



Industrial impact on sustainable dairy farms: Essential elements, hazardous metals and polycyclic aromatic hydrocarbons in forage and cow's milk

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ABSTRACT

Sustainable dairy farms are characterised by the self-production of forage for animal feed. These farms are sometimes located near industrial areas, entailing a risk of food chain contamination with hazardous metals and polycyclic aromatic hydrocarbons (PAHs). Accordingly, evaluating the impact of pollution on forage and milk is of great interest. In this study, the effects of industrial factors on sustainable forage from 43 dairy farms and possible correlations between inorganic elements and PAHs were studied. Spearman's correlation and principal component analysis (PCA) were performed for the forage and milk. Most of the inorganic elements in the forage were below the maximum residual limits for cadmium (Cd) and lead (Pb), established in EU 2013/1275 and EU 2019/1869, respectively. However, arsenic (As) and mercury (Hg) levels were above their respective limits in the forage (EU 2019/1869). No milk samples exceeded the maximum residual limits for Pb (EU 488/2014) or Cd (EU 1881/2006) in dairy products. Heavy-weight PAHs (HW-PAHs, four or more aromatic rings) were detected in forage but not in milk. In the forage samples, HW-PAHs were positively correlated with Zn and Cd. In addition, some hazardous metals (chromium (Cr), iron (Fe), As, Hg, and Pb) also were positively correlated with Zn and Cd. Interestingly, no correlations were found between forage pollutants and milk, suggesting that these pollutants have a low transfer rate to milk. The PCA results highlighted the predominant contribution of PAHs to the global variance in forage samples collected at different distances from industrial areas. In milk, the contributions of hazardous metals and PAHs were more balanced than in forages. Finally, when distances to potential pollution sources were included in the PCA of forage samples, a negative correlation was observed between the former and the concentrations of HW-PAHs, Cd, and Zn, suggesting that thermal power plants and steel factory emissions were the main sources of polluting forage in this area.

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1. Introduction

Hazardous metals (HMs) and polycyclic aromatic hydrocarbons (PAHs) are among the most abundant pollutants in atmospheric particulate matter (PM) [1] and often coexist in industrial areas, causing a severe threat to both human health and the environment [2]. Different types of anthropogenic sources, such as steelmaking factories [3,4], petrochemical industries [5,6], motor vehicle exhaust [6,7], coal refineries [8,9], and waste burning [10], are responsible for the combined emission of HMs and PAHs.

Furthermore, the simultaneous accumulation of HMs and PAHs in soil and environmental matrices is drawing increasing attention globally because human co-exposure to PAHs and toxic metals may result in synergistic toxic biological effects such as DNA oxidative damage [8]. In particular, HMs and metalloids, such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg), are toxic to humans and animals. Previous researchers have evaluated the risk to human health of these metals, and found that Zn and Cr in soil and dust falls increase the probability of cancer by up to 50% [11,12]. They are not biodegradable and can form free radicals that alter DNA and lipid peroxidation, eventually causing carcinogenic processes, autoimmune inflammation, and tissue toxicity in the nervous system, liver and kidneys [13]. Furthermore, owing to their chemical similarity, some HMs can displace essential elements (EEs) in organisms (i.e. sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca)), causing neuronal diseases [14]. Organisms can metabolise PAHs to form more soluble hydroxylated derivatives, which are more reactive, bind proteins and DNA, and cause cancer [15]. Although more than 200 PAHs are known, only 16 are listed as priority pollutants by the US Environmental Protection Agency [16].

Over the last few decades, a significant increase in industrial activities has promoted the emission of these pollutants into the agricultural environment. HMs and PAHs enter farm environments via similar mechanisms. HMs are transported into the atmosphere as gases, aerosols, and particulates. Volatile metalloids such as selenium (Se), Hg, and As can be transported in gaseous form or enriched in particles, whereas other metals such as copper (Cu), Pb, and Zn are only transported as particles [2]. Several studies have found increased HMs levels in soils, grasslands, and milk of dairy farms located in industrial areas [17–19] because grazing ruminants have been shown to accumulate hazardous metals [20,21]. In addition, HMs inhibit the uptake and distribution of EEs in plants [22], worsening the nutritional quality of animal feed. PAHs, in turn, are directed towards the soil and plant surfaces in the gaseous phase or as atmospheric PM [2]; therefore, PAH contamination of plant crops occurs mainly through atmospheric deposition, with root uptake being a minor route of contamination [23]. Under these circumstances, lactating ruminants may also be exposed to PAHs when polluted forage is ingested during grazing, whereas inhalation and skin absorption are less important [23]. Various studies have detected total PAH concentrations ranging from 120 to 950 $\mu\text{g kg}^{-1}$ in grass samples collected near roads and highways [24,25]. Furthermore, PAHs have been detected in milk, but at lower concentrations than in grasslands [26,27].

The association between metals and PAHs is another health risk that must be considered. Previous studies have revealed that the interactions between organic and inorganic compounds depend on pollutant sources and sinks [28]. Organic and inorganic contaminants in a specific area often originate from the same source and have similar absorption mechanisms, which can induce synergistic or antagonistic effects. Notably, these links between contaminants are responsible for human diseases, for example, Cd and Zn are associated with chronic obstructive pulmonary disease (COPD) [29].

According to EU policies, ensuring the sustainability of farms that harness natural resources is essential. Milk production in northern Spain is characterised by small local dairy farms which use their own resources in the form of forage produced on the farm [30]. In this context, dairy cows are highly dependent on their surrounding environment, which are sometimes near industrial areas, highways, and other pollution sources. Therefore, the surrounding pollution may impact the farm environment, increasing the risk of food chain contamination. Some studies have characterised the pollution levels in farms in this highly industrialised area of Spain, but they only addressed toxic metals in milk [31] or in cattle tissues [32] and did not offer an integral vision and correlations between HMs, essential minerals (EMs), and PAHs along the different matrices of the farm production chain. Although physicochemical and microbiological safety controls are well established in large-scale food industries through local and national food regulations, safety controls are sometimes overlooked. The nutritive value of forage-based dairy productions and the impact of over-industrialised areas on food security has been evaluated by exhaustive quantification of EEs, HMs, and PAHs in forage and milk samples from sustainable dairy farms which practice traditional management of land resources. However, correlation studies between these elements have not been conducted. The aim of this study was to determine the interaction of individual and combined pollutants in rural areas next to industrial zones and to develop and efficiently evaluate the risk zones of inorganic, organic, or combined pollutants. Subsequently, a comparative assessment of the safety of forage production, possible relationships between inorganic elements (HMs and EMs) and organic pollutants (PAHs), and the effects of potential sources of pollution in the farm environment was conducted.

2. Material and methods

2.1. Sample collection and storage

Forage (grass forage, silage, or total mixed ration depending on the farm stock) and milk samples were collected from 43 dairy farms in northern Spain located various distances from the pollution source (Table S1, Supplementary Information). These farms were near industrial facilities that were exposed to metal and PAH contamination. The criteria for farm selection were as follows: (1) small dairy farms (no more than 40 heads of cattle) with forage production and (2) close location (<20 km) to industries, roads, and other anthropogenic activities. After being homogenised in a mix cart, forage was collected from the trough where the cattle were fed. Milk was collected directly from the tanks to minimise variation between individuals from the same farm.

Freshly collected forage samples were freeze-dried (Coolsafe Pro 100-9 freeze-dry system, Labogene, Allerød, Denmark) at $-95\text{ }^{\circ}\text{C}$

for 2–3 days, ground, and stored in the dark at room temperature until analysis. Similarly, milk samples were lyophilised under the same conditions as the forage, but for 7 days or more, owing to their higher water content in comparison with the forage samples. Milk powder samples were stored in a -80°C freezer until analysis.

2.2. Determination of inorganic elements in forage and milk by ICP-MS

Forage or milk powder (0.5 g) was digested in 2 mL nitric acid (Suprapur analytical grade HNO_3 , 65 %, Merck, Germany) and 6 mL hydrochloric acid (Suprapur analytical grade HCl , 37 %, Merck, Germany) in closed polytetrafluoroethylene vessels using an Ethos One microwave digestion system (Milestone Shelton, CO, USA) according to conditions described in Table S2 (see Supplementary Information). The obtained solution was filtered through $0.22\ \mu\text{m}$ syringe filters and diluted to a final volume of 40 mL with ultrapure water (1:80 dilutions for forage and milk samples).

Samples were analysed using an Agilent 7800 ICP-MS equipped with an SPS4 autosampler and an Integrated Sample Introduction System (ISIS3, Agilent, Santa Clara, CA, USA). Measurement conditions were validated daily with a calibration solution ($1\ \mu\text{g L}^{-1}$ each of Li, Mg, Y, Ce, Tl, and Co in 2 % HNO_3 ; Agilent, Santa Clara, CA, USA) to improve sensitivity.

Na, K, Mg, and Ca Certified ICP-MS multi-element calibration solutions ($1\ \text{g L}^{-1}$, 1 % nitric acid, HPS, North Charleston, USA) and Cr, Zn, Fe, Cu, As, Se, Cd, and Pb Certified ICP-MS elemental calibration solutions ($1\ \text{mg L}^{-1}$, 1 % nitric acid, HPS, North Charleston, USA), which were prepared by dilution in 1 % nitric acid, were used as standard solutions. All dilutions were performed with ultrapure water ($\geq 18\ \text{M}\Omega\text{-cm}$ resistivity; $\leq 5\ \mu\text{g L}^{-1}$ TOC) obtained from a Milli-Q IQ 7000 purification system (Merck Millipore, Darmstadt, Germany).

^{45}Sc , ^{72}Ge , ^{103}Rh , and ^{193}Ir multi-elemental standard solutions were used as internal standards (ISTD) and injected simultaneously with the sample. The selected ISTD were: ^{45}Sc for Na, K, Ca, and Mg; ^{72}Ge for Cr, Fe, Cu, Zn, Se, and As; ^{103}Rh for Cd; and ^{193}Ir for Hg and Pb. The LOD and LOQ for each analyte quantified by ICP-MS were calculated as three and ten times the standard deviation (SD) of the ten blank samples, respectively (Table S3).

The analytical method was validated using the European Certified Reference Materials (ERMs): ERM-CD281 “Rye grass” and ERM-BD151 “Skimmed milk powder” (IRMM, Geel, Belgium). Some elements (whose concentration values were not provided by the manufacturer) were spiked into the ERMs aliquots to assess their recovery and validate the results. Accuracy quality control was performed by comparing reference and experimental values. Three aliquots of ERM were analysed to estimate precision, which was assessed using the relative standard deviation (RSD_r) of the ERM triplicates. Table S3 compares the certified values of ERM and those obtained using the proposed methodology. Recoveries from rye grass ranged from 93 % for Cd to 114 % for Ca, whereas those from skimmed milk powder ranged from 88 % for Pb to 113 % for Cr. The inorganic element content of the forage samples was based on dry weight (DW). For milk samples, it was based on wet weight (WW) after applying a correction factor based on the mean water content of milk samples (88 %).

2.3. Determination of polycyclic aromatic hydrocarbons in forage and milk by GC-MS/MS

Samples were treated according to the QuEChERS extraction method with some modifications. Briefly, 10 g of the sample was weighed in a 50-mL centrifuge tube, and 30 mL of acetonitrile was added and vortexed at 3000 rpm for 1 min. A total of 4 g anhydrous MgSO_4 and 1 g NaCl were added and immediately mixed using a vortex mixer for 1 min, followed by adding $50\ \mu\text{L}$ of an ISTD solution and mixing using a vortex mixer for another 30 s. The extracted mixture was centrifuged at 5000 rpm for 5 min. The supernatant (10 mL) was purified using dispersive solid-phase extraction (dSPE). A total of 5 mL of the supernatant was transferred to a flat-bottomed flask and concentrated in a 40°C water bath until it was almost dry. The concentrated sample was resuspended in 5 mL of cyclohexane [33].

An aliquot of $1\ \mu\text{L}$ was injected into a 7890B gas chromatograph equipped with a Select PAH CP7462 capillary column ($0.15\ \mu\text{m}$ film thickness, $0.25\ \text{mm} \times 30\ \text{m}$) and 7000D mass spectrometer detector (Agilent, Santa Clara, CA, USA). In each sample, 11 PAHs were identified: phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo(a)pyrene, indeno [1,2,3-c,d]pyrene, dibenz[a,h]anthracene, and benz[g,h,i]perylene. Certified standards of the individual compounds ($2\ \text{mg mL}^{-1}$ in cyclohexane; LGC Standards, Germany) were used to construct the external calibration curve. The method established in this study was validated using four different ISTDs (phenanthrene- d^{10} , chrysene- d^{10} , perylene- d^{12} and dibenzo[a,h]anthracene- d^{14} , $100\ \mu\text{g mL}^{-1}$ in cyclohexane; LGC Standards, Germany) which were added to each sample extract. The concentrations of the standards and samples were determined by comparing the standard peak areas with those of the internal standard. The concentrations of PAH obtained from the forage samples were based on DW. For milk samples, it was based on WW after applying a correction factor based on the mean water content of the milk samples (88 %).

2.4. Data treatment and statistical analysis

The mean, range (minimum and maximum values), standard deviation, and median of all observations for all parameters were calculated for forage and milk. The non-detected observations for a given parameter (named censored data) were imputed as $\text{LOD}/\sqrt{2}$, where LOD is the limit of detection of the parameter. This is a common method for dealing with low-to-mid-degree censored datasets [34,35]. The degree of skewness was calculated to verify the symmetry of the data [36]. Because the data were skewed for most pollutants (Fig. S1), the dataset was log-transformed before performing subsequent statistical analyses to approximate the data into a normal distribution. Bivariate correlation analyses were performed using the non-parametric Spearman’s method. A significance test,

at 99 % confidence level, was also conducted. In addition, the chemicals were grouped using hierarchical clustering based on the distance matrix $(1 - r)$, where r is the matrix of correlations between the log-transformed concentrations of chemicals. Ward's hierarchical agglomerative clustering algorithm [37,38] was used (i.e. the HCLUST function with the ward.D2 method in R). A principal component analysis (PCA) was also performed to reduce the dimensions of the data to try and spot patterns from the plots. Briefly, PCA constructs a new coordinate system formed by latent variables that are orthogonal and use only the most informative dimensions are used [36].

3. Results

The farms were located next to steel factories, chemical factories, and thermal power plants and near several highways and railways that provide logistics services to these industries. It was impossible to quantify each parameter's contribution to the studied parameters (inorganic elements and PAHs) based on industrial emissions. However, Figs. 1 and 2 show the median values of EEs, HMs, and PAHs in farms located at distances of less than or greater than 5 km.

As shown in Fig. 1, most of the studied EEs, Cu, Zn, Se and Cr, were below the maximum tolerable limits in animal feed [39]. Only Fe was an exception, with values higher than 500 mg kg^{-1} in forages from farms near an industry. With regard to HMs, it is important to point out that the median content was higher in farms next to industry than in those farther away. The widest gap between the near and far values in the forage was observed for Cd and Hg (Fig. 1). Similar results were obtained when analysing PAHs in forage. Furthermore, results showed that PAHs with more than four aromatic rings were enriched in forage collected near industries.

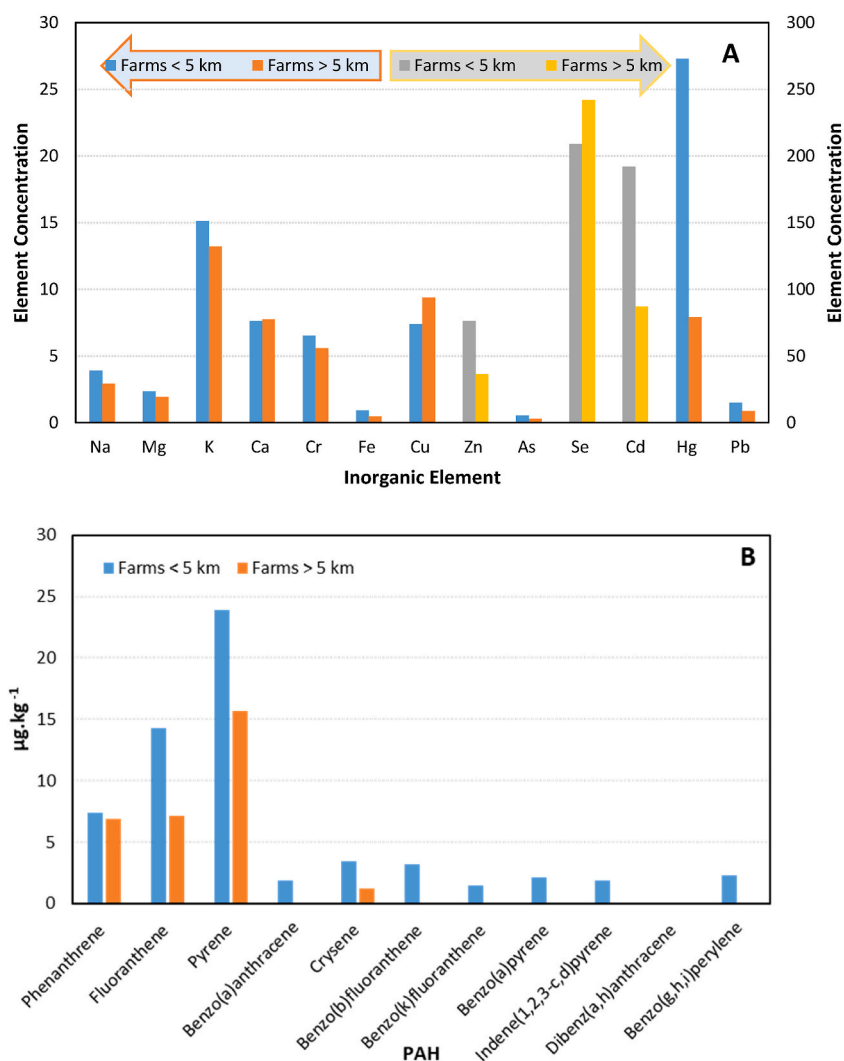


Fig. 1. Effect of distance from farm to pollution point sources into forages: A) Median of inorganic elements contents; B) Median of PAHs content. Concentration units: Na (g kg^{-1}); Mg (g kg^{-1}); K (g kg^{-1}); Ca (g kg^{-1}); Cr (mg kg^{-1}); Fe (g kg^{-1}); Cu (mg kg^{-1}); Zn (mg kg^{-1}); As (mg kg^{-1}); Se ($\mu\text{g kg}^{-1}$); Cd ($\mu\text{g kg}^{-1}$); Hg ($\mu\text{g kg}^{-1}$); Pb (mg kg^{-1}).

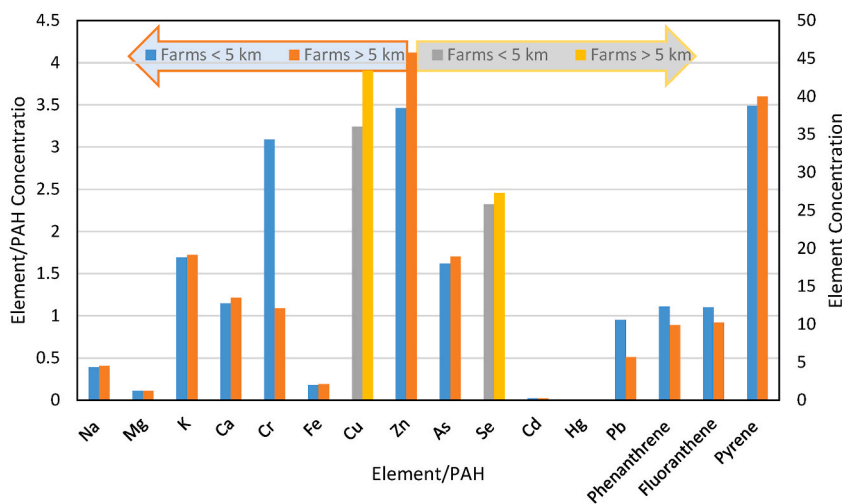


Fig. 2. Effect of distance from farm to pollution point sources into milk. Median of inorganic and PAHs content. Concentration units: Na (g kg^{-1}); Mg (g kg^{-1}); K (g kg^{-1}); Ca (g kg^{-1}); Cr ($\mu\text{g kg}^{-1}$); Fe (mg kg^{-1}); Cu ($\mu\text{g kg}^{-1}$); Zn (mg kg^{-1}); As ($\mu\text{g kg}^{-1}$); Se ($\mu\text{g kg}^{-1}$); Cd ($\mu\text{g kg}^{-1}$); Hg ($\mu\text{g kg}^{-1}$); Pb ($\mu\text{g kg}^{-1}$); PAHs ($\mu\text{g kg}^{-1}$).

No values above the maximum permitted level were observed in the milk samples. However, for Hg and Pb, an enrichment of 50 % confirmed the effect of distance to the pollution source point (Fig. 2). In contrast, the PAHs content in farther farms was slightly above the median of the nearby farms. The following sections detail and discuss the correlations between the obtained data.

3.1. Essential elements in forage and milk

The inorganic elements in the forage samples (HMs and EMs) are listed in Table 1. The EE concentrations were distributed normally throughout the forage sample population. For each EE, the concentrations ranges found in the forages were 0.2–6.5, 1.2–4.1, 4.9–40.9 and 0.3–16 g kg^{-1} for Na, Mg, K and Ca, respectively. Regarding essential trace minerals, the concentration ranges were 0.1–7.6 g kg^{-1} for Fe and 0.96–32.8, 4.4–22.8, 23–216 and 0.04–1.08 mg kg^{-1} for Cr, Cu, Zn, and Se, respectively.

The mineral composition of the analysed milk is presented in Table 2. Na, Mg, K, and Ca were found at the following concentrations: 287–519, 84–152, 1397–1967, and 996–1531 mg kg^{-1} , respectively. Trace minerals were detected in lower concentrations: 0.07–1.23, 0.02–0.07, 2.93–5.57 and 0.01–0.06 mg kg^{-1} for Fe, Cu, Zn, and Se were detected at low concentrations.

3.2. Hazardous metals in forage and milk

As shown in Table 1, the concentrations of the HMs in forage were 0.06–7.87, 0.01–0.86, 0.03–0.47 and 0.20–7.85 mg kg^{-1} for As, Cd, Hg and Pb. In contrast to EEs, the HMs (As, Cd, and Hg) and trace mineral (Cr and Fe) concentrations were positively skewed in the forage. In milk, HMs are found at trace levels, some of which are not detected in many samples. Cr was below LOD in nine samples, Cd in five samples, and Pb in one sample. Furthermore, Hg was only found in 14 of the 43 samples analysed. The maximum contents of these elements were 16 and 74 $\mu\text{g kg}^{-1}$ for As and Cr and 0.12, 0.28 and 2.70 $\mu\text{g kg}^{-1}$ for Cd, Hg, and Pb, respectively (Table 2). Similar

Table 1
Concentration of inorganic elements (mg kg^{-1} DW) in forage samples (N = 43).

Element	Min	Max	Mean	SD	Median	Skew	MRL ^a
Na	200	6475	3239	1643	3156	−0.03	–
Mg	1197	4149	2301	653	2300	0.46	–
K	4908	40951	16703	7475	13752	1.43	–
Ca	354	16046	7927	2145	7628	1.06	–
Cr	0.96	32.8	7.75	6.5	6.07	2.06	–
Fe	115	7630	1034	1174	817	4.36	–
Cu	4.4	22.8	9.6	4.6	7.5	0.91	–
Zn	22.99	216	71.05	46.99	62	1.49	–
As	0.06	7.87	0.82	1.3	0.42	4.21	2
Se	0.04	1.08	0.29	0.24	0.22	1.42	–
Cd	0.01	0.86	0.20	0.27	0.11	2.24	1
Hg	0.03	0.47	0.04	0.07	0.02	5.53	0.1
Pb	0.2	7.85	1.68	1.62	1.1	1.81	10

^a Maximum Residual Limit established in EU 2019/1869 (As, Hg, Pb) and EU 1275/2013 (Cd).

Table 2
Concentration (mg kg⁻¹ WW) of inorganic elements in bovine milk (N = 43).

Element	Min	Max	Mean	SD	Median	Skew	N < LOD ^b	MRL
Na	287	519	397	48	403	0.18	0	–
Mg	84	152	111	10	110	1.14	0	–
K	1397	1967	1683	127	1700	–0.39	0	–
Ca	996	1531	1181	82	1185	1.14	0	–
Cr	n.d.	0.074	0.007	0.015	0.002	3.18	9	–
Fe	0.070	1.23	0.26	0.268	0.183	3.02	0	–
Cu	0.02	0.074	0.039	0.011	0.04	1	0	–
Zn	2.93	5.57	3.72	0.51	3.67	0.99	0	–
As	0.001	0.016	0.002	0.002	0.002	4.72	0	–
Se	0.013	0.056	0.029	0.011	0.027	0.99	0	–
Cd ^a	n.d.	0.12	0.022	0.02	0.019	2.75	5	5 ^c
Hg ^a	n.d.	0.28	0.021	0.054	0.001	3.46	29	–
Pb ^a	n.d.	2.7	0.92	0.63	0.7	1.02	1	20 ^d

^a Concentration expressed in µg kg⁻¹ WW.

^b Samples below LOD were imputed as LOD/√2.

^c Maximum Residual Level established in EU 488/2014.

^d Maximum Residual Level established in EC 1881/2006.

to the forage, most of the toxic elements (As, Cd, Cr, and Hg) exhibited a right-skewed distribution in milk, in contrast to the more centred distributions of the EMs.

3.3. Polycyclic aromatic hydrocarbons in forage and milk

The results for the PAHs content in the forage samples are detailed in Table 3. It is also important to distinguish between low-weight PAHs (LW PAHs, 3–4 aromatic rings) which include phenanthrene, fluoranthene, and pyrene, and high-weight PAHs (HW PAHs, more than four aromatic rings), which include benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno [1,2,3-*c*,*c*]pyrene, dibenz[*a*,*h*]anthracene and benz[*g*, *h*, *i*]perylene. In general, 3 and 4 aromatic ring PAHs were present in higher amounts in forages than heavier molecular weight PAHs with five or six aromatic rings. Phenanthrene, fluoranthene and pyrene concentration ranges in forages were respectively 3.3–14.7, 1.8–37.4 and 1.1–120 µg kg⁻¹, and they were found in all samples. The maximum concentration of LW PAHs in the forage was 120 mg kg⁻¹, which was quantified in a forage sample collected from a farm located 4 km away from the steel industry. HW PAHs were detected in approximately half of the sample set, and their mean concentrations were lower than those of the LW PAHs. Furthermore, a significant right skew was observed for HW PAHs. The maximum concentration of HW-PAHs was observed in a forage sample produced on a farm at 0.4 km from a steel industry (sum of HW PAH = 329.7 µg kg⁻¹).

The PAH contents of milk are shown in Table 4. As can be seen, only phenanthrene, fluoranthene and pyrene (LW PAHs) were detected in the concentration ranges of 0.16–3.55, 0.22–7.22 and 0.43–39.4 µg kg⁻¹, respectively. Again, the dataset was right-skewed because there were punctual observations with high concentrations of certain PAHs.

3.4. Evaluation of relationships between inorganic elements and PAHs in forages and milk

Fig. 3 shows Spearman's correlation plot between pairs of variables (bivariate correlation plot). The level of correlation is scaled from blue to red for positive and negative correlations, respectively. Nonsignificant correlations ($p > 0.01$) were left blank. In addition, black rectangles were used to group the chemicals according to Ward's D2 hierarchical clustering.

Some of the essential minerals in milk (Na, Mg, Ca, Cu, and Zn) were clustered together. Another remarkable correlation cluster

Table 3
Concentration of PAHs (µg kg⁻¹ DW) in forage samples (N = 43).

Compound	N aromatic rings	Min	Max	Mean	SD	Median	Skew	N < LOD ^a
Phenanthrene	3	3.3	14.7	7.3	2.5	7.1	0.7	0
Fluoranthene	3	1.8	37.4	13.3	8.7	10.6	0.89	0
Pyrene	4	1.1	120	29.7	28.7	19.6	1.49	0
Benz[<i>a</i>]anthracene	4	n.d.	31.3	2.2	4.6	1	5.99	19
Chrysene	4	n.d.	51.9	3.9	7.7	2	5.68	4
Benzo[<i>b</i>]fluoranthene	5	n.d.	71.3	4.5	10.6	1.9	5.86	11
Benzo[<i>k</i>]fluoranthene	5	n.d.	30	2	4.4	0.8	5.97	22
Benzo[<i>a</i>]pyrene	5	n.d.	48.7	2.8	7.2	1.1	6.12	19
Indeno [1,2,3- <i>c</i> , <i>d</i>] pyrene	5	n.d.	41.6	2.6	6.1	1.1	6.06	14
Dibenz[<i>a</i> , <i>h</i>]anthracene	6	n.d.	10.2	0.9	1.4	0.6	6.37	37
Benz[<i>g</i> , <i>h</i> , <i>i</i>]perylene	6	n.d.	54.9	3.1	8.1	1.2	6.2	15

^a Samples below LOD were imputed as LOD/√2.

Table 4
Concentration of different PAHs ($\mu\text{g kg}^{-1}$ WW) in bovine milk (N = 43).

Compound	Min	Max	Mean	SD	Median	Skew
Phenanthrene	0.16	3.55	1.2	0.6	1.1	1.8
Fluoranthene	0.22	7.22	1.7	1.9	1	1.9
Pyrene	0.43	39.36	7	8.7	3.6	2.3

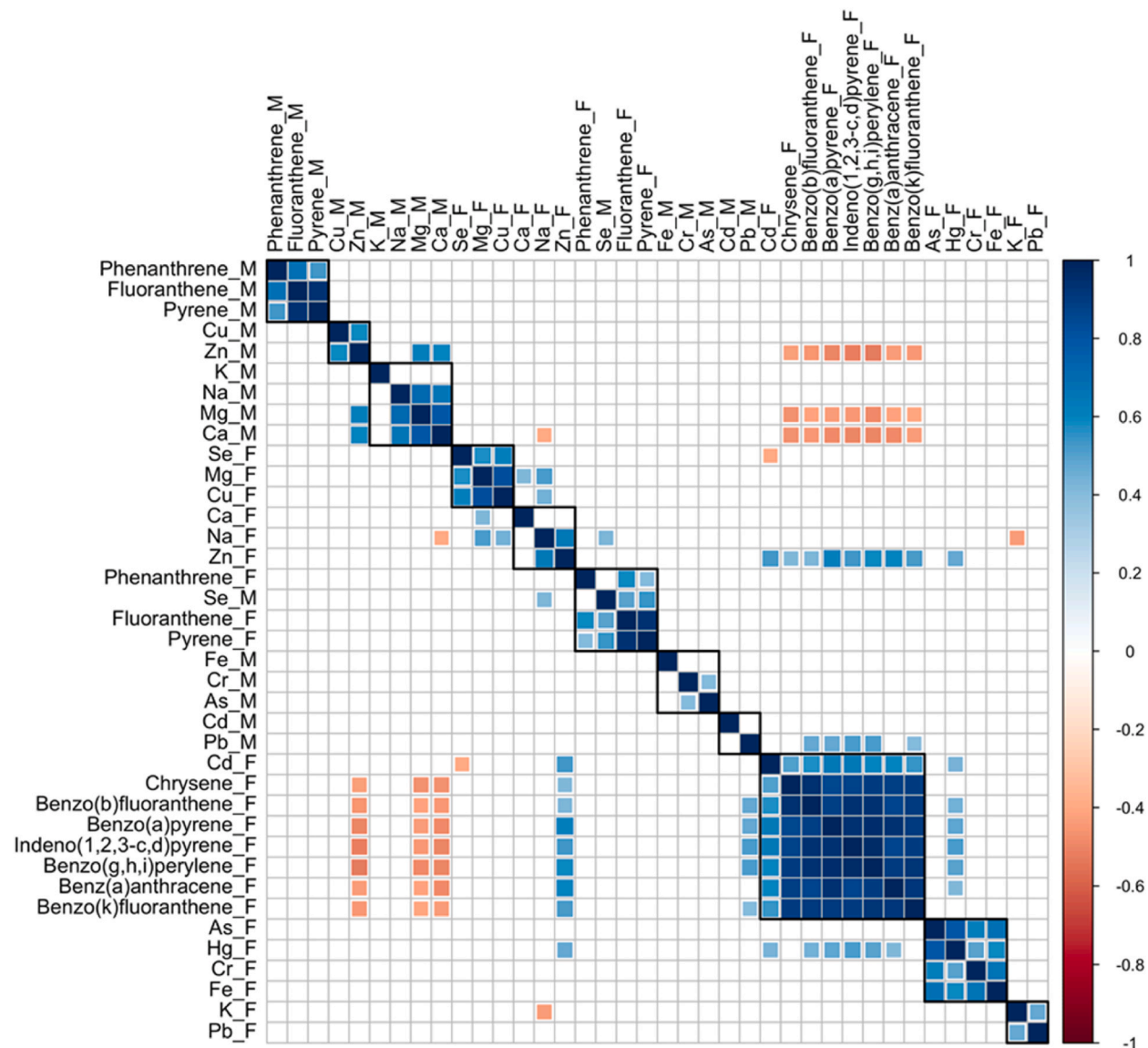


Fig. 3. Spearman's correlation plot for all metals and PAHs in both milk and cattle feed. Only significant correlations are shown ($p < 0.01$). F: forage, M: milk.

occurred in the forage containing HW PAHs, which also included three inorganic elements: Zn, Cd, and Hg. These elements positively correlated with HW PAHs; however, they did not cluster together. In addition, Pb in milk was positively correlated with HW PAHs in forage. The LW PAHs (phenanthrene, fluoranthene, and pyrene) were grouped in their respective clusters in forage and milk. In both matrices, pyrene and fluoranthene exhibited higher correlation levels than phenanthrene. Fig. 3 also shows high correlation levels between several hazardous metals such as Cr, Fe, As, and Hg in the forage, which were clustered together. However, Pb was not significantly correlated with other hazardous metals in the forage. Unlike forage, milk did not contain intra-correlated HMs, probably because of the negligible presence of these metals. However, a slightly significant correlation ($\rho = 0.41$) between Cr and As was

observed in milk. Furthermore, there were also non-clustered but significant negative correlations between the essential elements of milk (Mg, Ca, and Zn) and the HW PAHs in forage. Finally, no significant forage-milk correlation was found. After obtaining these results, PCA analyses were performed separately for each matrix.

3.5. Principal component analysis in forage and milk samples

The results of the PCA of the forage samples are summarised in Fig. 4. Seven dimensions (principal components) have been selected to cover almost 96 % of the total variance. As can be observed from the correlation circle plot (Fig. 4A), HW PAHs contributed to the first dimension in the same direction and had a major influence on the variability of the samples (49 %). As mentioned above, Cd was significantly correlated with the HW PAHs; therefore, it was again co-correlated in the first dimension. The second dimension had less influence on global variance (18.7 %), and it consisted of HMs (As, Hg), Cr, and Fe, ordered by level of correlation. Fig. 4B shows the correlation coefficients between the variables (pollutants) and dimensions (principal components). The percentage variability covered by each dimension is shown in Fig. 4B. As can be seen, the first two dimensions covered 68 % of the total variance, which suggests that pollutants, in particularly persistent organic pollutants such as HW PAHs, could significantly influence forage collected from these farms reared in an industrial area. Other less influential dimensions should also be appreciated. Dimension 3 (12.4 % explained variance) was formed by LW PAHs (with phenanthrene and fluoranthene having the main contributions), while dimension 4 (6.3 % variance explained) was solely composed of lead.

PCA of LW PAHs and HMs was also performed in milk. The results are shown in Fig. 5. The first dimension was mainly composed of LW PAHs, Cr, and Fe to a lesser extent. This first component explains approximately 35 % of the global variance, which could imply a lower contribution of organic pollutants to variability between milk samples compared to the forage samples. Concerning the second component, while Cr and Fe were still positively correlated, MW PAHs (MW PAHs: 3–4 aromatic rings) were negatively correlated, but the most remarkable aspect was the high correlation of Cd and As with this second dimension, which explained 29.2 % of the variance.

3.6. Potential sources of pollution in the farm environment

Fig. 6 shows the correlation plot obtained for the two main dimensions from a PCA, including the distance to potential sources of pollution as an additional factor, with the purpose of studying the contribution of these sources to the presence of pollutants determined in the forage samples. The distance of farms from potential sources of contamination (steel and zinc factories and thermal power plants) influenced the variability of forage samples in the opposite way to the concentrations of HW-PAHs, Cd, and Zn.

4. Discussion

4.1. Essential elements in forage and milk

EEs were generally present at higher concentrations in the forage samples than in milk. The concentrations of iron and copper were several orders of magnitude higher in forage than in milk, whereas the concentrations of essential macrominerals (Na, Ca, K, and Mg)

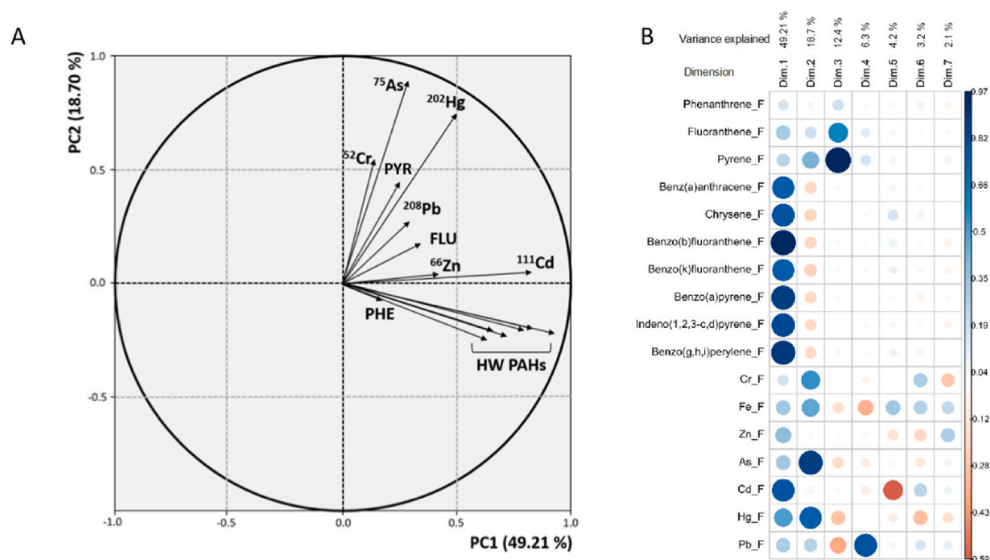


Fig. 4. PCA of pollutant variables in forage samples. (A) Correlation circle for the first two dimensions. (B) Correlation coefficients between variables and dimensions. PHE: phenanthrene, FLU: fluoranthene, PYR: pyrene, HW PAHs: heavy-weight polycyclic aromatic hydrocarbons, F: forage.

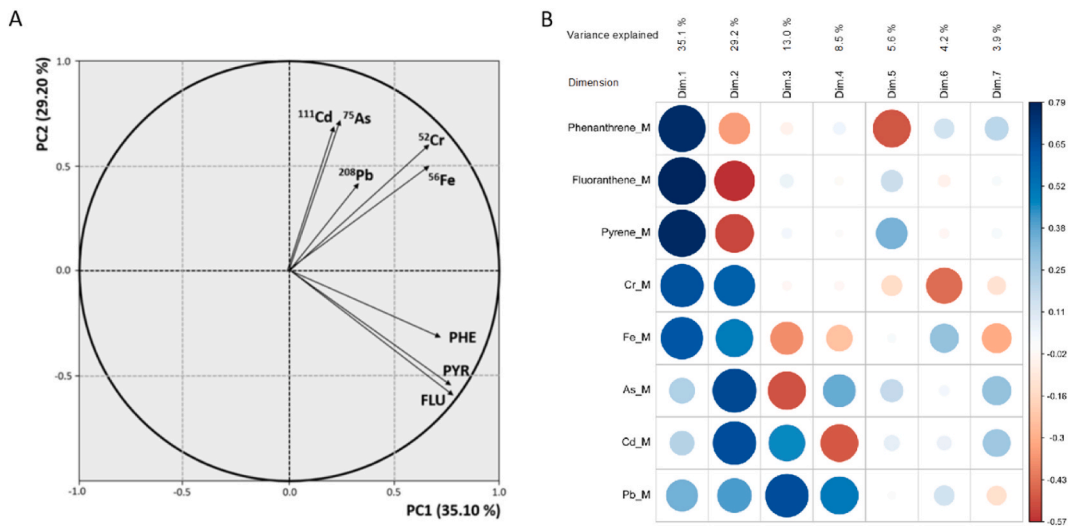


Fig. 5. PCA of pollutant variables in milk samples. (A) Correlation circle for the first two dimensions. (B) Correlation coefficients between variables and dimensions. PHE: Phenanthrene, FLU: fluoranthene, PYR: pyrene, M: milk.

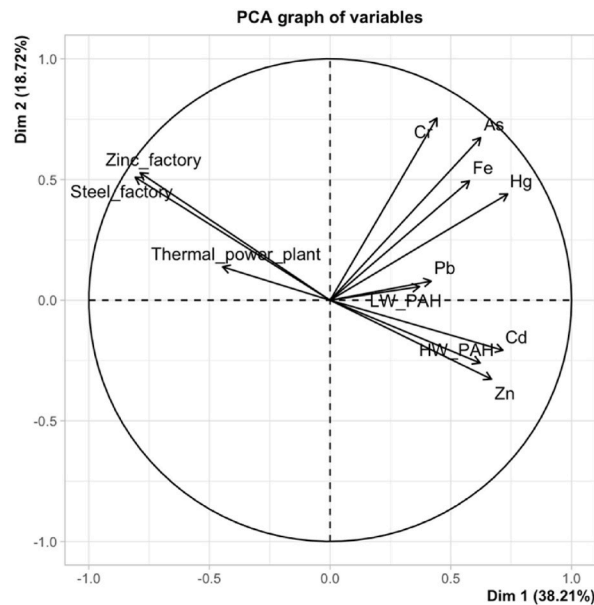


Fig. 6. Correlation circle for the first two dimensions of PCA of pollutant variables including distance to potential pollution sources in forage samples.

and Zn were of the same order of magnitude between milk and forage. These data indicate that milk is clearly a rich source of Ca, K, and Zn, but less satisfactory as a source of Fe or Cu [40]. The mean contents found in the forage (Table 1) complied with the optimal concentrations recommended for animal production [39,40] and with the usual levels previously reported in the literature [41–43]. The essential mineral composition of the milk samples (Table 2) also agreed with the typical levels found in commercial [44,45] and unprocessed farm milk [40,46,47].

Cr, Fe, Cu, Zn, and Se are also essential trace elements for cattle nutrition; however, at high concentrations, they can be toxic to animals (i.e. Cr or Fe) [48]. Accordingly, the National Research Council of the USA [39] proposed the following maximum tolerable concentrations of these microelements relative to cattle nutrition: 1000 mg kg⁻¹ for Cr, 40 mg kg⁻¹ for Cu, 500 mg kg⁻¹ for Fe, 500 mg kg⁻¹ for Zn, and 5 mg kg⁻¹ for Se. In this study, the trace mineral content of the analysed forage (Table 1) was below the above-mentioned limits, except for Fe, whose mean doubled the maximum tolerable limit for cattle. In fact, the median Fe concentration was five times higher than the usual concentration of Fe reported for grass forage [49]. This notorious presence of Fe may be due to the contribution of the steel industry to dairy farms. The Cr and Cu concentrations were in line with previously reported levels in forage

from conventional farms in England and Wales [50], but the median concentration of Zn was higher than that reported by Nicholson et al. [50], even in forage from farms near industrial areas [17,18]. Unlike forage, the zinc content in milk (Table 2) was below the levels reported by Yasotha et al., in 2021 around industrial areas in India [17] and in the same range as reported in commercial milk from Spain [45]. However, the concentration of Se in milk was slightly higher than that reported by Sola-Larrañaga et al., in 2019 [45].

4.2. Hazardous metals in forage and milk

Concerning hazardous elements in the forage, the maximum residual limits established in EU regulation no. 2019/1869 [51] were exceeded for As and Hg but not for Pb, whereas the maximum residual limit for Cd established in EU regulation no. 1275/2013 [52] was not exceeded (Table 1). Nevertheless, the forages exceeding the limits are an exception because only two samples were above the limit for As (2,19 and 7,87 mg kg⁻¹) and two other samples for Hg (0,113 and 0,471 mg kg⁻¹). In line with this, Cd and Pb concentrations in forage were below some of the previously reported levels in forages produced in areas near industrial facilities [17,18], but slightly above the levels reported in conventional farms [50].

There were significant differences in the concentrations of hazardous elements in forage and milk. The concentrations of Cr, As, Cd, Hg, and Pb were orders of magnitude lower in milk than in forage. Previous studies have revealed that hazardous elements tend to accumulate in cow tissues such as the kidney or liver [20] rather than in milk, which is secreted daily by lactating cows, and thus its hazardous mineral content is diluted. None of the samples exceeded the maximum residual limit for Pb in raw milk, as regulated by EC no. 1881/2006 [53] or Cd established by EU no. 488/2014 [54]. In addition, the maximum levels of Cr, Cd, and Pb in milk were negligible in comparison with the levels previously reported in areas near industrial facilities in India [17,55] and Romania [18]; however, they were similar to those reported in China by Zhou et al., in 2019 [19]. In the same year, Gonzalez-Montaña et al. [31] quantified several hazardous minerals in bovine milk from farms located close to a mining zone in Asturias (north of Spain) with important industrial traditions. They did not find Hg in any milk sample, whereas As was detected in four samples at concentrations double that of the median arsenic concentration found in this study.

4.3. Polycyclic aromatic hydrocarbons in forage and milk

In this study, higher concentrations of the four aromatic ring PAHs (LW PAHs) than HW PAHs were found in the forage (Table 3). This predominance of LW PAHs over HW PAHs was also reported two decades ago in grasslands near urban areas and highway roads [56]. This could be partially explained by the ability of plants to enzymatically degrade PAHs; therefore, HW PAHs may transform into MW PAHs in plant tissues [57,58].

HW PAHs were absent in the milk samples (Table 4), in agreement with previous studies [56,59], which did not detect PAHs with more than four aromatic rings in tank milk from both rural and urban farms. This could be partially explained by the higher water solubility of LW PAHs, which favours their absorption during digestion compared to HW PAHs. Previous food chain studies of compounds with chemical properties similar to those of PAHs support this hypothesis; polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) showed decreased absorption with increasing molecular weight [60]. The median concentration of pyrene detected in milk (Table 4) was consistent with that reported by Grova et al., in 2002 [59]. However, these authors have previously reported higher concentrations of pyrene and phenanthrene in milk [56,59]. More recently, higher concentrations PAHs with four aromatic rings (phenanthrene, fluoranthene, and pyrene) have been detected in milk from farms near industries [26] and in commercial milk [61].

4.4. Relationships between inorganic elements and polycyclic aromatic hydrocarbons

In this study, a strong correlation was found between HW PAHs and Zn and Cd; this common occurrence has also been reported in industrial areas as part of particulate air pollution [8,62]. The distribution of organic and inorganic compounds is related to the pollution source. When the same sources are related to both organic and inorganic contaminants, spatial homogeneity and interactions between PAHs and heavy metals are found [28,63].

In 2004, López-Alonso et al. [32] pointed out that the biological fluids of animals do not provide sufficient accuracy regarding the distribution and interactions of metals; therefore, tissues such as the kidney or liver should also be analysed owing to their role in HMs accumulation and the vulnerability of these organs to metal toxicity. These authors reported positive correlations between Cd and essential minerals in the kidneys; however, no correlations between these inorganic elements were found in the present study. Conversely, positive correlations were found in milk between Cd and other hazardous elements, such as Cr, Fe and Pb, which agrees with the results obtained by Yasotha et al., in 2021 [17] from milk produced in industrial areas in India, where Cr, Cd, and Pb were positively correlated. However, these authors reported higher levels of toxic metals than those reported in the present study.

Furthermore, the results of the PCA suggest that the contribution of PAHs and HMs to the global variance is more balanced in the case of milk than in forage, where HW PAHs seem to play a major role as environmental farm pollutants in this industrial area rather than HMs.

4.5. Potential sources of pollution in the environment

The results from the PCA, including distances to pollution sources in the study area, have shown a negative relationship between the former and the concentration of some pollutants, such as HM-PAHs, Zn, and Cd (see Fig. 6). Thus, the concentrations of these

compounds might decrease with the distance from farms to potential pollution sources. In addition, the enrichment of both PAHs and some HMs has been previously reported in soils from this area as a characteristic emission of Zn smelters [64]. Several studies have described the common origins of HW-PAHs, Cd, and Zn as products of incomplete combustion which are emitted in the form of PM [8, 62].

Despite the lack of data on atmospheric pollution in the surrounding area, the present study demonstrates the predominance of PAHs and HMs in forage crops intended for cattle nutrition rather than in the milk produced by animals. Finally, the proximity to pollution sources, such as steel factories or thermal power plants, might increase the concentrations of HM-PAHs, Cd, and Zn in forage. Therefore, this study offers a preliminary approach to address the chemical safety control on sustainable dairy farms.

5. Conclusions

This study provides new insights into the relationships among PAHs, essential metals, and hazardous metals in forage and milk in an industrialised farm environment.

In forage samples, the maximum concentrations of As (7.87 mg kg^{-1}) and Hg (0.47 mg kg^{-1}) exceeded the MRL established in EU regulations; however, the maximum content of Cd (0.86 mg kg^{-1}) and Pb (7.85 mg kg^{-1}) were lower than their respective EU MRLs, while EEs concentration were lower than normal levels. The maximum concentration of the sum of LW PAH was $161.6 \mu\text{g kg}^{-1}$, whereas the maximum of HW PAHs was $329.7 \mu\text{g kg}^{-1}$. In milk samples, the HMs concentrations were much lower; indeed, the maximum concentrations of Cd (0.12 mg kg^{-1}) and Pb (2.7 mg kg^{-1}) were under their respective EU MRLs. In addition, HW PAHs were not detected in milk, and the maximum concentration of LW PAHs in this product was $342.5 \mu\text{g kg}^{-1}$.

No significant forage-milk correlations for inorganic elements or PAHs were found; however, positive correlations between HW PAHs, Zn, and Cd, which are known to have a common steel industrial origin, were found in forage and in milk. In the absence of atmospheric pollution data for the area, this data partially confirmed the inclusion of the distances of the studied farms to potential sources of pollution in their surrounding areas.

In addition, the results from the individual PCAs of forage and milk highlighted the predominant contribution of HW PAHs to the variance of the forage samples rather than to the milk samples, where PAHs and toxic metals were balanced in terms of variance contribution.

These findings prove that, in terms of chemical pollution, milk produced in sustainable dairy farms near industries is safe for human consumption. However, as milk can have a dilution effect due to water consumption, future experiments addressing the accumulation of toxic minerals and PAHs in other environmental matrices, as well as in bovine tissues, should be conducted.

Data availability statement

All primary data and results obtained after analysing all samples (milk and forage) will be made available on request. The authors can not specify the exact location of each sampling point, due to data protection policy.

CRediT authorship contribution statement

Sergio Forcada: Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Mario Menéndez Miranda:** Formal analysis, Investigation, Methodology, Writing – original draft. **François Stevens:** Formal analysis, Methodology, Software, Writing – original draft. **Luis J. Royo:** Investigation, Methodology, Supervision. **Juan Antonio Fernández Pierna:** Formal analysis, Software, Supervision, Writing – original draft. **Vincent Baeten:** Formal analysis, Methodology, Resources, Supervision, Writing – original draft. **Ana Soldado:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ana Soldado reports financial support has been included in acknowledgements.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2023.e20977>.

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