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MINERAL OIL HYDROCARBONS (MOH) ANALYSIS IN ANIMAL FEED: A CHARACTERIZATION BASED ON MODERN POLLUTION

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Abstract

This research aims to confirm and quantify the presence of mineral oil hydrocarbons (MOHs) in feed, as well to investigate the contribution of modern pollution sources to the level of contamination. Through simultaneous processes of microwave assisted saponification (MAS), extraction and purification procedures, followed by the LC-GC-FID detection, 8 types of feeds from one of the most polluted areas of the country were analyzed. The results indicated contamination with MOH for most of the feed samples, mineral oil saturated hydrocarbons (MOSH) concentrations above the recommended limits (0.5 mg/kg) being recorded. The data indicated moderate to high contamination for MOSH, from 16.5 mg/kg to 77.3 mg/kg, while average values below the limit of quantification (< LOQ) were highlighted for mineral oil aromatic hydrocarbons (MOAH) content. Based on the results, was difficult to establish a clear relationship between feed contamination, crop location and different pollution sources. However, the information obtained by assessing the relationship between feed contamination and pollution, indicated that the pollution sources from the plotting area had an important contribution to the contamination of the analyzed feedstuffs.

Key words*: evolution, milk production, NW Region, Romania, trends.*

INTRODUCTION

In recent years, the characterization of the hazards associated with environmental pollution has focused on different modern pollutants, thus studies on the assessment of the exposure of environmental elements to modern pollution have seen important progress.

Mineral oil hydrocarbons (MOH) are a complex mixture of contaminants derived from chemical products from crude mineral oils and other solvents, following extensive distillation and refining processes (IARC, 2012), which resulted in various residual fractions, including fuels (diesel, jet fuel), motor oils, lubricants; MOH can be also synthetically produced from coal, natural gas or biomass (Zoccali et al., 2016; Bratinova & Hoekstra, 2019).

As a result of their chemical origin, the presence of MOH in environmental substrates represents a serious threat to the environment, as mineral oils come from a comprehensive chemical group, with complex structures and a varied number of carbon atoms (n-C_{10-50}).

According to EFSA (2012); Van Heyst et al. (2018), the complexity of each type of mineral oil include two main fractions that can be analytically separated: MOSH-saturated hydrocarbons from mineral oils, composed of alkanes (normal, isoalkanes and cycloalkanes), which includes, generally, a complex of paraffins and naphthenes, and MOAH - aromatic hydrocarbons from mineral oils, comprising alkylated fractions and non-alkylated cyclic systems, respectively polyaromatic compounds substituted with alkyl groups in the n-C_{10-50} range.

The non-polar character given by their chemical structure makes MOSH & MOAH to have a special affinity for fatty tissues; for this reason, their presence has been confirmed in numerous types of vegetable oils (Nestola, 2022; Menegoz Ursol et al., 2023) and other products with high fat content (Zhang et al., 2019; Bauwens et al., 2022; Srbinovska et al., 2023). The occurrence of MOH and possible sources of contamination have been extensively described in the literature (Purcaro et al., 2016; Gharbi et al., 2017).

Regarding the incidence and frequency of occurrence, MOH can appear as a result of contamination and various intentionally uses in the production chain (Grob, 2014; Bruhl, 2016; Zoccalli et al., 2016; Canavar et al., 2018)

through the products used for machinery and equipment (oils, lubricants) or as a component of packaging and transport materials (jute bags, cardboard packaging, waxed paper). More than contaminants, MOSH & MOAH are among the most important modern pollutants originating especially from emissions from urban area (transport, chemical and petrochemical industry), and also intensive agricultural processes (Van Heyst et al., 2018).

MOH contamination was mentioned for the first time around the 90s, when the first studies regarding the ecotoxicological safety and the origin of MOH contamination of different substrates has appeared (Grob et al., 1991; Moret et al., 1996; Wagner et al., 2001; Neukom et al., 2002). Since 2009, the analysis of MOH has become a routine through applied toxicological analysis methods. Biedermann et al. (2009) had published the first updated LC-GC-FID method that focused on the confirmation of MOSH and MOAH in different samples (Moret et al., 2016; Hochegger et al., 2021).

Although the effects on human or animal health are uncertain and insufficiently studied, the determination of MOH has gained more interest in recent years. For both, humans and animals, food is the main source of contamination of the body with MOSH and MOAH (Sdrigotti et al., 2021). Research carried out so far on animal tissues (Griffis et al., 2010; Boogaard et al., 2012; Barp et al., 2017; Cravedi et al., 2017) and human tissues (Barp et al., 2014; Biedermann et al., 2015; Adenuga et al., 2017; Carrillo et al., 2019) reported the ability of MOSH to accumulate especially in organs (liver, spleen) or in adipose tissues.

If for MOSH, according to the experiences carried out on animals and based on their structure and molecular weight, it has been shown that contamination can generally induce liver diseases, about MOAH, there are greater concerns given the aromatic structure, similar to polycyclic aromatic hydrocarbons (PAHs), with an alkylation degree of over 98% (Bratinova & Hoekstra, 2019) which thus imposes on them a genotoxic character and mutagenic actions, acting as promoters tumors (EFSA, 2012; Grob, 2018; Van Heyst et al., 2018; EFSA, 2019).

Despite the importance of feed for animal growth and development and for obtaining productions, and also the fact that the presence of MOSH & MOAH would represent a serious threat to feed and food safety considering the negative effects they could give, in the literature, there is no solid data on MOH contamination of feed are available to date.

Based on this background, the aim of this research was to quantify and confirm, for the first time, the presence of MOH in the feed representing the ration of a dairy farm, as well as to investigate the contribution of modern pollution sources to the final contamination level. Thus, processes of microwave assisted saponification (MAS) (Srbinovksa et al., 2021), followed by extraction step, modified according to Nestola & Schmidt (2017), were applied for 8 types of feeds, the samples thus prepared being analyzed by means of LC-GC-FID, having as a reference the method adapted according to Bauwens et al. (2022); Srbinovka et al. (2022).

The results from this work will contribute to the improvement of knowledge about MOSH and MOAH contamination of feed in relation to modern pollution, being a new topic for Romania and for the field of animal nutrition. Moreover, the results will create an overview of the understanding the importance of MOSH and MOAH contamination in animal nutrition and the importance of feed safety as an essential factor for the safety of animal production.

MATERIALS AND METHODS

Sample and sample collection

In order to quantify the level of contamination with MOSH and MOAH, were analyzed eight types of feed samples (raw materials and combined feed), taken from the feed base of a dairy cow farm located in an urban area in NE Romania, considered the second most polluted urban settlement in the country.

Excepting two samples: soybean meal (SM) and brewer's spent grain (BSG), which was purchased from external sources, all samples were obtained in the own vegetal farm of the unit, so that the feed sampling was carried out both directly, from the field, during harvesting, as well as from the storage area, with the necessary precautions to prevent any kind of modification or contamination.

The samples were collected in relation to the size of the sampling lots, in average quantities between 1-2 kg (depending on the type of sample). The selected samples were obtained by reducing the collective samples, according SR EN ISO 6497:2005 standard and Regulation (EC) 152/2009-Annex I.

Depending on the type of the feed, all the samples collected were prepared for analysis according to the guidelines mentioned in SR EN ISO 6498:2012 standard and Regulation (EC) 152/2009-Annex II, so as to avoid the contamination of the samples or the changes on their composition, using equipments that do not cause heating of the product mass or that do not be a source of contamination with mineral oils.

The feed samples were prepared for analysis according their type: cutting to 1-2 cm sizes, drying (moisture 8-12%) and grinding with the Grindomix GM 200 mill to fine powder. The samples were stored in aluminium packages until the analysis was carried out.

All the samples had a fat content < 4% and did not have in their composition other ingredients that could influence the results of the analyses. More information about the collected samples (types, quantities, plots) was included in Table 1.

Table 1. Characteristics of the samples and of the samples sites

Sample code	Area (ha)/ No.	% ration	Technological works			Sampling sites location	
			Phytosanitary (P) & Fertilising (F) treatments	Harvesting	Storage	d - roads/ trafic*	d - urban areas (no.)
Own production							
AH Alfalfa hay	30/6	5.5	F: Complex 16-16-16 (250 kg/ha) P: Corum (1.2 L/ha)	Mechanized/ (a) mowing and harvesting; (b) transport, (c) baling and wrapping press	Bales with plastic foil	~ 1 km / intense ~ 1 km/ medium**	~ 2 km (507.100)
AS Alfalfa silage		10.9		Mechanized/ (a) combine harvester; (b) transport; (c) crawler tractor	Concrete cell, polyethylene foil		
CS Corn silage	50/10	45.5	F: urea (100 kg/ha); NPK 20-20-0 (100 kg/ha); Ammonium nitrate (150 kg/ha) P: Henik (1.5 L/ha); Mustang (0.6 L/ha); Adengo (0.4 L/ha)			~ 4 km / intense ~ 1 km/ medium**	~ 2 km (507.100)
GrC Grain corn	23/4	6.4			Grain silos	~ 1 km / intense ~ 1 km/ medium**	~ 2 km (507.100)
T Triticale	8/2	4.5	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)	Mechanized/ (a) combine harvester; (b) transport	Grain silos	~ 5 km / medium	~ 4 km (2.067)
MF *Mixed feed*	-		Σ FL, SSL, SP, PB, SS, T, BB	Mechanized: technological trailer	-	∑ AH, AS, CS, GrC, T, SM, BSG	
External purchase							
SM *Soya meal*	-	18.2	-	Mechanized: transport	Grain silos	~ 1 km/ intense	~ 2 km (507.100)
BSG *Brewer Spent Grain*	-	6.5	-		Concrete platform	~ 1 km/ intense ~ 1 km/ medium**	~ 2 km (507.100)

Instrumentation and analysis conditions

For analysis of each carbon fraction: MOSH (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 MOAH (n-C_{10-16}; n-C_{16-25}; n-C_{25-35}; n-C_{35-50}), for all the samples, a method based on LC-GC-FID was applied, preceded by a microwave-assisted saponification (MAS) step.

The optimization of the method and the development of protocol belong entirely to the Food Chemistry Laboratory of the University of Udine (Department of Agrifood, Environmental and Animal Sciences), adapted and modified according to Biederman et al. (2009), Bierdemann & Grob (2012), and meets the requirements of JRC Guide (Bratinova & Hoekstra, 2019). The starting point in optimizing the method was the protocol developed by Moret et al. (2016), the method being subsequently applied with good results in numerous works (Menegoz Ursol et al., 2022; Srbinovska et al., 2022; Srbinovska et al., 2023). With some modifications, considering the specificity of the samples, the protocol corresponds to the method previously applied by Bauwens et al. (2022) for the determination of MOSH and MOAH in fish feed.

As a result of the interference of olefins, additional steps of clean-up of the samples (epoxidation, alluminium oxide clean-up) were necessary, being carried out according to the protocol described by Nestola & Schmidt (2017); CEN (2016).

RESULTS AND DISCUSSIONS

Regarding the level of contamination with MOSH and MOAH, in order to quantify and confirm the presence of MOH in relation to the modern sources of pollution, associated with the geographical area studied, were analyzed eight types of feed taken from plots located in one of the most polluted areas of the country (according World Air Quality Index, 2023).

Due to the complexity of the analyzed matrices, the monitoring and quantification of MOH proved to be difficult and required complementary analysis steps. However, the data obtained clearly indicated considerable MOH contamination for an important proportion of the analyzed feed samples.

By integrating the peaks in accordance with the principles and performance criteria established by the JRC Guide (Bratinova & Hoekstra, 2019) in correspondence with the European Commission (EC, 2022), was achieved the quantification for each carbon fraction included in the n-C_{10-50} range (6 MOSH C-fractions; 4 MOAH C-fractions) using CyCy as internal standard for MOSH quantification and average of 5B, 1-MN, 2-MN and TBB internal standards for MOAH quantification. Tables 2 and 3 summarizes the average values, expressed in mg/kg, showing the level of feed contamination with MOSH and MOAH for each carbon fraction (n-C) and for total content (n-C_{10-50}).

Table 2. MOSH content (mg/kg) of feed samples

Sample	% fat	IS MOSH	MOSH (mg/kg)						
			C_{10-16}	C_{16-20}	C_{20-25}	C_{25-35}	C_{35-40}	C_{40-50}	C_{10-50}
AH	1.67	CyCy	12.2	9.2	5.3	3.4	< LOQ (0.4)	< LOQ (0.4)	**31.0**
AS	0.55		9.9	10.1	8.1	11.0	1.5	1.0	**41.6**
CS	1.27		8.7	7.4	5.5	6.8	1.2	0.8	**30.5**
GrC	3.74		9.0	27.9	23.8	12.4	2.1	2.1	**77.3**
SM	1.08		4.1	4.1	3.0	5.0	< LOQ (0.2)	< LOQ (0.1)	**16.5**
T	1.37		2.3	3.2	2.0	12.5	4.7	3.3	**28.0**
BSG	2.24		14.6	8.4	8.5	27.2	1.5	0.7	**60.9**
MF	1.08		7.3	10.8	11.9	23.2	3.3	1.9	**58.4**

Absence of data labels (nd.) indicates levels below the LOQ (0.5 mg/kg for MOSH); CyCy = cyclohexylcyclohexane.

Table 3. MOAH content (mg/kg) of feed samples

Sample	% fat	IS MOAH	MOAH (mg/kg)				
			C_{10-16}	C_{16-25}	C_{25-35}	C_{35-50}	C_{10-50}
AH	1.67	5B/ 1-MN/ 2-MN/ TBB	< LOQ (0.06)	0.98	< LOQ (0.09)	< LOQ (nd.)	**1.13**
AS	0.55		< LOQ (0.09)	1.71	< LOQ (0.20)	< LOQ (nd.)	**2.00**
CS	1.27		< LOQ (nd.)	0.47	< LOQ (nd.)	< LOQ (nd.)	**0.47**
GrC	3.74		0.75	3.77	< LOQ (0.16)	< LOQ (nd.)	**4.68**
SM	1.08		0.24	0.73	< LOQ (nd.)	< LOQ (nd.)	**0.97**
T	1.37		< LOQ (nd.)	< LOQ (nd.)	< LOQ (nd.)	< LOQ (nd.)	**< LOQ**
BSG	2.24		< LOQ (0.20)	2.77	< LOQ (0.15)	< LOQ (nd.)	**3.13**
MF	1.08		< LOQ (nd.)	< LOQ (0.49)	< LOQ (0.02)	< LOQ (nd.)	**0.51**

Absence of data labels (nd.) indicates levels below the LOQ (0.5 mg/kg for MOAH); 5B = n-pentylbenzene; 1-MN = 1-methylnaphthalene; 2-MN = 2-metilnaftalenă; TBB = 1,3,5-tri-terț-butil-benzen.

Different from a compositional point of view, all analyzed feed samples, alfalfa hay (AH), alfalfa silage (AS), corn silage (CS), grain corn (GrC), soybean meal (SM), triticale (T), brewers spent grain (BSG) and mixed feed (MF) recorded MOSH concentrations above the limits recommended by the Guide JRC and the European Commission (2022) for products with a fat content < 4%, respectively 0.5 mg/kg. The data obtained indicating a moderate contamination, from 16.5 mg/kg (min. value) to a particularly high contamination 77.3 mg/kg (max. value). Instead, values below the limit of quantification (< LOQ) were highlighted regarding the MOAH content for the analyzed samples, 50 % of the samples recording values above the safety limits recommended previously; the highest MOAH contents were confirmed for BSG samples, 3.13 mg/kg and GrC samples, 4.68 mg/kg. The chromatograms in Figure 1 confirm the presence of MOSH and MOAH in the feed samples analyzed. Also, Figure 2 shows the contamination profile of the feed samples, by carbon fractions (n-C), that indicate the same hump on the same molecular rank, n-C_{10-35} for MOSH and n-C_{16-25} for MOAH, considered typical of contamination with lubricants, engine oil or hydraulic oil, as Srbinovska et al. (2023) mention in the similar study on MOH contamination of basil pesto.

However, the typical contamination was not considered representative for the high level of contamination found, so, that sources of contamination of a different origin than those already anticipated were associated with the analyzed fodder.

The literature generally shows that the presence of hydrocarbons from mineral oils as contaminants can come from various sources, from urban areas, car traffic or industrial sites (Neukom et al., 2002), but also from technological operations in farms (EFSA, 2012).

The information previously collected in the sampling sheets developed by Matei & Pop (2022) show that the farm is located in an urban area, in the immediate vicinity of numerous sources of pollution from industrial activities, construction and road infrastructure, municipal waste or road and air traffic, so that the pollution risk highlighted can be a probable cause of the high level of contamination with MOSH and MOAH identified for the analyzed feeds.

From the samples obtained in the own vegetable farm (AH, AS, CS, GrC, T, MF), excepting triticale samples (that will be discussed separately), all the other samples correspond to similar cultivation areas, being exposed to the same sources of pollution. Samples of alfalfa, corn for silage and for grains harvested near roads with high traffic (approx. 20.000 cars/day; Traffic Management Office, Iasi), air traffic runways (25 landing-takeoff cycles/day) and near areas of industrial activities (~1-2 km) contained average levels of MOSH above 30 mg/kg and MOAH above 1 mg/kg.

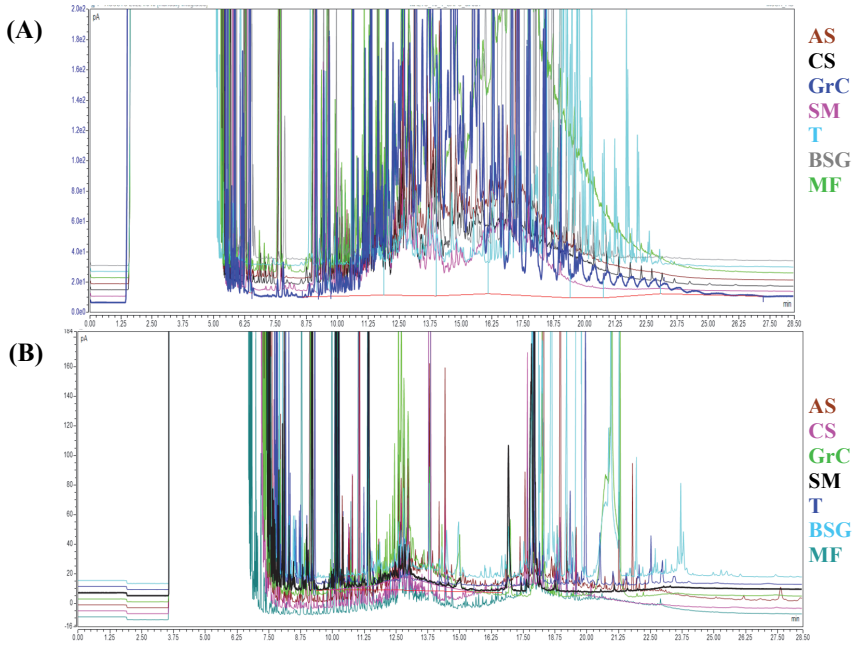

Figure 1. Overlay of MOSH (A) and MOAH (B) chromatograms of feed samples.
The figure legend is reffered the interpretation of the references to colour

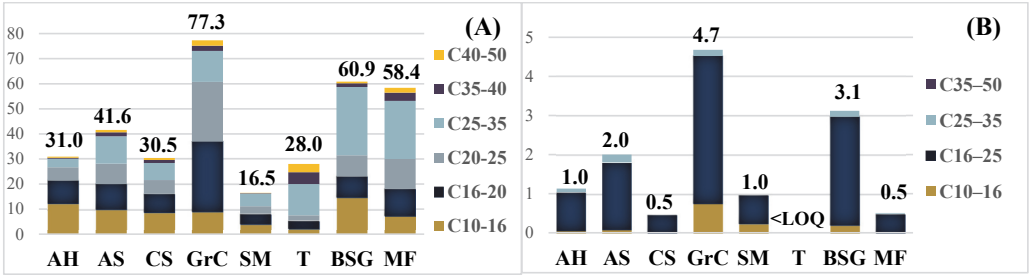

Figure 2. MOSH (A) and MOAH (B) distribution on C-fractions in feed samples

However, in terms of MOSH and MOAH concentrations detected for feed samples exposed to the same pollution sources, no uniformity was observed; giving an example, the MOSH and MOAH content for GrC samples (77.3 mg/kg and 4.68 mg/kg), was double that the content shown for AS (41.6 mg/kg MOSH, 2.0 mg/kg MOAH), AH (31.0 mg/kg MOSH, 1.13 mg/kg MOAH) or CS (30.5 mg/kg MOSH, < 0.5 mg/kg MOAH). These can be argumented bu the higher fat content of feed samples, therefore a higher concentration of contaminants in these types of samples, which can confirm the hypothesis developed by Matei et al. (2022) regarding the contribution of fats in the contaminant accumulation process. Also, the potential contamination of corn samples during processing cannot be keep out.

Figure 3 shows a selection of feed samples whose chromatograms obtained from contaminant analysis with LC-GC-FID system confirm the presence of MOSH and MOAH. The most representative samples were selected, with the most important levels of contamination (GrC; MF) and whose MOSH traces highlight the typical contamination profile previously mentioned. Carbon fractions with important contamination areas was also highlighted, but still unknown in origin, thus suggesting multiple contamination.

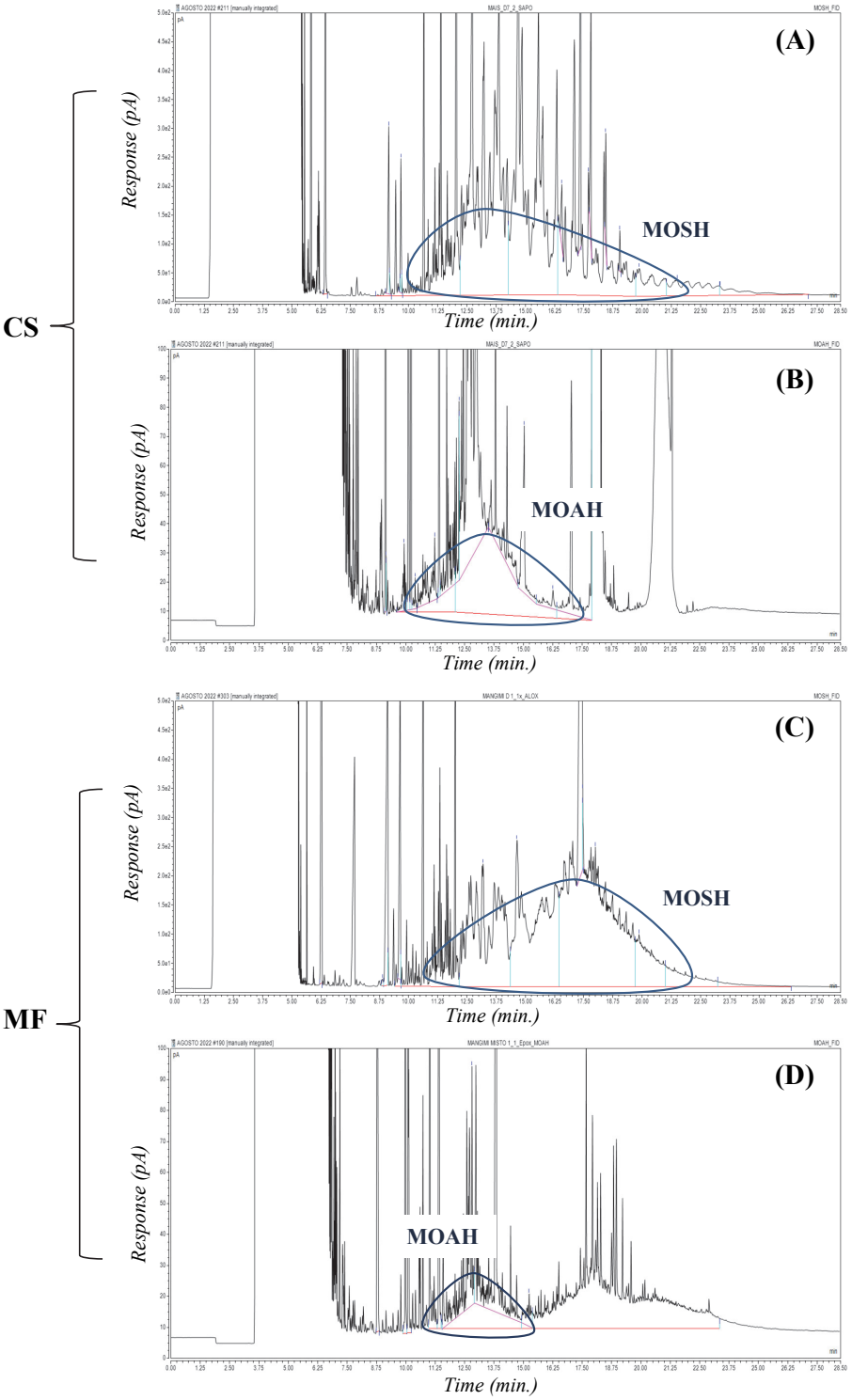

Figure 3. LC-GC-FID chromatograms of a selection of samples with existing contamination (CS, MF). (A), (C): MOSH traces and (B), (D): MOAH traces

Contrary to the previously samples, the T (triticale) samples located further from potential sources of pollution (the plot located in a rural area with low traffic density, ~ 5 km from populated areas), revealed an unexpectedly high level of MOSH contamination (28.0 mg/kg) whose origins still remain unknown. For MOAH instead contents below < LOQ were observed, which allows establishing a clear relation regarding the location of the crop relative to the sources of environmental pollution.

Regarding the compound feed samples, 58.4 mg/kg MOSH and 0.5 mg/kg MOAH concentrations obtained was considered proportional to the contribution of the raw feeds to the mixture and the content of MOSH and MOAH obtained for them.

Although they are outsourced samples, for which there is no information on crop location and associated potential pollution sources, the level of MOSH and MOAH contamination of SM (16.5 mg/kg MOSH; 1.0 mg/kg MOAH) and of BSG (60.9 mg/kg MOSH; 3.1 mg/kg MOAH) should not be considered minor as they indirectly contribute to the contamination level of the mixture formed.

The information obtained by evaluating the relationship between feed contamination and pollutant factors clearly indicated that the pollution sources associated with the analyzed sampling areas had an important contribution to the contamination of the analyzed feed. In addition, the possible contamination during feed care, harvesting and storage operations or the contribution of undeclared work practices by farmers that can substantially contribute to the contamination of feed with MOSH and MOAH have not been completely neglected.

CONCLUSIONS

In both MOSH and MOAH analysis, good quantitative and confirmatory results regarding contamination were generally obtained, important to prove the reliability of the LC-GC-FID detection method used, for which good performance was demonstrated (LOQ), according the requirements of JRC guideline.

Chromatographically, the contamination profiles confirmed the presence of different sources of pollution and contamination. In relation to the exposure to different sources of pollution founded, important clues were assigned to pollution from the urban area. The level of contamination observed was considered proportional to the degree and intensity of exposure to the associated modern pollution sources (urban traffic, air traffic, industrial activities). However, establishing a clear relation between the level of feed contamination, crop location and proximity to different sources of pollution has proven difficult.

In terms of identifying the most important sources of pollution and contamination, great efforts are needed to minimize contamination, although awareness of existing problems, and using good manufacturing practices can help to reduce the risks associated with high levels of contamination.

More studies are needed to assess the contribution of modern pollution to the contamination of feed with MOSH/MOAH. Although the contamination of feed with MOSH and MOAH does not currently cause significant problems, the potential cumulative risk may be sufficiently high, therefore, the economic consequences and the potential negative consequences on animal health and on the safety of consumers should not be considered minor.

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