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EXCUTIVE SUMMARY:

MAIN SOURCES OF MOH CONTAMINATION AND ENTRY POINTS INTO THE COCOA SUPPLY CHAIN

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Required by **JOINT RESEARCH FUND**

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ABSTRACT

Mineral oil hydrocarbons (MOH) are ubiquitous contaminants, mainly composed of two fractions, saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH), that have raised significant concern in the last years. The lack of definitive risk assessment, along with a lack in the occurrence data and analytical tools, has prevented the setting of an official limit. Nevertheless, the issue is open, and the European Union has asked for additional investigation.

Cocoa products are potentially exposed to MOH from the farm to the final product delivered to the consumer. Fuel or fumes from oil fired burners present potential contamination sources when cocoa beans are dried artificially or stored in warehouses. Other major sources are potentially packaging materials (jute bags) and the use of recycled paper/board, printing inks, and environmental contaminants (such as lubricants, fuels, debris from tires and road bitumen). Therefore, the ECA MOH Taskforce organized a study (divided into two parts) with **the aim to better understand the possible sources of contamination and entry points of MOH in the cocoa supply chain and the reliability of the analytical methods**.

- **Entry points**: Storage in origin and container transport to the final destination showed a critical role. Nevertheless, the high dependence of the contamination towards different batches of jute bags or cardboard, made it difficult to draw a definitive conclusion.
- **Contamination sources**: A lab-scale test, where cocoa beans were exposed to contaminated jute bags, showed that the migration occurred from the jute bags to the cocoa beans, with a preference of the lighter C-fractions. The shell seems to act as a barrier allowing only the lightest fraction to reach the nibs (~8% after 1 months and 20% after 2 months compared to the shells). Finally, contact time clearly plays an important role in the overall migration.

Reliability of the analytical methods: three ring tests were organized proving the necessity to still improve the methods for the determination of MOSH and MOAH, and as well of the chosen markers for assessing the contamination, namely phytane, pristane, and DIPN. Although the z'-scores were generally successful, the % uncertainty associated with the determination of MOSH and MOAH is not included in the (not specific for MOSH and MOAH) acceptable ranges set by the EU 2002/657/EC Commission Decision[1] (i.e., reproducibility <16% and trueness between +10% and -20 %). The LOQs reported were very different for the different laboratories contributing to increase the uncertainty of the final results, arising challenges related to the interpretation of MOSH/MOAH testing especially if close to LOQ of the methods also used for commercial testing.

PART 1A: SAMPLING EXERCISE

Main objective

In this part of the project the goal was to **identify the critical contamination points** in the supply chain: beans were sampled at arrival in the bean warehouse at origin ("after bushbag arrival") before shipment to Europe ("before shipment") and at arrival in the port in Europe ("after shipment"). Figure 1 reports a scheme of the sampling points.

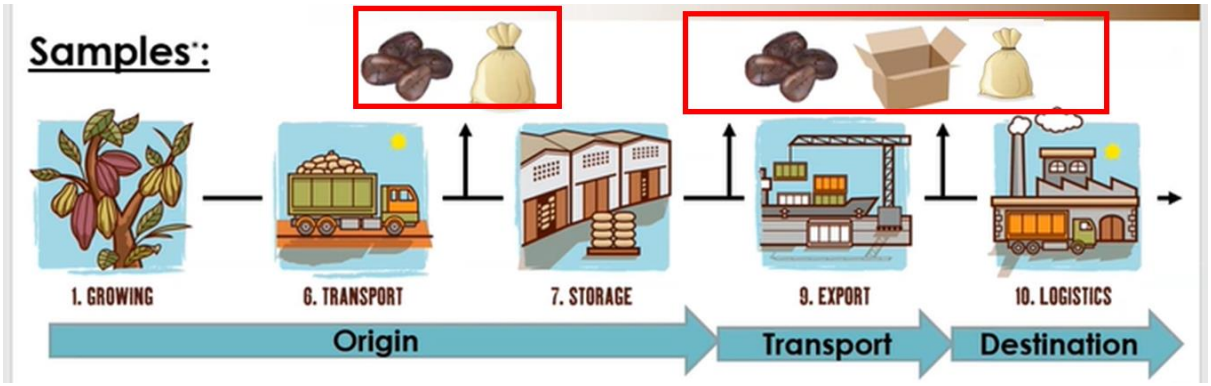

***Figure 1**. Scheme of the sampling points.*

Alongside that also the cardboard liners and the jute bags were sampled from the same containers at arrival in the ports in Europe. By sampling the same container before shipping and after arrival in the ports, the impact of the contact with jute and cardboard was evaluated. One container is typically filled with 385 bags. Each jute bags weighs 0.7 kg and contains 65 kg of cocoa beans. So in total, there are about 270 kg jute per container or about 10 g jute per kg of beans. The cardboard is located in two layers on the bottom and side of the container and one layer on top of all the jute bags (see Figure 2).

***Figure 2**. Photo of a) interior of the container; b) expansion of the contact between jute bags and cardboard on the bottom*

Approximately 400 samples (Table 1) at different stages were collected in the cocoa supply chain at origin. At the partner laboratory, samples were tested for MOH level using online-LC-GC-MS/FID system.

Table 1 *reports a summary of all the samples collected.*

Commodity	Origin	Stage in supply chain	N. Samples	
cardboard	CVI	-	**15**	10
	Nigeria			5
jutebags	Ghana	Bushbag	**20**	5
	CVI	Transport bag		10
	Nigeria			5
cocoa beans	CVI	after bushbag arrival	**326**	226
		before shipment		20
		after shipment		20
	Nigeria	after bushbag arrival		20
	Ghana			20
	Cameroon			20

Results and Discussion

1.1. Total MOSH and MOAH by FID

1.1.1. Packaging

The sum of the MOSH fractions (C10-C50) (= total MOSH) and of the MOAH fractions (C10-C50) (= total MOAH) were used for preliminary considerations. The ratio of MOSH/MOAH was evaluated. A ratio higher than 3-5 suggests contamination due to white mineral oil, while lower ratios suggest overestimation of the MOAH fraction due to the presence of interferences not adequately eliminated by the purification steps (11 samples out of 35 had MOSH/MOAH<3). Despite this doubts on the proper quantification of MOAH, all the data were further elaborated.

Similar MOSH and MOAH contamination can be observed in cardboard (327-1047 mg/kg for MOSH; 65-514 mg/kg MOAH) **and jute bag** (0-3537 mg/kg MOSH; 0-486 mg/kg MOAH) samples, although a larger variability is observed in jute bags.

The level of contamination of the jute bags seems more related to a random effect, probably linked to the production batch of the jute bag itself.

MS Data on packaging: Quantification of MOSH and MOAH using MS still needs a complete validation to understand how the different interferences contribute to the MS quantification. Nevertheless, a quantification using MS can serve as an indication to confirm the identity and to verify the presence of known markers of specific contamination sources.

Cardboard: 10 out of 15 cardboard samples were analyzed in MS as well. In 8 samples out of 10 the DIPNs peaks were identified over the LOQ. MOSH and MOAH contamination was confirmed by MS. Traces of sulphur-compounds, markers of jute bags contamination were found in some samples.

Jute bags: 10 out of 20 samples were analyzed by MS. As expected, sulphur-containing compounds were detected in all the samples. MOSH and MOAH contamination was confirmed.

1.1.2. Cocoa beans

In regard to the elaboration of the packaging samples, as a starting point, the evaluation was done on the total MOSH and total MOAH, and evaluating the MOSH/MOAH ratio. Only 11 samples had a MOSH/MOAH ratio ≥4.0 and 21 ≥3.0. This means that doubts on the MOAH quantification arise for all the samples that present a lower ratio.

- **A significant increase ($p<0.05$) in MOSH content can be observed between the cocoa beans arrived at the warehouse and before the shipment.** (No significant difference for the MOAH).

A critical step may occur during the cocoa beans handle after their delivery at the warehouse and before the shipment to the final destination. Most probably, the time factor (i.e., time of contact with jute bags before sampling) and possibly temperature at the warehouse may play an important role. It would be interesting to investigate further these variables.

Although not statistically significant due mainly to the high variability of the results, a trend of further contamination can be observed during the transport overseas.

CIV1 and CIV 2 sampling exercise:

Two separate containers were sampled before and after shipment from Côte d'Ivoire (CIV1 and CIV2). Before shipment and after shipment, beans were sampled at the top and at a random spot in the container. Cardboard and jute bags were sampled only after shipment. Very different results were obtained from CIV 1 and CIV 2 in particular regarding the jute bags and cardboard data, which suggests an **important effect of the different batches** of these products. MOSH and MOAH were not detected (<LOD) in any jute bags from the CIV 1 sampling.

- **The higher amount of MOSH in beans from the CIV2 sampling than for CIV 1, pointed towards the jute bags as the main contamination source.**

A general trend of increase during shipment can be observed for both MOSH and MOAH (**Figure 3**). No clear trend was observed in the relation of the sampling position in the container, *i.e.* top or random, suggesting a **non-significant contribution of the cardboard in the cocoa beans contamination**.

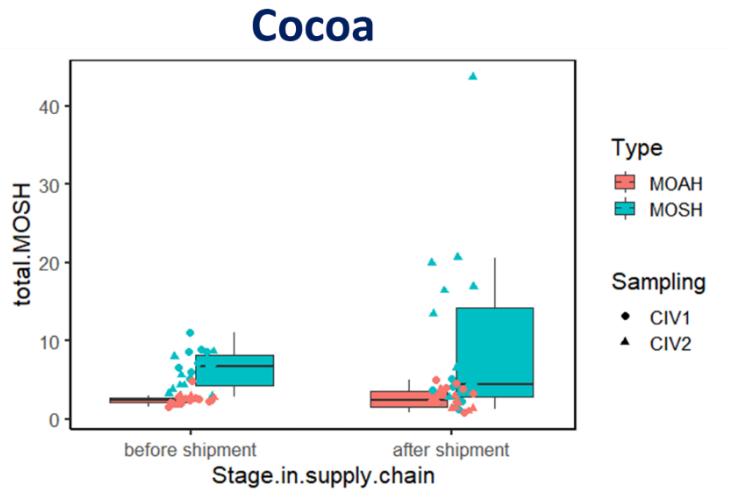

Figure 3. *Boxplot of the MOSH and MOAH content according to the Stage in the supply chain. Two sampling considered together, but different shapes indicate the sampling.*

C-Fraction profile:

Despite the high variability among samples (>20%) that make it difficult to have a clear trend, the distribution profile of the different C-fraction was investigated.

The MOAH C-fraction did not show any change regardless the comparison, and not following the trend of the MOSH (as it should be) suggesting again a misidentification. Regarding the MOSH, the profiles before shipment are different between CIV1 and CIV2. It needs to be recalled that the contribution of jute bags in CIV1 is virtually null, as the profile change before and after shipment is limited. A contribution of jute bags cannot be ruled out completely, with the contamination being randomly present (not all bags were analysed). Observing the profile of the samples from the CIV2 sampling, jute bags seem to be the main source of MOSH, as also the C-fraction profile is centered on lighter fractions compared to cardboard. The prevalence of lighter fractions also suggests a migration through volatility (**Figure 4**).

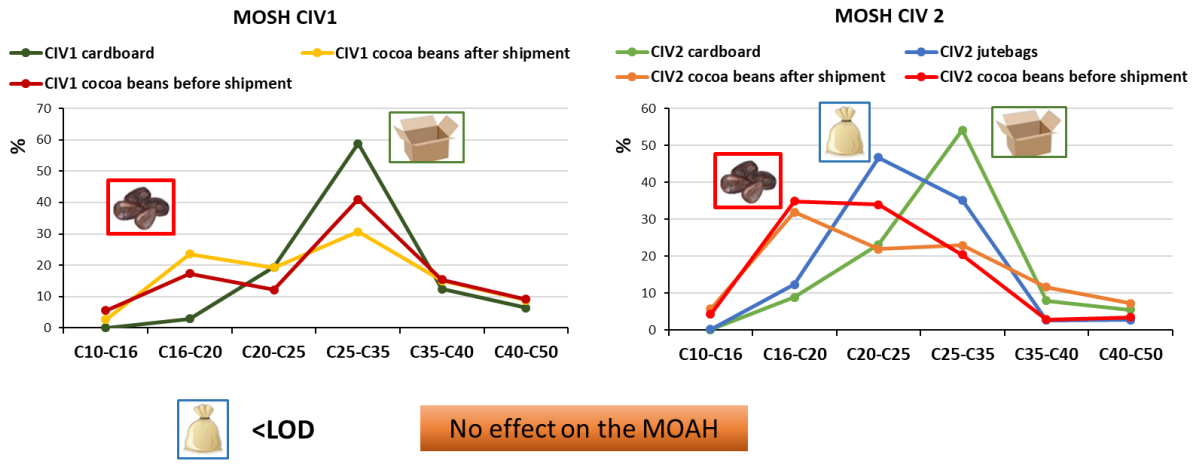

Figure 4. *MOSH and MOAH percentage profiles of the different C-fractions for the samples CIV1 and CIV 2 of cocoa beans before and after shipment.*

MS Data on cocoa beans: 42 Cocoa beans samples, all belonging to the CIV1 and CIV2 sampling plus two extra, were analysed by LC-GC-MS to verify the presence of specific markers of contamination.

- **Markers of petrogenic origin, of cardboard and jute bags migration were found:**

  Phytane and Pristane (markers of petrogenic origin of the MOSH) and 2,6-diisopropyl naphthalenes (DIPN, marker of cardboard contamination) were both found in any of the cocoa bean samples. Sulphur-compounds (markers of jute bags contamination) were found in traces in 6 samples of cocoa beans.

Conclusion

- **The contamination occurs during the storage in origin and the transport to the final destination**. Nevertheless, the high dependence of the contamination towards different batches of jute bags or cardboard, make it difficult to draw a definitive conclusion.
- The possible **presence of interferences not completely removed in the MOAH fraction hindered the identification of a clear trend for the MOAH**, although it is reasonable to conclude that the MOAH level follows the same trend of the MOSH.
- Cardboard and jute bags showed a similar overall contamination, while no trend of contamination was observed sampling the top (cocoa beans closer to cardboard) or randomly the cocoa beans. This observation suggests a **more significant role of jute bags** towards the overall contamination of the cocoa beans.
- The **contact time certainly plays an important role**, as the clear increase of the contamination in the three sampling points in the supply chain proved.

- Examination of the C-fraction profile suggested migration suggested migration of the lighter fraction, i.e. <C25 indicating a **migration mainly through volatility** and most probably originated from the jute bags.

PART 1B: LAB TEST

Main objective

The main goal was to understand the **behaviour of the migration from jute bags into the cocoa beans**, in regards to the time of storage and in terms of C-fraction distribution.

Results and Discussion

A lab test was performed to evaluate the migration from a contaminated jute bags into cocoa beans. Shells and nibs from cocoa were analysed before (named "**sample 1**") and after the contact with a jute. Two different proportions of cocoa beans and jute were used. The first, with the same proportion of cocoa and jute fibre as in real transport (named "**sample 2**"), which is usually 10 g per kg of cocoa beans, and the second increasing the proportion of jute by 10 times (100g per kg of cocoa beans) (named "**sample 3**"). The content of MOSH and MOAH, in the C10-C50 range, was measured after 1 month at 40°C and after 2 months. Beans were manually deshelled into shell and nibs, which were analysed separately. The results are presented in Figure 1 as the sum of total MOSH and MOAH in the C10-C50 range.

- **The contamination was mainly superficial** (i.e., on the shell), **but by increasing the contact time, a migration towards the inner of the cocoa, i.e., the nibs, can be observed** (Figure 1, sample 3 shell vs sample 3 nibs) especially after 2 months.

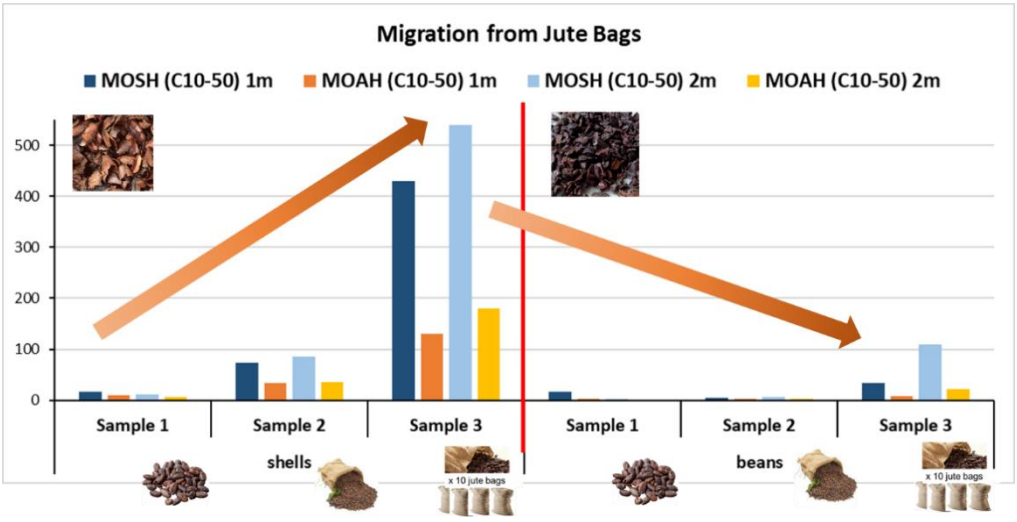

Figure 1. *Barplot of MOSH and MOAH content in shell and beans before and after exposure to jute bags for 1 and 2 months at 40°C. Sample 1: blank (no exposure to jute bags); sample 2: exposure to a proportion of jute bags as in the normal transport; Sample 3: 10-times higher quantity of jute bag.*

Considering samples 3, where a higher amount of MOSH and MOAH was observed, it can be seen how the migration from the jute bags to the shell already occurs mainly in the lighter C-fraction, <C35. Even more, the migration of the contamination from the external part (shell) towards the internal part of the cocoa (nibs) occurs mainly on the fraction <C20; indeed, the MOSH distribution profile over the C10-C50 range clearly changed (Figure 2). Moreover, by extending the contact time from 1 month to 2 months the extent of the MOSH in the C20-35 fraction had increased.

Different profile of the MOAH fraction (here centred on the C16-20), compared to the profile found in Part 1 can be observed. Here the MOAH profile followed the MOSH one suggesting a real contribution of the expected jute bag contamination.

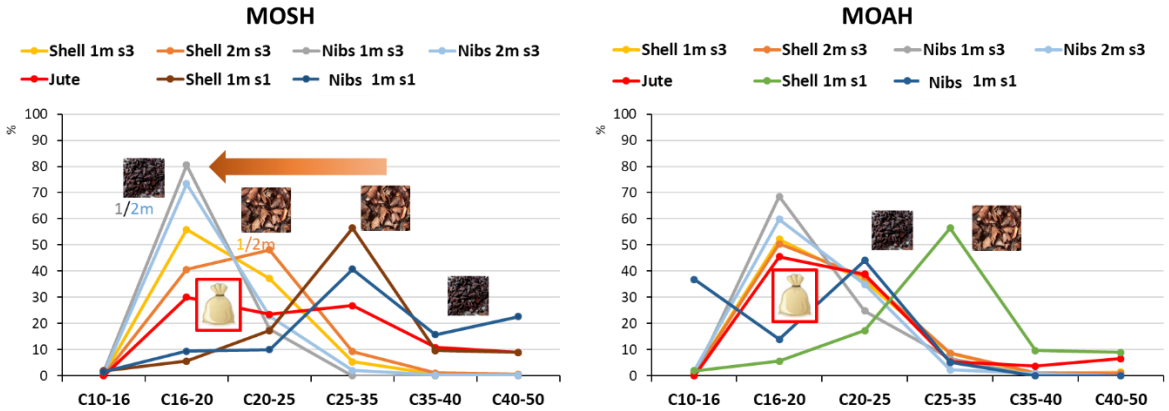

***Figure 2**. MOSH and MOAH percentage profile of the different C-fractions in the shell and in the beans after 1 and 2 months. Sample 1 after 1month and sample 3 after 1 and 2 months are reported.*

Conclusion

- The **migration occurred** from the jute bags to the cocoa beans, **with a preference of the lighter C-fractions**.
- **The shell seems to act as a filter**: the levels found in the nibs are much lower (~8% of the MOSH and MOAH amount in the shell was found in the nibs after 1 month and ~20% after 2 months) and in the nibs preferentially the lighter fraction, i.e., C16-20, is transferred.
- **Contact time, also played an important role** in increasing the absolute quantity of MOSH and MOAH migrate as well as in extending the C-range towards higher fractions, although lower than C35.

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PART 2: ECA MOH RING TEST

Main objective

The main goal was to evaluate the **reliability of the analytical methods used for MOSH and MOAH** determination.

Deutsches Referenzbüro für Ringversuche und Referenzmaterialien (DRRR) organized three proficiency tests (PT). The first two PTs involved 12 laboratories, 7 national and 5 international (thereof from the EU 1) and 6 and 6, respectively. The third PT involved 9 laboratories, 4 national and 5 international (thereof from EU 2). The main laboratories for performing routinely MOSH and MOAH analysis in EU and extra-EU were involved in this exercise (NOFALAB, SQTS, Institut Kirchhoff Berlin, Nestlé Research in Lausanne). Nevertheless, not all the laboratories returned the results (on average 10 laboratories returned all the results). The evaluation of the results followed a well-defined statistical scheme that is detailed in the DRRR website (https://drrr.de/en/).

The consensus value for determination of the assigned value (m_{best}) and not the result of a single "reference lab" is used. Therefore, the possible error of one lab does not influence m_{best} significantly.

Measurement of performance: The data provided by the different laboratories are evaluated according to the z´-score:

$$z' - score = \frac{m - m_{best}}{\sqrt{s_{best}^2 + s_{material}^2}}$$

z´ ≤ |2,00| performance is satisfactory
|2,00| < z´ < |3,00| performance is questionable
z´ ≥ |3,00| performance is unsatisfactory
The z'-score is rounded to two decimal places.

The value of z'-score is calculated based on the s_{best}, which leads to the paradox that the larger the uncertainty, the better the z'-score. Thus **the z'-score alone is not a sufficient estimation of the method**. The data were elaborated according to the EU Decision 2002/657 for value on the order of mg/kg a reproducibility <16% is expected (this is slightly stricter than the JRC Guidance (i.e., 20%), but were chosen as reference for this discussion to translate also the trueness values) and trueness between +10% and -20 %.

Figure 1 shows a summary of the results of the 3 PTs. An increased complexity of the sample led to increased uncertainty, with the cocoa mass (chocolate liquor) and the cocoa mass showing the highest uncertainty. High uncertainty and poor trueness were observed also for pristane, phytane and DIPN, suggesting the possibility that these single peaks were wrongly assigned when other interferences were present.

Conclusion

- The results of the 3 PTs proved the **necessity to improve further the method for the determination of MOSH and MOAH**, as well of the chosen markers for assessing the contamination. Although the z'-score was generally successful, the % uncertainty associated to the determination of MOSH and MOAH is out of the acceptable ranges set by the EU Decision 2002/657. No information on the sample preparation method was reported, but most of the laboratories used the same method, i.e. the LC-GC-FID.
- The LOQ reported were very different for the different laboratories contributing to increase the uncertainty of the final results, arising challenges related to interpretation of MOSH/MOAH testing especially if close to LOQ of the methods used also for commercial testing.

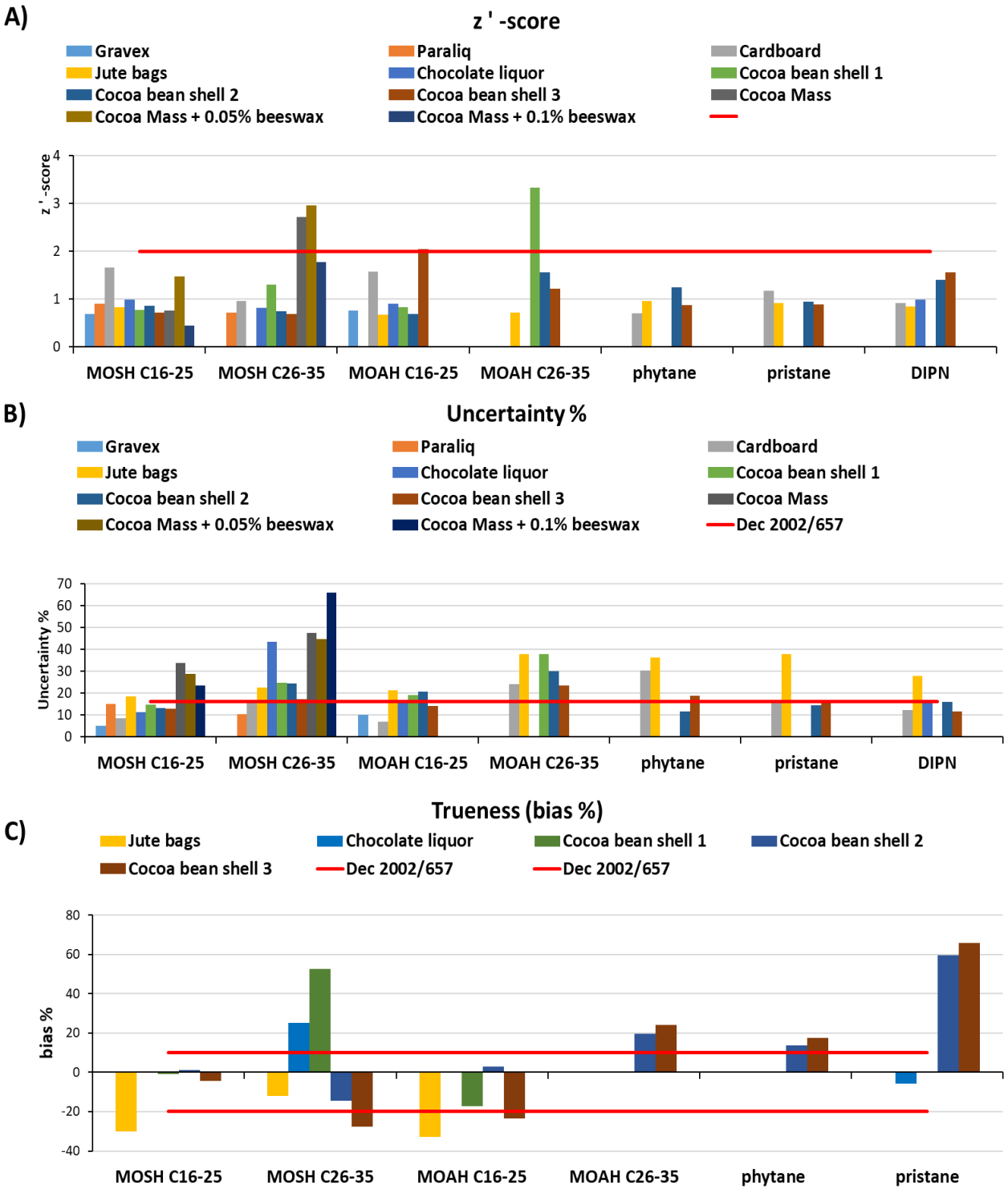

Figure 1. *Barplot of the average value per sample for A) z'-score, B) Uncertainty %, C) Trueness (as bias %).*