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# Trace elements in raw milk of buffaloes (*Bubalus bubalis*) from Campania, Italy

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ABSTRACT

The profile of 18 trace elements was traced in 68 milk samples collected from buffalo farms in the territory known as the "Land of Fires" in the Campania region (Italy). This area has been polluted by the illegal dumping in fields of industrial or domestic waste, wich is sometimes then burned spreading toxic contaminants. Milk from buffaloes raised on rural farms might be a good indicator of environmental contamination risk in the human food chain. Trace element analysis in milk was performed using mass spectrometry. One milk sample was found to be non-compliant due to high Pb concentration. In the absence of threshold values for the elements, established through legislation, the results were compared with similar studies from other countries, and in most cases the content determined in this study was in agreement with values reported elsewhere and do not represent a risk to human health.

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## 1. Introduction

Trace elements can be classified as toxic (arsenic, cadmium, lead, mercury), probably essential (nickel, cobalt) and essential (copper, iron, manganese, selenium, zinc). The dietary intake of toxic metals can cause serious harm, even at low concentrations over a long period, while essential metals can also produce toxic effects where their intake is excessive. Indeed, food is the main source of trace elements, and milk is one of the products with the highest risk of contamination, due to the environmental dispersion of toxic substances and potentially polluted feedstuffs (Ataro, McCrindle, Botha, McCrindle, & Ndibewu, 2008; Bilandžić et al., 2011; Rey-Crespo, Miranda, & López-Alonso, 2013).

Various studies suggest the importance of assessing trace element content in milk from animals reared in the vicinity of polluted areas, as their presence is due to various factors such as industrial activities, climatic factors, contaminated agricultural water for irrigation, accumulation along roads and motorways and the use of pesticide compounds (Esposito et al., 2010; Licata et al., 2012; Miedico, Tarallo, Pompa, & Chiaravalle, 2016; Pavlovic, Sikiric, Havranek, Plavljanic, & Brajenovic, 2004; Perugini et al., 2012; Sahayarai & Ayyadurai, 2009; Shahbazi, Ahmadi, & Fakhari, 2016).

While trace elements can enter cattle through inhalation of contaminated dust, they are more commonly, and more importantly for humans, absorbed orally from contaminated feed and water (Ren-ju, Hui-li, Jian-guo, & Xue-jun, 2015). Furthermore, ethological characteristics of buffaloes contribute to the risk through typical habits, such as rolling around in mud and licking fences: the dermal route might also be an important contamination source (Kalra, Chawla, Joia, & Tiwana, 1986).

Water buffalo accounts for the second most widely available milk source in countries around the world (Santillo, Caroprese, Marino, Sevi, & Albenzio, 2016). Within European countries, Italy accounts for 95% of all water buffalo, with 230,794 lactating buffalo (FAOSTAT, 2016) that are mainly reared in central and southern regions of Lazio, Campania, and Puglia (Borghese, Moioli, & Tripaldi, 2000). Therefore, the determination of toxic contaminants such as hazardous trace elements in buffalo milk might be an important issue for food safety. Indeed, buffalo milk is the basic ingredient for cheese production, in particular for *mozzarella di bufala*, a typical cheese produced in the Campania region of southern Italy (Zicarelli, 2004), where buffalo livestock is a cornerstone of the economy, providing widespread employment.

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There are very few data on trace element content in milk, especially in buffalo milk. Information on the presence of potentially toxic metals is very limited, except for lead and cadmium, which can affect various organs such as liver and kidney as well as the hematopoietic and nervous systems. Because of their recognized toxicity, EU legislation has set maximum tolerable limits (Regulation EU 1881/2006) in various types of food (Sahayarai & Ayyadurai, 2009), but in milk, a maximum limit has only been established for lead. Trace elements in milk could be due to rearing animals in areas affected by contaminated water or fodder and industrial waste disposal (Shailaja et al., 2014). The biological effects of other trace elements are unknown or not well known, due to the lack of toxicological data, although EFSA has issued a number of opinions to highlight the need for monitoring trace elements in various types of foods and so as to reduce exposure to them (EFSA, 2009, 2014, 2015).

The presence of trace elements in the environment at natural levels is often associated with human activity, which can cause increased levels in the different environmental compartments and inevitably lead to bioaccumulation in the food chain.

In the Campania region, the discovery of illicitly buried industrial waste and concomitant illegal burning (hence the name "Land of Fires") involving waste from various sources, from civil to manufacturing and industrial activities, led to a health alert due to a series of epidemiological studies correlating such phenomena with increases in cancer rates in some parts of Campania (Albanese, De Luca, De Vivo, Lima, & Grezzi, 2008; Altavista et al., 2004; Di Lorenzo, Federico, De Placido, & Buonerba, 2015; Fazzo et al., 2011; Martuzzi et al., 2009), which has in turn led to evident and growing concern among the public. In this area, some monitoring studies on the environmental quality of soil and water showed high levels of certain inorganic elements that could result from pollution (Albanese, De Vivo, Lima, & Cicchella, 2007). There are still no data on the presence of these elements in certain foods, in particular those of animal origin produced in this area. There is a clear need to understand whether such elements then enter the food chain, and to understand the degree of risk to consumers.

In this paper, we report the results of determining eighteen trace elements in buffalo milk. Buffalo milk was collected from farms in the Land of Fires (Campania region, Italy). Metals of known toxicity such as lead, cadmium, mercury, and arsenic were considered together with elements known to be essential but which may be toxic if assumed in high amounts, such as copper, zinc, chromium, cobalt, selenium, and tin; finally, we took into consideration those elements without any biological effects but which may be indicators of pollution of various origins, i.e. beryllium, uranium, manganese, molybdenum, thallium and strontium.

Determination of these trace elements is therefore important for the quality of dairy products derived from buffalo milk in order to ensure consumer safety but also because it constitutes an indirect way of monitoring the impact of anthropogenic activity on soil, water and air (Rahimi, 2013).

## 2. Materials and methods

### 2.1. Apparatus and materials

Trace elements were determined using an inductively coupled plasma mass spectrometer ICP-MS Elan DRC II (PerkinElmer, Waltham, USA) equipped with a concentric nebulizer (Meinhard Associates, Golden, USA), a cyclonic spray chamber (Glass Expansion, Inc., West Merbourne, Australia) and a quartz torch with a quartz injector tube (2 mm internal diameter). The instrumental conditions/parameters of ICP-MS and the isotopes monitored are reported in Table 1. To eliminate isobaric interferences, the Dynamic Cell Reaction (DRC) system was used with ammonia gas (100%, high purity) at 0.5 ml/min for the determination of As, Co, Cr, V, Ni, Zn, Mn and Cu, and methane gas (99.9995%) at 0.5 ml/min for the determination of Se. A solution of bismuth and rhodium (approximately 200 ng/ml) added on-line was used as internal standard.

**Table 1**
Instrumental operating conditions of ICP-MS and measurement parameters.

| | |
|---|---|
| Radiofrequency | 1200 W |
| Argon flow rate (L/min) | Plasma 15 – Nebuliser 0.97 |
| Sample flush speeding | 32.0 rpm |
| Read delay | 20 s |
| Read delay and analysis speeding | 20 rpm |
| Wash | 60 s |
| Wash speeding | 36 rpm |
| Dwell time | 50 ms |
| Sweeps/reading | 20 |
| Isotopes | $^{111}Cd$, $^{202}Hg$, $^{75}As$, $^{238}U$, $^{9}Be$, $^{118}Sn$, $^{205}Tl$, $^{98}Mo$, $^{59}Co$, $^{52}Cr$, $^{51}V$, $^{60}Ni$, $^{78}Se$, $^{66}Zn$, $^{55}Mn$, $^{63}Cu$, $^{88}Sr$ and the sum of isotopes $^{206}Pb$, $^{207}Pb$ and $^{208}Pb$ |
| N. of replicates per sample | 2 |

Argon gas (99.9999%) was supplied from SAPIO S.r.l. (Monza, Italy) and anhydrous ammonia from AIR Liquide S.p.a. (Milano, Italy).

Standard solution of, arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co),, mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), copper (Cu), selenium (Se), strontium (Sr), thallium (Tl), tin (Sn), uranium (U), vanadium (V) and zinc (Zn) at 1000 mg/l were obtained from CPA Ltd (Stara-Zagora, Bulgaria). Certified Reference Material NIST 1549 non-fat milk powder was purchased from NovaChimica S.r.l. (Cinisello Balsamo, Milano, Italy).

Superpure grade nitric acid 68% (v/v), hydrogen peroxide 30% (v/v) and ultrapure water (R > 18.0 MΩ) were obtained from Romil Ltd (Cambridge, UK).

It has been used only disposable glassware to avoid any cross-contamination.

### 2.2. Sampling

The milk samples were collected from 68 buffalo farms in the area corresponding to the so-called Land of Fires, in the Campania region of southern Italy (Fig. 1). The sixty-eight farms (11%) were selected randomly among the 602 total farms located in the municipalities involved in the present study. All the samples were collected from bulk milk obtained from two milkings. Farms were of different sizes, with a mean number of animals of about 550 and ranging from 46 to over 3000. A production capacity (number of animals) of 75% was generally estimated with a 8–12 *per die* and *pro-capite* milk production. The milk samples were directly collected into sterile screw-topped bottles (1.0 L). The samples were transported in a cooler (+4 °C) to the laboratory and were stored at −20 °C until chemical analysis. Sampling, storage and handling steps were set up to reduce all possible contamination, loss or alteration that couldnegatively affect data reliability.

### 2.3. Procedure

Before analysis, each entire milk sample was defrosted gradually at +4 °C. Two grams of sample were weighed inside Teflon vessels and 6 ml of nitric acid 68% (v/v) and 2 ml of hydrogen peroxide 30% (v/v) were added; then the vessels were put in the Milestone Ethos-One apparatus (FKV S.r.l., Torre Boldone, Bergamo, Italy) for microwave-assisted mineralization. The total sample digestion was obtained through the following program: up to 120 °C in 15 min and constant for 10 min; up to 190 °C in 15 min and

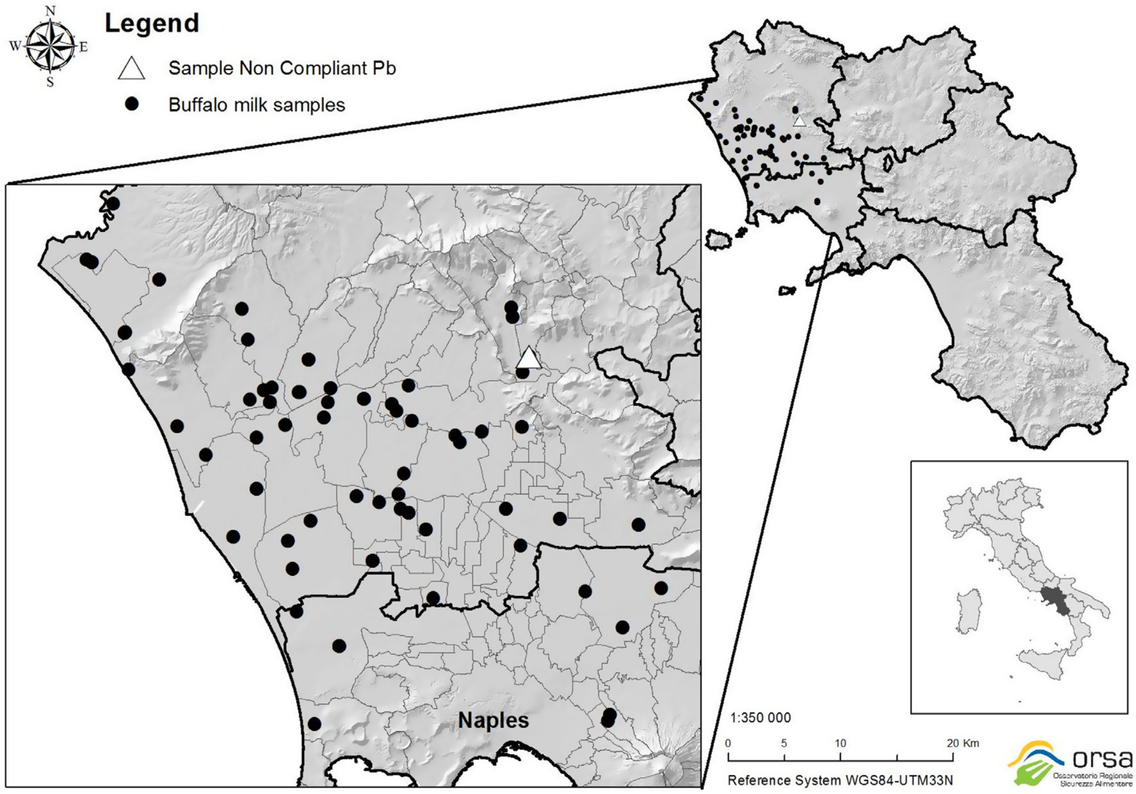


**Fig. 1.** Map of the Campania region (southern Italy) with the farms where buffalo milk samples were collected.

constant for 20 min; cooling stage (30 min) to reach room temperature. After acid digestion the mineralized solution was transferred into 50 ml polypropylene disposable tubes and filled to mark by addition of ultrapure water, for further analysis by ICP-MS.

The following elements/isotopes were detected: $^{75}$As, $^{9}$Be, $^{111}$Cd, $^{59}$Co, $^{52}$Cr, $^{63}$Cu, $^{202}$Hg, $^{55}$Mn, $^{98}$Mo, $^{60}$Ni, $^{78}$Se, $^{118}$Sn, $^{88}$Sr, $^{205}$Tl, $^{238}$U, $^{51}$V and $^{66}$Zn. In order to eliminate the intrinsic variability of lead isotope distribution and to improve the signal sensitivity, the sum of $^{206}$Pb, $^{207}$Pb and $^{208}$Pb were counted.

The analytical curves were performed by standard addition into the mineralized and diluted solution: for each element 5 addition levels, including a not added level, were used. A preventive and semi-quantitative analysis was carried out to state the order of concentration levels, for each element, in order to choice the most proper calibration range. The addition levels for each element were:

As, Pb, Cd, V, Ni, Se, Co, Mo, Sn and Cr (0.00 – 0.10 – 0.50 – 2.0 – 10.0 ng/ml); Be, U, Hg and Tl (0.000 – 0.001 – 0.005 – 0.020 – 0.10 ng/ml); Cu and Mn (0.0 – 0.5 – 2.5 – 10 – 50 ng/ml); Zn and Sr (0 – 4 – 20 – 80 – 400 ng/ml)

The correlation coefficient ($R^2$) of analytical curves for all the trace elements was always greater than 0.990, showing a good linear relationship throughout the selected ranges of concentration.

### 2.4. Quality assurance

Appropriate quality assurance procedures and precautions were implemented in order to ensure the reliability of the results in accordance with ISO/IEC 17025 (2005). The method used was validated by means of an in-house quality control procedure and through participation in several interlaboratory comparisons.

The accuracy of the method was checked by using the Certified Reference Materials NIST 1549 non-fat milk powder. The recovery for all analytes was close to 100% and therefore the results were not corrected for the recovery factor (Regulation EC 836/2011).

Chemical blank determinations were performed at each analytical batch, to check for possible contamination. The limits of detection (LOD) and quantification (LOQ) for all the elements were calculated on the basis of the standard deviation of the intensity of twenty reagent blanks, multiplying by a factor of three and ten, respectively. Validation parameters of the analytical procedure and the accuracy test by certified standard material are reported in Table 2.

### 2.5. Statistical analysis

The concentrations of the trace elements for each sample were evaluated as the mean of two determinations, with a repeatability of less than 10%. The results were expressed in mg kg$^{-1}$ wet weight. For each trace element mean concentration, standard deviation, median and range were calculated. Where the value was below the LOQ, a value equal to LOQ/2 was considered (Menichini & Viviano, 2004).

Statistical calculations were performed using SPSS Statistics for Windows (IBM Corporation, NY, USA). Kolmogorov–Smirnov test was applied to verify the normality of the distribution of trace elements in buffalo milk samples. Principal component analysis (PCA) study was carried out after the normalization by unit variance scaling method, using MetaboAnalyst 3.0 (Xia, Sinelnikov, Han, & Wishart, 2015).

## 3. Results and discussion

In the present study 68 milk samples were collected from as many buffalo farms in the area dubbed Land of Fires in the Campania region of southern Italy; descriptive statistics of the trace ele-

**Table 2**
Validation parameters and reference material analysis.

| Element | LOD (mg kg$^{-1}$) | LOQ (mg kg$^{-1}$) | Correlation Coefficient ($R^2$) | NIST SRM 1549 non-fat milk powder (mg kg$^{-1}$) | | RSD %(n = 6) |
|---|---|---|---|---|---|---|
| | | | | Certified Value ± U | Measured Value ± U | |
| As | 0.0013 | 0.0042 | 0.9997 | 0.0019# | <LOQ | 6.5 |
| Be | 0.00021 | 0.00071 | 0.9987 | – | <LOQ | 9.8 |
| Cd | 0.00013 | 0.00044 | 0.9975 | 0.0005 ± 0.0002 | 0.00057 ± 0.00011 | 5.3 |
| Co | 0.00016 | 0.00054 | 0.9999 | 0.0041# | 0.0037 ± 0.0007 | 6.4 |
| Cr | 0.00036 | 0.0012 | 0.9981 | 0.0026 ± 0.0007 | 0.0031 ± 0.0006 | 7.2 |
| Cu | 0.0030 | 0.010 | 0.998 | 0.7 ± 0.1 | 0.71 ± 0.12 | 4.1 |
| Hg | 0.00045 | 0.0015 | 0.9987 | 0.0003 ± 0.0002 | <LOQ | 8.4 |
| Mn | 0.016 | 0.052 | 0.9981 | 0.26 ± 0.06 | 0.30 ± 0.06 | 3.7 |
| Mo | 0.00045 | 0.0015 | 0.9986 | 0.34# | 0.33 ± 0.06 | 4.1 |
| Ni | 0.0060 | 0.020 | 0.998 | – | 0.39 ± 0.08 | 5.8 |
| Pb | 0.00075 | 0.0025 | 0.9992 | 0.019 ± 0.003 | 0.022 ± 0.004 | 5.7 |
| Se | 0.0014 | 0.0047 | 0.9875 | 0.11 ± 0.01 | 0.11 ± 0.02 | 6.8 |
| Sn | 0.00087 | 0.0029 | 0.9982 | <0.02# | <LOQ | 8.5 |
| Sr | 0.0028 | 0.0092 | 0.9982 | – | 3.7 ± 0.7 | 2.1 |
| Tl | 0.0000084 | 0.000028 | 0.9979 | – | 0.00066 ± 0.00015 | 7.7 |
| U | 0.0000045 | 0.000015 | 0.9991 | – | 0.00019 ± 0.00004 | 4.1 |
| V | 0.000075 | 0.00025 | 0.9995 | – | 0.0015 ± 0.0003 | 7.8 |
| Zn | 0.26 | 0.87 | 0.9994 | 46.1 ± 2.2 | 47.5 ± 8.1 | 2.9 |

#Information Values (Not Certified).

ment concentrations are shown in Table 3, while Fig. 2 depicts a boxplot with the distribution of groups of data through their quartiles.

By appling Kolmogorov–Smirnov test, trace elements in buffalo milk samples were in all cases not normally distributed, except for selenium, copper, zinc and strontium ($\alpha = 0.05$).

PCA was applied to verify any possible differentiation due to geographic factors, but it revealed no significant differences, showing a high degree of homegenity on the whole territory under investigation. Given the lack of statistical significance, PCA results are not shown.

### 3.1. Toxic trace elements

Due to the strong toxicity of some elements, also defined heavy metals, such as Pb, Cd, Hg and inorganic species of As, European legislation has set limits (maximum permitted level) for their presence in food (EU Regulation 1881/2006), but unfortunaltely, for milk only the content of Pb is regulated and fixed at 0.020 mg kg$^{-1}$. Therefore, with the exception of Pb, the lack of reference limits makes it difficult to assess the risk associated with the levels of these elements in buffalo milk. However, to get an estimate of the contamination level, where possible, a comparison of the present results with literature data of similar studies all around the world was carried out.

As regards Pb content, this study showed a mean concentration of 0.00522 mg kg$^{-1}$, considerably lower than the maximum limit above. Only one sample was found to be not compliant, with a Pb concentration of 0.0526 mg kg$^{-1}$ (expanded uncertainty 0.0079 mg kg$^{-1}$). This sample was collected in April 2015 at a farm in Castel di Sasso (province of Caserta, Italy, marked with a white triangle in Fig. 1). After reporting of this non-compliancee to the territorial competent authority, a control plan was set to check Pb levels in milk samples from other neighbouring farms within a radius of 3 km. Consequently, a further 18 milk samples were collected from as many herds, but no other case of Pb non-compliance was found, reporting most of the values below the LOQ. Controls were also carried out on feed, showing very low levels of Pb (ranged between 0.089 and 0.98 mg kg$^{-1}$ at 12% humidity). As outcome of the control it emerged the presence of a contamination source in that particular livestock: in 2014, hay was purchased from a neighbour in an area where quail-shooting activities are carried out and animals drink water from 3 corroded old enamel basins. In any case, further controls

**Table 3**
Trace element concentrations (mg kg$^{-1}$ wet weight) in 68 buffalo milk samples.

| | Samples > LOQ | Mean (mg kg$^{-1}$) | Std Dev (mg kg$^{-1}$) | Median (mg kg$^{-1}$) | Min (mg kg$^{-1}$) | Max (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| As | 18 | 0.00302 | 0.0017 | 0.00209 | 0.0042 | 0.00943 |
| Be | 7 | 0.00165 | 0.0047 | 0.000356 | 0.00071 | 0.022 |
| Cd | 30 | 0.000535 | 0.00053 | 0.00044 | 0.00044 | 0.00303 |
| Co | 68 | 0.00376 | 0.0013 | 0.00359 | 0.000867 | 0.00794 |
| Cr | 40 | 0.00258 | 0.0024 | 0.00163 | 0.0012 | 0.00967 |
| Cu | 67 | 0.0722 | 0.048 | 0.0674 | 0.01 | 0.332 |
| Hg | 2 | 0.000781 | 0.00015 | 0.000755 | 0.0015 | 0.00165 |
| Mo | 68 | 0.0449 | 0.02 | 0.0406 | 0.026 | 0.143 |
| Mn | 37 | 0.0493 | 0.06 | 0.0447 | 0.042 | 0.483 |
| Ni | 68 | 0.0724 | 0.054 | 0.0548 | 0.0212 | 0.332 |
| Pb | 49 | 0.00522 | 0.0067 | 0.00395 | 0.00126 | 0.0526 |
| Se | 67 | 0.0230 | 0.013 | 0.0212 | 0.0047 | 0.08 |
| Sn | 2 | 0.00180 | 0.00052 | 0.00147 | 0.0029 | 0.00463 |
| Sr | 68 | 1.07 | 0.35 | 1.01 | 0.276 | 2.09 |
| Tl | 67 | 0.000511 | 0.00065 | 0.000279 | 0.000028 | 0.00375 |
| U | 57 | 0.0000952 | 0.000095 | 0.0000644 | 0.000015 | 0.000407 |
| V | 40 | 0.000499 | 0.00056 | 0.000307 | 0.00025 | 0.00255 |
| Zn | 68 | 5.74 | 1.3 | 5.66 | 1.71 | 9.93 |

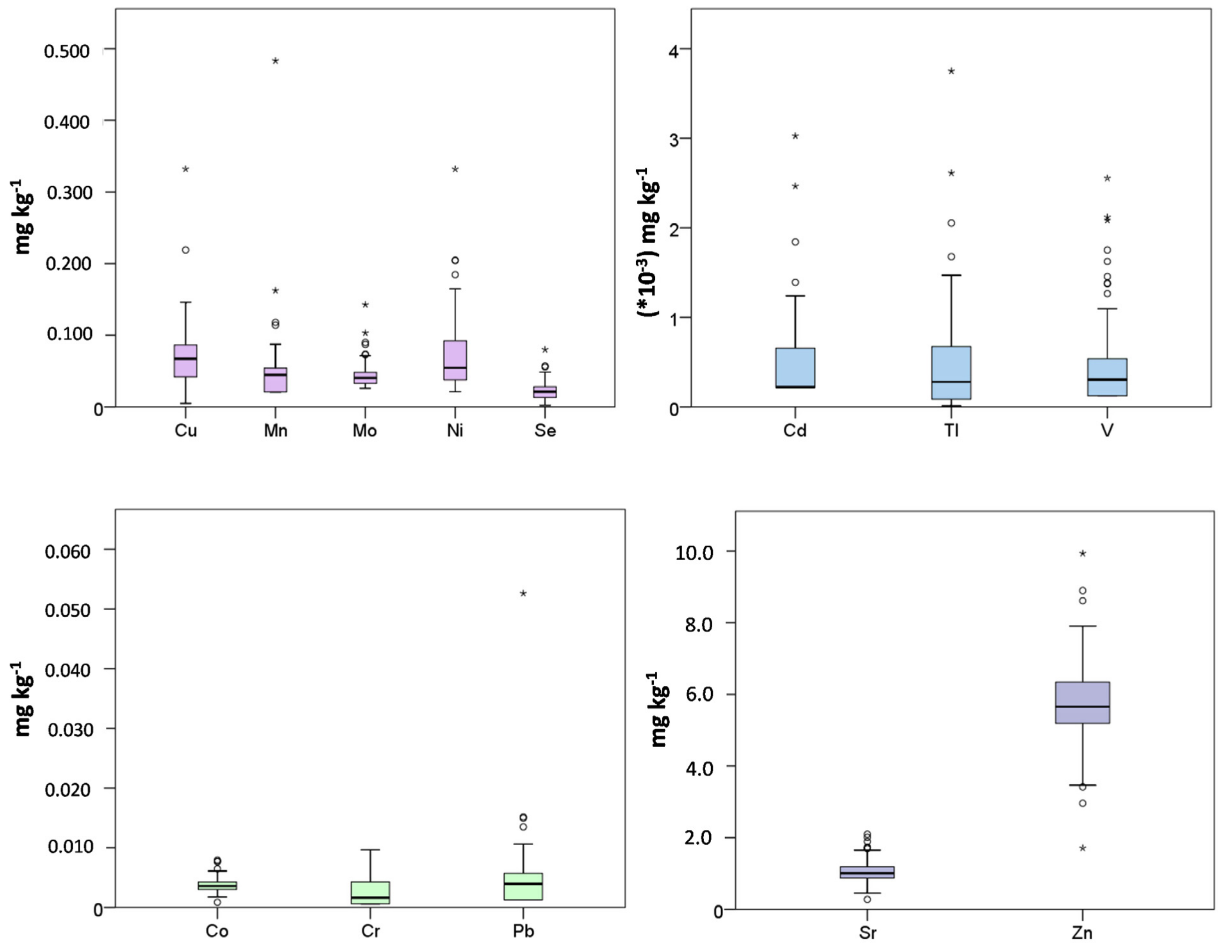


**Fig. 2.** Boxplot with the distribution of groups of data through their quartiles.

on milk produced in the farm showed that Pb values were within the maximum limits set by EU Regulation.

Table 4 reports a broad comparison of Pb content found in buffalo milk from around the world: these data indicate that environmental contamination by lead in the study area would seem to be very low. Considering that the uptake of lead into buffaloes occurs through ingestion, inhalation and dermal contact, and that all these sources are in most cases attributable to the presence of high volumes of vehicular traffic or to the contamination of fodder, a high level of contamination of the water can also be excluded, as described in the case report of India's buffalo reared near the contaminated watercourse, with which very high values of lead in milk are associated, confirming the important role of water and soil as a source of contamination (Dwivedi, Dey, & Swarup, 1995; Sahayarai & Ayyadurai, 2009).

Regarding Cd contamination in milk, although the mammary gland is an effective barrier to cadmium excretion in milk (Lawal, Mohammed, & Damisa, 2006), its presence in this matrix constitutes a risk to consumer health, since cadmium is carcinogenic and genotoxic to the point of being classified by IARC as a Group

**Table 4**
Comparison of trace element content in buffalo milk samples of previous works. Data are reported in mg kg$^{-1}$, as mean ± SD.

| Element (mg kg$^{-1}$) | Campania, Italy (present study) (n = 68) | West Bengal (Mondal et al., 2015) (n = 10) | Egypt (Enb et al., 2009) (n = 60) | Iran (Rahimi, 2013) (n = 11) | Italy (Benincasa et al., 2008) (n = 6) | Azerbaijan (Najarnezhad et al., 2015) (n = 100) | India (Guha et al., 2012) (n = 496) | India (Shailaja et al., 2014) (n = 30) |
|---|---|---|---|---|---|---|---|---|
| Pb | 0.00522 ± 0.0067 | | 0.084 ± 0.042 | 0.00616 ± 0.00316 | | 0.018 ± 0.001 | | |
| Cd | 0.000535 ± 0.00053 | | 0.118 ± 0.086 | 0.00074 ± 0.00041 | | 0.003 ± 0.001 | | |
| Cr | 0.00258 ± 0.0024 | | 0.042 ± 0.022 | | 0.00034 ± 0.007 | | | |
| Sn | 0.00180 ± 0.00052 | | 0.006 ± 0.010 | | | | | |
| Ni | 0.0724 ± 0.054 | | 0.006 ± 0.010 | | | | | |
| V | 0.000499 ± 0.00056 | | | | 0.0041 ± 0.017 | | | |
| Co | 0.00376 ± 0.0013 | | 0.008 ± 0.010 | | 0.00210 ± 0.020 | | 0.029 ± 0.019 | |
| Mo | 0.0449 ± 0.02 | | | | 0.0169 ± 0.0016 | | | |
| Sr | 1.07 ± 0.35 | | | | 0.749 ± 0.011 | | | |
| Cu | 0.0722 ± 0.048 | 0.33 ± 0.18 | 0.212 ± 0.102 | | | | 0.381 ± 0.032 | |
| Mn | 0.0493 ± 0.061 | 1.74 ± 0.39 | 0.076 ± 0.044 | | 0.00247 ± 0.024 | | 0.56 ± 0.11 | |
| Zn | 5.74 ± 1.3 | 3.75 ± 0.23 | 4.37 ± 0.81 | | 6.49 ± 0.020 | | 3.57 ± 0.27 | 3.97 ± 0.12 |

I carcinogen (IARC, 1993). Therefore, it must be emphasized that there is a need to monitor the presence of Cd in all foods, especially those of wide consumption (Recommendation 2014/193/EU). Mean cadmium concentration in the present buffalo milk samples was 0.000535 mg $kg^{-1}$, considerably lower than the concentration of 0.0030 mg $kg^{-1}$ reported by Najarnezhad, Jalilzadeh-Amin, Anassori, and Zeinali (2015) in buffalo milk from Azerbaijan, province of Iran; also, a very high value (0.118 mg $kg^{-1}$) was found in buffalo milk samples from Egyptian farms (Enb, Abou Donia, Abd-Rabou, Abou-Arab, & El-Senaity, 2009).

Moreover, the present data are very low even in comparison with milk samples from other animal species: two studies using cow milk samples in Egypt (El Sayed, Hamed, Badran, & Mostafa, 2011) and in Southern Croatia (Pavlovic et al., 2004) reported values of 0.086 mg $kg^{-1}$ and 0.00340 mg $kg^{-1}$, respectively.

The concentration of Hg was below the LOQ for all samples except for two, in which the values were very close to LOQ, further proof of Hg's low tendency to accumulate in milk and its negligible toxicological risk. In addition, Sn was quantifiable only in two milk samples, with values close to LOQ: the Egyptian study (Enb et al., 2009) found a fairly comparable mean level for Sn. The levels of Be were below the LOQ in most samples: two samples were quantifiable but very close to LOQ and only five samples showed a relatively high concentration, between 0.011 and 0.022 mg $kg^{-1}$. Regarding As content, about 74% of the samples were below the LOQ, while 18 samples showed values slightly above it. The concentrations of V were below the LOQ in 41% of the samples, with the remaining samples being very close to the LOQ value. As a comparative reference, the Italian study of 2008 in 6 samples of buffalo milk found mean V content to be about ten times higher (0.0041 mg $kg^{-1}$). Comparison with the milk samples from cattle farmed extensively adjacent to a vanadium processing plant is even more positive; those samples contained 0.23 mg $kg^{-1}$ of this non essential element (Gummow, Botha, Noordhuizen, & Heesterbeek, 2005). As regards Tl content, thanks to the high sensitivity of the analytical method, it was quantifiable in all samples (with the exception of one), with a very low mean concentration (0.000511 mg $kg^{-1}$): unfortunately, there are no reference data for this highly toxic element, whose presence is linked to human activities, both industrial and agricultural (it is in fact used as rat poison and as an insecticide). About half of the analyzed samples showed a Cr concentration below the LOQ, with an average of 0.00258 mg $kg^{-1}$, somewhat higher than the 2008 Italian study (0.00034 mg $kg^{-1}$) conducted in the Calabria region in milk of buffaloes reared in an area of low environmental impact. Furthermore, mean Cr content in the present study was much lower than in the Egyptian paper (0.042 mg $kg^{-1}$). The mean content of U was very low, at a concentration (0.0000952 mg $kg^{-1}$) such that its intake by ingestion may be considered not significant, and its health impact is such that it does not cause any damage (Khan et al., 2014). Also, the value found in this study was in agreement with similar assays in common foodstuffs: Anke, Seeber, Müller, Schäfer, and Zerull (2009) found mean total Uranium in cow milk of 0.00023 mg $kg^{-1}$. Regarding the mean content of Ni, a rather high value (0.0724 mg $kg^{-1}$) was found: in the Egyptian study, Ni values in buffalo milk were ten times lower, while other similar studies in sheep and goat milk have reported mean values of 0.0407 and 0.0440 mg $kg^{-1}$, respectively (Miedico et al., 2016).

### 3.2. Essential trace elements

Essential trace element levels in this study were found to be more or less different from those reported in the literature (Table 4), demonstrating their inherent variability. In particular, the concentration of Zn, the most abundant of the trace elements, with a mean content of 5.74 mg $kg^{-1}$ and a wide distribution, substantially overlaps the values obtained in other similar studies, with the exception of West Bengal (Mondal, Pyne, Samanta, & Roy, 2015) and India (Shailaja et al., 2014), where the levels of Zn were only about half. Sr was the second most relevant trace element, with a mean of 1.07 mg $kg^{-1}$, slightly higher than in the other Italian study, where mean Sr content in buffalo milk from Calabria was 0.749 mg $kg^{-1}$ (Benincasa, Lewis, Sindona, & Tagarelli, 2008). In contrast, mean Cu content in the present study (0.0722 mg $kg^{-1}$) was much lower (from 1/5 to 1/3) compared with other studies. Mean Mn concentration (0.0493 mg $kg^{-1}$) was quite similar to that of the Egyptian study (Enb et al., 2009) and much lower than in the Indian and West Bengal studies (Guha, Gera, & Sharma, 2012; Mondal et al., 2015; Shailaja et al., 2014), where levels were ten to thirty times higher. Mean content of Mo (0.0449 mg $kg^{-1}$) was about twice as high as in the other Italian study (0.0169 mg $kg^{-1}$, Benincasa et al., 2008), while Co average content in this study (0.00376 mg $kg^{-1}$) was substantially comparable with the value from the Calabria region, Italy (0.00210 mg $kg^{-1}$). No comparison can be made with regard to Se content in other studies regarding buffalo milk: we can only highlight that the mean value (0.0230 mg $kg^{-1}$) in our buffalo milk samples is higher than that reported in cow's milk at a farm in Spain (Rey-Crespo et al., 2013).

## 4. Conclusions

In this study, trace element levels in buffalo milk from the Campania region (Italy) were investigated. Average contents were found more or less comparable with those reported in other countries, showing greater variability mostly foressential trace elements, while toxic trace elements were comparable or lower than literature data. The buffalo milks had low contamination levels of toxic metals, minimizing any risk to health:even though one sample was found to be non-compliant due to its Pb content, the mean concentration of this toxic heavy metal was much lower than the maximum limit, leading to a very low risk of human exposure to Pb through dairy product consumption. The contamination level of Cr, that emerges from the comparison with other Italian regions, albeit very low extent, needs further and more exaustive studies.

As with other types of milk (i.e. bovine) more widely studied, this study may help to create reference values for trace elements (essential and non-essential) in buffalo milk, so as to be used in analogue monitoring studies. At the same time, further investigations need to determine any correlation with soil and water data and therefore whether the presence of these pollutants can be attributed to a natural background from geogenic activity or to the presence of sources of contamination such as industrial or civil waste.

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