Journal of Trace Elements and Minerals 3 (2023) 100046

Contents lists available at ScienceDirect

Journal of Trace Elements and Minerals

journal homepage: www.elsevier.com/locate/jtemin

Inorganic elements profiling in milk from buffalo farms of the Campania Region (Italy)

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A R T I C L E I N F O

Keywords:
Buffalo milk
Forages
Elements
Factor analysis
Campania Region

A B S T R A C T

Background: Bulk milk samples from 69 dairy buffalo farms of the Campania Region, Italy were drawn for the determination of 20 selected elements by ICP-MS. The main goal was to ascertain how the agricultural soil geochemistry could influence the milk pattern with respect to feeding practices.

Methods: Local forages (N = 207) and groundwater (N = 486) were analyzed too to recover the feeding-to-milk carry-over rates (COR) of selected elements and identify the main contributors to milk contamination. Left censored data >10% limited to 11 elements (Mn, As, Se, Sr, Zn, Cr, Fe, Co, Ni, Cu, and V) the multivariate system approach based on cluster and factor analysis.

Results: We identified 8/11 elements - Sr, Mn, Zn, Co, Se, Cr, Fe, and V - explaining the main quote of variation observed among farms milk pattern. In particular, Co resulted as discriminant for specific villages of the Salerno Province. On median values, cereals (34%) and feed supplement (31%) represented the main contributors to Cr intake; for V, hay (46%) and water (42%); for Fe, water (46%); for Se, Co and Zn, mineral supplements (67; 73; 82%); for Sr, hay (71%), for Mn, mineral supplements (36%) and water (30%). Median CORs rates (%) were the following: Se and Cr: 40; V: 13; Mn: 5.2; Sr: 4.7; Zn: 3.7; Co: 3.4; Fe: 1.6; respectively.

Conclusion: The factor analysis could trace the geographical origin of buffalo milk at farm level, according to similar management practices, partially irrespective of the municipality/province level.

1. Introduction

Buffaloes (*Bubalus bubalis*) are the second source of milk in the world after cows, and contribute up to 15% to the overall global dairy production [1].

The market of buffalo milk and dairy products represents a relevant economic sector well established in Central and Southern Italy [2]. In the period 2015–2018, the Italian production of buffalo milk increased by 27%, from 195.270 to 247.158 tons [3]; hence the need to extensive control in order to ensure quality, traceability, and safety of milk and dairy products. In Southern Italy, buffalo milk is mainly used for the production of "mozzarella di bufala", a soft cheese made with fresh buffalo milk and globally recognized for the good eating quality and peculiar taste [4]. The Protected Designation of Origin (PDO) recognized by official rules to produce mozzarella di bufala includes seven provinces; among them, Caserta and Salerno, settled in the Campania Region are the most productive areas, followed by part of the province of Benevento, Naples, Frosinone, Latina and Rome [5]. Most of the production lies on local forages feeding recovered from agriculture soils, some of them of volcanic origin [6].

From 2008, Campania Region has been intensively monitored for environmental contaminants in soil, water, and air, as consequence of high levels of persistent organic pollutants found in buffalo milk [7]. Within this frame, a wide characterization of the geochemical composition of Campania agricultural soils (Fig. 1) and well waters at farm level has been recently carried-out [8].

At present, recent studies on elements in buffalo milk are largely focused on the occurrence of As, Pb, Cd, Cr, Zn, Cu, Ni, and Co from restricted and possibly impacted areas [9–16]; an inventory referred more generally to dairy products is present in the database supporting intake estimates and risk assessment of selected elements (As, Pb, Cd, Cr, and Cu) in the European Union [17–23] (See Suppl Mat).

Only two papers considered the analysis of 22 and 17 elements, respectively in milk from buffalo and other species from Shandong

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https://doi.org/10.1016/j.jtemin.2023.100046
Received 29 August 2022; Received in revised form 6 January 2023; Accepted 7 January 2023
Available online 11 January 2023

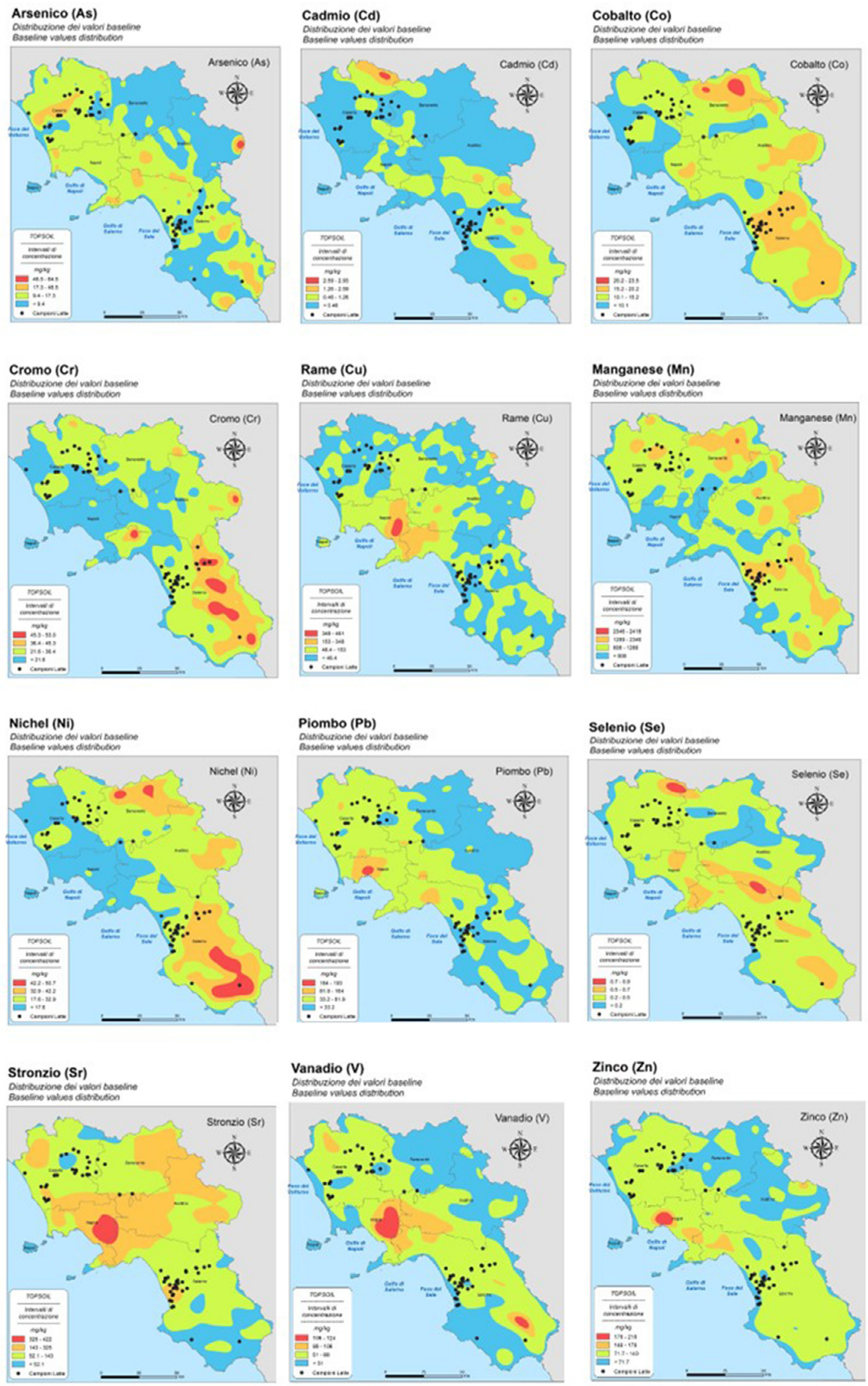

Fig. 1. Representation of the geochemical baseline contamination of selected elements in agricultural top soil in the Campania Region where dairy buffalo farming practices take place.

Table 1
List of the Provinces and Municipalities of Campania Region with the pertinent number of the selected dairy buffalo farms.

Province	Village	Farm frequency
Avellino (AV)	Cervinara	1
	Roccabascerana	1
Benevento (BN)	Faicchio	1
	S.Salvatore Telesino	1
Caserta (CE)	Alvignano	2
	Baia e Latina	3
	Caiazzo	4
	Calvi Risorta	1
	Cancello Arnone	2
	Castel Campagnano	1
	Grazzanise	2
	Piana di Monteverna	1
	Pietramelara	1
	Pontelatone	5
	Riardo	1
	Sessa Aurunca	1
	Sparanise	3
	Villa Literno	3
Salerno (SA)	Agropoli	3
	Albanella	5
	Altavilla Silentina	3
	Ascea	1
	B. Carillia	1
	Battipaglia	1
	Capaccio	7
	Eboli	5
	Oliveto Citra	1
	Postiglione	4
	Serre	3
	Sicignano degli A.	2

(157,100 km^2), Shaanxi (156,800 km^2), Guanxi (236,700 km^2), Sichuan (485,000 km^2) and Xinjiang (1,664,897 km^2) regions of the Popular Republic of China [24,25]; differences were found in element concentrations in milk based on species and considered regions.

Therefore, the aim of this study was to characterize the presence of 20 elements (Cr, Cu, Fe, Ga, Mn, Ni, Se, Sr, Zn, As, Be, Bi, Cd, Co, Cs, Pb, In, Tl, U, and V) in raw buffalo milks from 69 farms as representative of different agriculture areas of Campania Region in Italy (13.590 km^2).

The main goals were: a) to assess the impact of environmental and anthropogenic pressures on the element profiling of buffalo milk, and b) evaluate if the element profiling could provide evidences about the geographical origin of the product at Province, Municipality, or farm level.

To this respect, a "One Health" approach has been planned, to ascertain elements contribution from water, and feed & forages intake and their transfer to buffalo milk [26,27].

2. Material and methods

2.1. Study design and sample collection

Within "Campania trasparente" regional granted activities, between January and June 2018, 69 buffalo farms from 30 different villages of the Campania Region were selected randomly, recognizing differences in the geo-chemistry of the agricultural soils (Fig. 1; Table 1).

Sampling of milk was performed according to the provisions of law for chemical analysis (European Commission Regulation no. 333/2007). Bulk buffalo milk samples (pools from 2 milkings) were directly collected from the cooled tank into sterile screw-topped bottles (1.0 liter) previously cleaned with 2% (v/v) of nitric acid and rinsed with ultrapure water before use to reduce any exogenous contamination. Samples were transported in a cooler (+4 °C) to the laboratory and stored at −20 °C.

Similarly, forages were drawn at farm level according to the European Commission Regulation no.152/2009, while drinking water from farm wells was drawn according to the national guidelines for chemicals in groundwaters (SNPA 08/2018), after a 15′ purge. Sampling, storage and handling steps were set up to reduce all possible contamination, loss or alteration that could negatively affect data reliability.

2.2. Sample preparation and analysis

Milk samples were thawed at room temperature on a roller mixer to ensure complete homogenisation. Two grams of sample were weighed inside teflon vessels, then 6 mL of nitric acid 69% (v/v) and 2 mL of hydrogen peroxide 30% (v/v) were added. Microwave-assisted mineralization was carried out in the Milestone Ethos-One apparatus (FKV S.r.l., Torre Boldone, Italy) for. 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 constant for 20 min; cooling stage (30 min) to reach room temperature. After acid digestion the mineralized solution was transferred into 25 mL polypropylene disposable tubes and filled to mark by addition of ultrapure water, for further analysis by ICP-MS. The following elements were selected: ^{75}As, ^{9}Be, ^{209}Bi, ^{111}Cd, ^{59}Co, ^{52}Cr, ^{133}Cs, ^{63}Cu, ^{57}Fe, ^{69}Ga, ^{115}In, ^{55}Mn, ^{60}Ni, ^{208}Pb, ^{78}Se, ^{88}Sr, ^{205}Tl, ^{238}U, ^{51}V and ^{66}Zn. Each sample was analyzed in duplicate. Quantitative determination of trace elements was performed by using linear regression calibration curves obtained by analysis of standard solution of all elements at 5 concentration levels. To compensate for matrix effects and instrument drift, the same amount of rhodium (Rh) solution was added to all measured solutions (blanks, calibration standards, unknown samples, etc.). A mathematical correction factor is calculated from the relative internal standard response that is then applied to the analytes to correct for both matrix and drift effects. Certified multi-element standard solution of arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), copper (Cu), selenium (Se), strontium (Sr), thallium (Tl), uranium (U), vanadium (V) and zinc (Zn) at 10 mg L^{-1} was obtained from Perkin Elmer (Perkin Elmer, Shelton, CT).

The calibration standard solutions were prepared freshly each day of analysis by dilution of the standard solutions. The correlation coefficient (R^2) of calibration curves for all the trace elements was always greater than 0.99, showing a good linear relationship throughout the selected ranges of concentration. Procedural blanks were prepared using all reagents without the matrices and were digested together with milk samples and measured during each analytical run to reveal any accidental contamination.

The forage samples, once homogenized, were also analysed using a similar procedure. To two grams of sample weighed inside high-pressure Teflon (TFM) vessels, 6 mL of nitric acid 69% (v/v) and 2 mL of hydrogen peroxide 30% (v/v) were added then the vessels were put in a Milestone Ethos-One apparatus (FKW SrL, Torre Boldone, Bergamo, Italy) for microwave mineralization. After acid digestion, the samples were cooled to room temperature and brought to 50 mL with ultrapure water (resistivity of ca. 18.2 MΩ cm) produced in-house by means of an Arium® pro purification system (Sartorius, Göttingen, Germany).

Water samples were taken and prepared with filtration with a 0.45 µm MF-Millipore® Membrane Filter (Merck KGaA, Darmstadt, Germany) and stabilization with the addition of nitric acid up to pH below 2. Subsequently, samples were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS) for determination of trace elements of milk [28].

Contamination levels of forages were computed on 12% of moisture, according to the EU legislation (Directive 2002/32/EC).

2.3. Equipment and apparatus

Trace elements were determined using an inductively coupled plasma mass spectrometer ICP-MS NexION 350X (Perkin Elmer, Shelton, CT) equipped with concentric nebulizer baffled cyclonic spray chamber (Glass Expansion, Inc., West Melbourne, Australia) and quartz torch with quartz injector tube (2 mm internal diameter). The instrumental conditions/parameters of ICP-MS and the elements monitored are reported in Table 1S Supplementary materials. A solution of rhodium (approximately 200 ng/mL) added on-line was used as internal standard (Perkin Elmer, Shelton, CT). Superpure grade nitric acid 68% (v/v) and hydrogen peroxide 30% (v/v) were obtained from Romil Ltd (Cambridge, UK). It has been used only disposable glassware to avoid any cross-contamination. Calibrated pipettes (Eppendorf Multipette 100–1000 μL,) were used during blanks, reference materials, quality controls and samples preparation procedures. Details of the operating conditions are reported in the Supplementary Materials (SM, Table 1S).

2.4. Quality control and quality assurance

Appropriate quality assurance procedures and precautions were implemented in order to ensure the reliability of the results in accordance with UNI CEI EN ISO/IEC 17,025 (2017). The methods used were validated by means of an in-house quality control procedure and their satisfactory performance was demonstrated by participation in proficiency tests (NRL National reference laboratory for metals and nitrogenous compounds in food) with z score results within ±2.

The accuracy of the method was checked by using the Certified Reference Materials NIST 1549 non-fat milk powder (Table 5S) and ERM-CD281 rye grass (Table 6S). The recovery for all analytes was close to 100% and therefore the results were not corrected for the recovery factor.

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 based on the standard deviation of the absorbance of twenty reagent blanks, multiplied by a factor of three and ten, respectively.

2.5. Statistical approach in the analysis of elements pattern in buffalo milk

The database is represented by 69 samples and 11 variables (elements with left censored data < 7/69), therefore the ratio samples/variables is 69/11=6.3. To improve the multivariate system resolution a selection of most important variables has to be implemented with the aim to increase this ratio (Maldonado and Greenland, 1993). This process was carried out by a Cluster Analysis (CA) approach focused on finding the variables most representing of the database [29]. After this element selection, a Factor Analysis (FA) approach was implemented. The FA procedure is designed to extract common factors from a set of quantitative variables. In many situations, a small number of common factors may be able to represent a large percentage of the variability in the original database. Computing was performed using the Stratgraphics 18 centurion software from Stratgraphic Inc, Virginia, USA.

FA assumes that observed correlations are caused by some underlying pattern in the data resulting from the presence of a number of predetermined factors. The contribution of any variable is split into a common component, that part that contributes to the factors, and a unique component that can be thought of as noise. The sum of the common components is called communality. A communality of "0″ would arise if a variable was uncorrelated with all other variables and had no shared information. In FA the unique variability component is excluded from the analysis because it does not contribute to a shared factor [30]. To improve the FA output interpretability, a Varimax Rotation was applied. This is an iterative method which rotates the axes into a position such that the sum of the variances of the loadings is the maximum possible. This Varimax Rotation is recommended when the loadings, within each factor, are dissimilar, i.e. a combination of high and low values.

2.6. Elements intake and carry-over rates

The estimates of the dairy buffalo intake (external dose) were calculated according to a standardized diet for dairy buffalo adapted to local fodder availability and average production parameters as reported in Table 2, accounting for the median and mean contamination of local silages and hays, cereals, and water. The declared concentration (mg kg^{-1}) in commercial mineral supplements (Zn = 2500; Se = 5; Co = 25; *I* = 80; Mn = 800; Fe = 900), and commercially available soybean meals (Zn = 55; Fe = 170; Mn = 30 Se = 0.3; Co = 0.3; Cu = 20) was accounted for. Carry-over rates have been computed as ratio (%) between the dose excreted via milk (daily production on average 9 kg/head) and the computed intakes from forages, feeds and mineral supplements, and water, on median values. Left censored data have been replaced with half of the limit of quantification (LOQ) according to the medium-bound approach in computing.

3. Results

3.1. Occurrence of selected elements in buffalo milk

The occurrence of As, Be, Cd, Ga, Sr, In, Cs, Tl, Pb, Bi, Mn, Zn, Se, V, Cr, Fe, Co, Ni, Cu, and U in the 69 different buffalo milk samples is shown in Table 3.

Table 4 reports mean and median values of the contamination recorded in water (N = 486), silage (N = 27), hay (N = 137), cereals (N = 44), respectively for the determined elements Mn, Fe, Co, Cr, Ni, Se, Cu, Zn, V, Sr, As and Sn.

Carry-over rates (%) computed as ratio between amount excreted via milk and intakes on median values were the following in decreasing order: Se: 40; Cr: 40; Ni: 15; V: 13; Mn: 5.2; Sr: 4.7; Zn: 3.7 Co: 3.4; Fe: 1.6; Cu: 1.3; Pb: 1.0, respectively. More details in the supplementary material (Tables 2S, 3S).

On median value basis, mineral supplements represented the main source of intake for Zn, Fe, and Co (88%; 41%; 74%). Corn and feed supplement mostly contributed to the total Cr intake for the 34% and 31% respectively. For Ni, soy panel (61%) and hay (21%); For Cu, soybean meal (34%) and corn (31%); for As, water (87%), for Sr, hay (74%).

3.2. Province-specific buffalo milk contamination pattern

The Loadings and Communalities of the Varimax Rotated Factor considering the 8 variables that cluster analysis selected out the original 11 ones with quantified results are reported in Table 5. Three factors represent three possible sources of the variability and explain a variability quote of 75.8% of the measure values (Table 3). The most important positive loadings show a c value $^{>}$0.3 [30] and the variables showing higher absolute values (Table 5) for the first factor were Sr, Mn, Zn, Co and Se; for the second factor, Cr and Se.; and lastly, the highest loadings for the third factor where 0.410 for Co and 0.892 for V.

In addition, the estimated communality values (Table 5) give other important insights for data interpretation: in the Factor 1, Mn, Zn and Co values, ranging 0.821–0.856, appear to be correlated each other in explaining some underlying pattern in the database. This Factor 1 association appears to be widely diffused between the samples because the pertinent factor explains more than the 50% of the explained variability of the database. The factor 2 is weighted most heavily in a positive direction on Cr and Se which represent a further variability source for some samples; the third factor is weighted most heavily in a positive direction on Co and V.

Both factors 2 and 3 explain a corresponding variability quote of about 15% (Table 5). Cr and Se showing relatively low communality values indicate a low correlation degree between them. On the contrary, Co and V from the factor 3 appear to be correlated each other. Owing to the above, the two elements Se and Co seem to show a double influence on the samples because characterizing the first and second factor and the first and the third ones, respectively. Fig. 2ab shows the factor analysis distribution of the farms according to the Province of origin (Avellino, Benevento, Caserta, and Salerno, respectively). Because

Table 2
Standard dairy buffalo feeding regimen, tailored on average daily milk production.

Forage/feed	kg	kg dry matter	dry matter%	notes
Mais silage	16	5.6	35	
Hay	6.0	5.3	88	
Corn meal	4.0	3.5	88	
Soybean meal	1.0	0.9	88	
Mineral supplement	0.3	0.3	100	
Water	80			5 kg dry matter ingested per day
Total	107	16		

Table 3
Statistical values of trace element concentrations (mg kg^{-1}) in buffalo milk samples (N = 69). LCD = Left Censored Data.

	Be	Ga	As	Sr	Cd	In	Cs	Tl	Pb	Bi	Mn	Zn	Se	V	Cr	Fe	Co	Ni	Cu	U
mean	n.d.	0.007	0.012	0.947	0.008	n.d.	0.011	n.d.	0.003	0.003	0.035	4.310	0.044	0.016	0.141	6.850	0.008	0.150	0.106	0.039
median	n.d.	0.007	0.012	0.836	0.007	n.d.	0.005	n.d.	0.003	0.003	0.032	3.810	0.040	0.015	0.102	3.310	0.008	0.137	0.085	0.041
min	n.d.	0.003	0.005	0.235	0.007	n.d.	0.000	n.d.	0.001	0.001	0.009	1.830	0.020	0.004	0.059	1.150	0.002	0.049	0.029	0.001
max	n.d.	0.023	0.034	2.260	0.009	n.d.	0.099	n.d.	0.008	0.005	0.120	14.79	0.107	0.040	0.532	30.56	0.025	0.388	0.442	0.085
LOQ	0.005	0.003	0.010	0.020	0.005	0.005	0.005	0.005	0.005	0.005	0.010	0.020	0.020	0.005	0.020	0.100	0.010	0.020	0.020	0.005
LCD (%)	100	0	20	0	88	100	0	100	42	96	4	0	9	0	0	0	0	0	0	12

n.d. = not detected.

Table 4
Mean and median contamination (C) of selected elements in the considered feed materials and computed element intake (Int) according to the amount of forage/feed/water ingested (Ing). Carry-Over Rates (COR) computed as ratio between the total amount excreted via milk (T Excr) and the total intake (T Int).

	Silage (N = 27)			Hay (N = 137)			Cereals (N = 44)			min suppl*			soy meal*			Water (N-486)							
Element	C mg/kg	Ing kg	Int mg	C mg/kg	Ing kg	Int mg	C mg/kg	Ing kg	Int mg	C mg/kg	Ing kg	Int mg	C mg/kg	Ing kg	Int mg	C mg/kg	Ing kg	Int mg	T Int mg	Milk mg/kg	Prod Kg	T Excr mg	COR %
Cr ***mean***	0.282	5.6	1.581	0.135	5.3	0.716	0.675	3.5	2.364	0.700	0.3	0.210	0.706	1.0	0.706	0.004	80.00	0.286	5.863	0.141	9	1.273	21.7
P50	0.007	5.6	0.037	0.067	5.3	0.353	0.217	3.5	0.760	0.700	0.3	0.210	0.706	1.0	0.706	0.003	80.00	0.200	2.265	0.102	9	0.915	40.4
Ni ***mean***	0.126	5.6	0.705	0.449	5.3	2.380	0.418	3.5	1.463		0.3		5.544	1.0	5.544	0.001	80.00	0.092	10.18	0.150	9	1.348	13.2
P50	0.055	5.6	0.310	0.326	5.3	1.726	0.135	3.5	0.473		0.3		5.544	1.0	5.544	0.001	80.00	0.080	8.133	0.137	9	1.237	15.2
Cu ***mean***	1.392	5.6	7.795	4.641	5.3	24.60	5.822	3.5	20.38		0.3		20.16	1.0	20.16	0.008	80.00	0.647	73.57	0.106	9	0.956	1.30
P50	1.338	5.6	7.491	2.460	5.3	13.04	5.040	3.5	17.64		0.3		20.16	1.0	20.16	0.003	80.00	0.200	58.53	0.085	9	0.765	1.31
Zn ***mean***	11.31	5.6	63.32	11.39	5.3	60.38	35.59	3.5	124.6	2500	0.3	750,0	20.16	1.0	20.16	0.049	80.00	3.936	1022	4.315	9	38.833	3.80
P50	3.931	5.6	22.01	4.026	5.3	21.34	28.89	3.5	101.1	2500	0.3	750.0	20.16	1.0	20.16	0.001	80.00	0.040	914.7	3.808	9	34.270	3.75
As ***mean***	0.005	5.6	0.027	0.010	5.3	0.054	0.017	3.5	0.058		0.3		0.000	1.0		0.002	80.00	0.149	0.289	0.012	9	0.108	37.6
P50	0.001	5.6	0.006	0.001	5.3	0.005	0.001	3.5	0.004		0.3		0.000	1.0		0.001	80.00	0.098	0.113	0.012	9	0.105	93.5
Se ***mean***	0.006	5.6	0.034	0.038	5.3	0.001	0.112	3.5	0.391	5.00	0.3	1.500	0.302	1.0	0.302	0.008	80.00	0.647	2.876	0.100	9	0.900	31.3
P50	0.005	5.6	0.028	0.005	5.3	0.000	0.059	3.5	0.208	5.00	0.3	1.500	0.302	1.0	0.302	0.003	80.00	0.200	2.239	0.100	9	0.900	40.2
Co ***mean***	0.011	5.6	0.064	0.023	5.3	0.001	0.043	3.5	0.152	5.00	0.3	1.500	0.252	1.0	0.252	0.003	80.00	0.200	2.168	0.008	9	0.073	3.35
P50	0.005	5.6	0.025	0.005	5.3	0.000	0.022	3.5	0.078	5.00	0.3	1.500	0.252	1.0	0.252	0.003	80.00	0.200	2.055	0.008	9	0.070	3.39
V ***mean***	0.059	5.6	0.328	0.297	5.3	1.576	0.169	3.5	0.593		0.3			1.0		0.010	80.00	0.803	3.300	0.000	9	0.002	7.00
P50	0.018	5.6	0.102	0.145	5.3	0.769	0.036	3.5	0.127		0.3			1.0		0.010	80.00	0.800	1.798	0.000	9	0.002	13.0
Sr ***mean***	63.44	5.6	355.2	53.61	5.3	284.1	44.0	3.5	154.1		0.3			1.0		0.015	80.00	1.200	794.7	0.002	9	0.014	0.16
P50	3.851	5.6	21.56	1.109	5.3	5.877	7.55	3.5	26.44		0.3			1.0		0.015	80.00	1.200	55.08	0.002	9	0.014	2.45
Mn ***mean***	5.850	5.6	32.76	26.00	5.3	137.8	21.0	3.5	73.5	800	0.3	240.0	30.00	1.0	30.00	2.500	80.00	200	614.0	4.310	9	38.79	5.43
P50	3.870	5.6	21.67	22.10	5.3	117.1	15.5	3.5	54.25	800	0.3	240.0	30.00	1.0	30.00	2.500	80.00	200	773.0	3.810	9	34.29	5.17

* Concentration declared on the information sheet of the commercial feed administered.

farms from the two Provinces, Benevento and Avellino (4/69) are underrepresented, we focused our attention on the groups from Caserta and Salerno. With respect to the factor 1 and 2 scores (Fig 2a). The samples from the Caserta province appear to be clustered mainly in three subgroups according to Factor 2 ordinate. The first subgroup ranges from −2.7 to −1.2, the second from −0.92 to 0.026, and the third one from 0.72 to 2.54. On the contrary, no clear clusters are seen on Factor 1 abscissa. The first subgroup includes 12 on 30 samples which are not characterized by the Factor 2 (negative values), while the second includes 8 on 30 and overlaps the distribution of 25/35 samples from the Salerno Province. The third group includes 7 on 30 samples from the Caserta Province and 6 from the Salerno one showing full positive scores even if quite scattered, and therefore well characterized. Relatively to the Salerno Province, Fig. 2a shows a main group consisting of 25/35 farms ranked in the range −0.82 – 0.51, thus overlapping the second subgroup from Caserta, while the remaining 10 samples appear to be more scattered across the graph. The Fig. 2b shows high overlapping between the Factor 1 and Factor 3 score ranges from −5.3 to 4.99, and from −3 to 3.2, respectively, of both Salerno and Caserta farms. Even in this situation, some differences between the two Provinces can be argued: the Salerno Province samples show a tendency to be distributed along the Factor 3 ordinate, while the major part of samples from the Caserta Province do not show any specific tendency. It is also worth noting some samples from the Caserta Province share the highest score values from Factor 1 and 3.

4. Discussion

In this paper we aimed to explore if an extended elements characterization in dairy buffalo milk from 69 farms within the Campania Region (13,595 km^2) could support a municipality-based resolution power of their geographical traceability. Previous papers reported the feasibility of such approach comparing different macro-regions of the Popular Republic of China, as already represented by Zhou et al., 2017 [24].

In carrying out this study it was taken into account that milk, local forages, and feed contamination (Tables 3 and 4) can reasonably reflect the 2–3 months time frame exposure of dairy buffaloes, thus reasonably bringing minor uncertainties in factor analysis due to potential day-by-day variation and in the intake assessment and related carry-over rates in milk. This claim is supported by the presence of rumen as forage stor-

Table 5
Loadings, communality and the specific variance from factor analysis on the 69 buffalo milk samples for those elements that were screened as discriminant. The loadings or weights of each variable control the contributions that they make to the factor scores. The communality is the variance attributable to factors that all the X variables have in common, while the specific variance is specific to a single factor. Bolded values represent the most contributing elements to explain the variability.

	Factor 1	Factor 2	Factor 3	Estimated Communality	Specific Variance
Sr	**0.827**	0.047	−0.189	0.722	0.278
Mn	**0.906**	0.005	−0.008	0.821	0.179
Zn	**0.925**	−0.008	−0.002	0.856	0.144
Co	**0.810**	0.091	**0.410**	0.833	0.167
Se	**0.557**	**0.470**	−0.091	0.540	0.460
Cr	0.155	**0.694**	**−0.419**	0.681	0.319
Fe	0.124	**−0.835**	**−0.326**	0.820	0.180
V	0.017	0.012	**0.892**	0.796	0.204

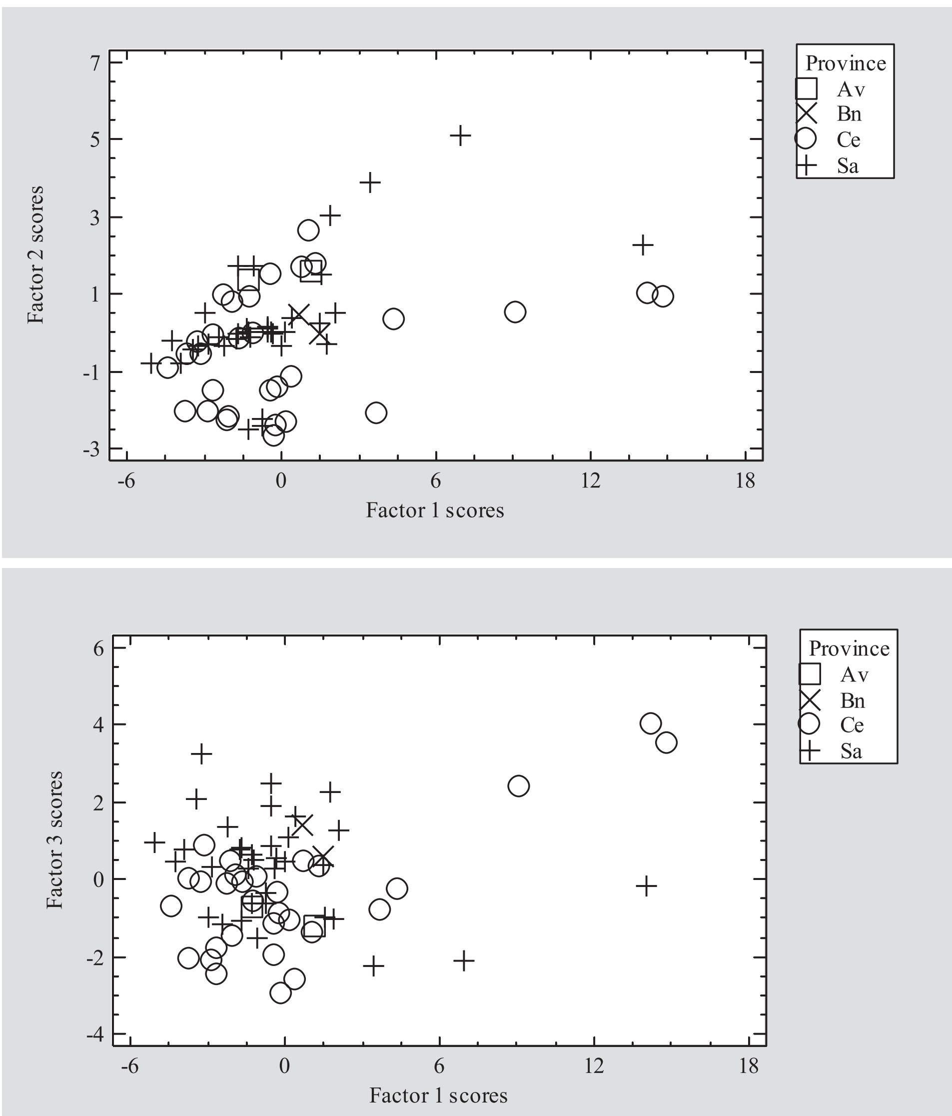

Fig. 2. Factor analysis distribution of the 69 buffalo milk samples belonging to the Salerno (SA), Caserta (CE), Avellino (AV), and Benevento (BN) provinces for the first two principal components **(a)** and for the first and third component **(b)**.

age compartment that makes bulk milk profile less susceptible of sudden day-by-day variation. Moreover, harvested fodders (hay, *Zea mais* for silage) usually guarantee a 3 month-long supply with the same forage. The "one day" approach in water sampling is mitigated by the larger number of observation (N = 486; Table 2S) where the computed median value may smooth seasonal variations in elements concentration.

Feeding regimen may acknowledge seasonal differences i.e. with respect to water consumption (winter-summer period range: from 60 to 120 L head day^{-1}) and possibly in the silage/hay balance. During the summer period, greater intakes are expected for those elements for which water represents the main source of exposure.

Strontium values revealed important presence of this element both in forages and drinking water (Table 4). However, carry-over rates estimated < 0.1% should determine appreciable variation in milk only in presence of outliers in the feeding regimen.

Left censored data (LCD) in the occurrence of some elements in water and/or forages under the medium bound approach may bring overestimation in the intakes and consequently lower carry-over rates. Larger LOQs for some elements definitively hampered the possibility to estimate intakes and CORs for all the elements analyzed in buffalo milk.

Not for all considered farms there was a tight pair between milk, water, forages, and feed samples, because sampling was framed under different monitoring programs.

The following confounder factors can be envisaged: (a) the presence of Mn, Co, Zn, Se in the mineral supplements routinely administered via buffalo feeding; (b) forages recovered from agriculture areas different from each farm settlement; (c) groundwater used for buffalo watering originating from geochemical springs different from that of the well location; and d) bulk milk as pool from possibly different farms.

Almost all the selected elements (Zn, V, Cu, Ni, Cr, As, Mn, Sr, Co, Fe, and Se) follow a log-norm distribution (significance level 0.05, not quantified values and outliers excluded) in hay ($N = 137$), thus suggesting forages rely on baseline values for the Region. In drinking water, Zn, As, Fe, and Se only show quantified data enabling to assess the presence of a log-norm distribution, with the exception of Cu. Therefore, the median values chosen to calculate carry-over rates seem more robust than those refereed to the mean.

4.1. Province-specific buffalo milk contamination pattern via factor analysis

Considering together the frequency of the mentioned farms with their pertinent province (Table 1) and the factor analysis shown in Fig. 2ab, two Factors (2 and 3) explaining a quote of 30.09% of the database variability on a total of explained variability of 75.6% could discriminate farms according to the provinces of origin and, possibly, to similar management practices at each farm level.

By overlapping the province specific pattern of bufaline milk samples with the baseline element agricultural soil maps (Fig. 1), we can see that 61% of the database variability characterizing mainly the Province of Caserta, does not appear to reflect the highest Sr, Mn, Co, Zn, and Se levels indicated by the pertinent maps. Indeed, all the aforesaid elements show a relatively wide diffusion of baseline values in the Campania Region. In addition, Cr levels showing at geochemical level a relative specificity for the Province of Salerno, still does not appear to influence specifically the milk from the pertinent farms (Fig. 2ab)

Cobalt and Vanadium levels in soil, showing a relative specificity for the Salerno milk samples (Fig. 1), suggest for Co, a possible minor influence of the “geochemical factor” also because the Factor Analysis distinguishes two contributions orthogonal each other, only for Co: one can be due to a common influence between the farms such as Co administration to buffaloes *via* the mineral supplements; and another one, linked to Factor 3 (Fig. 2b), characterizing mainly the farms from the Salerno Province (See Supplementary Materials). In the pertinent map (Fig. 1), the reported highest Co levels in soils of the Province of Salerno appear to be consistent with the localization of the farms and the related milk pattern.

For the other farms whose variability is not explained (24.2%), the strong presence of confounding factors can be envisaged:

Occurrence of selected elements in local forages and water.

Despite their relevance in the transfer of elements to food commodities, for forages there are not so consolidated database as those available for food commodities. The ability of some fodders such as *Zea mais* to bio-remediate metal polluted soils [31] however highlights the relevance of the environmental quality of agriculture soil to guarantee both animal welfare and safe products. It is worth noting that the highest concentration found in corn silage and hay (Tables 4, 2S) are targeted on Cu and Zn, two elements that in their organic forms are currently used as fertilizer and pesticide in agriculture, respectively. Hay contamination could also acknowledge the contribution of top soil, as matter of the very ground-level cutting height of fodders at the harvesting. Therefore, for those elements, where hay represents one of the main sources of intake, such as Sr, V, and Cu (Table 3S) we could expect also a contribution from the associated soil contamination in addition to that represented by fodder uptake.

Carry-over rates

According to the carry-over rates (%) computed on mean and median values from milk excretion, and animal nutrition intakes, differences in the environmental pressure from As (38; 93), Se (31; 40), Cr (22; 40); Hg: (15; 43), Ni (13; 15), should be more easily reflected in milk, with respect to those from Sr (0.05) Zn (3.8; 3.7), Co (3.4), Pb (2.3; 1.0), Cu (1.3; 1.3), V (0.07; 0.13), A (0.02; 0.07), Be (0.02,; 0.07), Fe (0.01; 0.02) and Sn (<0.01; 0.02), respectively (Table 5).

With respect to the factor analysis (Table 5) and matching the geochemical pattern of agriculture soil in Campania, it seems reliable that for Co, even in presence of COR of 3.4% the difference geochemistry of agriculture soil (Fig. 1) is reflected in milk samples according to the Factor Analysis (Table 2, Factor 1 and 3). On the contrary, this seems not the case of Zn (COR% 3.8), because considered farms are dislocated in almost the same baseline areas. Despite larger Carry-Over Rates, Cr is not identified as a factor to explain observed variability among milk samples, while Se partially.

Comparison of elements occurrence in buffalo milk with respect to literature data.

On mean and median values, As, Pb, Cd, Zn, Cu, and Co concentration found in the 69 buffalo milk samples and water are aligned with most of the baseline values reported in three different Provinces of the RPC (Zhou et al., 2017; Chou et al., 2020 (C) (Table 6) and in the literature (Table 4S in the Supplementary Materials), while Cr and Ni ones fall in the upper range of the inventoried data. This could be explained with the location of most of the buffalo farms in area with Cr and Ni in the 22–45 and 18–42 mg kg^{-1} range in agriculture top soil, as matter of the volcanic geochemistry of the Region, and of the good carry-over rates to milk above reported (Fig. 1).

5. Conclusion

In this work we explore if the broadening of the panel of elements to be analyzed in buffalo dairy milk via ICP-MS could represent a tool for the georeferenced traceability, up to small scale level, in presence of regional baseline soil levels. This could be feasible in those farms where most of the forages production is autochthonous. To this respect, mineral supplements in the feeding regimen represent the main confounding factor. Because the easy implementation with sustainable costs, the ICP-MS analysis represent also an useful tool to monitor the time trends of legacy heavy metals in milk (Pb) and of other elements of toxicological and nutritional interest. The opportunity to link the dairy milk from local family farms to the territory would support sustainable goals related to food safety& food security, environment, social and economic issues. In the near future, the same factor analysis approach will be applied to elements in other feed and food commodities of plant origin, to strength the link between the soil geochemistry and Campania Region local productions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Giuseppe Rofrano: Conceptualization, Methodology, Visualization, Investigation, Writing – review & editing. **Mauro Esposito:** Supervision, Writing – review & editing. **Antonio Pizzolante:** Data curation. **Roberto Miniero:** Software, Validation. **Amalia Danese:** Formal analysis, Visualization, Investigation. **Daniel Signorelli:** Software, Validation, Data curation. **Luigi Jacopo D'Auria:** Formal analysis. **Alfonso Gallo:** Formal analysis. **Antonio Di Stasio:** Formal analysis. **Pasquale Gallo:** Supervision. **Pellegrino Cerino:** Conceptualization, Methodology. **Gianfranco Brambilla:** Conceptualization, Methodology, Writing – review & editing.

Acknowledgements

We acknowledge the Campania Region for the support in this research activity (PAC Funding - Action B4).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jtemin.2023.100046.

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