

Article

Mineral Oil Hydrocarbons in Feed: Corn Silage Contamination in a Romanian Dairy Farm

Mădălina Matei , Daniel Simeanu *  and Ioan Mircea Pop 

“Ion Ionescu de la Brad” Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700489 Iasi, Romania; madalina.matei@iuls.ro (M.M.); mircea.pop@iuls.ro (I.M.P.)

* Correspondence: daniel.simeanu@iuls.ro

Abstract: This study investigates the presence of mineral oil hydrocarbons (MOHs) in corn silage, aiming to assess contamination levels and identify potential sources, including technological and environmental factors. Given the increasing concern regarding the presence of MOHs all over the food chain, this research provides important data on feed safety. A total of 15 corn silage samples were collected from the feed base of a dairy farm. Sampling was performed systematically across silos (top, middle, bottom layers). The analysis was conducted using LC-GC-FID to quantify mineral oil saturated hydrocarbon (MOSH) and aromatic hydrocarbon (MOAH) fractions. Statistical evaluation was applied to determine contamination patterns and potential influencing factors. The findings confirmed the presence of MOSH and MOAH in the analyzed silage, averaging 23.3 mg/kg MOSH and 1.4 mg/kg MOAH, exceeding European Commission guideline limits. Notably, the MOAH fraction, known for its potential toxicity, was detected at significant levels in several samples. The study highlights that corn silage can act as a source of MOSH/MOAH contamination in livestock feed. Technological processes, especially mechanized harvesting and ensiling, and environmental pollution factors appear to be likely the main contributors, emphasizing the need for improved monitoring and preventive measures to mitigate risks in the feed-to-food chain.

Keywords: feed safety; environmental pollution; farming practices; animal nutrition



Academic Editor: Zoltán Györi

Received: 26 February 2025

Revised: 30 March 2025

Accepted: 1 April 2025

Published: 3 April 2025

Citation: Matei, M.; Simeanu, D.; Pop, I.M. Mineral Oil Hydrocarbons in Feed: Corn Silage Contamination in a Romanian Dairy Farm. *Agriculture* **2025**, *15*, 777. <https://doi.org/10.3390/agriculture15070777>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The negative impact of feed contamination has become a main concern for both the quality and safety of animal feed, as well as for production efficiency [1]. Ensuring safe consumption and nutritionally balanced feeding strategies for animals requires a thorough understanding of feed composition, quality, and safety characteristics to optimize animal health, improve production performance, and minimize contamination risks [2]. The nutritional value of feed is closely related to its chemical composition, which has a direct impact on the nutritional requirements of animals. The chemical composition of feed is highly variable and influences the behavior of contaminants within the animal body, ultimately affecting the safety of animal products as well [3]. The chemical characteristics of feed can influence both the level of contamination and the way in which contaminants are available for absorption in the animal body. Growing concerns regarding the presence of mineral oil hydrocarbons (MOHs) in the food chain have led to an increased focus on their occurrence in feed. Due to their lipophilic nature, MOH compounds have a high affinity for the lipid phase of feed, raising concerns about their potential transfer into animal products [4].

Mineral oil hydrocarbons (MOHs) are a class of contaminants of petrogenic origin, present in the environment because of pollution or as by-products of industrial processes [5–8]. Structurally, MOHs are a complex mixture of saturated hydrocarbons (MOSHs—mineral oil saturated hydrocarbons) and aromatic hydrocarbons (MOAHs—mineral oil aromatic hydrocarbons). While MOSHs have not yet been confirmed to present high toxicity, MOAHs are considered the concerning fraction, because of their carcinogenic potential and toxicological risks to living organisms [1,9–11]. The presence of MOSH/MOAH contamination has been confirmed for several plants, with the non-polar character of MOSH/MOAH, given by their chemical structure, leading to a particular affinity for fatty plant parts. The occurrence and possible sources of contamination, including industrial emissions or technological operations, have been described and discussed in the literature across similar studies [12–16]. Considering the complex structure and associated risks of MOHs, especially the carcinogenic potential of the aromatic fractions, it becomes essential to investigate the pathways through which these substances may enter the animal body, in order to predict the potential contamination of animal-derived products. From a regulatory perspective, European authorities currently provide only a set of guidelines and recommendations regarding acceptable MOH levels in both animal feed and animal products. However, beyond all these regulatory aspects, there are ethical considerations related to ensuring the safety of animal nutrition and consumer health. In the future, the formalization of these provisional recommendations into binding legislation is expected to have important economic implications, perhaps exerting a more pronounced impact on producers.

Corn silage is a widely used feed, having an important role in dairy cattle rations [17,18]. The quality of silage primarily depends on its nutritional value and the chemical composition of the raw material. Corn silage has an important role in dairy cows' rations, due to its nutritional value and elevated digestibility of organic compounds [19]. Its inclusion allows for the formulation of a balanced diet, while supporting economically viable milk production [20–22]. Corn is known for its high biomass yield and suitable nutritional value for ensiling, owing to its high content of easily fermentable sugars and well-balanced composition of organic compounds [23]. The nutritional quality of silage can vary considerably based on the cultivated hybrids or growing conditions [24], while its overall safety can be strongly influenced by factors such as harvesting, processing, or storage conditions [25]. Thus, the production of high-quality and economically viable feed depends, among other factors, on the complexity of equipment and its efficiency [26]. Technological advancements available to producers and corn growers have contributed to improved crop management, increased harvest efficiency, and optimized nutritional modeling of this feed resource. Thus, ensiling can be an ideal process for the temporary storage of high-energy density crops and for minimizing nutrient losses from processed raw materials. However, these advancements have also introduced a heightened risk of inherent contamination during technological processing [18,25].

Given its production and handling process, this research is based on the hypothesis that corn silage may be susceptible to MOSH and MOAH contamination. In many areas of the world, while corn silage is not always the least expensive feed option, it remains a cost-effective choice compared to other forage alternatives with similar nutritional value. However, the production of corn silage requires technical expertise and involves substantial investments in machinery and labor. Therefore, the presence of any contaminants can have major economic consequences, compromising both production efficiency and the quality and safety of the animal feed obtained.

This study aims to evaluate contamination of corn silage with mineral oil saturated hydrocarbons (MOSHs) and aromatic hydrocarbons (MOAHs), and to analyze the influence

of technological processes or environmental pollution on this risk. Specifically, our work aims to determine whether corn silage can be an important source of contamination for cattle. Additionally, the study investigates the susceptibility of corn silage to MOSH and MOAH contamination compared to other feed types, considering the technological operations and mechanized interventions involved in its production and processing.

2. Materials and Methods

2.1. Sampling Framework

A total of 15 corn silage samples were collected from the feed base of a dairy farm, serving as the research material of this study. The farm maintains a lactating herd of approximately 400 cows, including German Friesian cattle and Romanian Grey Steppe breeds, the latter being under genetic conservation. Notably, the farm also holds ownership of the National Genealogical Register of Romania for this breed. In addition to livestock, the farm operates a crop production unit, covering an agricultural area of approximately 650 ha. This includes 515 ha of arable land cultivated with alfalfa, grain corn, corn for silage production, and triticale, along with 90 ha of pastures. Of the total arable land, 150 ha is dedicated to corn for silage production. Corn silage represents 45.5% of the total feed ration administered on the farm. The corn silage was obtained during the 2022/2023 agricultural campaign, using the farm's own processing system. The analysis of the corn silage was conducted in 2023. The number of collected samples was determined depending on the size of the sampling area.

The sampling, the analytical protocol, and the reporting of the results followed established standards and validated analytical methods, incorporating relevant protocols adapted from previous research [27]. Corn silage samples were collected following the SR EN ISO 6497:2005 [28] standard and Regulation (EC) No. 152/2009, Annex I [29]. Sample preparation complied with the SR EN ISO 6498:2012 [30] standard and Regulation (EC) No. 152/2009, Annex II [29]. The samples were dried to a moisture content of 8–12% at 60 °C, using a thermoregulated oven (model ESAC-100, Electronic April S.R.L., Cluj-Napoca, Romania), and were subsequently finely ground with a Grindomix GM 200 laboratory mill (Verder GmbH, Vienna, Austria). To prevent contamination, the prepared samples were stored in polypropylene and aluminum containers until analysis.

In line with the study's objectives, preliminary assessments were conducted to evaluate the crop-specific factors related to exposure to various sources of pollution and contamination. Experimental variables were selected based on the model developed by Matei and Pop [27]. These included both environmental pollution factors affecting plant crops (such as the presence and type of pollution sources, the positioning of the feed base relative to these sources, and the farm's proximity to urban or industrial areas) and technological operations involved in corn silage production. The technological factors considered included the type and frequency of chemical and phytosanitary treatments, the agricultural operations and equipment used, and also harvesting, storage, and processing methods. These assessments were carried out through direct observation, supplemented by structured interviews aimed at identifying possible sources of contamination at the farm level. Data collection involved participation in the entire process of harvesting and processing the raw corn plant, with regular visits and observations ensuring comprehensive monitoring. Additional details on the characteristics of the corn silage samples in relation to technological processing methods or contamination risks are provided in Table 1.

The assessment of potential contamination risks considered both the intensity of polluting activities and the distance between the pollution sources and the analyzed crop. As detailed in Table 1, major pollution sources included waste combustion, industrial activities (chemical and steel industry), and road and air transport, as well as construction

sites and one sewage treatment plant. The estimated risk level was determined based on proximity, with sources located closer to the crop posing a potential higher risk, and based on the intensity of the polluting activities. While no deliberate sampling strategy was applied to differentiate between high- and low-risk areas, the study included all potential contamination sources identified that could have contributed to MOSH/MOAH levels in the feed. Technological operations applied to crops are also documented in Table 1 as potential contributors to contamination.

Table 1. Preliminary assessment of potential technological and environmental pollution sources for corn silage samples.

Production and Processing Technologies					
Technological Operations Applied to Crops			Equipment	Storage	
Chemicals/ Phytosanitary/Fertilization	Crop Rotation	Harvesting/ Handling			
Organic: Animal waste Chemical: Urea (100 kg/ha, Azomureş, Târgu Mureş, Romania); NPK 20-20-0 (100 kg/ha, Azomureş, Târgu Mureş, Romania); Ammonium nitrate (150 kg/ha, Azomureş, Târgu Mureş, Romania) Henik (1.5 L/ha, Innvigo Agro Srl, Cluj-Napoca, Romania); Mustang (0.6 L/ha, Corteva Agriscience, Ilfov, Romania); Adengo (0.4 L/ha, Bayer Crop Science, Iaşi, Romania)	Monoculture	Mechanized	Sprinkler pump Harvesting machine Transport vehicle Crawler tractor Technological trailer	Concrete cell covered with polyethylene film	
Pollution factors					
Crop location					
Sources of pollution			Distance/Activity intensity		
Waste combustion			~4 km/intensive		
Chemical and steel industry			~4 km/medium		
Road transport (car aerosols, tire wear)			~1 km/intensive *		
Air transport			~1 km/medium **		
Construction industry			~1 km/intensive		
Sewage treatment plant			~4 km/medium		

* Urban traffic conditions; ** 25 landing–takeoff sequences.

The corn for silage was sourced from internal production and processed using the unit's own infrastructure. Harvesting was carried out with a combine harvester, and the raw corn was transported by truck to a concrete storage facility near the farm. The green corn mass was then leveled and compacted using a tracked tractor, before being left to ferment for approximately two months. The silage produced in September was introduced into the cows' ration starting in January of the following year. For corn for silage production, monoculture is practiced, meaning crop rotation is not practiced. As a result, the risk of contamination from chemical residues associated with other phytosanitary and fertilization treatments, apart from those applied to this crop, can be excluded.

2.2. Reagents and Chemicals

n-Hexane ($\geq 95\%$), methanol ($\geq 99.9\%$), potassium hydroxide (KOH), and sodium thiosulphate were purchased from Merck Millipore (Burlington, MA, USA). Metachloroperoxybenzoic acid (mCPBA) (70–75%, 200 mg/mL in ethanol) was obtained from Acros Organics (Thermo Fisher Scientific, Waltham, MA, USA). Aluminum oxide and sodium sulfate were supplied by Sigma-Aldrich (Saint Louis, MO, USA), and ethanol was procured from Supelco (Bellefonte, PA, USA). Ultrapure water was prepared using a Milli-Q filtration system (Millipore, Bedford, MA, USA). To avoid any additional contamination, all glass-

ware used during sample preparation was meticulously cleaned and rinsed with acetone and *n*-hexane prior to use. *n*-Hexane was also distilled and tested for purity before use.

For the evaluation of LC-GC performance and MOSH/MOAH separation and quantification, the internal standards (150–600 µg/mL in 99% toluene) from Restek (Bellefonte, PA, USA) were used. The standard used specifically contained a mixture consisting of *n*-undecane (*n*-C₁₁; 99%), cyclohexylcyclohexane (CyCy), *n*-pentylbenzene (5B; 99%), 1-methylnaphthalene (1-MN; 98%), 2-methylnaphthalene (2-MN; 96%), and 1,3,5-tri-tert-butylbenzene (TBB; 99%) at 0.30 mg/mL, as well as 5- α -cholestane (Cho; 99%) and perylene (Per; 99%) at 0.6 mg/mL, and *n*-tridecane (*n*-C₁₃; 99%) at 0.15 mg/mL.

A standard mixture containing even-numbered *n*-alkanes (0.05 mg/mL each), specifically, a *n*-C_{10–40} *n*-alkane mixture supplemented with *n*-C₅₀ (supplied by Restek), was used to evaluate GC performance for the LC–GC system. According to Bratinova and Hoekstra's [6] model, we integrated chromatograms by specific carbon fractions using a retention time standard mixture (*n*-C₁₀, *n*-C₁₁, *n*-C₁₃, *n*-C₁₆, *n*-C₂₀, *n*-C₂₄, *n*-C₂₅, *n*-C₃₅, *n*-C₄₀, and *n*-C₅₀) sourced from Restek.

2.3. LC–GC–FID Instrumentation and Analytical Conditions

MOSH and MOAH analysis in corn silage samples was carried out using an LC-GC 9000 system (Brechtbühler AG, Zurich, Switzerland); specifically, a Phoenix 9000 HPLC coupled to a Trace 1310 GC series (Thermo Fisher Scientific, Waltham, MA, USA) was used. The HPLC elution program began with 100% *n*-hexane, followed by a gradient switch to a 70/30 *n*-hexane/dichloromethane (*v/v*) mixture at a flow rate of 300 µL/min. The fractions were transferred to the GC between 2.1–3.6 min (MOSH) and 3.8–5.3 min (MOAH). After injection, the column was backwashed with dichloromethane (500 µL/min, 9 min), and then reconditioned with hexane (700 µL/min, 6.5 min; 300 µL/min, 1.5 min). For the HPLC analysis, a Lichrospher Si 60 column (25 cm × 2.1 mm, 5 µm) from DGB (Schlossboeckelheim, Germany) was used.

The fractions of the analyzed sample, separated using the HPLC system, were transferred to the GC system for detailed analysis. During this process, the solvent used in the HPLC for separation was relatively evaporated to remove excess solvent. A “Y” interface connected the two systems, allowing analytes to transfer from the HPLC to the GC system, while controlling solvent evaporation. To protect the main GC column and ensure sample transition to the gas phase, a retention gap technique was employed. For validation and context, the methodology is described in detail by Biedermann et al. [31]. The GC system consisted of two independent channels with distinct configuration, a H₂ carrier gas line, and a 10 m × 0.53 mm i.d. deactivated retention gap (Mega, Milan, Italy). This setup enabled the simultaneous and differential analysis of the two fractions. The GC's dual channel was equipped with a 15 m × 0.25 mm i.d. PS-255 column (0.15 µm film thickness, 1% vinyl, 99% methyl polysiloxane), also from Mega, connected to gap pre-columns by a steel T-piece. A solvent vapor removal system (SVE = solvent vapor exit) was used to prevent solvent interference during the analysis. The GC oven temperature was programmed to rise from 51 °C to 350 °C at a rate of 20 °C/min. The carrier gas (H₂) was maintained at a constant pressure of 60 kPa, increased to 90 kPa for enabling the transfer. To prevent condensation before detection, the flame ionization detector (FID, 50 Hz) was heated at 350 °C, with a data acquisition rate of 10 Hz, while the SVE was heated to 140 °C.

The data were processed using Chromeleon 7.3 software (Thermo Fisher Scientific, Waltham, MA, USA). Quantification was performed with the internal standard CyCy for MOSHs, and 5B, 1-MN, 2-MN, and TBB for MOAHs. The quantification and integration of the entire chromatographic signal were made between the retention times of *n*-C₁₀ and *n*-C₅₀. Corresponding areas were integrated, and interferences were eliminated by

performing blank runs for each batch of samples. The analytical method performance was assessed according to the JRC [6,32] and Eurachem [33] guidelines, using blind samples. The limit of quantification (LOQ) was determined following the SANTE/12682 guidelines [34], meeting the JRC criteria. Recovery rates ranged between 70% and 120%, and intermediate repeatability was deemed appropriate for method validation.

The saponification was carried out using a MARS 5 microwave extraction system (CEM Corporation, Bergamo, Italy), equipped with 14 Teflon vessels. Sample concentration was performed using a Uniequip centrifugal evaporation system (UNIVAPO-100H model, 24 positions) sourced by Büchi AG (Flawil, Switzerland), coupled with a V-700 vacuum pump and V-850 controller, also from Büchi AG (Flawil, Switzerland).

For data analysis, we used statistical methods to assess the distribution and significance of contamination in feed samples. A descriptive analysis was conducted to determine the mean values and dispersion of the data. To assess the normality of the distribution, we used the Kurtosis and Skewness parameters. To test the differences between sample groups, we performed analysis of variance (ANOVA), and to examine the correlations between MOSH and MOAH fractions, Pearson correlation analysis was applied. All statistical analyses were conducted using GraphPadPrism 9.3.0 (GraphPad Software Inc., Boston, MA, USA) and Microsoft® Excel® LTSC MSO 2021 version 16.0.14332.21007 (Microsoft Corporation, Redmond, WA, USA).

2.4. MOH Analysis

The working method was adapted and modified based on Biederman et al. [31] (2009) and Biedermann and Grob [35,36], optimized based on Moret et al. [37], and detailed further based on Menegoz Ursol et al. [38], Srbinovska et al. [39], and Srbinovska et al. [40]. With minor adjustments, giving thought to the specificity of the samples, the protocol aligns closely with the method previously applied by Bauwens et al. [41] for determining MOSHs and MOAHs in fish feed. The separation of the organic phase followed the same protocol used for the extraction of MOHs from cereal-based products, as optimized by Moret et al. [37].

A 5 g sample of feed was placed in a vial, along with 10 mL of 40% KOH solution, 10 mL of *n*-hexane, and 20 µL of internal standard (IS). The mixture was subjected to microwave-assisted extraction at 120 °C for 20 min. After the extraction, we added 40 mL of ultrapure water (Milli-Q) and 2 mL of methanol to the solution, then left it to allow phase separation. The organic phase was then concentrated under a vacuum until a final volume of 4 mL was achieved. To ensure the purity of the extract, a washing step with a methanol–water mixture (2:1, *v/v*) was conducted. Samples were vortexed, centrifuged, and further concentrated to 700 µL. Because of the specificity of the samples, additional purification (epoxidation, AlOx purification) was necessary to remove substantial amounts of natural *n*-alkanes. This purification was conducted according to the method developed by Nestola and Schmidt [42]. It involved adding 500 µL of a solution of *m*-chloroperbenzoic acid (*m*-CPBA) in ethanol (20% *w/v*) to the 700 µL concentrated extract. The mixture was placed on an agitator at room temperature, with a stirring speed of 500 rpm, for 15 min. After epoxidation, the reaction was stopped by adding 2 mL of aqueous sodium thiosulphate solution (10% *w/v*) to neutralize any remaining oxidant (*m*-CPBA), preventing further unwanted reactions. A 500 µL volume of ethanol was added for mixing and to stabilize the solution, and then 500 µL of the mixture was transferred to an autosampler vial. A spatula tip of anhydrous sodium sulfate (used to remove any trace of water from the extract) was also put into this vial. A 100 µL volume of this purified solution was injected into the LC-GC-FID analysis system. For the AlOx purification step, 40 µL of the epoxidized extract was diluted with *n*-hexane and passed through a cartridge with activated aluminum oxide

(AlOx) and sodium sulfate. The purified extract was then concentrated to 250 µL, from which 75 µL was injected into the LC-GC-FID system for analysis.

2.5. Chemical Composition

The crude chemical composition of the corn silage samples was determined according to national and international standards, following the guidelines provided in Regulation (EC) No. 152/2009, Annex III [29]. SR ISO 6496:2001 [43] was followed for moisture content; SR EN ISO 5983-1:2006 [44] was followed for crude protein content; SR ISO 6492:2001 [45] was used for ether extract; and SR EN ISO 6865:2002 [46] was used for crude fiber content.

3. Results

The chemical composition of corn silage was determined to assess its potential influence on the contamination and bioavailability of MOSHs/MOAHs. The analysis included key organic components, such as crude protein (CP), crude fat (EE, ether extract), crude fiber (CF), and non-fiber carbohydrate (NFC) content. These parameters are relevant for evaluating the matrix effect on the mobility of hydrocarbons within the feed. The average values are presented in Table 2. The MOH distribution (mg kg^{-1}) detected in the 15 corn silage samples collected from different points within the same storage unit is summarized in Table 3. According to the JRC guidance [6], quantification was performed for each C fraction. The internal standard CyCy was used for MOSHs, while 5B, 1-MN, 2-MN, and TBB were used for MOAHs. To ensure accurate MOSH quantification, the high concentration of naturally occurring *n*-alkanes in the samples necessitated the mandatory AlOx step, as previously described. Table 3 also details the individual values per carbon sub-fraction and the total *n*-C_{10–50} concentrations.

Table 2. Average data on chemical composition of corn silage.

Item	Chemical Components				
	¹ DM	² EE	³ CP	⁴ CF	⁵ NFC
	%	% DM			
Mean (x) ± s _x	34.93 ± 0.02	3.64 ± 0.09	8.77 ± 0.10	18.42 ± 0.09	65.85 ± 0.21
Min.	34.90	3.46	8.51	18.16	65.31
Max.	34.98	3.91	9.13	18.68	66.57
SEM	0.04	0.21	0.23	0.21	0.48
V (%)	0.11	2.71	2.63	1.14	0.73

¹ DM = dry matter; ² EE = ether extract; ³ CP = crude protein; ⁴ CF = crude fiber; ⁵ NFC = non-fiber carbohydrate; s_x = standard deviation; SEM = standard error of mean; V (%) = variability coefficient.

The analytical results indicated a notable presence of MOHs in the tested samples. All corn silage samples showed an average contamination with MOSHs of 23.3 mg/kg, with values ranging from 20.3 mg/kg to 27.7 mg/kg, while MOAH contamination varied between 1.1 mg/kg and 1.8 mg/kg, with an average of 1.4 mg/kg. However, the variability of these values was considerable, as evidenced by the descriptive statistical analysis. The high variability between samples for MOSHs was evident from the minimum and maximum values (20.3–27.7 mg/kg) and the relatively high standard error of the mean. Also, the contamination distribution was not uniform, exhibiting a rightward skewness (e.g., values of 2.147 and 2.746). This suggests the presence of samples with significantly higher contamination levels compared to the average. MOAH contamination was significantly lower than MOSH contamination, with less variability between samples. The relatively low standard error of the mean (e.g., 0.026–0.288) indicated a more uniform contamination distribution, suggesting a more constant source of pollution. The contamination profile showed that

over 90% of the MOSH fraction was concentrated in the $n\text{-C}_{10-35}$ range, with more than 30% found in the $n\text{-C}_{16-20}$ range in particular. Similarly, over 70% of MOAH contamination was detected in the $n\text{-C}_{16-25}$ molecular range, a pattern consistent with contamination with hydraulic oils or lubricants. Menegoz Ursol et al. [7] reported this in a recent investigation when they highlighted the MOH distribution of various technical oils on the Italian market used for different agricultural equipment.

Table 3. Statistical distribution and significance of MOSH/MOAH contamination levels (mg kg^{-1}) in corn silage samples (individual and mean values), structured by carbonic sub-fractions and total contamination level.

Samples		MOSHs (mg/kg)						MOAHs (mg/kg)				
C-Fractions	$n\text{-C}_{10-16}$	$n\text{-C}_{16-20}$	$n\text{-C}_{20-25}$	$n\text{-C}_{25-35}$	$n\text{-C}_{35-40}$	$n\text{-C}_{40-50}$	$n\text{-C}_{10-50}$	$n\text{-C}_{10-16}$	$n\text{-C}_{16-25}$	$n\text{-C}_{25-35}$	$n\text{-C}_{35-50}$	$n\text{-C}_{10-50}$
S1	6.3	9.0	5.7	4.3	0.5	0.2	26.0	0.1	0.9	<0.5	nd.	1.4
S2	4.0	5.3	3.7	8.5	0.4	0.3	22.2	nd.	1.5	<0.3	nd.	1.8
S3	5.2	7.2	4.7	3.9	0.5	0.3	21.6	nd.	1.2	<0.3	nd.	1.5
S4	8.5	8.9	5.1	3.1	0.3	0.4	26.3	nd.	1.4	<0.3	nd.	1.6
S5	10.2	8.1	4.8	2.1	0.1	0.2	25.5	<0.1	1.0	<0.1	nd.	1.1
S6	3.9	8.5	5.0	2.6	0.2	0.3	20.4	0.1	1.0	0.1	nd.	1.2
S7	7.1	9.4	5.9	4.6	0.5	0.2	27.7	<0.1	1.0	0.1	nd.	1.2
S8	4.7	5.6	5.4	3.8	0.4	0.4	20.3	nd.	1.5	<0.3	nd.	1.8
S9	5.9	7.5	5.0	4.2	0.5	0.3	23.3	nd.	0.9	0.7	<0.1	1.7
S10	7.6	6.4	3.9	3.2	0.8	0.7	22.6	nd.	1.1	0.2	nd.	1.3
S11	4.1	4.1	3.8	6.0	2.2	0.1	20.3	nd.	0.5	0.7	nd.	1.2
S12	4.7	4.2	3.3	9.4	0.3	0.1	22.0	nd.	1.2	0.5	<0.1	1.7
S13	5.1	6.2	3.2	7.2	0.3	0.1	22.0	<0.3	0.9	nd.	nd.	1.1
S14	2.3	3.2	2.0	8.5	4.7	3.3	24.0	nd.	0.6	<0.5	nd.	1.1
S15	2.4	3.2	2.8	10.2	5.7	1.8	26.1	0.1	1.1	<0.1	nd.	1.2
Mean ($\bar{x}$) $\pm$ s_x	5.5 $\pm$ 0.57	6.4 $\pm$ 0.55	4.3 $\pm$ 0.30	5.4 $\pm$ 0.69	1.2 $\pm$ 0.44	0.6 $\pm$ 0.22	23.3 $\pm$ 0.63	<0.05 $\pm$ 0.02	1.0 $\pm$ 0.08	<0.3 $\pm$ 0.06	<0.1 $\pm$ 0.01	1.4 $\pm$ 0.07
Min.	2.3	3.2	2.0	2.1	0.1	0.1	20.3	0.0	0.5	0.0	0.0	1.1
Max.	10.2	9.4	5.9	10.2	5.7	3.3	27.7	0.2	1.5	0.7	0.1	1.8
SEM	2.188	2.132	1.143	2.656	1.722	0.8621	2.437	0.062	0.288	0.220	0.026	0.266
CI (95%)	1.211	1.180	0.633	1.471	0.954	0.477	1.350	0.034	0.159	0.122	0.014	0.147
Kurtosis	0.1603	−1.308	−0.6667	−1.107	3.582	7.613	−1.191	2.625	−0.028	−0.546	1.500	−1.520
Skewness	0.611	−0.206	−0.4414	0.601	2.147	2.746	0.35	1.792	−0.165	0.647	3.873	0.425
ANOVA: Single Factor												
Source of Variation	SS	df	MS	F	p -value	F_{crit}	SS	df	MS	F	p -value	F_{crit}
Between Groups	47.0765	14	3.363	0.0535	0.9999	1.8032	0.795	14	0.057	0.1315	0.9999	1.8602
Within Groups	5652.729	90	62.808	-	-	-	25.928	60	0.432	-	-	-
Total	5699.805	104	-	-	-	-	26.723	74	-	-	-	-

Values are mean of 2 replicates/sample. Performance criteria—total LOQ ($n\text{-C}_{10-50}$): MOSH = 0.5 mg/kg ; MOAH = 0.5 mg/kg (EU/ScoPAFF recommended limit for food with fat/oil $\leq$ 4%). nd. = not detected; s_x = standard deviation; SEM = standard error of mean; CI = confidence interval; SS = sum of squares; df = degrees of freedom; MS = mean squares; F = Fisher's statistic; p = error probability—significance level.

Figure 1 shows the distribution of MOSH (a) and MOAH (b) contamination in the 15 analyzed corn silage samples and the final average, structured by the carbon sub-fractions of MOSHs ($n\text{-C}_{10-16}$, $n\text{-C}_{16-20}$, $n\text{-C}_{20-25}$, $n\text{-C}_{25-35}$, $n\text{-C}_{35-40}$, $n\text{-C}_{40-50}$) and MOAHs ($n\text{-C}_{10-16}$, $n\text{-C}_{16-25}$, $n\text{-C}_{25-35}$, $n\text{-C}_{35-50}$) and the total contamination level ($n\text{-C}_{10-50}$). Above-average MOH concentration values were highlighted for some samples; specifically, 6 out of the 15 samples (S1, S4, S5, S7, S14, S15) clearly showed higher values of MOSHs and MOAHs (S2, S3, S4, S8, S9, S12), with increases of approximately 20% in some cases. Notable differences between samples were identified, especially in the $n\text{-C}_{10-16}$, $n\text{-C}_{16-20}$, and $n\text{-C}_{25-35}$ sub-fractions, and partially in the $n\text{-C}_{35-40}$ sub-fractions, for MOSHs, as well

as in the n -C_{16–25} and, partially, in the n -C_{25–35} sub-fractions for MOAHs, where certain samples exceeded the overall average. Despite these variations, the ANOVA results indicated that the observed differences between groups were not statistically significant (MOSH: $F = 0.0535$, $p \approx 1$; MOAH: $F = 0.1315$, $p \approx 1$). This suggests that while certain samples exhibited higher contamination levels, the overall distribution of MOSHs and MOAHs did not show significant group-related trends. Instead, the variability appeared to be driven especially by internal fluctuations among samples, rather than systematic differences attributable to external factors.

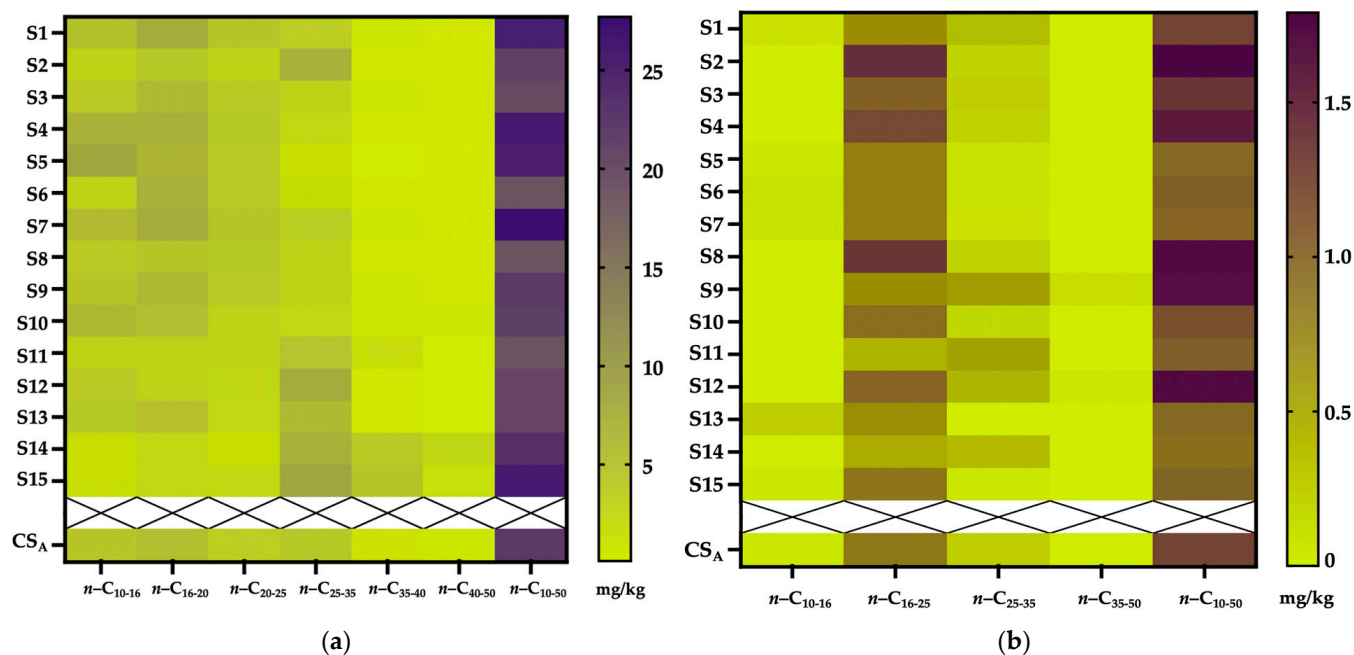


Figure 1. The distribution of MOSH (a) and MOAH (b) contamination in corn silage samples and the overall average. The horizontal axis indicates the carbon sub-fractions for MOSHs (n -C_{10–16}, n -C_{16–20}, n -C_{20–25}, n -C_{25–35}, n -C_{35–40}, n -C_{40–50}) and MOAHs (n -C_{10–16}, n -C_{16–25}, n -C_{25–35}, n -C_{35–50}) and the total contamination (n -C_{10–50}). The vertical axis indicates the individual samples analyzed (S1–S15) and the final average (CS_A) calculated for the entire sample set. Contamination is visually represented by a colorimetric scale ranging from 0 to 30 mg/kg (a, MOSH) and 0 to 2 mg/kg (b, MOAH); the color intensity reflects the level of contamination: deep purple shades (close to the upper limit) indicate higher contamination levels, while lighter green shades (close to the lower limit) indicate lower contamination levels.

The analysis of correlations between MOSH and MOAH fractions provides insight into potential contamination sources and the distribution of these compounds in the analyzed samples. Statistical relationships between the fractions were examined to determine whether a common contamination pattern existed or whether the sources were distinct. Table 4 presents the correlation coefficients between individual MOSH and MOAH fractions, as well as their correlation with total mineral hydrocarbons. High correlation coefficients suggest a possible common origin of the contaminants, while low or negative correlations may indicate different contamination sources or variations in the accumulation behavior of these fractions.

The correlation analysis of MOSH fractions revealed a positive relationship between short- and medium-chain hydrocarbons (n -C_{10–25}), suggesting a common contamination source, likely linked to air pollution, industrial emissions, or road traffic near the farm. These fractions are more volatile and prone to airborne contamination. In contrast, long-chain fractions (n -C_{25–50}) exhibited strong correlations among themselves, indicating a more specific contamination source, possibly associated with the use of industrial oils

or lubricants in agricultural equipment. The total MOSH level did not show significant correlations with the individual fractions, suggesting high variability between samples and the absence of a dominant contamination source. For MOAHs, a negative correlation was observed between short-chain ($n\text{-C}_{10-16}$) and long-chain fractions ($n\text{-C}_{25-50}$), implying differences in contamination sources or the stability of these compounds; this pattern likely reflects differential degradation of fractions or competition between contamination sources.

Table 4. Correlation analysis between carbon fractions of MOSH and MOAH contaminants.

Samples	MOSH (mg/kg)							MOAH (mg/kg)					
C fractions	<i>n</i> -C ₁₀₋₁₆	<i>n</i> -C ₁₆₋₂₀	<i>n</i> -C ₂₀₋₂₅	<i>n</i> -C ₂₅₋₃₅	<i>n</i> -C ₃₅₋₄₀	<i>n</i> -C ₄₀₋₅₀	<i>n</i> -C ₁₀₋₅₀		<i>n</i> -C ₁₀₋₁₆	<i>n</i> -C ₁₆₋₂₅	<i>n</i> -C ₂₅₋₃₅	<i>n</i> -C ₃₅₋₅₀	<i>n</i> -C ₁₀₋₅₀
<i>n</i> -C ₁₀₋₁₆	1.00	-	-	-	-	-	-	<i>n</i> -C ₁₀₋₁₆	1.00	-	-	-	-
<i>n</i> -C ₁₆₋₂₀	0.7161	1.00	-	-	-	-	-	<i>n</i> -C ₁₆₋₂₅	−0.1878	1.00	-	-	-
<i>n</i> -C ₂₀₋₂₅	0.5885	0.8613	1.00	-	-	-	-						
<i>n</i> -C ₂₅₋₃₅	−0.7086	−0.7947	−0.7778	1.00	-	-	-	<i>n</i> -C ₂₅₋₃₅	−0.4726	−0.372	1.00	-	-
<i>n</i> -C ₃₅₋₄₀	−0.6164	−0.6938	−0.6715	0.6129	1.00	-	-	<i>n</i> -C ₃₅₋₅₀	−0.1494	−0.1475	0.5021	1.00	-
<i>n</i> -C ₄₀₋₅₀	−0.5015	−0.5537	−0.6450	0.4461	0.8419	1.00	-						
<i>n</i> -C ₁₀₋₅₀	0.4172	0.3629	0.1970	−0.001	0.204	0.2035	1.00	<i>n</i> -C ₁₀₋₅₀	−0.4208	0.7059	0.3658	0.3191	1.00
Pearson r													
r	−0.4768	−0.7125	−0.7171	0.5742	0.6372	0.5114	−0.1265		0.2070	−0.4277	0.1015	0.0617	−0.3725
95% CI	−0.7949	−0.8973	−0.8991	0.0877	0.1855	−0.0015	−0.5999		−0.3415	−0.7711	−0.4333	−0.4651	−0.7430
	0.0467	−0.3154	−0.3238	0.8395	0.8666	0.0811	0.4125		0.6503	0.1082	0.5834	0.5565	0.1727
R-squared	0.2273	0.5077	0.5142	0.3297	0.4060	0.2615	0.016		0.0429	0.1830	0.0103	0.0039	0.1388
<i>p</i> (one-tailed)	0.0362 *	0.0014 **	0.0013 **	0.0126 *	0.0053 **	0.0257 *	0.3267 ns		0.2296 ns	0.0559 ns	0.3595 ns	0.4133 ns	0.0858 ns

CI = confidence interval; r = correlation coefficient; R = determination coefficient; p = error probability: ** $p < 0.01$ highly significant, * $p < 0.05$ significant, ns = not significant.

Figure 2 illustrates a model of the MOSH/MOAH contamination profile in corn silage samples, confirming the presence of these contaminants. The observed contamination pattern is similar to that reported for common technological contamination sources, but it does not entirely explain the high concentrations detected, namely, 23.3 mg/kg for MOSHs and 1.7 mg/kg for MOAHs for this sample. HPLC-GC-FID chromatogram analysis revealed that MOSH contamination extended from $n\text{-C}_{10}$ to $n\text{-C}_{35}$, with an important hump between $n\text{-C}_{10}$ and $n\text{-C}_{25}$, while MOAH contamination was identified at levels of 17–20% within the molecular range of $n\text{-C}_{16-25}$. This profile suggests possible contamination associated with lubricant residues, engine oil, hydraulic oil, or vehicle emissions. However, the relatively low amount of MOAHs detected could indicate that this fraction might have a different origin, which remains unclear. The similarities between this contamination profile and that of certain lubricants used in technological equipment suggest that part of the contaminants could originate from technical oils used in the processing stages. Nevertheless, the high levels detected do not exclude the possibility of additional contamination resulting from feed exposure to external pollution sources. Although these findings provide valuable insights, further investigations are needed to more clearly confirm the mechanisms involved.

To provide a broader perspective on MOSH and MOAH contamination, the average data for corn silage were compared with contamination levels previously determined for different feed components within the same ration. The study carried out by Matei et al. [25], conducted on the same farm, considered both technological contamination and external pollution sources. Figure 3 presents the average contamination levels of MOSHs (a) and MOAHs (b) structured by carbon sub-fractions ($n\text{-C}_{10-16}$, $n\text{-C}_{16-20}$, $n\text{-C}_{20-25}$, $n\text{-C}_{25-35}$, $n\text{-C}_{35-40}$, $n\text{-C}_{40-50}$) and the total contamination ($n\text{-C}_{10-50}$). The data include the average values for corn silage (45.5% participation rate in the ration) and different feeds within the same

ration, with different participation rates (alfalfa hay—5.40%, alfalfa silage—10.9%, corn grain—6.35%, soya meal—7.1%, triticale—4.5%, brewer’s grains—18.2%).

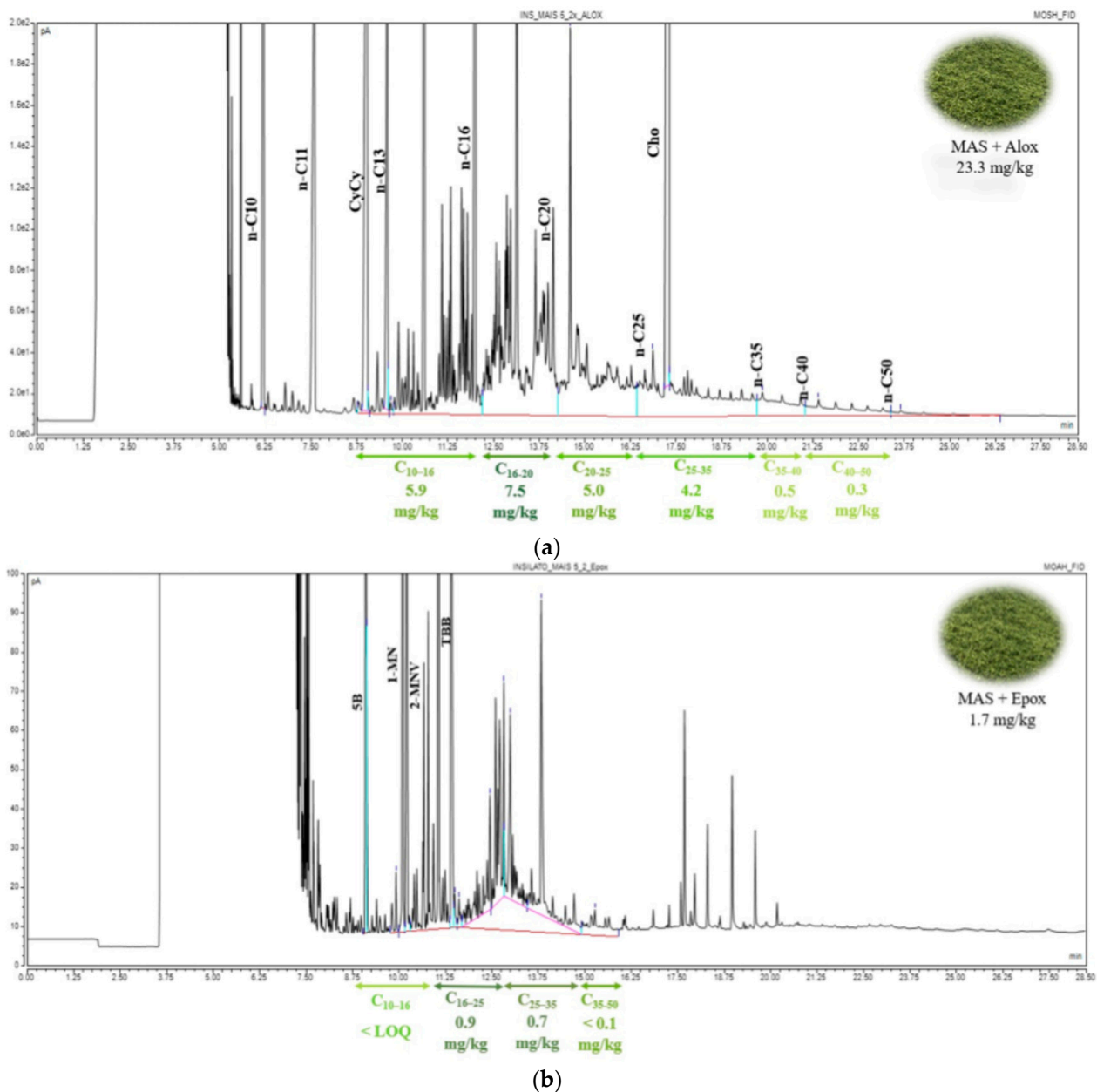


Figure 2. HPLC-GC-FID chromatograms of MOSHs (a) and MOAHs (b), confirming overall contamination in a representative corn silage sample (S9). The chromatographic humps indicate the presence and concentration of contaminants. Contamination levels are visually represented by shades of green: deep green denotes higher contamination levels, while lighter green indicates lower contamination levels. The red line marks the baseline for peak integration. The horizontal axis represents the retention time (x): 0–28.5 min, while the vertical axis corresponds to the detector signal (y): 0–200 pA MOSH/0–100 pA MOAH.

Although all the feed crops originated from the same geographical area and had been exposed to similar pollution sources (e.g., traffic emissions, industrial activities), it is important to note that some feeds with comparable or even lower levels of technological processing than corn silage clearly exhibited higher contamination levels. Comparatively, although corn silage showed notable MOH contamination, other feeds may pose an even more worrying risk to animals. Additionally, across all feed types, the dominant carbon

sub-fractions were $n\text{-C}_{16-35}$ for MOSHs and $n\text{-C}_{16-20}$ for MOAHs, suggesting the existence of a common source of contamination.

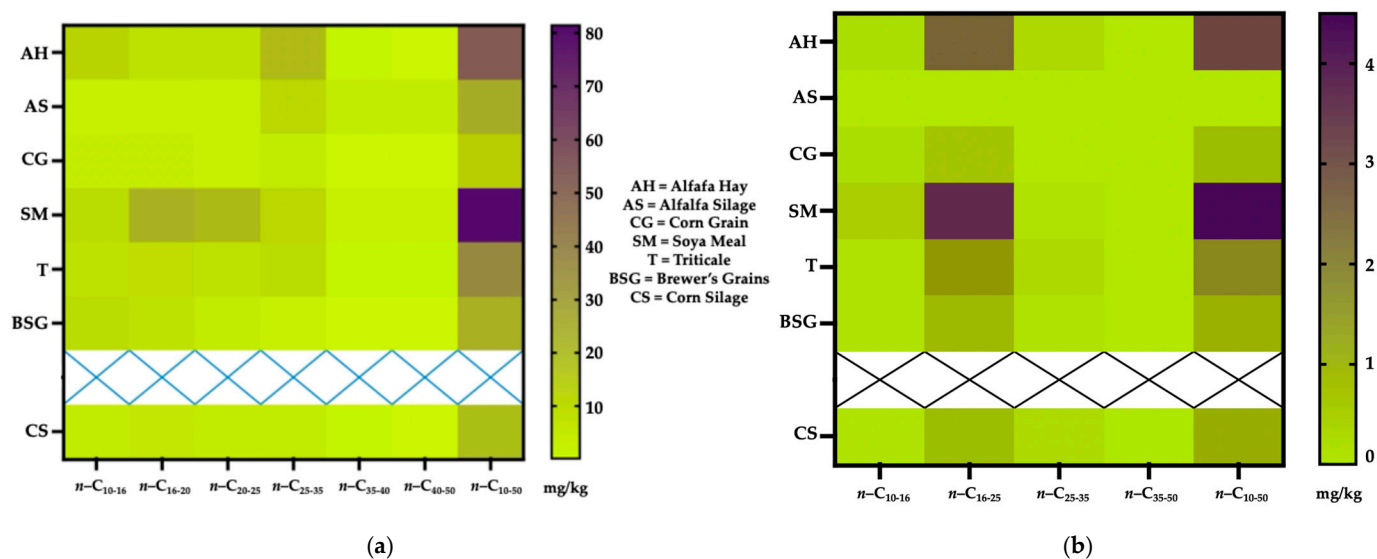


Figure 3. The average contamination levels of MOSHs (a) and MOAHs (b) in corn silage and feed from the same ration. The horizontal axis indicates the carbon sub-fractions for MOSHs ($n\text{-C}_{10-16}$, $n\text{-C}_{16-20}$, $n\text{-C}_{20-25}$, $n\text{-C}_{25-35}$, $n\text{-C}_{35-40}$, $n\text{-C}_{40-50}$) and MOAHs ($n\text{-C}_{10-16}$, $n\text{-C}_{16-25}$, $n\text{-C}_{25-35}$, $n\text{-C}_{35-50}$) and the total contamination ($n\text{-C}_{10-50}$). The vertical axis represents the contamination level in feed samples. Contamination is visually represented by a colorimetric scale, ranging from 0 to 80 mg/kg for MOSHs (a) and 0 to 4.5 mg/kg for MOAHs (b). The intensity of color reflects the contamination level: deep purple (near the upper limit) indicates higher contamination, while pale green (near the lower limit) indicates lower contamination levels.

4. Discussion

This study focused exclusively on the characteristics of corn silage, providing an overall assessment of contamination at this stage in the production chain. The results account for both technological contamination and pollution linked to atmospheric factors, which are further discussed in the following dedicated sections.

The detection of above-average contamination levels raises concerns about potential localized contamination sources within the silo, likely linked to technological processes such as technical oil leaks, prolonged contact with agricultural equipment, or materials used throughout the processing steps. Since sampling was conducted to characterize overall contamination—collecting samples from multiple points of the batch (upper, middle, and lower layers), but analyzing them collectively, without differentiating them by location—the results do not allow for conclusions on contamination distribution across different silo layers. However, there was a clear suggestion that technological factors and storage conditions may differentially influence contaminant accumulation in corn silage, warranting further studies to assess the associated risks more precisely. Statistical analysis not only confirmed the presence of MOSH and MOAH contamination, but also showed significant variations between samples. The high variability suggests the potential influence of external factors on contaminant distribution. The greater data dispersion for MOSHs points to multiple contamination sources and varying impacts of technological processes on hydrocarbon distribution. Also, the contamination pattern may indicate sporadic pollution, possibly influenced by technological factors such as equipment type, harvesting methods, or storage conditions. Furthermore, the lack of a strong correlation between the total contamination level and the individual MOSH fractions suggests that the pollution sources are not uniform, resulting in heterogeneous contamination. In contrast, the more uniform

distribution of MOAHs suggests more systematic contamination, likely associated with the use of lubricants and agricultural equipment. For MOAHs, this aspect is particularly important in assessing the associated risks, as MOAHs are considered more problematic from a toxicological standpoint. However, both for MOSHs and MOAHs, ANOVA results indicated that the observed variability between samples was primarily driven by internal fluctuations, rather than well-defined external factors. These findings suggest that under the conditions of this study, no clear patterns emerged to indicate a direct influence of specific contamination sources on mineral hydrocarbon levels. Nevertheless, the presence of samples with above-average values warrants further investigation into other factors that may contribute to these localized increases.

The concentration of contamination within a single fraction, along with the absence of MOAHs in other fractions, suggests exposure to a contamination source specifically affecting compounds in the $n\text{-C}_{16-25}$ range. This possibly facilitated the accumulation of MOAHs in this specific range. A plausible explanation, as reported by Matei et al. [25], is that contamination occurred all along the ensiling process, most likely due to contact with lubricants of similar composition or with the agricultural equipment used, such as harvesting machines, transport vehicles, or crawler tractors. No proportional relationship was observed between MOSH and MOAH levels across samples, indicating that the sources or mechanisms of contamination were not entirely shared; each fraction appeared to be influenced by distinct factors. MOSH contamination is typically associated with lubricants and technical oils used in agricultural machinery or packaging materials [47], whereas MOAH—recognized for its higher toxicity—is more commonly associated with contamination from fuels throughout transportation or technological processing, as well as industrial pollution [39,48].

Regarding agricultural crops and livestock farms, they are traditionally situated in rural areas, where the risk of contamination is often considered minimal. However, in this case, environmental pollution sources can no longer be dismissed as unlikely or insignificant. A previous study on the same subject [25] gathered data through sampling sheets and documented several plant crops, including the corn crop intended for obtaining the silage analyzed in this study. In terms of territorial administration, the studied farm is located within the metropolitan area of Iași city, Romania, with its agricultural fields located within a 3–5 km radius. Based on the collected data, the corn crop was exposed to environmental contamination not only due to its proximity to the urban center (Iași, Romania), but also because of its location near multiple industrial facilities from Iași, Romania (Arcelor Mittal Products, Technosteel, Rancon SA, Chemical Company—chemical and steel industry), municipal landfills (Țuțora, Romania—waste combustion), and air transport activities (Iași International Airport, with 25 daily flight cycles). Additionally, extensive construction and road infrastructure projects have been carried out in recent years at a short distance from the farm (approx. 2–3 km). Establishing a clear and direct correlation between corn silage contamination levels and the proximity of the farm's agricultural crops to these potential pollution sources remains challenging. Although the exact contribution of environmental contamination is difficult to quantify, there is strong evidence that some of the detected MOSH and MOAH concentrations can be associated with these environmental pollution sources. The findings of this study align with previous research on feed contamination; for example, Rychen et al. [49] highlighted that exposure of crops to pollutant emissions may be responsible for feed contamination. These conclusions reinforce the hypothesis that nearby pollution sources played a role in the contamination observed in this study.

Although there are some noteworthy indications, a direct relation between MOH contamination levels and the use of fertilizers or phytosanitary treatments remains unclear.

Certain crop protection products may contain mineral oils, making them a potential contamination source [5]. Additionally, liquid fertilizers applied during the intensive growth stages of maize could further contribute even more to MOSH accumulation in the soil and on plant surfaces. Based on the available data, three types of fertilizers were used on the maize crop: 100 kg/ha urea ($\text{CO}(\text{NH}_2)_2$) for seedbed preparation; 100 kg/ha NPK 20-20-0 (20% total N; 20% total P_2O_5 ; 60% water-soluble P_2O_5 ; 98% P_2O_5 soluble in citric acid 2%; max. 0.6% water) at sowing; and 150 kg/ha ammonium nitrate (27% N; 7% CaO; 5% MgO) in the vegetation phase with 5–6 leaves. Post-sowing, the first phytosanitary treatment included 0.4 L/ha Adengo herbicide (225 g/L Isoxaflutol; 90 g/L Thiencarbazone-methyl; 150 g/L Cyprosulfamides). In the vegetation phase with 4–6 leaves, two more phytosanitary treatments with herbicides were applied: 1.5 L/ha Henik (40 g/L Nicosulfuron) to combat monocotyledonous weeds, and 0.6 L/ha Mustang (6.25% Florasulfam; 30% Acid 2,4D EHE) to combat dicotyledonous weeds. None of these products explicitly list mineral oil-based substances on their labels. However, this does not entirely rule out the presence of MOHs as co-formulants, since current regulations do not require their disclosure. A similar observation was made by Menegoz Ursol et al. [7], who analyzed the impact of harvesting operations on MOH contamination in olive oil.

Another plausible hypothesis is that mineral oil leaks from equipment used for maintenance treatments (e.g., sprinkler pump) could contribute to MOSH and MOAH contamination. Thus, these practices cannot be entirely ruled out as potential contamination sources. Nevertheless, a direct connection between contamination levels and fertilizers or phytosanitary treatments has not been clearly established for other feed types either. In a study by Matei et al. [25], feeds that underwent multiple phytosanitary treatments did not necessarily exhibit high contamination levels, whereas some feeds treated only with products reported elevated MOSH and MOAH concentrations; this lack of proportionality between these variables suggests that other factors have a more interesting role in contamination. A comprehensive perspective on contamination sources emerges when considering the technological operations involved in feed production. Certain feed processing techniques are more susceptible to introducing these types of contaminants into the product. It is well known that corn silage production involves several mechanical stages, including harvesting and crushing, transport, and compaction. The choice of processing methods prioritizes both economic efficiency and operational quality. Given that silage production is completely mechanized, various types of technical oils, lubricants, and hydraulic fluids—mostly composed of mineral oils—are inevitably used for the equipment or machines involved. Potential contamination may also occur through accidental leaks of oils into the silage mass, as well as through prolonged contact with lubricated mechanical parts of machinery. Contamination through emissions from equipment or from the material (polyethylene foil) used for silo covering was considered negligible in this study.

Despite the clarity of the results, there are still some limitations in precisely identifying contamination sources. As noted by Menegoz Ursol et al. [38], confirming environmental contamination is especially challenging for the MOAH fraction, due to its susceptibility to oxidation upon air exposure. Although current legislation on MOH contamination is not fully standardized, all the analyzed corn silage samples exhibited MOSH concentrations exceeding the indicative limits set by the JRC Guide [6,32] and the European Commission [50]. The high contamination levels raise concerns about potential consequences for the food chain and food safety. In particular, the presence of the aromatic fraction MOAH—known for its toxic potential—represents a serious warning signal. This highlights the striking risk these contaminants pose in animal feed rations and, subsequently, in animal-derived products. To address this issue at the farm level, immediate preventive measures should focus on regular maintenance of agricultural equipment and the use of high-technical-quality,

safe, and adequate materials. Measures to prevent MOSH/MOAH contamination should focus on optimizing the technological processes for feed production and processing, particularly by reducing excessive mechanical contact by improving the silo compaction process. Given the identified potential contamination sources, a priority may be the management of technical liquids used in agricultural equipment. An effective strategy could involve using refined or partially refined (MOAH content below 30%) food-safe lubricants and oils, or replacing them with MOH-free alternatives [5,38]. Proper maintenance of machinery plays an important role in reducing contamination risks. Regular cleaning and inspection of harvesting and storage equipment can prevent residue accumulation and enable early detection of potential leaks. Replacing worn seals and components helps to reduce the risk of accidental contamination, thereby ensuring feed safety. In the long term, another viable solution could be optimizing the design of agricultural equipment to prevent direct contact between technical components and feed. While this approach involves structural changes and investments, its implementation could ensure a considerable reduction in contamination risks. Although requiring additional effort, periodic monitoring of contamination levels in both feed and animal products is recommended, thus providing valuable data to support adjustments in technological processes, ultimately mitigating contamination risks.

5. Conclusions

The analyses conducted on corn silage revealed high levels of contamination with mineral oil hydrocarbons (MOSHs and MOAHs), with levels exceeding the guideline limits set by the European Commission. For all samples analyzed, the average concentrations detected were 23.3 mg/kg for MOSHs and 1.4 mg/kg for MOAHs, raising concerns about potential risks to the food chain and food safety, especially due to the toxicity associated with the aromatic MOAHs. The primary contributing factors to this appear to be technological processes involved in ensiling, especially the use of agricultural equipment and machinery throughout corn harvesting and processing. The production of corn silage involves multiple mechanized steps, each presenting a potential source of contaminants introduced into the final product. Agricultural equipment operating with lubricants and technical oils can introduce MOSH and MOAH contaminants into feed, either through accidental leaks or prolonged contact with mechanical components.

The proximity of corn crops to industrial and urban pollution sources, such as steel plants, landfills, or areas with intense air traffic, could also contribute to environmental contamination and, implicitly, to the contamination of crops. Various statistical reports highlight these common sources of contamination, which are likely linked to air pollution that can carry particles and chemicals, including mineral oils. In this context, MOSH/MOAH contamination may occur due to continuous exposure to these sources, with considerable transfer of pollutants from the environment to the feed, ultimately affecting feed quality. Although this study did not establish a definitive link between contamination levels and the use of phytosanitary products, some of these products may contain mineral oils, representing a potential source of contamination. Thus, corn silage may be susceptible to contamination due to both its exposure to environmental pollutants and the complexity of the technological processes involved in its production. To mitigate contamination risks, preventive maintenance of agricultural machinery is recommended, ensuring proper upkeep and the use of materials and fuels of appropriate technical quality. Additionally, periodic monitoring of feed could enable early detection of contamination, allowing for adjustments to technological processes. This research underscores the need for further investigations to precisely identify contamination sources and ensure long-term feed monitoring. Moreover, updating existing regulations or introducing new guidelines on MOSH/MOAH contamination in feed is also needed for enhancing agro-food chain safety.

Author Contributions: Conceptualization, M.M. and I.M.P.; methodology, M.M.; software, M.M.; validation, D.S. and I.M.P.; formal analysis, M.M.; investigation, M.M. and D.S.; resources, D.S.; data curation, I.M.P.; writing—original draft preparation, M.M.; writing—review and editing, M.M. and D.S.; visualization, I.M.P.; supervision, I.M.P.; project administration, M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data supporting the reported results are available from the authors upon request.

Acknowledgments: The present study is part of a broader research initiative aiming to investigate feed contamination and the transfer of contaminants into animal-derived products. The research is a result of a collaboration between Iasi University of Life Sciences (Romania) and the University of Udine (Italy). We acknowledge the Food Chemistry Laboratory team for providing the necessary facilities, resources, data analysis support, and technical assistance.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

MOH	Mineral oil hydrocarbons
MOSH	Mineral oil saturated hydrocarbons
MOAH	Mineral oil aromatic hydrocarbon
LC-GC-FID	Liquid chromatography–gas chromatography–flame ionization detection
LOQ	Limit of quantification
IS	Internal standard

References

- Grob, K. Toxicological Assessment of Mineral Hydrocarbons in Foods: State of Present Discussions. *J. Agric. Food Chem.* **2018**, *66*, 6968–6974. [PubMed]
- Pop, I.M.; Halga, P.; Avarvarei, T. *Animal Nutrition and Feeding*, 1st ed.; TipoMoldova: Iași, Romania, 2006.
- Postolache, A.N.; Chelmu, S.S.; Arition, A.M.; Ciorpac, M.; Pop, C.; Ciobanu, M.M.; Creangă, Ș. Analysis of RASFF notifications on contaminated dairy products from the last two decades: 2000–2020. *Rom. Biotechnol. Lett.* **2020**, *25*, 1396–1406. [CrossRef]
- Matei, M.; Pop, I.M. Mineral oil hydrocarbons (MOH) analysis in animal feed: A characterization based on modern pollution. *Sci. Papers Ser. D Anim. Sci.* **2023**, *LXVI*, 113–122.
- EFSA European Food Safety Authority (EFSA). Scientific opinion on mineral oil hydrocarbons in food. *EFSA J.* **2012**, *10*, 2704.
- Bratinova, S.; Hoekstra, S. *Guidance on Sampling, Analysis and Data Reporting for the Monitoring of Mineral Oil Hydrocarbons in Food and Food Contact Materials*, 1st ed.; Publications Office of the European Union: Luxembourg, 2019.
- Menegoz Ursol, L.; Conchione, C.; Peroni, D.; Carretta, A.; Moret, S. A study on the impact of harvesting operations on the mineral oil contamination of olive oils. *Food Chem.* **2023**, *406*, 135032.
- Canavar, Ö.; Kappenstein, O.; Luch, A. The analysis of saturated and aromatic mineral oil hydrocarbons in dry foods and from recycled paperboard packages by online HPLC–GC–FID. *Food Addit. Contam. Part A* **2018**, *35*, 2471–2481.
- European Food Safety Authority (EFSA); Arcella, D.; Baert, K.; Binaglia, M. Rapid risk assessment on the possible risk for public health due to the contamination of infant formula and follow-on formula by mineral oil aromatic hydrocarbons (MOAH). *EFSA Support. Publ.* **2019**, *16*, 1741E.
- Nestola, M. Automated workflow utilizing saponification and improved epoxidation for the sensitive determination of mineral oil saturated and aromatic hydrocarbons in edible oils and fats. *J. Chromatogr. A* **2022**, *1682*, 463523.
- Carrillo, J.C.; van der Wiel, A.; Danneels, D.; Kral, O.; Boogaard, P.J. The selective determination of potentially carcinogenic polycyclic aromatic compounds in lubricant base oils by the DMSO extraction method IP346 and its correlation to mouse skin painting carcinogenicity assays. *Regul. Toxicol. Pharmacol.* **2019**, *106*, 316–333.
- Moret, S.; Populin, T.; Conte, L.S. La contaminazione degli oli vegetali con oli minerali. *Riv. Ital. Delle Sostanze Grasse* **2009**, *LXXXVI*, 3–14.
- Bruhl, L. Occurrence, determination, and assessment of mineral oils in oilseeds and vegetable oils. *Eur. J. Lipid Sci. Technol.* **2016**, *118*, 361–372.

14. Gharbi, I.; Moret, S.; Chaari, O.; Issaoui, M.; Conte, L.S.; Lucci, P.; Hammami, M. Evaluation of hydrocarbon contaminants in olives and virgin olive oils from Tunisia. *Food Control* **2017**, *75*, 160–166.
15. Purcaro, G.; Barp, L.; Moret, S. Determination of hydrocarbon contamination in foods. A review. *J. Anal. Methods* **2016**, *8*, 5755–5772.
16. Matei, M.; Petrescu, S.I.; Flocea, E.I.; Lăpușneanu, D.M.; Simeanu, D.; Pop, I.M. Variation in mineral oil hydrocarbons content of milk during processing. *Sci. Papers Ser. D Anim. Sci.* **2024**, *LXVII*, 490–499.
17. Khan, N.; Shabbir, A.; George, D.; Hassan, G.; Adkins, S.W. Suppressive fodder plants as part of an integrated management program for *Parthenium hysterophorus* L. *Field Crops Res.* **2014**, *156*, 172–179.
18. Ferraretto, L.F.; Shaver, R.D.; Luck, B.D. Silage review: Recent advances and future technologies for whole-plant and fractionated corn silage harvesting. *J. Dairy Sci.* **2018**, *101*, 3937–3951.
19. Kung, L., Jr.; Moulder, B.M.; Mulrooney, C.M.; Teller, R.S.; Schmidt, R.J. The effect of silage cutting height on the nutritive value of a normal corn silage hybrid compared with brown midrib corn silage fed to lactating cows. *J. Dairy Sci.* **2008**, *91*, 1451–1457.
20. Khan, N.A.; Khan, N.; Tang, S.; Tan, Z. Optimizing corn silage quality during hot summer conditions of the tropics: Investigating the effect of additives on in-silo fermentation characteristics, nutrient profiles, digestibility and post-ensiling stability. *Front. Plant Sci.* **2023**, *14*, 1305999.
21. Rațu, R.N.; Ciobanu, M.M.; Radu-Rusu, R.M.; Usturoi, M.G.; Ivancia, M.; Dolis, M.G. Study on the chemical composition and nitrogen fraction of milk from different animal species. *Sci. Papers Ser. D Anim. Sci.* **2021**, *LXIV*, 374–379.
22. Schmidt, P.; Novinski, C.O.; Junges, D.; Almeida, R.; de Souza, C.M. Concentration of mycotoxins and chemical composition of corn silage: A farm survey using infrared thermography. *J. Dairy Sci.* **2015**, *98*, 6609–6619.
23. Neumann, M.; Baldissera, E.; Jenke, L.A.; de Souza, A.M.; de Oliveira, P.E.P.; Bumbieris Junior, V.H. Nutritional Value Evaluation of Corn Silage from Different Mesoregions of Southern Brazil. *Agriculture* **2024**, *14*, 1055. [\[CrossRef\]](#)
24. Krause, K.M.; Oetzel, G.R. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Anim. Feed Sci. Technol.* **2006**, *126*, 215–236.
25. Matei, M.; Petrescu, S.I.; Mădescu, B.M.; Lăpușneanu, D.M.; Simeanu, D.; Boișteanu, P.C.; Pop, I.M. The Impact of Feed Management Technologies on Mineral Oil Hydrocarbons (MOH) Contamination: A Comparative Farm Level Approach. *Agriculture* **2024**, *14*, 2008. [\[CrossRef\]](#)
26. Valge, A.; Sukhoparov, A.; Papushin, E.; Dobrinov, A.V. Evaluation effectiveness of forage harvesters in silage preparation. In Proceedings of the IOP Conference Series: Earth and Environmental Science, Yekaterinburg City, Russia, 15–16 October 2021; Volume 699, p. 012050.
27. Matei, M.; Pop, I.M. Monitoring of dairy farms to assess the potential level of pollution of animal feed and animal production. *Sci. Papers Ser. D Anim. Sci.* **2022**, *LXV*, 129–136.
28. SR EN ISO 6497:2005; Animal Feeding Stuffs. Sampling. International Organization for Standardization: Geneva, Switzerland, 2005.
29. European Commission. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Off. J. Eur. Union* **2009**, *52*, 1–130.
30. SR EN ISO 6498:2012; Animal Feeding Stuffs. Guidelines for Sample Preparation. International Organization for Standardization: Geneva, Switzerland, 2012.
31. Biedermann, M.; Fiselier, K.; Grob, K. Aromatic hydrocarbons of mineral oil origin in foods: Method for determining the total concentration and first result. *J. Agric. Food Chem.* **2009**, *57*, 8711–8721.
32. Bratinova, S.; Robouch, P.; Hoekstra, E.; Bratinova, S. *Guidance on Sampling, Analysis and Data Reporting for the Monitoring of Mineral Oil Hydrocarbons in Food and Food Contact Materials*, 2nd ed.; Publications Office of the European Union: Luxembourg, 2023.
33. Magnusson, B.; Ormemark, U. *Eurachem Guide: The Fitness for Purpose of Analytical Methods—A Laboratory Guide to Method Validation and Related Topics*, 2nd ed.; Eurachem: Middlesex, UK, 2014; Available online: https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf (accessed on 2 April 2023).
34. SANTE/12682/2019. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed. Available online: https://www.eurl-pesticides.eu/userfiles/file/EurlALL/AqcGuidance_SANTE_2019_12682.pdf (accessed on 5 May 2023).
35. Biedermann, M.; Grob, K. On-line coupled high performance liquid chromatography-gas chromatography for the analysis of contamination by mineral oil. Part 1: Method of analysis. *J. Chromatogr. A* **2012**, *1255*, 56–75.
36. Biedermann, M.; Grob, K. On-line coupled high performance liquid chromatography-gas chromatography for the analysis of contamination by mineral oil. Part 2: Migration from paperboard into dry foods: Interpretation of chromatograms. *J. Chromatogr. A* **2012**, *1255*, 76–99.
37. Moret, S.; Sclaro, M.; Barp, L.; Purcaro, G.; Conte, L.S. Microwave assisted saponification (MAS) followed by on-line liquid chromatography (LC)-gas chromatography (GC) for high-throughput and high-sensitivity determination of mineral oil in different cereal-based foodstuffs. *Food Chem.* **2016**, *196*, 50–57.

38. Menegoz Ursol, L.; Conchione, C.; Srbinovska, A.; Moret, S. Optimization and validation of microwave assisted saponification (MAS) followed by epoxidation for high-sensitivity determination of mineral oil aromatic hydrocarbons (MOAH) in extra virgin olive oil. *Food Chem.* **2022**, *370*, 130966.
39. Srbinovska, A.; Conchione, C.; Celaj, F.; Menegoz Ursol, L.; Moret, S. High sensitivity determination of mineral oils and olefin oligomers in cocoa powder and related packaging: Method validation and market survey. *Food Chem.* **2022**, *396*, 133686.
40. Srbinovska, A.; Gasparotto, L.; Conchione, C.; Menegoz Ursol, L.; Lambertini, F.; Suman, M.; Moret, S. Mineral oil contamination in basil pesto from the Italian market: Ingredient contribution and market survey. *J. Food Compos. Anal.* **2023**, *115*, 104914.
41. Bauwens, G.; Conchione, C.; Sdrigotti, N.; Moret, S.; Purcaro, G. Quantification and characterization of mineral oil in fish feed by liquid chromatography-gas chromatography-flame ionization detector and liquid chromatography-comprehensive multidimensional gas chromatography-time-of-flight mass spectrometer/flame ionization detector. *J. Chromatogr. A* **2022**, *1677*, 463208. [PubMed]
42. Nestola, M.; Schmidt, T.C. Determination of mineral oil aromatic hydrocarbons in edible oils and fats by online liquid chromatography–gas chromatography–flame ionization detection—Evaluation of automated removal strategies for biogenic olefins. *J. Chromatogr. A* **2017**, *1505*, 69–76.
43. SR ISO 6496:2001; Animal Feeding Stuffs. Determination of Moisture and Other Volatile Matter Content. International Organization for Standardization: Geneva, Switzerland, 2001.
44. SR EN ISO 5983:1-2006; Animal Feeding Stuffs. Determination of Nitrogen Content and Calculation of Crude Protein Content. Part 1: Kjeldahl method. International Organization for Standardization: Geneva, Switzerland, 2006.
45. SR ISO 6492:2001; Animal Feeding Stuffs. Determination of Fat Content. International Organization for Standardization: Geneva, Switzerland, 2001.
46. SR EN ISO 6865:2002; Animal Feeding Stuffs. Determination of Crude Fibre Content. International Organization for Standardization: Geneva, Switzerland, 2002.
47. Hochegger, A.; Moret, S.; Geurt, L.; Gude, T.; Meitner, E.; Mertens, B.; O'Hagan, S.; Pocas, F.; Simat, T.; Purcaro, G. Mineral oil risk assessment: Knowledge gaps and roadmap. Outcome of a multi-stakeholders workshop. *Trends Food Sci. Technol.* **2021**, *113*, 151–166.
48. Van-Heyst, A.; Goscinny, S.; Bel, S.; Vandevijvere, S.; Mertens, B.; Elskens, M.; Van Hoeck, E. Dietary exposure of the Belgian population to mineral oil. *Food Addit. Contam. Part A* **2020**, *37*, 267–279.
49. Rychen, G.; Jurjan, S.; Toussaint, H.; Feidt, C. Dairy ruminant exposure to persistent organic pollutants and excretion to milk. *Anim. Consort.* **2008**, *2*, 312–323.
50. European Commission. Summary Report. Standing Committee on Plants, Animals, Food and Feed. Section Novel Food and Toxicological Safety of the Food Chain, 21 April 2022. Available online: https://food.ec.europa.eu/system/files/2022-11/reg-com_toxic_20221019_sum.pdf (accessed on 31 March 2023).

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.