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The Impact of Feed Management Technologies on Mineral Oil Hydrocarbons (MOH) Contamination: A Comparative Farm Level Approach

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Abstract: Legislative frameworks about contamination are often limited to foods and underestimate the role of animal nutrition for safe production. This study aims to assess mineral oil hydrocarbon (MOH) contamination in feed and identify the technological factors that are contributing to this issue, particularly focused on mechanised harvesting and processing. Three dairy farms, classified by contamination risk (low, medium, and high), were selected, and fifteen feed samples were analysed using the coupled liquid chromatography–gas chromatography–flame ionisation detection (LC–GC–FID) method, with a microwave-assisted saponification (MAS) step to determine mineral oil saturated (MOSH) and aromatic (MOAH) hydrocarbon levels. Important contamination levels were observed depending on the technological development of each farm. MOSH levels ranged from 11.4 mg/kg to 81.40 mg/kg, while MOAH levels ranged from 0.5 mg/kg to 4.6 mg/kg. MOAH accounted for 4.74% of the total MOH content. The results showed a connection between feed production technologies and MOH contamination levels. Factors such as the mechanisation, the machinery used, and the storage conditions were potentially contributors to contamination, while chemical treatments had no direct impact but some potential risks. The contamination levels varied across farms, indicating certain contamination sources beyond technological factors. Advanced technological measures and proper equipment maintenance are suggested to mitigate MOH contamination risks in feed.

Keywords: animal feed; contamination; food safety; toxicity; public health

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1. Introduction

In the agrifood system, the study of the traceability of contaminants is not well addressed. Legislative frameworks are often limited to the final food sector and underestimate the major role of animal nutrition in ensuring safe production. Monitoring potential sources of contamination and implementing specific prevention measures could contribute positively to food safety [1,2]. However, these efforts are often hampered by legislative gaps and a lack of standardised practices at the farm level.

Approaches to contamination of animal products must include the entire production chain, from soil and crops to feed and livestock. The presence and accumulation of contaminants in any of these components can lead to the transfer and bioaccumulation of residues in the animals' bodies, affecting their health [3]. The negative repercussions of the presence of contaminants in the animal body are reflected both in terms of food security and animal productions' quality and safety [4]. The accumulation of foreign substances compromises the safety and innocuity of the products, generating considerable risks and negative effects on consumers' health [5,6].

Among the products of animal origin, milk holds a special place in the human diet due to the important supply of nutrients. As a result of the high fat content, milk has the unwanted ability to retain contaminants, making it vulnerable to their accumulation. Therefore, ensuring milk innocuity becomes essential to guaranteeing food safety.

In particular, the relationship between the consumption of contaminated feed and the safety of animal production, especially milk, has been highlighted in numerous studies [7–10]. These works show us that the massive presence of contaminants in animal feed can lead to their transfer during production, affecting milk safety and consumer health. Also, long-term exposure to contaminants can have serious consequences for both animals and humans, but also for the productivity and economic viability of farms, highlighting the importance of suitable feed management [11].

Mineral oil hydrocarbons (MOHs) are contaminants of petrogenic origin, complex mixtures of saturated (mineral oil saturated hydrocarbon (MOSH)) or aromatic (mineral oil aromatic hydrocarbon (MOAH)) hydrocarbons.

The presence of MOH is the result of environmental pollution or contamination that occurs throughout the production cycle [12–14].

In the food chain, the presence of MOH has been a persistent problem for more than a decade, raising concerns about its ecotoxicological health impact [15–17]. Recent findings, based on various contamination situations [18,19], have led to a deeper awareness of the problems related to the incidence of MOH in the environment and food [20–23].

In the agrifood system, MOHs mainly contaminate when they are released as residues, especially from using lubricating oils in various technological processes [14,24].

MOH contamination of feed can be greatly influenced by the technological factors carried out when obtaining them, especially through mechanised agricultural machinery. This can result from incorrect harvesting and processing practices of plant raw materials, from intentional use of mineral oils as lubricants or release agents, from accidental contamination, but also from contact materials containing mineral oils [25]. Moreover, feed storage and handling techniques are other technological factors that can influence their MOSH and MOAH contamination.

Using lubricating oils for the agricultural machinery and equipment operation presents a considerable risk of contamination, since the oils used (engine oils and hydraulic oils) can enter the environment in unrefined or partially refined form, bringing with them a wide range of secondary compounds. Grob et al. [26] underlined the alarming realities of these oils, because, in addition to mineral hydrocarbons, they also contain polyalphaolefins, high-molecular-weight compounds ($n\text{–}C_{25\text{–}30}$, and they can reach up to $n\text{–}C_{45}$), polyesters, and different proportions of additives with toxic potential. This contamination is also compounded by the presence of other chemicals, including cleaning solvents and thinners from machine maintenance processes, as well as paints or other protective substances.

Recent studies by Hoccheger et al. [24] and Van Heyst et al. [27] highlighted concerns related to MOH contamination, including the licensed use of these oils as ingredients in various products. According to Regulation (EC) 1107/2009 [28] and the Commission Implementing Regulation (EU) 540/2011 [29], paraffinic mineral oils are permitted as components in substances intended for agricultural crop protection, such as pesticides, insecticides, and acaricides. This authorised use can contribute to feed contamination and, by extension, food product contamination, raising serious concerns about food safety and consumer health. In addition, the presence of these compounds in the food chain can generate long-term risks; therefore, careful assessment of the risks associated with the use of mineral oils in agriculture is insisted upon.

MOHs' toxicity remains a distinct concern in specialist research because of the uncertainties related to their effects on living organisms. Recent studies indicated an increased capacity of MOSH to accumulate in human organs and tissues, which may generate health risks [30–32]. More, MOAHs are structurally similar to polycyclic aromatic hydrocarbons (PAHs), have carcinogenic potential, and can exert genotoxic effects, especially the variants with three or more aromatic rings [22,33].

The toxicity of mineral oils has been studied for about three decades, but the subject is still open due to the chemical complexity of these substances. MOHs are characterised by a diversity of constituents and structures, so the variable toxicological profile contributes to the uncertainty regarding their health effects. The risks associated with mineral oils are influenced by the molecular weight distribution of hydrocarbons and the presence of MOAH, which is considered the most toxic of the fractions [34].

Their high fat content and the absence of specific functional barriers make products of animal origin vulnerable to MOSH and MOAH contamination through the migration of these compounds from the animal tissues [21]. Over time, varying proportions of MOH have been identified in products of animal origin as well as other food products [35]. According to Bratinova and Hoekstra [36] and Bratinova et al. [37], Commission Recommendation (EU) 2017/84 [38] emphasises the importance of monitoring the presence of these contaminants, especially in fats, meat, dairy, fish, and derived products.

In relation to food safety, although their toxicological impact is well documented [22,30–34], up to the present, very few specific approaches regarding MOH contamination of feed have been developed. This gap highlights the need to investigate the sources and mechanisms by which these contaminants can affect the food chain, especially in the context of their negative effects on animal and consumer health.

Limited data and the harmful nature of these contaminants have led to the need to critically investigate the incidence of MOHs in animal production.

The aim of this research is to assess the mineral oil hydrocarbon (MOH) contamination of feed and to understand the impact on food safety and consumer health. The study aims to identify the sources and the risky technological factors that contribute to feed contamination, with a particular focus on mechanised feed harvesting and processing.

Through the analysis of MOH compounds and contamination mechanisms, this study aims to contribute to the awareness of feed safety issues in relation to food safety, providing a basis for the development of more efficient production practices in the agrifood system. The results will have the final objective of proposing measures and recommendations to reduce the risks associated with feed contaminants and their sustainable management at the farm level.

2. Materials and Methods

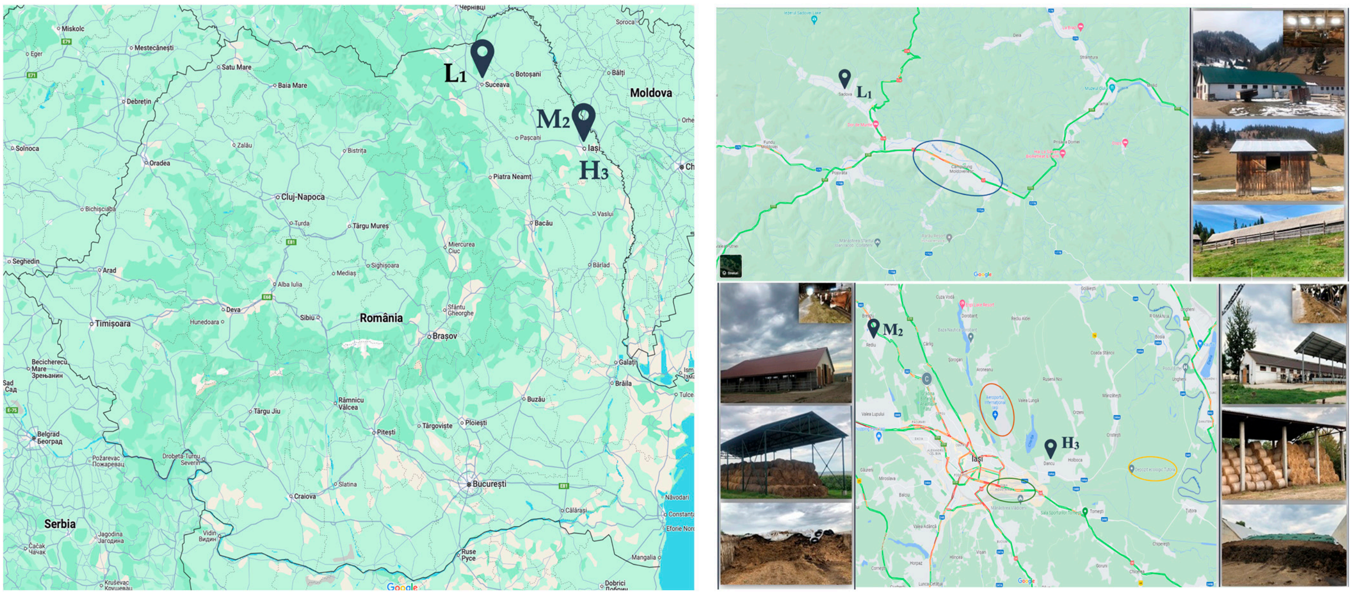
2.1. Samples and Sampling Sites

For this study, three dairy farms were selected by the levels of potential contamination, established on the basis of some preliminary assessments. These evaluations were carried out through the observation method, structured interviews, and an evaluation questionnaire designed to identify possible sources of contamination for each farm. For the objectivity of the results, the farms were monitored and analysed during the study according to the protocol developed by Matei and Pop [39].

The questionnaire focused on (1) the feed base and the diet specificity, (2) the traceability of agricultural practices, soil fertilisation, and phytosanitary treatments applied to crops, (3) the use of equipment and technical oils with contamination risk, (4) feed processing, storage, and handling practices, and (5) farms' proximity to urban or industrial areas.

Figure 1 shows the locations of the farms and some brief images illustrating the specific feed base. The feed base of the farms was provided mostly from internal production, obtaining feeds directly from the crop land using its own infrastructure. For some farms, the external purchase of feed from different sources was performed for supplementation when the internal production was not sufficient.

Our hypothesis was focused on the different levels of contamination from each farm, which may directly influence the contamination levels of the feed samples. Table 1 summarises some characteristics of the sampling sites (area, number of inhabitants, and traffic distance) and of the feed samples (origin and participation rate in the ration).



(**a**) (**b**)

Figure 1. Sampling sites: (**a**) Farms' locations within Romania, with codes indicating the contamination risk level: low (L), medium (M), and high (H). (**b**) The location of L_1 farm (mountain area—low contamination) in relation to the nearest major city in the region (Suceava County); the locations of M_2 farm (rural area—medium contamination) and H_3 farm (urban area—high contamination), highlighting the proximity to the nearest urban area (Iasi city). Symbols show the main sources of pollution in the vicinity of the farms. The colour indicates the estimated pollution risk level of the sources near the sampling sites: red (high pollution risk, intense air transport), yellow (moderate risk, small-scale industry), green (low pollution risk, sporadic pollutant emissions), and blue (urban pollution).

Farms were grouped into three categories of expected contamination risk, based on the responses and assessments from the evaluation questionnaire. Each farm was coded (L_1, M_2, and H_3) to indicate its contamination category and risk: low (L), medium (M), and high (H). Farm L_1 is located in the mountainous area and is represented by a low use of machinery in feed management, semi-intensive animal husbandry and agricultural activities, potentially improper feed handling and storage practices, and a large distance from sources of pollution and urban traffic. Farm M_2 is located in a rural area and is represented by a moderate use of agricultural machinery and potentially improper feed handling and storage practices. Farm H_3 is closer to the urban area and is characterised by intensive use of machinery, potentially improper feed handling and storage practices, and is positioned closer to industrial areas.

Information on some possible sources of pollution (road distances, traffic intensity, distance from urban areas, and number of inhabitants) is also provided. H_3 farm sites were located close to one of the biggest cities in Romania (Iasi), near an industrial area, with an airport positioned in the vicinity of the farm as well as other potential sources of contamination (municipal waste, construction activities, and traffic infrastructure). Samples from M_2 farm, such as alfalfa hay (M_2–AH), corn grains (M_2–C), and corn silage (M_2–CS), were also located ~5 km from the urban centre. There were no important sources of contamination for L_1 farm, even though it is located 6 km from one important urban centre of Suceava County, except the mountain pasture (L_1–MP), which is located in an ex-mining area (activity stopped in 2002).

For some samples, information about technological processes (crop-care treatments, the harvesting mode, equipment, and storage area) was also reported. Table 2 lists the main substances used in these treatments.

Table 1. Characteristics of the feed samples in relation to sampling sites.

Farm and Category	Sample Code	Sample Name	% of the Ration	Origin	Crop Location	
					d—Traffic/Roads *	d—Inhabited Areas (No.)
L_1 Low risk	L_1–NH	Natural hay	100 (summer)	Internal	500 m/medium ~6 km/intensive (urban)	500 m/medium ~6 km/intensive (urban)
	L_1–MP	Mountain pasture	100 (winter)	Internal	~5 km/medium	>7 km (269)
TOTAL (L_1): 2						
M_2 Medium risk	M_2–AH	Alfalfa hay	22.4	Internal	>10 km/intensive (S_1) ~2 km/intensive (S_2)	~6 km (4.577)/rural ~4 km (271.692)/urban
	M_2–CS	Corn silage	56	Internal	~6 km/intensive	~6 km (271.692)/urban
	M_2–C	Corn grains	11.20	Internal	<15 km/intensive	~4 km (4.577)/rural
	M_2–S	Soya	8.40	External purchase	-	-
	M_2–CF	Combined feed ***	100	Internal	AH, CS, C, S	
TOTAL (M_2): 5						
H_3 High risk	H_3–AH	Alfalfa hay	5.45	Internal	~1 km/intensive ~1 km/medium **	~2 km (271.692)
	H_3–AS	Alfalfa Silage	10.9	Internal	~1 km/intensive ~1 km/medium **	~2 km (271.692)
	H_3–CS	Corn silage	45.45	Internal	~4 km/intensive ~1 km/medium **	~2 km (271.692)
	H_3–C	Corn grains	6.35	Internal	~1 km/intensive ~1 km/medium **	~2 km (271.692)
	H_3–S	Soya	7.1	External purchase	-	-
	H_3–T	Triticale	4.54	Internal	~5 km/medium	~4 km (2.067)
	H_3–BSG	Brewer's grains	18.18	External purchase	-	-
	H_3–CF	Combined feed ***	100	Internal	AH, AS, CS, C, S, T, BSG	
TOTAL (H_3): 8						

* Traffic conditions associated with urban areas (national roads). ** Air transport (25 landing–take-off sequences). *** Supplements are also included in the combined feed. d = distance; $S_{1,2}$ = parcelling area; L_1, M_2, H_3 = farm codes indicating contamination risk levels: low (L), medium (M), and high (H); AH, AS, BSG, C, CF, CS, MP, NH, S, T = abbreviations used for feed sample types derived from the sample name (column 3).

Different types of samples (green fodder, dry fodder, pickled fodder, concentrated fodder, and combined fodder) were collected for analysis from the feed base of the farms during the feed-obtaining campaign in 2021–2022. Some of the samples were taken directly from the crop area, and others were taken after harvesting steps, from their transport machine, or from the storage areas. A total of 15 feed samples were obtained for the laboratory (2 samples from the L_1 farm, 5 samples from the M_2 farm, and 8 samples from the H_3 farm) after dividing the elemental samples taken depending on the size of the sampling area and the specific ration from each farm. The samples were packaged, labelled, and transported to the laboratory for processing.

The sampling, the protocol, but also the reporting of the results followed the standards and performance criteria of the analytical approaches, but also different working protocols used by different authors [11].

Table 2. Market formula and active ingredients for crop-care treatments.

Sample	Market Formula	Active Ingredient
M_2–AH	Pulsar 40	40 g/L Imazamox
M_2–CS	Sulfammo–25–APPM–1	25% N (18% ammoniacal N; 7% N nitric); 31% SO_3; 2% MgO
	Principal Plus	9.2% Nicosulfuron; 55% Dicamba; 2.3% Rimsulfuron
M_2–C	DAP 18–46–0	18% NH_4; 46% P_2O_5
	Sulfammo–25–APPM–1	25% N (18% ammoniacal N; 7% N nitric); 31% SO_3; 2% MgO
	Principal Plus	9.2% Nicosulfuron; 55% Dicamba; 2.3% Rimsulfuron
H_3–CS and H_3–C	Urea	$CO(NH_2)_2$
	NPK 20–20–0 Complex	20% total N; 20% total P_2O_5; 60% P_2O_5 water soluble; 98% P_2O_5 soluble in citric acid 2%; max. 0.6% water
	Ammonium nitrate	27% N; 7% CaO; 5% MgO
	Henik	40 g/L Nicosulfuron
	Mustang	6.25% Florasulfam; 30% Acid 2,4D EHE
	Adengo	225 g/L Isoxaflutol; 90 g/L Thiencarbazone-methyl; 150 g/L Cyprosulfamides
H_3–AH and H_3–AS	16–16–16 Complex	16:16:16 N:P:K
	Corum	480 g/L Bentazon; 22.4 g/L Imazamox
H_3–T	Urea	$CO(NH_2)_2$
	Ammonium nitrate	27% N; 7% CaO; 5% MgO
	Lebosol	1.6% Cu—$Cu_2Cl(OH)_3$ 25 g/L; 11.5% Mn—MnO_2 183 g/L; 4.9% Zn—ZnO 78 g/L
	Pixxaro Super	12 g/L Halauxifen-methyl; 280 g/L Fluroxy-pyr meptyl; 12 g/L Cloquintocet-mexyl
	Orius	250 g/L Tebuconazole
	Falcon Pro	53 g/L Prothioconazole; 224 g/L Spiroxamine; 148 g/L Tebuconazole
	Mospilan	20% Acetamiprid

M_2, H_3 = farm codes indicating contamination risk levels: medium (M) and high (H). AH, AS, C, CS, T = abbreviations used for feed sample types derived from sample names: AH = alfalfa hay; AS = alfalfa silage; C = corn grain; CS = corn silage; t = triticale. APPM = activated poly-phenolic molecules; DAP = diammonium phosphate; NPK = nitrogen (N), phosphorus (P), and potassium (K) fertiliser; SO_3 = sulphur trioxide; MgO = magnesium oxide; NH_4 = ammonium; P_2O_5 = phosphorus pentoxide; CaO = calcium oxide; EHE = ethylhexyl ester; $Cu_2Cl(OH)_3$ = dicopper chloride trihydroxide; MnO_2 = manganese dioxide; ZnO = zinc oxide.

Feed samples were collected according to the SR EN ISO 6497:2005 [40] standard and Regulation (EC) 152/2009 Annex I [41]. Sample preparation was carried out according to the SR EN ISO 6498:2012 [42] and Regulation (EC) 152/2009 Annex II [41]. Samples were manually cut to 1–2 cm, dried to 8–12% moisture (60 °C, ESAC–100 model thermo-adjustable oven, Electronic April s.r.l., Cluj–Napoca, Romania), then finely ground using a Grindomix GM 200 (Verder GmbH, Vienna, Austria) laboratory mill. The samples were stored in polypropylene and aluminium packaging to prevent contamination until analysis.

2.2. Protocol, Reagents, and Standards

A method based on liquid chromatography–gas chromatography–flame ionisation detection (LC–GC–FID), including a microwave-assisted saponification (MAS) step, was applied to the feed samples for determining MOSH and MOAH fractions.

The protocol was adjusted to indicate the specificity of our samples, being similar to the methodology used by Bauwens et al. [21] for MOSH and MOAH analysis from fish feed. Due to the presence of natural n-alkanes and olefins, additional sample purification steps were required, such as epoxidation and passage through aluminium oxide (AlOx), according to the protocol developed by Nestola and Schmidt [43]. Method optimisation and protocol development were performed with adjustments based on the work of Biederman et al. [44] and Biedermann and Grob [45,46]. The method met the analytical performance criteria set out in the Joint Research Centre (JRC) Guide [36]. The protocol used for optimisation was proposed by Moret et al. [47] and applied with good results in other studies [25,48,49].

The following solvents and reagents were used for the preparation and analysis of feed samples: n-hexane (≥95%), methanol (≥99.9%), saturated KOH, metachloroperoxybenzoic acid (mCBPA; 70–75%, 200 mg/mL ethanol), anhydrous sodium thiosulphate, aluminium oxide, and sodium sulphate.

All reagents used were purchased from Merck Millipore (Burlington, MA, USA), Sigma-Aldrich (Saint Louis, MO, USA), or Supelco (Bellefonte, PA, USA). The mCBPA reagent was obtained from Acros Organics (Thermo Fisher Scientific, Waltham, MA, USA). Ultrapure water was obtained using a Milli-Q filtration system (Millipore, Bedford, MA, USA). To prevent contamination during sample preparation, all glassware was carefully cleaned and rinsed with pure solvents (acetone and n-hexane) before use.

A standard mixture purchased from Restek (Bellefonte, PA, USA) was the Internal Standard (IS) #31070 (150–600 µg/mL in toluene 99%) used for LC–GC performance evaluation, MOSH/MOAH separation, integration, and quantification of the results. This standard includes the following: n-Undecane (n–C_{11}; 99%; 0.3 mg/mL), cyclohexylcyclohexane (CyCy; 0.3 mg/mL), n-pentylbenzene (5B; 99%; 0.3 mg/mL), 1–methyl naphthalene (1–MN; 98%; 0.3 mg/mL), 2–methyl naphthalene (2–MN; 96%; 0.3 mg/mL), 1,3,5–tritertbutylbenzene (TBB; 99%; 0.3 mg/mL), n-tridecane (n–C_{13}; 99%; 0.15 mg/mL), 5-α-cholestane (Cho; 99%; 0.6 mg/mL), and perylene (Per; 99%; 0.6 mg/mL).

2.3. Sample Preparation

Organic phase separation was performed according to the protocol optimised by Moret et al. [47] for MOH extraction from cereal-based products. Saponification was performed using a microwave system (MARS 5, CEM Corporation, Bergamo, Italy), equipped with Teflon-lined cartridges. Each vial was filled with 5 g of feed sample, 10 mL of KOH (40%), 10 mL of n-hexane, and 20 µL of IS, then subjected to microwave extraction for 20 min at 120 °C. After extraction, the mixture was diluted with 40 mL of Milli-Q ultrapure water and 2 mL of methanol and set aside for phase separation. The extract was concentrated under vacuum up to 4 mL (Uniequip centrifuge, UNIVAPO–100H model, coupled with a V-700 vacuum pump and V-850 controller, Büchi AG, Flawil, Switzerland).

For a pure extract, a washing step with a mixture of methanol and water (2:1 v/v) was applied. The samples were vortexed and centrifuged, and the purified extract was concentrated to 700 µL. For alfalfa hay, corn silage, and compound feed samples, additional purification was required due to the presence of large amounts of natural n-alkanes. This step was performed according to the method described by Nestola and Schmidt [43]. The extract was epoxidised with mCBPA, to which sodium thiosulphate and ethanol were added, and 500 µL of this mixture was transferred for injection into the LC–GC–FID system. For the AlOx purification step, 40 µL of the epoxidised extract was diluted with n-hexane and passed through an AlOx and sodium sulphate cartridge. The extract obtained was concentrated to 250 µL, of which 75 µL was injected into the LC–GC–FID system.

2.4. LC–GC–FID Analysis and Instrument Conditions

MOSH and MOAH analysis was performed with a LC–GC 9000 Brechbuhler system (Zurich, Switzerland) composed of a Phoenix 9000 HPLC coupled to a Trace 1310 GC (Thermo Fisher Scientific, Waltham, MA, USA), configured with a dual channel for simultaneous analysis of the fractions. MOH fractions were transferred from LC to GC via a Y-interface using partial eluent evaporation, according to the method of Biedermann et al. [44].

For HPLC, a Lichrospher Si 60 column (25 cm × 2.1 mm, 5 µm) from DGB (Schlossboeckelheim, Germany) was used. The dual channel of the GC was composed of two PS-255 columns (15 m × 0.25 mm, 0.15 µm, Mega, Milan, Italy) connected to gap pre-columns and a solvent vapour removal system.

The HPLC elution program started with 100% n-hexane, followed by a switch to a 70/30 n-hexane/dichloromethane mixture at a flow rate of 300 µL/min. MOSH and MOAH fractions were transferred to GC between 2.1 and 3.6 min and 3.8 and 5.3 min, respectively. The carrier gas (H_2) was set at a constant pressure of 60 kPa, and the GC temperature was increased from 51 °C to 350 °C at a rate of 20 °C/min. The FID detector was heated to 350 °C with a collection rate of 10 Hz.

2.5. MOH Quantification and Method Validation

Data were processed using Chromeleon 7.3 software (Thermo Fisher Scientific, Waltham, MA, USA). The quantification was based on the internal standard CyCy for MOSH and average values of 5B, 1–MN, 2–MN, and TBB for MOAH. Methodologically, the total mass fractions for MOSH and MOAH were expressed in mg, related to the mass of the sample (expressed in kg), after the separation and removal of all possible interferences from the extract and the quantification and integration of the entire chromatographic signal between n–C_{10} and n–C_{50} retention times. Associated areas were integrated, and interferences were eliminated by running blanks for each batch of samples.

Analytical method performance was evaluated according to the JRC [36] and Eurachem [50] guidelines, using blind samples. These allowed for checking the possible contributions of the reagents used in the analytical process of the measurement signal, thus eliminating the possibility of external interferences. The limit of quantification (LOQ) for each n–C fraction, as well as for total MOSH and MOAH, was determined according to the recommendations in the SANTE/12682 guidelines [51]. Method performance met the JRC guideline criteria, with recovery values between 70 and 120%, as well as suitable intermediate repeatability for method validation.

3. Results

The feeds were analysed based on the technological development degree of each farm to assess the level of MOSH/MOAH contamination and identify the technological factors contributing to this contamination.

4. MOSH and MOAH in Animal Feed

MOSH and MOAH contamination for all feeds is indicated by the hydrocarbon range (n-C; 6 MOSH sub-fractions and 4 MOAH sub-fractions) and the total area (n–$C_{10–50}$). The data synthetically present the average results and summarise the overall situation of contamination levels with MOSH (Figure 2) and MOAH (Figure 3) of each type of feed. MOSH and MOAH concentrations are expressed in mg/kg. The quantification in the n-$C_{10–50}$ range was achieved by integrating the peaks, respecting the performance criteria described in the JRC guide [36], according to the European Commission regulations [52].

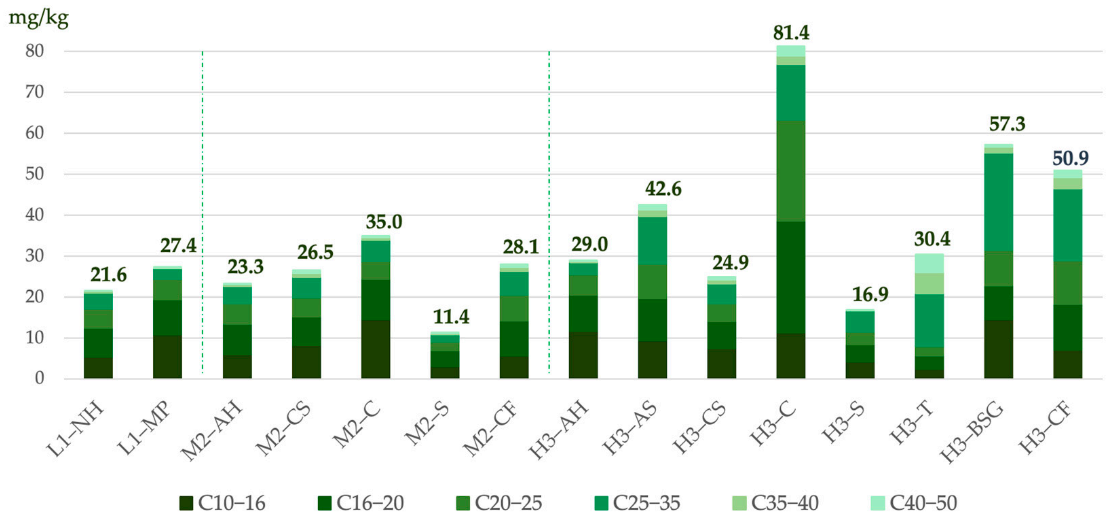


Figure 2. MOSH concentrations of feed sampled from each farm. L_1, M_2, H_3 = farm codes indicating contamination risk levels: low (L), medium (M), and high (H). AH = alfalfa hay; AS = alfalfa silage; BSG = brewer's grain; C = corn grain; CF = combined feed; CS = corn silage; MP = mountain pasture; NH = natural hay; S = soya; T = triticale. Values are the mean of two replicates per sample, shown as carbon fractions (n–$C_{10–50}$).

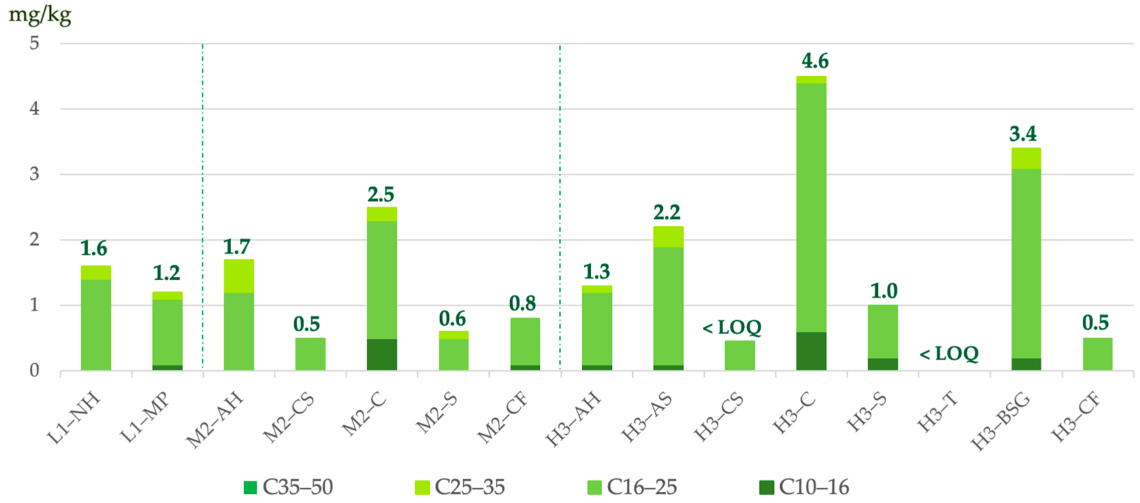


Figure 3. MOAH concentrations of feed sampled from each farm. L_1, M_2, H_3 = farm codes indicating contamination risk levels: low (L), medium (M), and high (H). AH = alfalfa hay; AS = alfalfa silage; BSG = brewer's grain; C = corn grain; CF = combined feed; CS = corn silage; MP = mountain pasture; NH = natural hay; S = soya; T = triticale. Absence of data labels indicates levels below the limit of quantification (LOQ; 0.5 mg/kg). Values are the mean of two replicates per sample, shown as carbon fractions (n–$C_{10–50}$).

The results following the analyses performed confirmed the MOH contamination of most of the feed samples. Important value differences were observed depending on the degree of technological development of each farm.

The L_1 farm, practicing semi-intensive agricultural and livestock activities, presented important levels of MOH contamination of feed, on average between 21.6 mg/kg and 27.4 mg/kg MOSH and 1.2 mg/kg and 1.6 mg/kg MOAH. These results show a serious potential contamination determined by applying some rudimentary agricultural and technological practices. The lack of effective contamination prevention measures also contributes.

In the feed from the medium-risk farm (M_2), MOSH levels ranged from 11.4 mg/kg to 35.0 mg/kg, while MOAH levels ranged from 0.5 mg/kg to 2.5 mg/kg. These results indicate a remarkable contamination linked to the higher degree of technological development, likely due to inadequate monitoring of technological processes.

Compared to the less advanced farms, the farm utilising intensive machinery and possibly inadequate feed handling or storage practices (high-risk farm, H_3) exhibited higher MOH contamination levels. This may suggest a greater exposure to contamination sources during cultivation, harvesting, transport, and storage. The average MOSH contamination ranged from 16.9 mg/kg to 81.4 mg/kg, and MOAH levels reached a maximum of 4.6 mg/kg.

Related to the technological specifics of each farm, the contamination levels were relatively proportional to the degree and intensity of exposure to various sources of contamination, as we found in the sampling sheets. Different numbers of samples were collected from the three farms (two samples from L_1 farm, five samples from M_2 farm, and eight samples from H_3 farm). While this variation in sample size could suggest differences between results, all samples were analysed according to the same methodological standards and validation criteria to ensure data comparability and consistency.

In analysing MOH contamination of feed, particular attention was especially focused on the proportion of the MOAH fraction, as it poses a risk factor to the safety of animal products. MOAH is considered more toxicologically concerning because it may include polycyclic aromatic compounds, some of which may have carcinogenic potential.

Figure 4 highlights the lack of uniformity among the 15 feed samples, with variability accentuated by the differing technological levels of the farms. The differences in MOSH and MOAH proportions can be attributed to specific feed-processing technologies, including the technical quality and maintenance of the machinery or processing equipment, the quality of mineral oils, but also the origin of raw materials, particularly those sourced from industrialised areas.

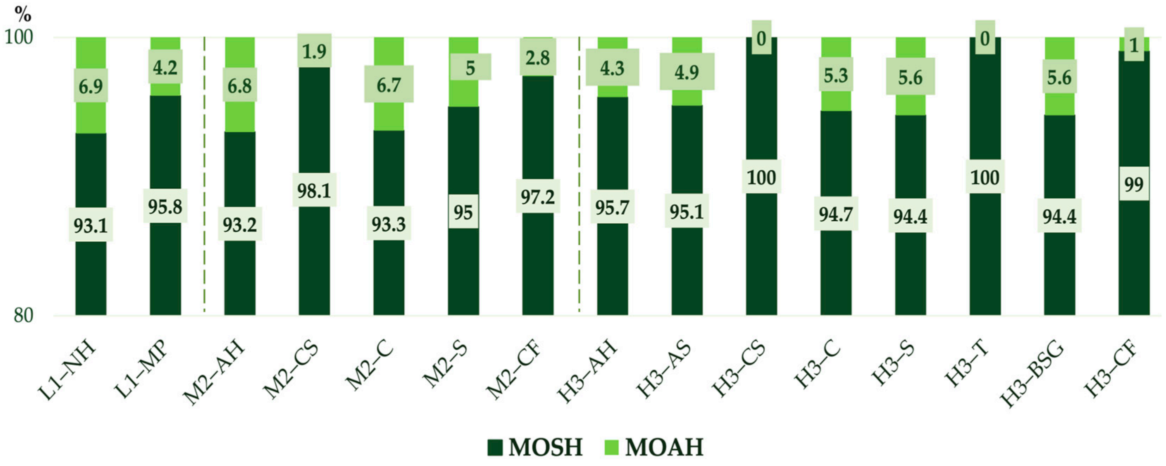
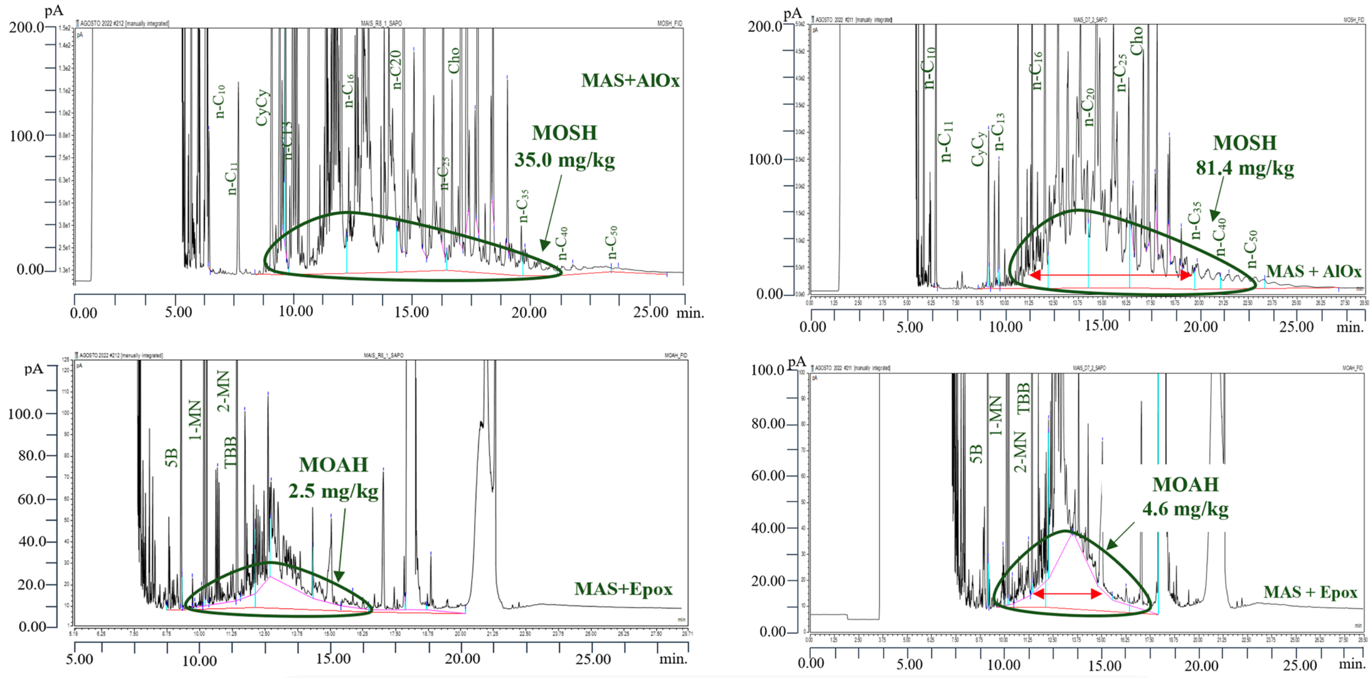


Figure 4. Proportion (%) of MOSH and MOAH in feed relative to total MOH content. L_1, M_2, H_3 = farm codes indicating contamination risk levels: low (L), medium (M), and high (H). AH = alfalfa hay; AS = alfalfa silage; BSG = brewer's grain; C = corn grain; CF = combined feed; CS = corn silage; MP = mountain pasture; NH = natural hay; S = soya; T = triticale.

MOAH concentrations showed some variability, with certain samples, such as H_3–AH (12.7%) and H_3–BSG (5.6%), presenting higher proportions. On average, in our samples, MOAH accounted for 4.74% of the total MOH content, which raises concerns because of the toxic nature of this fraction. Although this percentage may appear small, even the small amounts of MOAH in feed can be alarming for food safety if transferred into food products. Considering the lack of clearly defined European limits for MOAH in food or feed, it is recommended to minimise contamination as much as possible, ideally reaching an absence of MOAH in feed, to reduce the risk of transfer into the food chain.

A selection of chromatograms that confirmed MOSH and MOAH presence in various feed samples are shown in Figures 5 and 6. The contamination profiles were analysed for natural hay (L_1–NH), corn grain (M_2–C and H_3–C), alfalfa hay (M_2–AH), and corn silage (M_2–CS).

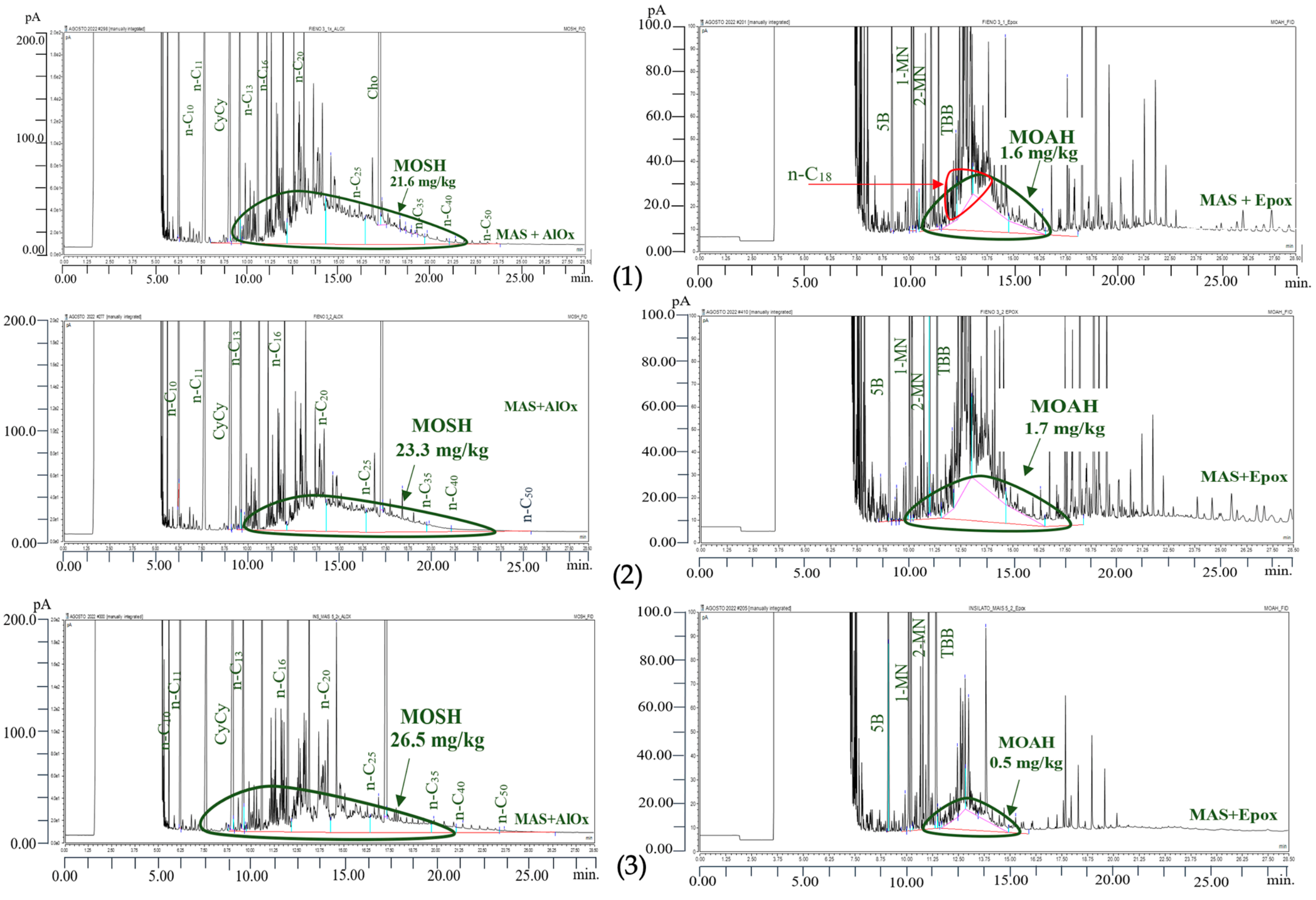


(**a**) (**b**)

Figure 5. MOSH (**top**) and MOAH (**bottom**) HPLC–GC–FID chromatograms confirming contamination of corn grain samples M_2–C (**a**) and H_3–C (**b**) collected from the storage area. The green arrows and circles highlight the contamination concentrations and common profiles for MOSH and MOAH, particularly around specific molecular humps (n–$C_{10–35}$ MOSH and n–$C_{16–25}$ MOAH). The red arrow in (**b**) evidences the boundary distinguishing the MOSH and MOAH areas. Retention time (x): 0–28.5 min, and detector signal (y): 0_200 pA MOSH/0_100 pA MOAH.

Corn grain samples (M_2–C and H_3–C) indicated the highest levels of MOSH and MOAH, with a common contamination profile focused on the same molecular humps (n–$C_{10–35}$ MOSH and n–$C_{16–25}$ MOAH). This increased contamination can be attributed to factors such as mechanised harvesting, handling, and long-term storage, where mineral oils from equipment likely served as contamination sources. Additionally, the mineral oils used in post-harvest treatments to prevent infestation and preserve the feeds, along with the specific structure and chemical composition of corn, making it more prone to absorbing contaminants, also contributed to this contamination profile.

For the natural hay (L_1–NH), alfalfa hay (M_2–AH), and corn silage (M_2–CS) samples, a common contamination profile was observed, probably associated with the mineral oils used in agricultural machinery, as was also reported by Srbinovska et al. [49] in a study on certain plant products. Contamination can be further attributed to the specific technological processing methods. Mowing, baling, and compacting hay and silage involve direct contact with different equipment and materials (e.g., polythene sheeting), which can also be factors in MOSH and MOAH contamination (Figure 6).



(**a**) (**b**)

Figure 6. MOSH (**a**) and MOAH (**b**) HPLC–GC–FID chromatograms confirming contamination of natural hay L_1–NH (**a1**,**b1**), alfalfa hay M_2–AH (**a2**,**b2**), and corn silage M_2–CS (**a3**,**c3**). The green arrows and circles highlight the contamination concentrations and the common profiles between samples for MOSH and MOAH. The red line in (**b1**) evidences the contamination profile centred around a specific molecular hump (n–$C_{16–35}$). Retention time (x): 0–28.5 min, and detector signal (y): 0_200 pA MOSH/0_100 pA MOAH.

5. Technological Operations

We considered it important to assess the potential feed contamination factors, some predictable factors, focusing on those typically associated with the specific technological processes and the level of mechanisation within the analysed farms. Table 3 summarises the main activities evaluated for their potential contribution to MOSH/MOAH contamination against the level of feed contamination.

The high contamination levels in the feed samples, as indicated by sampling sheets, suggest, to a certain extent, a direct connection between the MOH content in the feed and the diversity and complexity of the technological operations carried out on the farm.

An overview of the data from the sampling sheets reported that typical fertilisation or pest-control treatments were applied for crop protection on farms M_2 and H_3. The treatments applied did not seem to have a direct and express connection with the contamination levels detected. According to their labels, the substances used did not specifically contain MOH risk compounds. However, within a legislative framework that lacks strict regulation, the absence of specific mentions on the labels does not exclude the possible presence of MOH as co-formulants, since current regulations do not require their declaration. Moreover, the diversity of treatments applied (on average, 6–7 different substances) suggests that these practices cannot be entirely dismissed as potential sources of MOSH and MOAH contamination.

Table 3. Feed contamination factors linked to technological processes and mechanisation levels in analysed farms.

Sample	MOSH	MOAH	Feeding	Technological Operations Applied to Crops				Storage	
				Phytosanitary Treatments (P) and Fertilisation (F)		Harvesting/ Handling	Equipment	Area	Type/ Material
	mg/kg			Type	Formula */ Quantity (ha)				
L_1–NH	21.6	1.6	Manually	Organic/Manually	-	Mechanised	(a) Mowing/harvesting machine (b) Transport vehicle	Half-open	Traditional wooden construction
L_1–MP	27.4	1.2	Manually	-	-	-	-	-	-
M_2–AH	23.3	1.7	Technological trailer	Organic and Chemical/Mechanised	P: Pulsar 40 (1.1 L/ha)	Mechanised	(a) Sprinkler pump (b) Mowing/harvesting machine (c) Transport vehicle (d) Baler	Open	Unwrapped bales
M_2–CS	26.5	0.5	Technological trailer	Organic and Chemical/Mechanised	F: Sulfammo–25–APPM–1 (170 kg/ha) P: Principal Plus (440 g/ha)	Mechanised	(a) Sprinkler pump (b) Harvesting machine (c) Transport vehicle (d) Crawler tractor	Open	Concrete cell covered with polyethylene film
M_2–C	35.0	2.5	Technological trailer	Organic and Chemical/Mechanised	F: DAP 18–46–0 (250 kg/ha); Sulfammo–25–APPM–1 (250 kg/ha) P: Principal Plus (440 g/ha)	Mechanised	(a) Sprinkler pump (b) Harvesting machine (c) Transport vehicle	Closed	Polypropylene bags
M_2–S	11.4	0.6	Technological trailer	-	-	Mechanised	Transport and unloading vehicle	Closed	Polypropylene bags
M_2–CF	28.1	0.8	Technological trailer	-	-	Mechanised	Technological trailer	-	-
H_3–AH	29.0	1.3	Technological trailer	Organic and Chemical/Mechanised	F: Complex 16–16–16 (250 kg/ha) P: Corum (1.2 L/ha)	Mechanised	(a) Sprinkler pump (b) Mowing/harvesting machine (c) Transport vehicle (d) Baler and foil press	Open	Polyethylene foiled bales
H_3–AS	42.6	2.2	Technological trailer	Organic and Chemical/Mechanised	F: Complex 16–16–16 (250 kg/ha) P: Corum (1.2 L/ha)	Mechanised	(a) Sprinkler pump (b) Harvesting machine (c) Transport vehicle (d) Crawler tractor	Open	Concrete cell covered with polyethylene film
H_3–CS	24.9	<LOQ **	Technological trailer	Organic and Chemical/Mechanised	F: urea (100 kg/ha); NPK 20–20–0 (100 kg/ha); Ammonium nitrate (150 kg/ha) P: Henik (1.5 L/ha); Mustang (0.6L/ha); Adengo (0.4 L/ha)	Mechanised	(a) Sprinkler pump (b) Harvesting machine (c) Transport vehicle (d) Crawler tractor	Open	Concrete cell covered with polyethylene film
H_3–C	81.4	4.6	Technological trailer	Organic and Chemical/Mechanised	F: urea (100 kg/ha); NPK 20–20–0 (100 kg/ha); Ammonium nitrate (150 kg/ha) P: Henik (1.5 L/ha); Mustang (0.6L/ha); Adengo (0.4 L/ha)	Mechanised	(a) Sprinkler pump (b) Harvesting machine (c) Transport vehicle	Closed	Silo
H_3–S	16.9	1.0	Technological trailer	-	-	Mechanised	Transport and unloading vehicle	Closed	Silo

Table 3. *Cont.*

Sample	MOSH	MOAH	Feeding	Technological Operations Applied to Crops				Storage	
				Phytosanitary Treatments (P) and Fertilisation (F)		Harvesting/ Handling	Equipment	Area	Type/ Material
	mg/kg			Type	Formula */ Quantity (ha)				
H_3–T	30.4	<LOQ **	Technological trailer	Organic and Chemical/Mechanised	F: urea (150 kg/ha); Ammonium nitrate (150 kg/ha); Lebosol (1.5 L/ha); P (I): Pixxaro Super (0.3 L/ha); P(II): Orius, Falcon Pro (0.5 L/ha) P(III): Mospilan (0.15 L/ha)	Mechanised	(a) Sprinkler pump (b) Harvesting machine (c) Transport vehicle	Closed	Silo
H_3–BSG	57.3	3.4	Technological trailer	-	-	Mechanised	Transport and unloading vehicle	Open	Concrete platform
H_3–CF	50.9	0.5	Technological trailer	-	-	Mechanised	Technological trailer	-	-

* The treatments and active substances can be found in the previous sections. ** Absence of data labels indicates levels below the limit of quantification (LOQ; 0.5 mg/kg). L_1, M_2, H_3 = farm codes indicating contamination risk levels: low (L), medium (M), and high (H). AH, AS, BSG, C, CF, CS, MP, NH, S, T = abbreviations used for feed sample types derived from sample names: AH = alfalfa hay; AS = alfalfa silage; BSG = brewer's grain; C = corn grain; CF = combined feed; CS = corn silage; MP = mountain pasture; NH = natural hay; S = soya; T = triticale. APPM = activated poly-phenolic molecules; DAP = diammonium phosphate; NPK = nitrogen (N), phosphorus (P), and potassium (K) fertiliser.

Although no clear connection was established between the number or type of treatments applied and the level of MOH contamination, several noteworthy observations and trends emerged.

Samples H_3–C and H_3–BSG showed the highest contamination levels, with 81.4 mg/kg MOSH and 4.6 mg/kg MOAH, and 57.3 mg/kg MOSH and 3.4 mg/kg MOAH, respectively, despite undergoing relatively few chemical treatments. Even for crops with fewer treatments, such as alfalfa, similarly high contamination levels were reported (29.0 mg/kg to 42.6 mg/kg MOSH, and 1.3 mg/kg to 2.2 mg/kg MOAH) as for crops with more diversified treatments (24.9 mg/kg to 81.4 mg/kg MOSH, and 0.5 mg/kg to 4.6 mg/kg MOAH). This suggests that other factors, such as mechanised handling, equipment, and storage conditions, may play an important role in contamination.

Samples such as L_1–NH and L_1–MP, which were treated organically without using chemicals, showed lower contamination levels compared to those treated with both organic and chemical methods, such as H_3–C. While a direct causal connection could not be established, it can be inferred that organic treatments did not seem to have an important contribution to MOSH and MOAH contamination.

Some of the primary sources of MOSH and MOAH contamination are the residues, emissions, and technical oils from agricultural equipment and machinery used in feed processing. On the studied farms, the moderate to high technological level indicated an important contamination with MOSH and MOAH. Specifically, on farms M_2 and H_3, the extensive use of mechanised agricultural processes stood out as a major technological factor contributing to contamination. The variety of agricultural machinery used for harvesting and processing feed, such as harvesters, balers, or crawler tractors, presented a high risk of contamination due to the use of engine oils, lubricants, or hydraulic oils, which are known sources of MOH contamination. Moreover, for some feeds, such as hay or silage, the risk of contamination increased even more because of the specific processing methods involved, particularly direct contact of the feed with mechanised equipment and materials used in the process (e.g., polyethylene film).

Samples collected and transported using mechanised methods, such as L_1–NH, M_2–C, and H_3–C, showed high levels of MOH contamination. This confirmed that mechanised equipment can be an important source of contamination. Furthermore, the use of multiple complex machines for samples M_2–CS, M_2–C, H_3–AS, and H_3–C appeared to be associated with high contaminations (ranging from 26.5 mg/kg to 42.6 mg/kg MOSH and 0.5 mg/kg to 2.2 mg/kg MOAH). This reinforces the hypothesis that prolonged contact between feed and various mechanised components of equipment during harvesting and transport processes may introduce contaminants. Nevertheless, in the case of the L_1-MP sample, where animals grazed freely, no mechanised operations were involved that could have influenced the level of contamination; thus, the hypothesis of technological contamination was excluded in this case.

Regarding the causal connection between contamination levels and feed storage conditions, both the type of storage (closed or open) and the materials used (polyethylene, polypropylene, and concrete) seemed to have a certain influence on MOSH and MOAH contamination. Prolonged exposure to open environments and the use of synthetic materials appeared to be associated with higher contamination. Although various chemicals were used on some farms to sanitise animals or shelters, they did not seem to have a marked impact on the level of MOH contamination. No clear results could be directly linked to these substances, suggesting that other sources are likely more important to feed contamination.

6. Discussion

The results of our research reported varying levels of MOSH and MOAH contamination in the analysed feeds, influenced by technological factors on the selected farms.

Although the topic of this paper has gained attention in recent years and relevant studies have been conducted, it is possible that existing data are limited to food contamination or only to certain types of feed and technologies. However, similar data to those in the present research have been reported in other studies. Jaén et al. [53] obtained comparable conclusions regarding MOSH and MOAH contamination levels in food, assigning this to contaminant migration from packaging materials and direct contact with processing equipment. In the study of Srbinovska et al. [25], part of the contamination was also attributed to the technological equipment and materials used during production. High levels of MOH were detected in feed stored in polyethylene bags, similar to the H_3–C and H_3–S samples in this study, which were maintained under similar conditions. In contrast, L_1 samples were stored in different conditions, without polyethylene materials, which may account for their contamination levels.

Recently, Menegoz Ursol et al. [33] investigated the impact of certain agricultural technologies on MOH contamination. The risks associated with the use of mechanised equipment, such as a mechanised comb, straddle harvester, or pneumatic comb, were highlighted, and their findings seem to support our hypothesis regarding the role of advanced equipment in feed harvesting, especially the use of hydraulic oils and other technical oils as sources of contamination. The same studies mentioned the critical factors involved and mechanisms through which feed-processing technologies influence MOH contamination. Various conclusions suggested that hydrocarbon migration from contact materials into feed can occur through direct contact or infiltration of residues from the storage spaces, especially under conditions of material degradation or exposure to extreme conditions [53]. These data aligned with other studies investigating feed contamination from packaging and coating materials. In particular, research emphasised the risk of hydrocarbon infiltration from polymers during prolonged storage or in environments exposed to temperature and humidity fluctuations [54].

The confirmation of MOSH and MOAH presence in animal feed can have serious implications for the safety of animal products. Albendea et al. [55] showed that MOH-contaminated feed can be a direct source of contamination in animal productions. The authors further detailed how MOH from feed can be transferred into animal tissues, directly affecting the safety of meat. The presence of MOH in products such as dairy is even more concerning, as these are regularly consumed by vulnerable groups, including children, the elderly, or the ill. In addition to the toxic risks for consumers, high levels of contamination can lead to non-compliance with food safety standards established by international organisations, such as the European Food Safety Authority [23]. In the

European Union, strict limits are recommended for MOSH and MOAH contamination in food, and producers who exceed these limits face economic sanctions or even the withdrawal of their products from the market [2,20,23,52].

The results of this study, along with the importance of assessing technological risk in preventing MOSH and MOAH contamination, are supported by the literature. However, more extensive monitoring may be necessary to fully understand the implications for food safety and public health.

The contamination levels identified in the analysed feed emphasised the need for certain measures to minimise contamination risks, especially during the harvesting and processing stages. Adopting good working practices is an effective solution, as contamination can occur at all stages of the feed production chain. More awareness of the contamination risks caused by technical oils used in agricultural equipment is necessary.

To prevent MOSH/MOAH contamination, it is recommended to replace lubricants and oils with high-grade, refined, or food-grade products that do not contain MOH. For enhanced safety, completely MOH-free alternatives should be considered. Furthermore, proper maintenance of agricultural machinery and equipment is particularly important for reducing the risk of contamination. Regular sanitation and proper usage of equipment can help prevent the accumulation and transfer of contaminants to feed. Additionally, improvements in the design and development of agricultural equipment that minimise the impact on feed could provide long-term solutions, even though they may require substantial technological changes.

7. Conclusions

This study showed a clear connection between the technologies employed in feed production and the levels of MOSH and MOAH contamination. Specifically, the storage conditions, as seen in certain samples, appeared to be associated with elevated contamination levels, underscoring the importance of storage materials and methods in the technological process.

The comparative assessment of contamination levels generally indicated the presence of MOSH and MOAH across all types of feed at each studied farm. The proportional differences were attributed to the specific characteristics of each unit concerning their exposure to various contamination sources, including the degree of technologisation in farm operations, the location of agricultural crops, and the technological practices employed.

Factors such as the mechanisation of the agricultural process, the machinery used, and the storage conditions considerably influenced contamination levels, highlighting the essential requirement of implementing advanced technological measures to mitigate this issue. The data clearly showed that the use of mechanised equipment, such as combines, balers, crawler tractors, and transport vehicles, was associated with increased contamination, particularly when the equipment came into direct contact with feed. Storage conditions and the materials used further exacerbated contamination, indicating that other stages of the technological processes may also contribute to the transfer of contaminants. The chemical treatments applied did not appear to have a direct impact on MOH contamination; however, the presence of unclarified co-formulants and the mechanised application processes could represent indirect sources of contamination.

In the context of the findings concerning MOSH and MOAH contamination in feed, it is necessary to embrace optimal technological measures to mitigate these risks. Farmers and producers are encouraged to replace the lubricants and technical oils used in agricultural equipment with food-grade products or MOH-free alternatives. They should also ensure regular and appropriate maintenance of mechanised equipment to prevent leaks of technical oils and the accumulation of contaminants on surfaces. Moreover, using well-protected storage areas and avoiding materials that may contribute to contamination is important. Lastly, supporting improvements in the design of agricultural equipment to minimise direct contact with feed can help reduce contamination risks. As a forward-looking solution, transitioning towards the electrification of agricultural machinery by replacing combustion engines with electric motors could further reduce MOH contamination, enhancing overall safety in feed production.

The elevated levels of contamination in feed samples cannot be totally attributed to technological factors. The MOSH and MOAH concentrations suggested the presence of multiple sources of contamination that are likely more diverse than initially expected.

To further substantiate the research findings, future studies could explore in depth the impact of additional technological steps on contamination, including processing methods and the influence of atmospheric conditions, as well as exposure to urban pollution sources. A more comprehensive approach should also extend to analysing MOSH and MOAH contamination in various types of feed, but especially in animal products. Such an approach would provide a broader perspective on risks throughout the food chain and have a direct impact on food safety.

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