zn accumulates more rapidly in plants than other heavy metals.

Table 3. Indicators of pollution risk according to the various livestock types.

Indicators	EL	IL	PUL	<i>p</i>	
	N	17	14	17	
PWPA (3) *	$\mu \pm \sigma$	0.882±0.332	1±0.00	1.294±0.771	0.048
	Average rank	20.652 ^a	23.00 ^{ab}	29.594 ^b	
	N	17	14	17	
CMGP (2) *	$\mu \pm \sigma$	0.882±0.332	0.857±0.363	1±0.00	0.321
	Average rank	23.683	23.072	26.501	
	N	17	14	17	
PRN (2) *	$\mu \pm \sigma$	0.647±0.492	0.714±0.468	0.941±0.242	0.109
	Average rank	21.531	23.146	28.592	
	N	17	14	17	
DPCM (3) *	$\mu \pm \sigma$	2.294±0.771	2.50±0.650	2.529±0.514	0.671
	Average rank	22.328	25.754	25.652	
	N	17	14	17	
DAZ (1) *	$\mu \pm \sigma$	0.941±0.242	1.00±0.00	0.823±0.393	0.195
	Average rank	25.091	26.507	22.267	
	N	17	14	17	
SI (11) *	$\mu \pm \sigma$	5.647 ^y ±0.996	6.071 ^{xy} ±0.828	6.588 ^x ±0.712	0.01

*: theoretical maximum; SI: Synthetic index; n: number of livestock; $\mu \pm \sigma$: mean \pm SD. Different letters (superscripts) in the same column indicate the significant difference between the mean contents of different livestock ($p < 0.05$) by Kruskal-Wallis test, followed by the Dunn test (a and b), and by ANOVA, followed by LSD-test (x and y).

Based on the values presented in Table 4, average concentrations of Fe, Cu, Zn and Cd far exceed the maximum recommended by the International Dairy Federation (IDF). Concerning chromium, there was not sufficient evidence to set an Estimated Average Requirement (EAR) or a Tolerable Upper Intake Level (UL). Therefore, from literature an Adequate Intake (AI) was set based on estimated mean intakes. The AI is 35 $\mu\text{g/day}$ and 25 $\mu\text{g/day}$ for young men and women, respectively. Few serious adverse effects have been associated with excess intake of chromium from food (IOM, 2001).

These exceedances of IDF standards result from the various sources of pollution that have affected this region, and especially for water quality, which in recent years has deteriorated considerably by uncontrolled industrial releases (Chaoui *et al.*, 2013; Abda *et al.*, 2015). Also, the intensive use of chemical fertilizers in agriculture and the uncontrolled use of contaminated water have contributed to a worsening of the environmental situation, which impact the quality of agricultural products such as milk (Chaoui, *et al.*, 2013).

Zinc: Zn concentration was highest in the milk samples analyzed; it varies from 3.488 to 11.00 mg/L, an average of 5.983 ± 2.040 mg/L was recorded. These concentrations were higher compared to results obtained by other authors, across several countries of the world such as Lante *et al.*, (2006); El-Gendy *et al.*, (2007); El Sayed *et al.*, (2011), with respective averages of 3.6 mg/L, 2.152 mg/kg and 1.209 mg/L. Nevertheless, the mean Zn concentration was lower than that of 7.20 mg/L, obtained by Malhat *et al.*, (2012) in Egypt.

The lowest mean Zn concentrations were significantly recorded in extensive and intensive farms, with a mean of 5.230 ± 0.961 mg/L and 5.342 ± 1.512 mg/L, respectively, compared to peri-urban farming (Figure 1A), while the highest Zn concentration was equal to 7.262 ± 2.645 mg/L, which exceeding the standard recommended by the International Federation of Products (IDF, 1979). These results were in concordance with those obtained by Simsek *et al.*, (2000) who found a very high Zn content equal to 5.01 mg/L in farms near industrial areas, compared to the rural area of 3.77 mg/L.

Table 4. The average heavy metals content of raw milk samples (mg/L).

Metal (mg/L)	N	$\mu \pm \sigma$	CV (%)	IDF ML (mg/L)
Zn	144	5.983±2.040	34.096	3.28
Fe	144	1.432±0.582	40.642	0.037
Cu	144	0.239±0.166	69.456	0.01
Cd	144	0.030±0.013	43.333	0.0026
Cr	144	0.086±0.114	167,441	/

N: number of samples; $\mu \pm \sigma$: mean \pm SD; CV: coefficient of variation; IDF ML: Maximum level according to International Dairy Federation (IDF, 1979).

Iron: Fe was the second most recovered element in milk samples with an average of 1.432 ± 0.582 mg/L. This average was lower than the values obtained by Malhat *et al.*, (2012) in Egypt and Abdul *et al.*, (2012) in Palestine which reported an average of 16.38 mg/L and 8.23 mg/L respectively. However, iron concentration was well above

the value reported by Khalil and Seliem (2013) in Saudi Arabia which is equal to 1.13 mg/L. High iron concentrations can be a problem in milk processing due to its catalytic effect on lipid oxidation, with the development of unpleasant odors affecting proteins and membrane lipoproteins of milk fat globules (Lante *et al.*, 2006).

Fe concentrations indicated a significant difference between extensive livestock vs peri-urban livestock and intensive livestock vs peri-urban livestock, with $p < 0.05$. The highest mean level was recorded in intensive farms (1.665 ± 0.684 mg/L), while the lowest concentration was observed in peri-urban farms with a concentration equal to 1.112 ± 0.436 mg/L (Figure 1B).

These differences in Fe concentrations could be related to feeding practices. In addition, the results of the survey on the functioning of different types of farms show that intensive livestock farming is very marked by fertilization, complementation and the use of veterinary medicinal products. Results are in concordance with data obtained by Dey and Swarup (1996), which have shown relationships between heavy metal concentrations in raw milk and feed.

Copper: The copper values obtained in this study vary between 0.133 and 1.275 mg/L, with an average of 0.239 ± 0.166 mg/L. This concentration is well below the values obtained by Malhat *et al.*, (2012) and Islam *et al.*, (2015) in Egypt and in Bangladesh respectively, which reported an average of 2.836 mg/L and 2.3 mg/L respectively. However, copper concentrations were lower compared to results obtained by Alem *et al.*, (2015) in Ethiopia, which reported an average of 0.206 mg/L.

According to Mitchell (1981), animal feed, and more particularly drinking water, is the most important factor in the contamination of Cu milk. Copper levels indicate almost equal concentrations in intensive and peri-urban farms with respective values of 0.255 ± 0.104 mg/L and 0.254 ± 0.264 mg/L. In addition, the average copper concentration remains low, on the order of 0.209 ± 0.049 mg/L in extensive farms. However, statistical analysis showed no significant differences between the three systems ($p = 0.231$) (Figure 1C). Thus, these results are in concordance with those observed by Simsek *et al.*, (2000) who reported that copper milk cows level was higher in the industrial zone (0.96 mg/L) than in the road zone (0.58 mg/L), itself higher than in rural areas (0.39 mg/L).

Cadmium: From literature, exposure to Cd can affect human health: low birth weight, fertility and semen quality, cancer development and progression (Larsson *et al.*, 2015; De Angelis *et al.*, 2017; Wai *et al.*, 2017). In this study, the mean of Cd concentration found was significantly lower than the values reported by Ali *et al.*, (2011) in Nigeria (0.59 mg/L); Abdul *et al.*, (2012) in Palestine (0.66 mg/L) and Malhat *et al.*, (2012) in Egypt

(2.836 mg/L). However, Cd concentration was higher than result reported by Alem *et al.*, (2015) in milk collected from Ethiopia, with concentration equal to 0.206 mg/L. Cd concentration in this study was far from the recommendations set by Lante *et al.*, (2006) indicating a cadmium-free milk.

The highest average Cd concentration was recorded in peri-urban farms with an average of 0.037 ± 0.017 mg/L, which is significantly higher ($p < 0.05$) compared to those recorded in intensive and extensive livestock production, with values of 0.024 mg/L and 0.028 mg/L respectively (Figure 1D).

Analysis of survey showed that the majority of peri-urban livestock farms are nearby to intense road traffic on the one hand, and a high correlation between “distance from high traffic road” in km and “Cd concentration” in mg/L ($r = 0.32$, $p < 0.05$). These results are in concordance with results reported by Bhatia and Choudhri (1996); Farmer and Farmer (2000); Simsek *et al.*, (2000); Cai *et al.*, (2009), which have shown that milk produced by cows grazing along a road axis contains high cadmium levels with “positive correlation” manner.

Chromium: Chromium levels vary widely from one rearing to another with an average of 0.086 ± 0.114 mg/L. This concentration was well below the value reported by Islam *et al.*, (2015) in Bangladesh (1.6 mg/L), and slightly higher than that found by Alem *et al.*, (2015) in Ethiopia (0.064 mg/L). However, the average chromium content found in each type of farm was almost the same in the three types of farming (Figure 1E).

Assessment of risks to human health related to the consumption of milk produced by the different livestock farming types: The estimated daily intake (EDI) of metals in cow milk for human consumption was compared with the permissible values (Table 5). The EDI data for the different heavy metals were in the following order: Zn > Fe > Cu > Cr > Cd. The highest daily intake was calculated for Zn (27.32 mg/kg bw/d), while the lowest value was for cadmium (0.137 mg/kg bw/d). The calculated EDI for Fe was of the order of 6.538 mg/kg bw/day.

Compared to the maximum permissible values for the various heavy metals, Cd has the metal farthest from the recommendation value, i.e. 297.82% of the maximum permissible value. This percentage reaches 365.21%, which is equivalent to an EDI 0.168 mg/kg bw/d in peri-urban farming, which exceeds the value reported by Salah *et al.*, (2013), which was in the order of 0.158 mg/kg bw/d. The EDI for chromium was in the range of 0.392 mg/kg bw/d, exceeding the maximum permissible value of 0.2 mg/kg bw/d among the highest at the permissible value of 196% (Table 5).

Taking into account the increased demand for milk and dairy products, and the concentration of heavy

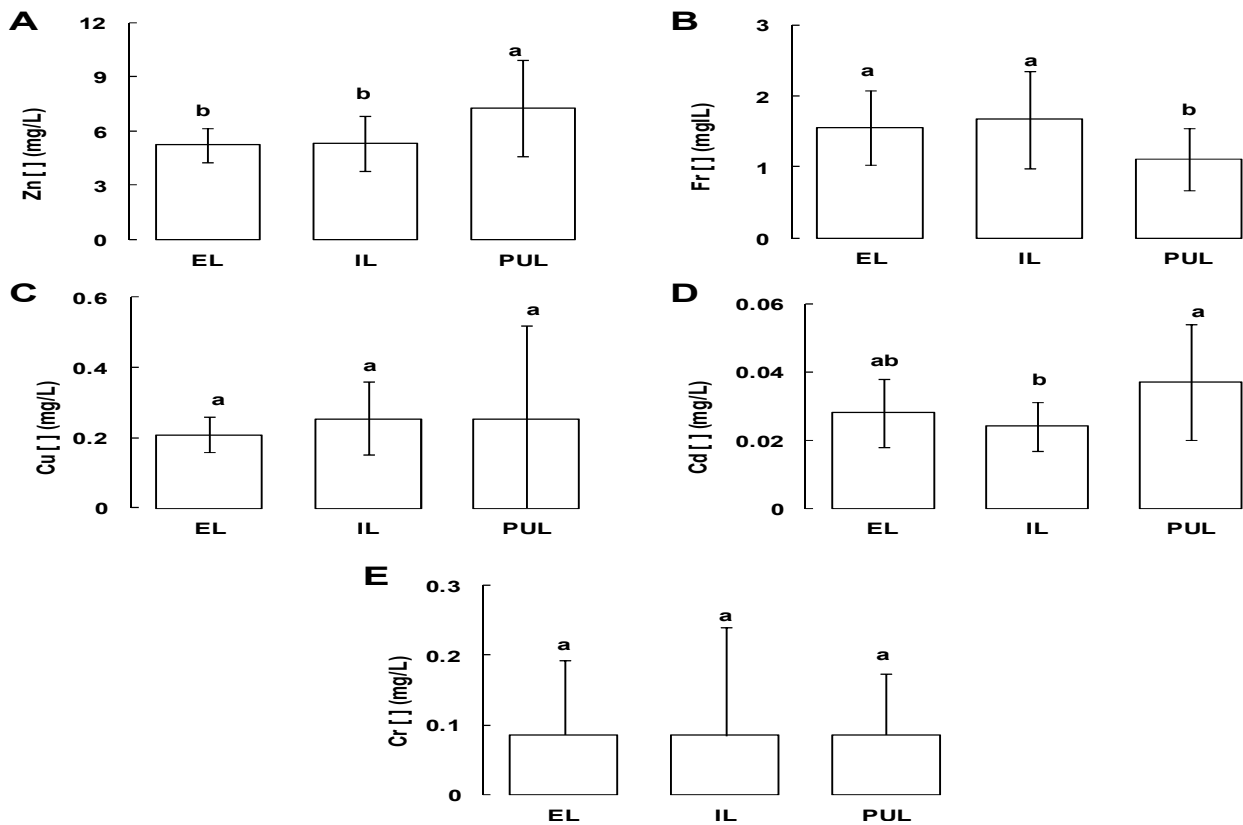
metals compared to the permissible values for, the assessment of the toxic effects for consumers is a necessity. Mean concentrations of Fe and Cu are not toxic to humans as long as Fe and Cu EDIs have been found below permitted limits and have also been approved with previous work (Islam *et al.*, 2015; Ademola, 2014; Salah and Ahmed, 2012; Anita *et al.*, 2010), while Cd, Cr, and

Zn have fairly high concentrations compared to permissible values and far exceed the lower limits. On the basis of these data, it can be concluded that Cd, Cr and Zn were the main contributors to the potential risk incurred by consumers of raw cow milk collected in this study region.

Table 5. Permissible value for metals studied in cow milk samples of different types of livestock, mean EDI values and their contributions to PV for adult consumers.

Metal	Total (n =144)		EL (n=51)		IL (n=42)		PUL (n=51)		(PV) (mg/day)	standard References
	EDI (mg/kg bw/d)	C. to PV (%)	EDI (mg/kg bw/d)	C. to PV (%)	EDI (mg/kg bw/d)	C. to PV (%)	EDI (mg/kg bw/d)	C. to PV (%)		
Zn	27.320	182.130	23.881	159.206	24.392	162.613	33.159	221.060	15	(FAO/WHO, 2002)
Fe	6.538	16.345	7.112	17.780	7.6	19.0	5.077	12.692	40	(FAO/WHO, 2002)
Cu	1.091	3.636	0.954	3.180	1.164	3.880	1.159	3.863	30	(JECFA, 2003)
Cd	0.137	297.826	0.127	276.086	0.109	236.956	0.168	365.217	0.046	(JECFA, 2003)
Cr	0.392	196.0	0.392	196.0	0.388	194.0	0.392	196.0	0.2	(Oliver, 1997)

PV: Permissible Value; EDI: estimated daily intake; C: Contribution; d: day; n: number of samples; bw: body weight



[EL (17 farms), IL (14 farms) and PUL (17 farms)]. Results are mean values \pm SD (n=51, 42, 51/milk samples from IL, EL and PUL farms respectively). Each bar represents Mean \pm SD. Means which are denoted by different letters (a and b) indicate significantly different values (Kruskal-Wallis test followed by Dunn test, $p < 0.05$).

Fig.1. Metals concentrations (Zn, Fe, Cu, Cd and Cr) in cow’s raw milk collected from three types of livestock farming.

Conclusion: This study carried out an environmental analysis of pollution risks around cattle breeding

belonging to the three types of dairy cattle farms in the region of Guelma-Algeria. In one hand, extensive

livestock farming was less affected by the different sources of pollution compared to other types of livestock. On the other hand, the milk produced in the peri-urban system was more exposed to pollution and can therefore be a vector of contamination.

Heavy metals analysis in cow raw milk showed high levels of iron, zinc, copper, chromium, and cadmium compared to the International Dairy Federation standard. As a result, most levels of heavy metals in milk were above the maximum limit for consumers except of iron and copper in all types of farming, with a higher degree of risk in peri-urban farming.

Particular attention should be given to heavy metal contamination when they are present in higher concentrations, than the standard daily intake. It is therefore necessary to monitor the feeding and environment of dairy females.

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