BEFORE THE HON'BLE NATIONAL GREEN TRIBUNAL PRINCIPAL BENCH, NEW DELHI

Original Application No. 1094/2024

All Indian salt Sugar brands contain microplastics reveals study" appearing in the Business Standard dated 13.08.2024

.....Petitioner

VS

Central Pollution Control Board, & Ors.

.....Respondents

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Through

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Date: 28.04.2025
Place: New Delhi

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BEFORE THE HON'BLE NATIONAL GREEN TRIBUNAL PRINCIPAL BENCH, NEW DELHI

Original Application No. 1094/ 2024

All Indian salt Sugar brands contain microplastics reveals study" appearing in the Business Standard dated 13.08.2024

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VS

Central Pollution Control Board, &Ors.

.....Respondents

COUNTER AFFIDAVIT ON BEHALF OF RESPONDENT NO.4

1. I, Ms. Smita Singh, D/o Sh. Vinod Kumar Singh, aged about 34 Years, presently holding the position of Assistant Director of the Food Safety and Standards Authority of India (hereinafter referred to as "***FSSAI***"), a statutory authority established under the provisions of the Food Safety and Standards Act, 2006, do hereby solemnly affirm and state that I am well-versed

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with the facts and circumstances of the present proceedings. My knowledge is derived from official records maintained by FSSAI in its ordinary course of business and from my personal understanding of the issues involved in the instant matter.It is further submitted that vide order dated 23.08.2024, passed by the Hon'ble National Green Tribunal, Principal Bench, New Delhi, Respondent No. 4, i.e., FSSAI was impleaded as a necessary party to the present suomotu proceedings. The said impleadment has been effected in light of the allegations pertaining to the presence of microplastics in food products, specifically salt and sugar, as purportedly reported in the news article published in Business Standard dated 13.08.2024.

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2. That I, being duly authorized to represent FSSAI in these proceedings, place on record the considered position of the Respondent Authority

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and submit that FSSAI, as the apex regulatory body for food safety in India, has been actively discharging its statutory mandate to ensure the safety and quality of food products in accordance with the provisions of the Food Safety and Standards Act, 2006 and the regulations framed thereunder.

3. That itis most respectfully submitted that the Hon'ble National Green Tribunal, Principal Bench, New Delhi, has, in the exercise of its suomotu jurisdiction, taken cognizance of a news article titled "All Indian Salt and Sugar Brands Contain Microplastics, Reveals Study", which was published in *Business Standard* on 13.08.2024. The said news report purports to highlight the findings of a study conducted by an entity named Toxics Link, wherein it is alleged that various brands of salt and sugar available in India contain microplastic and

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nanoplastic particles. The article further suggests that the presence of such contaminants in essential food commodities may have adverse, cumulative, and long-term implications on human health and the environment.

4. That at the very outset, it is most respectfully submitted that the FSSAI is a statutory regulatory body constituted under the Food Safety and Standards Act, 2006, operating under the aegis of the Ministry of Health and Family Welfare, Government of India. FSSAI is vested with the exclusive mandate to regulate, monitor, and ensure the safety, quality, and standards of food products across the entire food value chain within the territory of India. In the discharge of its statutory obligations, FSSAI formulates scientific, evidence-based food safety regulations, prescribes permissible limits for contaminants

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and additives, and ensures strict compliance through enforcement mechanisms and surveillance measures.

5. It is further submitted that FSSAI is committed to upholding the highest standards of food safety and public health protection by implementing scientifically validated risk assessment protocols. The regulatory functions of FSSAI extend to framing and enforcing standards, conducting research, surveillance, and testing, and providing policy recommendations to safeguard consumer interests. FSSAI also works in close collaboration with national and international scientific bodies to ensure that food safety measures in India are aligned with global best practices and evolving scientific developments.

6. That in the instant matter, this Hon'ble Tribunal vide its order dated 23.08.2024 was pleased to

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issue notice to FSSAI to file its response by way of an affidavit concerning the allegations raised in the news article published in Business Standard on 13.08.2024. The said news article, purportedly based on a study conducted by Toxics Link, claims to have examined 10 types of salt, including table salt, rock salt, sea salt, and local raw salt, as well as five types of sugar procured from online and local markets, wherein the presence of microplastics and nanoplastics was allegedly detected.

7. It is respectfully submitted that FSSAI, being the principal regulatory authority for food safety in India, is actively engaged in policy formulation concerning the issue of microplastic contamination in food products.

8. That it is most respectfully and unequivocally submitted that FSSAI, in the discharge of its

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statutory functions, has at all times exercised due diligence, vigilance, and regulatory oversight to ensure the highest standards of food safety in India. At no point has FSSAI exhibited any laxity, omission, or lackadaisical approach in fulfilling its obligations under the Food Safety and Standards Act, 2006, and the regulations framed thereunder.The Respondent Authority has consistently ensured the effective implementation, monitoring, and compliance of all prescribed food safety regulations across the food supply chain. That FSSAI has not only adhered to the statutory framework in letter and spirit but has also undertaken proactive measures to strengthen food safety standards by conducting scientific research, surveillance, policy interventions, and stakeholder engagement initiatives. It is, therefore, submitted that any inference or allegation suggesting regulatory

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inaction or lack of enforcement by FSSAI is misplaced, untenable, and devoid of merit.

9. That FSSAI, in its capacity as the statutory regulatory authority for food safety in India, has been cognizant of the emerging concerns pertaining to the presence of microplastics and nanoplastics in food matrices and has proactively undertaken necessary measures to scientifically assess, regulate, and mitigate potential risks associated with such contaminants. In furtherance of its statutory mandate and regulatory obligations under the Food Safety and Standards Act, 2006, the Respondent Authority has not only taken cognizance of this issue but has also initiated a comprehensive scientific research project aimed at evaluating the prevalence, detection methodologies, and potential health implications of micro- and nanoplastics in food products.

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10. That it is submitted that even prior to the publication of the impugned news article dated 13.08.2024, the Respondent Authority had already embarked upon a structured, evidence-based research initiative to formulate scientifically validated methodologies for the detection, identification, and quantification of microplastics and nanoplastics in food matrices. The said initiative was conceptualized and launched in March 2024, underscoring the Respondent Authority's proactive and preemptive approach in addressing emerging food safety concerns.

11. That in furtherance of its regulatory and research mandate, the Respondent Authority has initiated a multi-institutional research project titled "Micro-and Nano-plastics as Emerging Food Contaminants: Establishing Validated

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Methodologies and Understanding the Prevalence in Different Food Matrices" (hereinafter referred to as *"the Project"*). The Project was officially launched in March 2024 as part of FSSAI's broader efforts to strengthen scientific research, risk assessment mechanisms, and regulatory frameworks concerning food safety in India.

12. That the primary objectives of the Project are as follows:

i) To develop standardized, validated, and internationally benchmarked methodologies for the detection and quantification of microplastics and nanoplastics (MNPs) in food matrices,

ii) To conduct inter and intra-laboratory comparison of developed methods to establish their workability.

iii) To scientifically evaluate the prevalence, nature, and composition of microplastics and

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nanoplastics across various food categories, thereby generating empirical, data-driven insights that shall contribute to evidence-based policymaking and regulatory interventions.

13. That the initiation of the Project underscores the Respondent Authority's unwavering commitment to upholding public health and food safety by fostering high-quality scientific research, regulatory preparedness, and industry compliance.

14. That the Project is being implemented in collaboration with three leading research institutions, namely:

i) CSIR-Indian Institute of Toxicology Research, Lucknow, pursuant to Memorandum of Understanding dated 19.04.2024.

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ii) ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Kochi, pursuant to Memorandum of Understanding dated 25.04.2024.

iii) Birla Institute of Technology and Science (BITS), Pilani, pursuant to Memorandum of Understanding dated 30.04.2024.

15. That for the effective execution of the Project, a total of Rs. 1,35,69,920/- (Rupees One Crore Thirty-Five Lakh Sixty-Nine Thousand Nine Hundred and Twenty Only) has been allocated, ensuring the successful implementation of research activities in accordance with the defined scope, deliverables, and compliance benchmarks. Atrue and correct copy of the relevant Memorandum of Agreement (MOAs) executed between FSSAI and the aforementioned research

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institutions is annexed herewith and marked as **Annexure-R-4/1 (Colly).**

16. That the conceptualization, execution, and scientific rigour of this research initiative align with the Respondent Authority's overarching statutory objective of ensuring that food products available in India adhere to the highest safety and quality standards. The outcome of this scientific initiative is expected to make significant contributions towards the evolution of regulatory guidelines, risk assessment protocols, and statutory policy frameworks governing the presence of microplastics and nanoplastics in the food supply chain.

17. That in furtherance of this initiative, subsequent to the execution of the MOAs with the collaborating research institutions, the CEO of the Respondent Authority officially launched the

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project on 21.06.2024 in Lucknow. A true copy of the Minutes of Meeting dated 21.06.2024 is annexed herewith and marked as **Annexure-R-4/2**.

18. That the Respondent Authority, in furtherance of its statutory obligations and regulatory mandate under the Food Safety and Standards Act, 2006, has been diligently pursuing the scientific research initiative concerning the presence of microplastics and nanoplastics in food matrices. The Respondent has consistently undertaken necessary steps, periodic evaluations, and scientific deliberations to assess and enhance the scope of the Project while ensuring methodological accuracy, regulatory compliance, and adherence to internationally recognized analytical standards.

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19. That the commitment of the Respondent Authority to scientific integrity and evidence-based policymaking is exemplified by the deliberations held during the 'Record of Discussion on the Report of Toxics Link on Microplastics in Salt & Sugar', convened on 20.08.2024 at FSSAI, FDA Bhawan, New Delhi. During this scientific review meeting, it was categorically observed by the Respondent that:

    i) The data presented by Toxics Link lacks scientific rigor, quantitative accuracy, and methodological transparency, thereby rendering the findings inconclusive for any meaningful regulatory or policy-level determination.

    ii) The study fails to comply with internationally recognized analytical protocols, particularly ISO 24187:2023

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(Principles for the Analysis of Microplastics in Environmental Matrices), which is a globally acknowledged standard for the identification, quantification, and characterization of microplastic contamination in food and environmental matrices.

iii) The conclusions drawn in the Toxics Link study are not supported by adequate empirical evidence or robust statistical validation, thereby raising concerns regarding scientific ethics, data reliability, and the risk of misinformation.

iv) The extrapolation of inadequately substantiated data could mislead the public, potentially causing unwarranted alarm and regulatory confusion, particularly in the absence of standardized

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testing methodologies or established permissible limits for microplastics in food.

20. That in view of the foregoing, the Respondent Authority, in the said scientific deliberation, recommended that any future studies concerning microplastics in food matrices must conform to the prescribed methodologies under ISO 24187:2023 to ensure scientific accuracy, transparency, and credibility. Atrue copy of the 'Record of Discussion on the Report of Toxics Link on Microplastics in Salt & Sugar' held on 20.08.2024 at 11:30 AM at FSSAI, FDA Bhawan, New Delhi, is annexed herewith and marked as **Annexure-R-4/3**.

21. That it is most respectfully submitted that the Minutes of the Meeting dated 24.11.2024, and 25.11.2024 convened to review the First Phase of the Project titled 'Micro-and Nano-plastics as

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Emerging Food Contaminants: Establishing Validated Methodologies and Understanding the Prevalence in Different Food Matrices", serves as documentary evidence of the continuous efforts, scientific rigor, and regulatory diligence exercised by the Respondent Authority in implementing and monitoring the said initiative.

22. That the deliberations recorded in the said meeting reflect the systematic and methodical approach adopted by the Respondent Authority, wherein key stakeholders, provided a comprehensive status update on the Project, highlighted ongoing research developments, and the respondent authority proposed strategic recommendations to enhance the efficiency and effectiveness of the Project's execution.

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23. That the Minutes of the Meeting conclusively establish that the Respondent Authority has undertaken proactive, data-driven, and evidence-based measures to ensure that the Project achieves its intended objectives in a scientifically robust and regulatory-compliant manner. A true copy of the Minutes of the Meeting on the First Phase of Review on the status of the Project, held on 24.11.2024 and 25.11.2024, is annexed herewith and marked as **Annexure-R-4/4**.

24. That it is most respectfully submitted that on 11.12.2024, the CSIR-Indian Institute of Toxicology Research (CSIR-IITR), being one of the principal collaborating research institutions in the execution of the Project titled "Micro-and Nano-plastics as Emerging Food Contaminants: Establishing Validated Methodologies and Understanding the Prevalence in Different Food Matrices," submitted its Six-Month Progress

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Report, providing a detailed update on the scientific advancements, methodological developments, and ongoing research activities under the Project.

25. That the said Progress Report serves as documentary evidence of the structured, data-driven, and scientifically rigorous approach adopted by the Respondent Authority and its collaborating research institutions in ensuring that the study adheres to internationally accepted analytical standards and regulatory best practices. Some of the key developments recorded in the Six-Month Progress Report are enumerated as follows:

A. Establishment of a Standardized Fluorescence Microscopy Protocol for Microplastic Detection: That a standardized fluorescence microscopy technique, coupled with Nile Red staining, has

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been successfully developed and implemented for the rapid and cost-effective identification of microplastics, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and nylon-6. The developed protocol has been scientifically validated through Fourier Transform Infrared Spectroscopy (FT-IR) and Raman Spectroscopy, ensuring accuracy and reproducibility in microplastic detection.

B. Development of Ultra-High-Performance Liquid Chromatography (UHPLC) Based Depolymerization and Detection Methods: That UHPLC-based depolymerization methodologies have been developed and optimized for the detection and quantification of PET, polycarbonate (PC), and PS in food matrices. The said methodologies have been further validated using FT-IR and Raman

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Spectroscopy, reinforcing their scientific reliability.

C. Implementation of LC-MS/MS-Based Analytical Techniques for Polymer Monomers and Bisphenols:

That an advanced Liquid Chromatography–Mass Spectrometry/Mass Spectrometry (LC-MS/MS) method has been developed for the precise analysis of polymer monomers derived from PET and bisphenol compounds.

D. Development of a Microwave-Assisted Extraction Method for Biological Matrices:

That a microwave-assisted extraction method has been successfully developed and validated for biological matrices, specifically for the detection of polypropylene (PP) and high-density polyethylene (HDPE) in fish tissue samples. The method has significantly reduced

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the extraction time to 30 minutes while ensuring an analytical recovery rate of 90%, thereby enhancing the efficiency of contaminant detection in biological samples.

E. Implementation of Geographically Zoned Sampling Strategies for Diverse Food Matrices: That a comprehensive, geographically zoned sampling strategy has been outlined and implemented to systematically collect and analyze various food matrices, including bottled water, packaged beverages, fresh and dried fish, and fish meals, thereby ensuring broad-spectrum assessment and data representation in the study of microplastic contamination in food products.

26. That the Six-Month Progress Report unequivocally establishes that the Project has been progressing in a structured, scientifically

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rigorous, and methodologically sound manner. A true copy of the Progress Report is annexed herewith and marked as **Annexure-R-4/5**.

27. That it is most respectfully submitted that the Respondent Authority, in the discharge of its statutory mandate under the Food Safety and Standards Act, 2006, has consistently and diligently undertaken proactive measures to ensure the effective implementation, monitoring, and completion of the ongoing scientific research initiative concerning the detection, prevalence, and regulation of microplastics and nanoplastics in food matrices.

28. That the Respondent Authority has been actively engaged in periodic scientific evaluations, structured review meetings, and continuous oversight mechanisms to facilitate the successful execution of the Project. These review

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mechanisms have involved regular assessments, technical deliberations, and consultative engagements with collaborating research institutions and expert bodies to ensure that the Project adheres to established scientific methodologies, regulatory frameworks, and international best practices.

29. That there exists no recorded instance, finding, or material evidence to suggest any regulatory lapse, inaction, or non-compliance on the part of the Respondent Authority concerning the subject matter of these proceedings. On the contrary, the Respondent Authority has exercised due diligence, regulatory foresight, and scientific rigor in addressing concerns related to microplastic contamination in food products.

30. That it is further submitted that the Respondent

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Authority has been cognizant of emerging scientific developments and evolving global discourse on the issue of microplastics in food matrices, and accordingly, has taken necessary preemptive and remedial actions through the initiation of the aforementioned Project. The said scientific study is being pursued in a structured and time-bound manner, with clear milestones, deliverables, and compliance measures to ensure its successful execution.

31. That the continued follow-up and systematic monitoring of the Project by the Respondent Authority unequivocally establishes its commitment to upholding food safety, regulatory compliance, and public health standards, thereby negating any unsubstantiated claims of regulatory oversight or non-implementation.

32. That That in view of the foregoing submissions

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and the documentary evidence placed on record, it is unequivocally established that the Respondent Authority has, at all times, diligently discharged its statutory functions and regulatory obligations under the Food Safety and Standards Act, 2006, and has exercised continuous oversight, scientific diligence, and procedural compliance in the execution of the ongoing Project on Microplastics and Nanoplastics in Food Matrices. That it is manifestly evident that the Respondent Authority has neither engaged in regulatory inaction nor exhibited any lapse in fulfilling its mandate. On the contrary, the Respondent has been actively involved in structured scientific research, policy formulation, and regulatory enforcement to ensure that the issue of microplastic contamination in food is addressed through scientifically validated methodologies

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and internationally recognized best practices. That the Respondent Authority shall abide by the directions and instructions of the Hon'ble Tribunal as and when issued. Any other relief, which this Hon'ble Tribunal deems just and equitable in the facts and circumstances of the present case, may also be granted in the interest of justice.

I identified the executant who has signed in my presence

FOOD SAFETY & STANDARDS AUTHORITY OF INDIA ★ fssai

DEPONENT

VERIFICATION

Verified on this ___ day of April, 2025 at New Delhi that the contents of the above Affidavit have been explained to me in vernacular language and are true and correct to my knowledge, no part thereof is false and nothing material has been there from.

DEPONENT

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FOOD SAFETY & STANDARDS AUTHORITY OF INDIA ★ fssai

ATTESTED

NOTARY PUBLIC DELHI

28 APR 2025

उत्तर प्रदेश UTTAR PRADESH GP 564963

MEMORANDUM OF AGREEMENT

This MEMORANDUM OF AGREEMENT (MoA) is made on this 19 day of April , 2024 BY AND BETWEEN Food Safety & Standards Authority of India, Ministry of Health & Family Welfare, Government of India, New Delhi, hereinafter referred to as the 'FSSAI (which expression unless excluded by or repugnant to the subject shall mean and include its successor in office and assigns) of the ONE PART

AND

CSIR-Indian Institute of Toxicology Research, having its registered office in/at Vishvigyan Bhawan, 31, Mahatma Gandhi Marg, Lucknow, Uttar Pradesh, India hereinafter referred to as Grantee (which expression shall where the context so admits include its successors and permitted assigns) of the OTHER PART

WHEREAS FSSAI, being desirous of developing standards for pure, nutritious, and safe food, decided to support a project submitted by the grantee for the attainment of the objectives, hereinafter described in the Annexure I annexed hereto;

This MoA defines the role and responsibilities of the participating agencies, monitoring and other matters related to the research project on "Micro-and nano-plastics as emerging food contaminants: Establishing validated Methodologies and understanding the prevalence in different food matrices".

19/4/24
डॉ0 के0 सी0 खुल्बे/Dr. KC Khulbe
प्रमुख, अनुसंधान योजना एवं व्यापार विकास विभाग
Head, Research Planning & Business Development Division
सी.एस.आई.आर.–भारतीय विषविज्ञान अनुसंधान संस्थान
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Vishvigyan Bhawan 31, Mahatma Gandhi Marg, Lucknow-226001 INDIA

डी वेणुगोपाल/D. VENUGOPAL
उप निदेशक/Deputy Director
गुणवत्ता आश्वासन प्रभाग/Quality Assurance Division
भारतीय खाद्य सुरक्षा एवं मानक प्राधिकरण
Food Safety and Standards Authority of India
(स्वास्थ्य एवं परिवार कल्याण मंत्रालय)
(Ministry of Health & Family Welfare)
भारत सरकार/Govt. of India
एफ.डी.ए. भवन, कोटला रोड, नई दिल्ली–११०००२, भारत
FDA Bhavan, Kotla Road, New Delhi-110002, INDIA

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NOW THE PARTIES HERETO AGREE AS FOLLOWS: -

1.0 ROLE OF FOOD SAFETY & STANDARDS AUTHORITY OF INDIA, NEW DELHI

To provide funds to the extent of 1,35,69,920/- (One Crore Thirty-Five Lakhs Sixty-Nine Thousand Nine Hundred and Twenty Rupees only) over a period of two years from the date of sanction of the project, to the grantees (CSIR-IITR, Lucknow; ICAR-CIFT, Kochi and BITs, Pilani) for undertaking activities as detailed in Annexure A (Technical Details of the project with work allocated to each institution) and details of the funds to be provided to each institutions as given in Annexure B.

2.0 Role of CSIR-Indian Institute of Toxicology Research

2.1 To provide their contribution of Nil for Nil years from date of sanction of the project as detailed in Annexure (if a jointly supported project).

2.2 To provide existing facilities as mentioned in the project document.

2.3 To be responsible for accomplishing objectives identified and activities listed

2.4. To allow the Scientists authorized by FSSAI to work with the Research & Development team of the center in all stages of process development and production.

2.5 To recruit all scientific and non-scientific staff as sanctioned by FSSAI

2.6 To prepare and submit all periodical reports and other documents that would be required by FSSAI

2.7. To maintain a separate audit head of account for the grants received from FSSAI for the project.

2.8. To submit an annual audited statement of expenditure incurred under the project.

2.9. To ensure effective utilization of the grant given by FSSAI for the purpose for which it was granted and to ensure timely progress of project work

2.10. The manpower, both scientific and non-scientific, recruited shall be purely on contractual terms & conditions such that the contract for engagement of the manpower shall run concurrently with the said project period only.

3.0 DURATION OF PROJECT

3.1. Duration of project shall be two years from the date the Project has been sanctioned by FSSAI.

4.0 RIGHTS OF OWNERSHIP/TECHNOLOGY TRANSFER AND UTILIZATION

19/4/24

डॉ0 के0 सी0 खुल्बे/Dr. KC Khulbe
प्रमुख, अनुसंधान योजना एवं व्यापार विकास विभाग
Head, Research Planning & Business Development Division
सी.एस.आई.आर.–भारतीय विषविज्ञान अनुसंधान संस्थान
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भारतीय खाद्य सुरक्षा एवं मानक प्राधिकरण
Food Safety and Standards Authority of India
(स्वास्थ्य एवं परिवार कल्याण मंत्रालय)
(Ministry of Health & Family Welfare)
भारत सरकार/Govt. of India
एफ.डी.ए. भवन, कोटला रोड, नई दिल्ली–110002, भारत
FDA Bhavan, Kotla Road, New Delhi-110002, INDIA

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4.1. The know-how generated from the project by **the grantee** will be the Joint property of CSIR-Indian Institute of Toxicology Research and FSSAI, Government of India. It shall be the responsibility of **the grantee** to take necessary action for protection of the intellectual property arising out of the PROJECT through proper instruments, such as, patents, copy rights etc.

4.2. The know-how developed may be transferred to other entrepreneurs on a non exclusive basis on such terms and conditions as may be determined by FSSAI

4.3. All the assets including the equipment and produce acquired will be the property of FSSAI and shall not be utilized for purposes other than those for which the grant has been sanctioned. The rights of grantee under this MoA shall not be transferred to any other party without prior approval in writing of FSSAI

4.4. It shall be the responsibility of **the grantee** to ensure that support of FSSAI is suitably acknowledged in the publications (papers, reports. Etc.) arising out of the PROJECT.

5. SECRECY

It is hereby agreed that the participating agencies shall keep information and data collected completely secret provided that the right to transfer the technology shall rest with the FSSAI.

6. MONITORING

6.1. The progress of implementation of the project and proper utilization of grant shall be reviewed by the FSSAI and by the Monitoring Committee set up by FSSAI.

6.2. The periodic progress of physical achievements and the utilization of funds, statement of expenditure shall be evaluated by the Monitoring Committee.

6.3. The Comptroller and Auditor General of India, at his discretion shall have the right of access to the books and accounts of the grantee for the grants received from FSSAI for this project.

6.4. The FSSAI may terminate the grant at any stage if it is convinced that the grant has not been properly utilized or appropriate progress has not been made. In the event, FSSAI terminates the grant, shall hand over all documents including technical details and equipment purchased related to the project.

7.0 DURATION OF MEMORANDUM OF AGREEMENT

19/4/24

डॉ0 के0 सी0 खुल्बे/Dr. KC Khulbe
प्रमुख, अनुसंधान योजना एवं व्यापार विकास विभाग
Head, Research Planning & Business Development Division
सी.एस.आई.आर.–भारतीय विषविज्ञान अनुसंधान संस्थान
CSIR-Indian Institute of Toxicology Research
विषविज्ञान भवन 31, महात्मा गांधी मार्ग, लखनऊ–226001 भारत
Vishvigyan Bhawan 31, Mahatma Gandhi Marg, Lucknow-226001 INDIA

डी वेणुगोपाल/D. VENUGOPAL
उप निदेशक/Deputy Director
गुणवत्ता आश्वासन प्रभाग/Quality Assurance Division
भारतीय खाद्य सुरक्षा एवं मानक प्राधिकरण
Food Safety and Standards Authority of India
(स्वास्थ्य एवं परिवार कल्याण मंत्रालय)
(Ministry of Health & Family Welfare)
भारत सरकार/Govt. of India
एफ.डी.ए. भवन, कोटला रोड, नई दिल्ली–110002, भारत
FDA Bhavan, Kotla Road, New Delhi-110002, INDIA

3/4

This MoA will remain in force for the duration of the project and until all claims are settled between FSSAI and **the grantee**.

8.0 ARBITRATION

In the event of any question, dispute or difference whatsoever arising between the parties to this Agreement out of or relating to the construction, meaning, scope, operation or effect of this Agreement or the validity of the breach thereof shall be referred to an Arbitrator to be appointed by mutual consent of both the parties herein. If the parties cannot agree on the appointment of the Arbitrator within a period of one month from the notification by one party to the other of existence of such dispute, then the Arbitrator shall be nominated by the Secretary, Department of Legal Affairs, Ministry of Law & Justice, Government of India. The provisions of the Arbitration and Conciliation Act, 1996 will be applicable and the award made thereunder shall be final and binding upon the parties hereto, subject to legal remedies available under the law. Such differences shall be deemed to be a submission to arbitration under the Indian Arbitration and Conciliation Act, 1996, or of any modifications or re-enactments thereof.

9.0 GOVERNING LAW

This Contract shall be governed by the Law of India for the time being in force.

IN WITNESS WHEREOF the parties hereto have signed, sealed and delivered this Agreement on the day, month and year first above written in presence of:

Witnesses:

1. HEMALATHA P, TECHNICAL OFFICER, QUALITY ASSURANCE DIVISION, FSSAI.

2. Jyotsana Singh
Jyotsana Singh, Food Analyst
QA Division, FSSAI

Signed by

डी वेणुगोपाल/D. VENUGOPAL
उप निदेशक/Deputy Director
गुणवत्ता आश्वासन प्रभाग/Quality Assurance Division
भारतीय खाद्य सुरक्षा एवं मानक प्राधिकरण
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एफ.डी.ए. भवन, कोटला रोड, नई दिल्ली–११०००२, भारत
FDA Bhavan, Kotla Road, New Delhi-110002, INDIA

For and on behalf of FSSAI

Witnesses:

1. Kausar Mahmood 19.4.2024
Dr. Kausar Mahmood Ansari
Principal Scientist
CSIR-Indian Institute of Toxicology Research
Vishvigyan Bhawan, 31, Mahatma Gandhi Marg,
Lucknow-226001, Uttar Pradesh, India

2. 19.4.24
डॉ० अंकुर गोयल/Dr. Ankkur Goel
वरिष्ठ प्रधान वैज्ञानिक
Senior Principal Scientist
आरपीबीडी विभाग/RPBD Division
सी.एस.आई.आर.-आई.आई.टी.आर., लखनऊ
CSIR-IITR, Lucknow

Signed by 19/4/24
(Designation)

For and on behalf of Grantee

डॉ० के० सी० खुल्बे/Dr. KC Khulbe
प्रमुख, अनुसंधान योजना एवं व्यापार विकास विभाग
Head, Research Planning & Business Development Division
सी.एस.आई.आर.–भारतीय विषविज्ञान अनुसंधान संस्थान
CSIR-Indian Institute of Toxicology Research
विषविज्ञान भवन 31, महात्मा गांधी मार्ग, लखनऊ–226001 भारत
Vishvigyan Bhawan 31 Mahatma Gandhi Marg, Lucknow-226001 INDIA

കേരളം केरल KERALA CU 908867

MEMORANDUM OF AGREEMENT

This MEMORANDUM OF AGREEMENT (MoA) is made on this 25th day of April (month), 2024 BY AND BETWEEN Food Safety & Standards Authority of India, Ministry of Health & Family Welfare, Government of India, New Delhi, hereinafter referred to as the 'FSSAI (which expression unless excluded by or repugnant to the subject shall mean and include its successor in office and assigns) of the ONE PART

AND

ICAR-Central Institute of Fisheries Technology (Name of Institute) having its registered office in/at Cochin, Matsyapuri P.O., W. Island, Kerala, India (Address of Institute) hereinafter referred to as Grantee (which expression shall where the context so admits include its successors and permitted assigns) of the OTHER PART

WHEREAS FSSAI being desirous of developing standards for pure, nutritious and safe food decided to support a project submitted by the grantee for the attainment of the objectives, hereinafter described in the Annexure I annexed hereto;

No: 3371 Date: 23/4/2024
Value Rs
Sold To Dr. Nladrisekhar chatt
Senior Scientist,
ICAR CIFT
Cochin - 29

V N SATHYAN
Sub Court Stamp Vendor
Kochi- 682005

डी वेणुगोपाल/D. VENUGOPAL
उप निदेशक/Deputy Director
गुणता आश्वासन प्रभाग/Quality Assurance Division
भारतीय खाद्य सुरक्षा एवं मानक प्राधिकरण
Food Safety and Standards Authority of India
(स्वास्थ्य एवं परिवार कल्याण मंत्रालय)
Ministry of Health & Family Welfare
एफ.डी.ए. भवन, कोटला रोड, नई दिल्ली-110002, INDIA
FDA Bhawan, Kotla Road, New Delhi-110002, INDIA
19 APR 2024
MATTANCHERY

This MoA defines the role and responsibilities of the participating agencies, monitoring and other matters related to the research project on 'Micro-and nano-plastics as emerging food contaminants: Establishing validated methodologies and understanding the prevalence in different food matrices.'

NOW THE PARTIES HERETO AGREE AS FOLLOWS: -

1.0 ROLE OF FOOD SAFETY & STANDARDS AUTHORITY OF INDIA, NEW DELHI

To provide funds to the extent of 1,35,69,920/- (One Crore Thirty-Five Lakhs Sixty-Nine Thousand Nine Hundred and Twenty Rupees only) over a period of two years from the date of sanction of the project, to the grantees (CSIR-IITR, Lucknow; ICAR-CIFT, Kochi and BITs, Pilani) for undertaking activities as detailed in Annexure A (Technical Details of the project with work allocated to each institution) and details of the funds to be provided to each institutions as given in Annexure B.

2.0 ROLE OF ICAR-Central Institute of Fisheries Technology, Cochin

2.1 To provide their contribution of Nil for Nil years from date of sanction of the project as detailed in Annexure (if a jointly supported project)

2.2 To provide existing facilities as mentioned in the project document.

2.3 To be responsible for accomplishing objectives identified and activities listed

2.4. To allow the Scientists authorized by FSSAI to work with the Research & Development team of the center in all stages of process development and production.

2.5 To recruit all scientific and non-scientific staff as sanctioned by FSSAI

2.6 To prepare and submit all periodical reports and other documents that would be required by FSSAI

2.7. To maintain a separate audit head of account for the grants received from FSSAI for the project.

2.8. To submit an annual audited statement of expenditure incurred under the project.

डी वेणुगोपाल/D. VENUGOPAL
उप निदेशक/Deputy Director
गुणवत्ता आश्वासन प्रभाग/Quality Assurance Division
भारतीय खाद्य सुरक्षा एवं मानक प्राधिकरण
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भारत सरकार/Govt. of India
एफ.डी.ए. भवन, कोटला रोड, नई दिल्ली-११०००२, भारत
FDA Bhavan, Kotla Road, New Delhi-110002, INDIA

2.9. To ensure effective utilization of the grant given by FSSAI for the purpose for which it was granted and to ensure timely progress of project work

2.10. The manpower, both scientific and non-scientific, recruited shall be purely on contractual terms & conditions such that the contract for engagement of the manpower shall run concurrently with the said project period only.

3.0 DURATION OF PROJECT

3.1. Duration of project shall be two years from the date the Project has been sanctioned by FSSAI.

4.0 RIGHTS OF OWNERSHIP/TECHNOLOGY TRANSFER AND UTILIZATION

4.1. The know-how generated from the project by **the grantee** will be the Joint property of ICAR-CIFT (Name of institute) and FSSAI, Government of India. It shall be the responsibility of **the grantee** to take necessary action for protection of the intellectual property arising out of the PROJECT through proper instruments, such as, patents, copy rights etc.

4.2. The know-how developed may be transferred to other entrepreneurs on a non exclusive basis on such terms and conditions as may be determined by FSSAI

4.3. All the assets including the equipment and produce acquired will be the property of FSSAI and shall not be utilized for purposes other than those for which the grant has been sanctioned. The rights of grantee under this MoA shall not be transferred to any other party without prior approval in writing of FSSAI

4.4. It shall be the responsibility of **the grantee** to ensure that support of FSSAI is suitably acknowledged in the publications (papers, reports. Etc.) arising out of the PROJECT.

5. SECRECY

It is hereby agreed that the participating agencies shall keep information and data collected completely secret provided that the right to transfer the technology shall rest with the FSSAI.

6. MONITORING

6.1. The progress of implementation of the project and proper utilization of grant shall be reviewed by the FSSAI and by the Monitoring Committee set up by FSSAI.

6.2. The periodic progress of physical achievements and the utilization of funds, statement of expenditure shall be evaluated by the Monitoring Committee.

6.3. The Comptroller and Auditor General of India, at his discretion shall have the right of access to the books and accounts of the grantee for the grants received from FSSAI for this project.

6.4. The FSSAI may terminate the grant at any stage if it is convinced that the grant has not been properly utilized or appropriate progress has not been made. In the event, FSSAI terminates the grant, shall hand over all documents including technical details and equipment purchased related to the project.

7.0 DURATION OF MEMORANDUM OF AGREEMENT

This MoA will remain in force for the duration of the project and until all claims are settled between FSSAI and **the grantee**.

8.0 ARBITRATION

In the event of any question, dispute or difference whatsoever arising between the parties to this Agreement out of or relating to the construction, meaning, scope, operation or effect of this Agreement or the validity of the breach thereof shall be referred to an Arbitrator to be appointed by mutual consent of both the parties herein. If the parties cannot agree on the appointment of the Arbitrator within a period of one month from the notification by one party to the other of existence of such dispute, then the Arbitrator shall be nominated by the Secretary, Department of Legal Affairs, Ministry of Law & Justice, Government of India. The provisions of the Arbitration and Conciliation Act, 1996 will be applicable and the award made thereunder shall be final and binding upon the parties hereto, subject to legal remedies available under the law. Such differences

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उप निदेशक/Deputy Director
गुणवत्ता आश्वासन प्रभाग/Quality Assurance Division
भारतीय खाद्य सुरक्षा एवं मानक प्राधिकरण
Food Safety and Standards Authority of India
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(Ministry of Health & Family Welfare)
भारत सरकार/Govt. of India
एफ डी ए भवन, कोटला रोड, नई दिल्ली-११०००२, भारत
FDA Bhavan, Kotla Road, New Delhi-110002, INDIA

shall be deemed to be a submission to arbitration under the Indian Arbitration and Conciliation Act, 1996, or of any modifications or re-enactments thereof.

9.0 GOVERNING LAW

This Contract shall be governed by the Law of India for the time being in force.

IN WITNESS WHEREOF the parties hereto have signed, sealed and delivered this Agreement on the day, month and year first above written in presence of:

Witnesses:

1. P. Hemalatha, Technical officer, Quality Assurance Division, FSSAI.
2. Jyotsana Singh, Food Analyst QA Division, FSSAI

Signed by

डी वेणुगोपाल/D. VENUGOPAL
उप निदेशक/Deputy Director
गुणवत्ता आश्वासन प्रभाग/Quality Assurance Division
भारतीय खाद्य सुरक्षा एवं मानक प्राधिकरण
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FDA Bhawan, Kotla Road, New Delhi-110002, INDIA

(Designation)

For and on behalf of FSSAI

Witnesses:

1. (CHILADRI S. CHATTERJEE)
2. (PANKAJ KISHORE)

Signed by

(Designation)

For and on behalf of Grantee

Dr. GEORGE NINAN
DIRECTOR
ICAR-CIFT

5/5

राजस्थान RAJASTHAN

BS 005797

MEMORANDUM OF AGREEMENT

This MEMORANDUM OF AGREEMENT (MoA) is made on this **30th day of April, 2024** BY AND BETWEEN Food Safety& Standards Authority of India, Ministry of Health & Family Welfare, Government of India, New Delhi, hereinafter referred to as the 'FSSAI (which expression unless excluded by or repugnant to the subject shall mean and include its successor in office and assigns) of the ONE PART

AND

Birla Institute of Technology and Science, Pilani is a society incorporated under the Rajasthan Societies Registration Act, 1958, and is a deemed to be a University established vide Sec.3 of the UGC Act, 1956 under notification # F.12-23/63.U-2 of June 18, 1964 and have been granted the status of Institute of Eminence by Ministry of Education and having its registered office at VidyaVihar, Pilani-333031, Rajasthan, India] (hereinafter referred to as "BITS (which expression shall unless repugnant to the context or meaning thereof be deemed to mean and include its successor /s in business and permitted assign/s) of the **OTHER PART.**

WHEREAS FSSAI being desirous of developing standards for pure, nutritious and safe food decided to support a project submitted by the grantee for the attainment of the objectives, hereinafter described in the Annexure I annexed hereto;

डी वेणुगोपाल/D. VENUGOPAL
उप निदेशक/Deputy Director
गुणवत्ता आश्वासन प्रभाग/Quality Assurance Division
भारतीय खाद्य सुरक्षा एवं मानक प्राधिकरण
Food Safety and Standards Authority of India
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(Ministry of Health & Family Welfare)
भारत सरकार/Govt. of India
एफ.डी.ए. भवन, कोटला रोड, नई दिल्ली-११०००२, भारत
FDA Bhavan, Kotla Road, New Delhi-110002, INDIA

REGISTRAR
Birla Institute of Technology & Science
PILANI (Rajasthan)

This MoA defines the role and responsibilities of the participating agencies, monitoring and other matters related to the research project on Nutritional quality and safety evaluation of common processed products of grape

NOW THE PARTIES HERETO AGREE AS FOLLOWS: -

1.0 ROLE OF FOOD SAFETY & STANDARDS AUTHORITY OF INDIA, NEW DELHI

To provide funds to the extent of 1,35,69,920/- (One Crore Thirty-Five Lakhs Sixty-Nine Thousand Nine Hundred and Twenty Rupees only) over a period of two years from the date of sanction of the project, to the grantees (CSIR-IITR, Lucknow; ICAR-CIFT, Kochi and BITs, Pilani) for undertaking activities as detailed in Annexure A (Technical Details of the project with work allocated to each institution) and details of the funds to be provided to each institutions as given in Annexure B.

2.0 ROLE OF BITS Pilani

2.1 To provide their contribution of Nil for Nil years from date of sanction of the project as detailed in Annexure (if a jointly supported project)

2.2 To provide existing facilities as mentioned in the project document.

2.3 To be responsible for accomplishing objectives identified and activities listed

2.4. To allow the Scientists authorized by FSSAI to work with the Research & Development team of the centre in all stages of process development and production.

2.5 To recruit all scientific and non-scientific staff as sanctioned by FSSAI

2.6 To prepare and submit all periodical reports and other documents that would be required by FSSAI

2.7. To maintain a separate audit head of account for the grants received from FSSAI for the project.

2.8. To submit an annual audited statement of expenditure incurred under the project.

डी वेणुगोपाल/D. VENUGOPAL
उप निदेशक/Deputy Director
गुणवत्ता आश्वासन प्रभाग/Quality Assurance Division
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REGISTRAR
Birla Institute of Technology & Science
PILANI (Rajasthan)

2.9. To ensure effective utilization of the grant given by FSSAI for the purpose for which it was granted and to ensure timely progress of project work

2.10. The manpower, both scientific and non-scientific, recruited shall be purely on contractual terms & conditions such that the contract for engagement of the manpower shall run concurrently with the said project period only.

3.0 DURATION OF PROJECT

3.1. Duration of project shall be two years from the date the Project has been sanctioned by FSSAI.

4.0 RIGHTS OF OWNERSHIP/TECHNOLOGY TRANSFER AND UTILIZATION

4.1. The know-how generated from the project by **the grantee** will be the Joint property of BITS and FSSAI, Government of India. It shall be the responsibility of **the grantee** to take necessary action for protection of the intellectual property arising out of the PROJECT through proper instruments, such as, patents, copy rights etc.

4.2. The know-how developed may be transferred to other entrepreneurs on a non exclusive OR exclusive basis on such terms and conditions as may be determined by BITS in consultation with FSSAI

4.3. All the assets including the equipment and produce acquired will be the property of FSSAI and shall not be utilized for purposes other than those for which the grant has been sanctioned. The rights of grantee under this MoA shall not be transferred to any other party without prior approval in writing of FSSAI

4.4. It shall be the responsibility of **the grantee** to ensure that support of FSSAI is suitably acknowledged in the publications (papers, reports. Etc.) arising out of the PROJECT.

5. SECRECY

डी वेणुगोपाल/D. VENUGOPAL
उप निदेशक/Deputy Director
गुणवत्ता आश्वासन प्रभाग/Quality Assurance Division
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REGISTRAR
Birla Institute of Technology & Science
PILANI (Rajasthan)

3/5

It is hereby agreed that the participating agencies shall keep information and data collected completely secret provided that the right to transfer the technology shall rest with the FSSAI.

6. MONITORING

6.1. The progress of implementation of the project and proper utilization of grant shall be reviewed by the FSSAI and by the Monitoring Committee set up by FSSAI.

6.2. The periodic progress of physical achievements and the utilization of funds, statement of expenditure shall be evaluated by the Monitoring Committee.

6.3. The Comptroller and Auditor General of India, at his discretion shall have the right of access to the books and accounts of the grantee for the grants received from FSSAI for this project.

6.4. The FSSAI may terminate the grant at any stage if it is convinced that the grant has not been properly utilized or appropriate progress has not been made. In the event, FSSAI terminates the grant, shall hand over all documents including technical details and equipment purchased related to the project.

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REGISTRAR
Birla Institute of Technology & Science
PILANI (Rajasthan)

Secretary, Department of Legal Affairs, Ministry of Law & Justice, Government of India. The provisions of the Arbitration and Conciliation Act, 1996 will be applicable and the award made thereunder shall be final and binding upon the parties hereto, subject to legal remedies available under the law. Such differences shall be deemed to be a submission to arbitration under the Indian Arbitration and Conciliation Act, 1996, or of any modifications or re-enactments thereof.

9.0 GOVERNING LAW

This Contract shall be governed by the Law of India for the time being in force.

IN WITNESS WHEREOF the parties hereto have signed, sealed and delivered this Agreement on the day, month and year first above written in presence of:

Witnesses:

1. HEMALATHA P, TECHNICAL OFFICER, QUALITY ASSURANCE DIVISION, FSSAI.

2. Jyotsana Singh
Jyotsana Singh, Food Analyst
QA Division, FSSAI

Signed by

डी वेणुगोपाल/D. VENUGOPAL
उप निदेशक/Deputy Director
गुणवत्ता आश्वासन प्रभाग/Quality Assurance Division
भारतीय खाद्य सुरक्षा [illegible] प्राधिकरण
Food Safety and Standards Authority of India
(स्वास्थ्य एवं परिवार कल्याण मंत्रालय)
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FDA Bhavan, Kotla Road, New Delhi-110002, INDIA

(Designation)

For and on behalf of FSSAI

Witnesses:

1. Kuldeep Dudi
Grants Consultancy & Industrial Research Division
BITS Pilani

2. DALEEP SWAMI
GCIR, Bits Pilani

Signed by

REGISTRAR
Birla Institute of Technology & Science
PILANI (Rajasthan)
(Designation)

For and on behalf of Grantee

5/5

Annexure-R4/2

Minutes of the meeting on revamping of NetSCoFAN activities with various Research Institutions and launching, evaluation of Micro plastic project at CSIR-Indian Institute of Toxicology Research Lucknow (CSIR-IITR), Uttar Pradesh held on 21st June 2024 at 9:30 AM

The CEO, FSSAI launched (virtual mode) the activities of FSSAI funded project on "Micro-and nano-plastics as emerging food contaminants: Establishing validated methodologies and understanding the prevalence in different food matrices" on 21st June 2024 at 9:30 at CSIR-Indian Institute of Toxicology Research Lucknow (CSIR-IITR), Uttar Pradesh.

Followed by launching event, the meeting was convened on revamping of NetSCoFAN activities with various Research Institutions at CSIR-Indian Institute of Toxicology Research Lucknow (CSIR-IITR), Uttar Pradesh on 21st June 2024.

Representatives also attended the meeting from NetSCoFAN Research Institutions. A list of participants from Research Institutions & NetSCoFAN Secretariat is provided in Annexure I.

The Advisor, QC FSSAI welcomed all the members of Research Institutions and gave a brief explanation on revamping of NetSCoFAN activities.

A brainstorming session was conducted during the meeting on the way forward and revitalization plan for revamping / re-structuring the NETSCOFAN initiative.

During brainstorming session, there was deliberation on usage of plastic, innovations in packaging sector, ban on single use of plastics, alternative of plastics and environmental remediation strategies on microplastics.

The following points were discussed during the brainstorming session:

Advisor (QA) made detailed deliberation on various activities to be carried out as part of revamping of NetSCoFAN.

1. **Structural Modification**
   a. Revising the existing nine groups in terms of assigned activities and their linkage to various scientific panels.
   b. Addressing the overlapping mandates of each group while incorporating emerging food safety concerns.
2. **Incorporating More Scientific Institutions**
   a. Identifying and including leading academic and research organizations in the network.
   b. Establishing partnerships with international organizations working on food safety.
3. **Streamlining Scientific Opinion**
   a. Formalizing the process of receiving scientific opinions from research organizations.
   b. FSSAI will seek opinions from the selected NetsCoFAN groups on issues synthesized from internal discussions or scientific panel observations.
   c. Each group can present scientific challenges for regulatory attention. These points will be discussed quarterly in national steering committee meetings.

4.	**Clear-cut Objectives**	a. Identifying research gaps in respective areas, helping formulate research proposals, and recommending funding by FSSAI. b. Conducting horizon-scanning for existing and emerging food safety risks and issues. c. Collecting, collating, and developing a database on food safety issues to facilitate risk assessment and standards development. d. Organizing periodic brainstorming sessions, workshops, and seminars on emerging food safety issues. e. Providing immediate technical support to FSSAI on urgent food safety issues through short surveillance or risk assessment studies.
5.	**Pre-defined Activities**	a. Organizing brainstorming sessions, seminars, and workshops on pressing food safety issues. b. Holding stakeholder consultation meetings. c. Creating citizen-centric dissemination materials. d. Preparing advisories and guidance notes. e. Vetting research proposals for funding by FSSAI.
6.	**Revision of Funding Pattern**	a. Funding lead and partner institutes for organizing national-level brainstorming sessions, seminars, workshops, or stakeholder consultation meetings. b. Funding for content creation of food safety publicity materials. c. Funding for publishing specific research articles that safeguard national interests and highlight Indian initiatives in food safety.

It was decided to share the PPT on revamping NetSCoFAN activities and get inputs/suggestions from lead research institutes on the above points on the revamping of NetSCoFAN activities.

Signed by
Venugopal Dubakula
Date: 26-07-2024 16:54:48
D. Venugopal
Deputy Director
Quality Assurance Division

Annexure-I

List of Participants

1. Dr. Bhaskar Narayan, Director, CSIR-IITR, Vishvigyan Bhawan, Lucknow
2. Dr. Kausar,Mahmood Ansari, Principal Scientist, CSIR-IITR, Vishvigyan Bhawan, Lucknow.
3. Dr Parathasharty - Principal Scientist, CSIR-IITR, Vishvigyan Bhawan, Lucknow.
4. Dr Satyakam - Principal Scientist, CSIR-IITR, Vishvigyan Bhawan, Lucknow.
5. Dr Akhilesh Yadav, CSIR-IITR, Vishvigyan Bhawan, Lucknow.
6. Dr. Shobi Veleri Ph.D. Scientist – E, ICMR-NIN (National Institute of Nutrition), Beside Tarnaka Metro Station, Jamai-Osmania PO, Hyderabad-500 007, Telangana.
7. Dr M Muthukumar, Principal Scientist, ICAR - National Research Centre on Meat (NRCM), Chengicherla, Boduppal, Hyderabad-S00092, Telangana.
8. Dr. Komal Chauhan, Dean Research & Outreach, National Institute of Food Technology Entrepreneurship and Management (NIFTEM), PIot No. 97, Sector 56, HSIIDC Industrial Estate, Sector 56, Kundli, Sonepat, Haryana.
9. Dr. Madhumala, CSIR IICT-(Indian Institute of Chemical Technology), Uppal Road, Tarnaka, Hyderabad, 500007 Telangana.
10. Dr. Niladri Chatterjee, Sr. Scientist, ICAR-Central Institute of Fisheries Technology (CIFT), Matsyapuri P.0., Willingdon Island, Cochin-68Z 02g, Kerala.
11. Dr Tanweer Alam, Indian Institute of Packaging, plot EZ, MIDC Area, Andheri East, Road No.8, Post Box No. g432, Mumbai 400093, Maharashtra.
12. Dr. Sridevi Annapurna Singh, Director, CSIR- Central Food Technological Research Institute (CFTRI), Mysuru- ST OOZO, Karnataka.
13. Dr. Bhaskar Dutta, Professor, Civil Engineering, Indian Institute of Technology (IIT) Gandhinagar, Block 3, Room 301, IIT Gandhinagar, palaj, p.0. Simkheda, Gandhinagar 382355, Gujarat.
14. Dr. Rahul Banerjee, ICAR-IASRI (Indian Agricultural Statistics Research Institute), Library Ave, Pusa, New Delhi, Delhi 110012.

Attended in virtual mode:

15. Dr Ravi Kant Agrawal, Principal Scientist, Indian Veterinary Research Institute (IVRI) , lzatnagar, Bareilly, 243122, Uttar Pradesh (Attended in virtual mode).
16. Swapan Ray, Hon. Secretary, Indian Center for Plastics in the Environment. Sr. VP, Reliance Industries Limited, India

NetSCoFAN Secretariat members, QA Division, FSSAI:

1. Dr. Satyen Kumar Panda, Advisor, Quality Assurance Division, FSSAI, New Delhi-Chairperson
2. Ms. Sweety Behera, Director, Quality Assurance, FSSAI
3. Sh. D. Venugopal, Deputy Director ,QA Division, FSSAI

Annexure-R4/3

भारतीय खाद्य संरक्षा एवं मानक प्राधिकरण
(स्वास्थ्य एवं कल्याण परिवार मंत्रालय)
(विज्ञान एवं मानक विभाग)
एफडीए भवन, कोटला रोड, नई दिल्ली --110002

Dated: 30th August, 2024

Record of discussion on the report of Toxics Link on Microplastics in Salt & Sugar held on 20.08.2024 at 11.30 AM at FSSAI, FDA Bhawan, New Delhi

A Meeting of the Food Safety and Standards Authority of India (FSSAI) and publishers of the report of Toxics Link on Microplastics in Salt & Sugar was held on 20.08.2024 from 11:30 AM onwards under the chairmanship of Advisor (Science & Standards and Regulations) at FSSAI HQ, FDA Bhawan, New Delhi to understand the study conducted, protocol used and outcome highlighted in the report. The list of participants is placed at ***Annexure-I.***

At the outset, Advisor (Science & Standards and Regulations) express the welcome to all the scientific experts of FSSAI and the representatives from the Toxics Link. The published report on Microplastics in Salt and Sugar was placed for discussion before the experts.

The following points emerged during meeting;

1. Microplastics are a significant emerging issue, potentially contaminating ocean and soil, and subsequently entering the food chain through various ways. Microplastics may be present in salt due to its source from evaporated seawater and its presence in sugar is particularly undesirable.
2. It was highlighted that Microplastics can vary in size and in polymeric form, which makes challenging its identification and quantification without the use of advanced and robust technology.
3. The authors informed that 10 samples of Salt and 5 samples of Sugar including branded, packed and loose samples were purchased from the local market. These samples then repacked in Ziploc polythene covers and sent to Manipal Institute of Technology, Manipal, Karnataka for analysis. Further, FTIR-ATR spectroscopy used for analysis to determine the polymer type.
4. Scientific experts emphasized that the storage and transportation conditions of the samples are important and critical care has to be taken for source of sample (raw material, process, transportation or atmosphere), nature of sample, sample size, sample preparation method, working condition of storage or transportation of sample, analysis methodology etc. during conduct of such study.
5. Scientific experts highlighted that ISO 24187:2023 *'Principles for the analysis of microplastics present in the environment'* shall be referred as standard protocol for microplastics analysis. Further, combination of test methods is required to identify/quantify the microplastics in food matrices.
6. The Scientific experts opined that the report has several limitation as follows:

a) The sample size is too small. Further, samples were packed in ziploc polythene covers for transportation with uncontrolled temperature conditions which may contribute microplastics in the samples and affect the results of the study.
b) Further, FTIR-ATR spectroscopy is only a qualitative method and may not be applicable to detect which polymer of microplastics present in the samples. Single method is not feasible for identification of microplastics. Internationally adopted methodology (ISO 24187:2023) shall be adopted for analysis and authenticity of the data.
c) The size distribution of microplastics reported is quite doubtful. The data on quantification is not sufficient to draw a conclusive remark.
d) Colour of microplastics cannot be properly identified through the visual by naked eyes.
e) The report does not having substantiating data for setting standards.

7. It was informed to the Toxics Link that Food Safety and Standards Authority of India (FSSAI) launched a project entitled "Micro-and nano-plastics as emerging food contaminants: Establishing validated Methodologies and understanding the prevalence in different food matrices". The project was started in March, 2024 to develop and validate analytical methods to detect micro and nano-plastics in various food products and assess their prevalence and exposure levels in the country. The project duration is for 02 years and being implemented in collaboration with 03 research institutes viz. CSIR-Indian Institute of Toxicology Research, Lucknow; ICAR-Central institute of Fisheries Technology (ICAR-CIFT), Kochi and Birla Institute of Technology and Science (BITS), Pilani.
8. Non-profit organisations such as Toxics Link are suggested that they may discuss with scientific experts of relevant field before publishing such report in public domain. FSSAI is open to discuss such issues with Toxics Link in future.
9. It was concluded that data presented by Toxics Link is not convincing in terms of quantitative analysis and scientific ethics and currently conclusion on the report cannot be drawn. Toxics Link may carry study using ISO 24187: 2023 guidelines. The extrapolation of inadequately substantiated data could mislead public upon publishing such reports.

Meeting ended with vote of thanks.

Annexure-I

LIST OF PARTICIPANTS

Scientific Experts empanelled with FSSAI:

1. Dr. Alok Dhawan, Chair of SP on Contaminants in Food Chain
2. Dr. Vimal Katihar, Chair of SP on Food Packaging
3. Dr. Rajiv Diwedi, Chair of EC on Packaging
4. Dr. Kausar Mahmood Ansari, Member of SP on Contaminants in Food Chain

FSSAI Officials:

1. Dr. Alka Rao, Advisor (Science & Standards Division and Regulation Division)
2. Dr. Amit Sharma, Director (Science & Standards Division)
3. Sh. Perumal Karthikeyan, Joint Director (Science & Standards Division)
4. Sh. Pankaj Kumar Meena, Assistant Director (Science & Standards Division)
5. Sh. Kishor Shedage, Technical Officer (Science & Standards Division)

Toxics Link Representatives:

1. Dr. Anish Kumar, Assistant Professor, Manipal Institute of Technology, Karnataka
2. Mr. Ravi Agarwal, Director (Toxics Link)
3. Dr. Satish Sinha, Associate Director (Toxics Link)
4. Ms. Priti Mahesh, Chief Programme Coordinator (Toxics Link)
5. Dr. Anjali Nair, Programme Officer (Toxics Link)
6. Dr. Amit, Ex-Programme Coordinator (Toxics Link)
7. Dr. Deepak Marathe
8. Dr. Piyush Mahapatra

Annexure-R4/4

Minutes of the meeting on First phase of Review on status of project titled "Micro-and nano-plastics as emerging food contaminants: Establishing validated methodologies and understanding the prevalence in different food matrices" held on 24th November 2024 at 4:00 PM

List of Participants:

1. Dr. Kausar,Mahmood Ansari, Principal Scientist, CSIR-IITR, Vishvigyan Bhawan, Lucknow.
2. Dr Satyakam Patnaik - Principal Scientist, CSIR-IITR, Vishvigyan Bhawan, Lucknow.
3. Dr Niladri Sekhar Chatterjee, Sr. Scientist, ICAR-Central Institute of Fisheries Technology (CIFT), Matsyapuri P.0., Willingdon Island, Cochin-68Z 02g, Kerala.
4. Dr Paul Atish Tulshiram, Birla Institute of Technology and Science, Pilani

NetSCoFAN Secretariat members, QA Division, FSSAI:

1. Dr. Satyen Kumar Panda, Advisor, Quality Assurance Division, FSSAI, New Delhi- Chairperson
2. Ms. Sweety Behera, Director, Quality Assurance, FSSAI
3. Sh. D. Venugopal, Deputy Director ,QA Division, FSSAI

Dr. Satyen Kumar Panda, Advisor (QA) at FSSAI, chaired a virtual meeting to review the status on project titled "Micro-and nano-plastics as emerging food contaminants: Establishing validated methodologies and understanding the prevalence in different food matrices" on 24th November 2024 at 4PM.

The meeting was attended by Principal Investigator and Co-Principal Investigator of project and NetSCoFAN Secretariat, QA Division FSSAI.

The agenda was to discuss the status on project proposal titled "Micro-and nano-plastics as emerging food contaminants: Establishing validated methodologies and understanding the prevalence in different food matrices"" submitted by CSIR-IITR, Lucknow.

The meeting commenced with a welcome address by the Advisor (QA) and welcomed all the members**.**

Dr. Kausar, Mahmood Ansari, Principal Investigator of Project, CSIR-IITR, Lucknow presented the progress of project work. He presented the following points:

- Compounds database for the monomers of PET, Nylon-6 (PA-6) and PC have been created and validated with published literature.
- Conducted pyrolysis of PET and monomers (Benzoic acid and p-terphthanic acid) were identified on LC-MS/MS and
- LC-MS/MS based method has been developed for simultaneous analysis of 5 Bisphenols.

It was also presented the progress of project.

1. Pyrolysis study for the Nylon-6 and Polycarbonate will be conducted and a LC-MS/MS based method will be established for the analysis of their monomers.
2. Study on the real samples for identification and quantification of PET, Nylon and Polycarbonate microplastics will be conducted.

Advisor (QA) suggested to prepare a sampling plan commodity wise and regional wise for testing microplastics. Also requested to accommodate additional matrix such as salt etc. The LC-MS/MS and GC-MS/MS methods are to be validated for application in various matrices selected for the study.

Dr Niladri Sekhar Chatterjee informed that CIFT has developed a microwave assisted rapid extraction method for six different types of microplastics (PP, HDPE, LDPE, PVC, PET, and Polystyrene) for extracting them from tissue samples such as fish, dried fish, fishmeal, and meat. The method reduced the sample preparation time to 30 minutes as compared to traditional sample preparation method that takes 48 to 72 h.

Advisor (QA) suggested for development of a single method for characterizing multiple types of microplastics. The research team apprised that the Raman spectroscopy based method can possibly characterize multiple types of microplastics. At the present GC- MS/MS and LC-MS/MS based methods are not capable to determine multiple plastic types simultaneously. The same sample has to be analysed using different method.

Advisor (QA) suggested:

- To make a sampling plan so as to include statistically significant number of samples for each product category.
- that LoD (size) and LoQ (concentration) for various matrices must be ascertained.

Finally, the Advisor (QA)/ Chairperson, NetSCoFAN Secretariat concluded the session by expressing gratitude to all participants for their valuable contributions and engagement.

Signed by
Venugopal Dubakula
Date: 29-11-2024 19:03:21

Minutes of the Meeting: Meeting to review the status of the project titled "Micro-and Nano-plastics as Emerging Food Contaminants: Establishing Validated Methodologies and Understanding Their Prevalence in Different Food Matrices."

Date & Time: 25.11.2024 at 04.30 PM

1. **Project Name:** Micro-and Nano-plastics as Emerging Food Contaminants: Establishing Validated Methodologies and Understanding Their Prevalence in Different Food Matrices
2. **Institutes involved:** CSIR-IITR, ICAR-CIFT, BITS Pilani
3. **The List of Participants:**

S No.	Name	Organization / Institute
1	Dr Satyen Kumar Panda, Advisor (QA)	FSSAI
2	Ms. Sweety Behera, Director (QA)	FSSAI
3	Shri D. Venugopal, Deputy Director (QA)	FSSAI
4	Ms. Hemalatha P, Technical Officer (QA)	FSSAI
Institutes – Nodal Officers		
1	Dr Bhaskar Narayan, Director	CSIR-IITR
2	Dr Kausar Mahmood Ansari, Principal Investigator	CSIR - IITR
3	Dr Paul Atish Tulshiram	BITS, Pilani
4	Dr Niladri Sekhar Chatterjee, Co-Principal Investigator	ICAR-CIFT

The meeting commenced with a welcome address by the Director (QA).

Presentation on the Status of the Project:

1. Dr. Kausar Mahmood Ansari from CSIR-IITR, Lucknow, presented the status of the project titled **"Micro- and Nano-plastics as Emerging Food Contaminants: Establishing Validated Methodologies and Understanding Their Prevalence in Different Food Matrices."**
2. In his presentation, he outlined the task-wise work progress for the three

objectives:

i. **Objective 1:** Development and validation of analytical methods for identifying, characterizing, and quantifying micro-/nano-plastics in food matrices.
ii. **Objective 2:** Inter- and intra-laboratory comparison of the developed methods.
iii. **Objective 3:** Conducting a survey to determine the prevalence of micro-/nano-plastics in identified food matrices.

3. Details on the **tasks, timelines, and progress status** for each objective were also provided.
4. Dr. Ansari highlighted that the **Nile Red Staining method** has been optimized and successfully applied to detect plastics, including **polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), nylon, and polyvinyl chloride (PVC),** across all three participating institutes: **CSIR-IITR, ICAR-CIFT,** and **BITS Pilani.**
5. He also mentioned that the **Raman Spectroscopy method** has been optimized and successfully used to analyse the spectra of **PE, PP, PET, PS, nylon, and PVC** at **CSIR-IITR.** The method is now being prepared for sharing with **BITS Pilani** and **ICAR-CIFT.** Currently, the plan is to collect samples from **BITS Pilani** and **ICAR-CIFT** and conduct spectroscopy analyses at **CSIR-IITR.**
6. Additionally, he reported that the **microwave-assisted digestion method** has been optimized for various types of fish samples. He further noted that **pyrolysis of PET** was conducted, leading to the identification of monomers, including **benzoic acid** and **p-terephthalic acid,** using **LC-MS/MS.**
7. Dr. Ansari emphasized that all optimized and developed methods are currently undergoing validation at both the inter-laboratory and intra-laboratory levels
8. Dr. Ansari shared comprehensive information on zone-wise sample collection, including specifics such as **Sample Name, Brand, Batch Number, Manufacturing Date, Expiry Date, Date of Sampling, Weight, Regulatory Authority, Location, Number of Samples, Temperature/Humidity,** and **Packaging Material:**

- **Water, Cold Drink, and Tea Samples** were collected from **Zone West (Rajasthan).**
- **Fresh Fish Samples** were collected from **Zone 2 South (Kochi).**
- **Dried Fish Samples** were collected from **Zone 1 West (Veraval and**

Mumbai) and **Zone 2 South (Munambam).**

- **Fish Meal Samples** were collected from **Zone 2 South (Andhra Pradesh).**

9. He shared the procedure for Microplastic analysis of packaged water following by CSIR-IITR and he shared the images of **Optical Microscopy of Microplastics**, **Nile Red Staining of Microplastics, FESEM Images of Microplastics.**
10. The Director of IITR informed that, following the development of a method for microplastics analysis, IITR will impart training and facilitate the transfer of knowledge to the FSSAI notified laboratories.

11. Finally, the Advisor (QA) recommended the institutes to submit the progress report with the NetSCoFAN Secretariat and concluded the meeting.

Signed by
Venugopal Dubakula
Date: 29-11-2024 19:01:48

Annexure-R4/5
Six monthly Project Report

on

Micro-and nano-plastics as emerging food contaminants: Establishing validated methodologies and understanding the prevalence in different food matrices

Submitted to

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA

Inspiring Trust, Assuring Safe & Nutritious Food

Ministry of Health and Family Welfare, Government of India

By

Kausar Mahmood Ansari, Ph.D
Principal Scientist
Food Toxicology Laboratory
CSIR-Indian Institute of Toxicology Research, M G Road, Lucknow, India

CSIR-Indian Institute of Toxicology Research, Lucknow, India

6- Month Report

(To be submitted to the Chief Executive Officer, Food Safety and Standard Authority of India, FDA Bhawan, Kotla Road, New Delhi-110 002)

1. Title of the project: Micro-and nano-plastics as emerging food contaminants: Establishing validated methodologies and understanding the prevalence in different food matrices

2. Date of Commencement: 13/03/2024

   Date of termination: 12/03/2026

3. Name and address of Principal Investigator:

Dr Kausar Mahmood Ansari
CSIR-Indian Institute of Toxicology Research, Vishvigyan Bhawan, 31, M. G. Marg, Lucknow-226001, India

Name and address of Co-Principal Investigator's:

Dr Satyakam Patnaik
CSIR-Indian Institute of Toxicology Research, Vishvigyan Bhawan, 31, M. G. Marg, Lucknow-226001, India

Dr Niladri Sekhar Chatterjee
ICAR-Central Institute of Fisheries Technology, Matsyapuri P.O., Willingdon Island, Cochin-682029, India

Dr Atish Paul
Birla Institute of Technology and Sciences, Pilani, India

4. Total grant sanctioned and expenditure during the entire tenure

S.No.	Expenditure Head	Amount (Rs in lakh)		
		1st Instalment	2nd Instalment	3rd Instalment
1.	Equipments	Nil	Payment not received	Payment not received
2.	Man power	Rs. 13,92,000/-		
3.	Consumables	Rs. 25,00,000/-		
4.	Travel	Rs. 30,000/-		
5.	Contingency	Rs. 70,000/-		
6.	Overheads 12%	Rs. 4,79,040/-		
	TOTAL	Rs. 44,71,040/-		

Man power is co terminous with the project. Authority will not be responsible for any legal implication,for any further employment / payment of salary / wages etc and it will solely responsibility of the concerned University / Institution where he/she is working or employed.

5. Equipment(s) purchased out of FSSAI assistance: None

Name	Cost (Rs. in lakh)

6. Research fellows associated with project

Name & Designation	Date of Joining	Date of Leaving
Vishal Narayan Soni, Project Associate -1	16.04.2024	
Sakshi Singh, Project Associate -1	16.04.2024	
Sauhard Kushwaha, Project Associate -1	15.04.2024	
Sana Bano, Project Associate -1	13.06.2024	

7. List of research papers published/communicated, based on the research work done under the project (Name(s) of author(s), Title, Journal, Volume number, Year and Pages should be given for each paper published and a copy of each of them should be enclosed; reprints/copies of papers appearing after submission of PCR should also besent to Authority):

NIL

8. Details of patents filed if any.

NIL

9. Detailed account of the work carried out in terms of the objective(s) of the projectand how far they have been achieved;(results and discussion should be presented in the manner of a scientific paper/project report in about 5000 words)

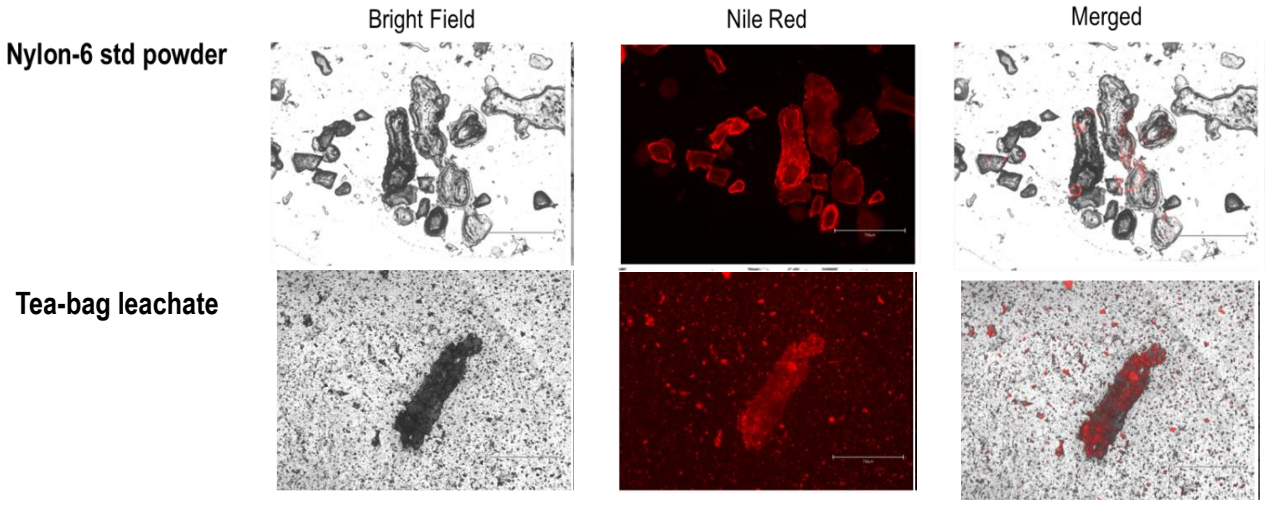
9.1 Approved Objectives of the Proposal:

1. Development and validation of analytical methods for identification and quantification micro-/nano-plastics in food matrices
2. Inter- and intra-laboratory comparison of developed method in identified in food matrices
3. Survey study to determine the level of micro-/nano-plastics in identified food matrices

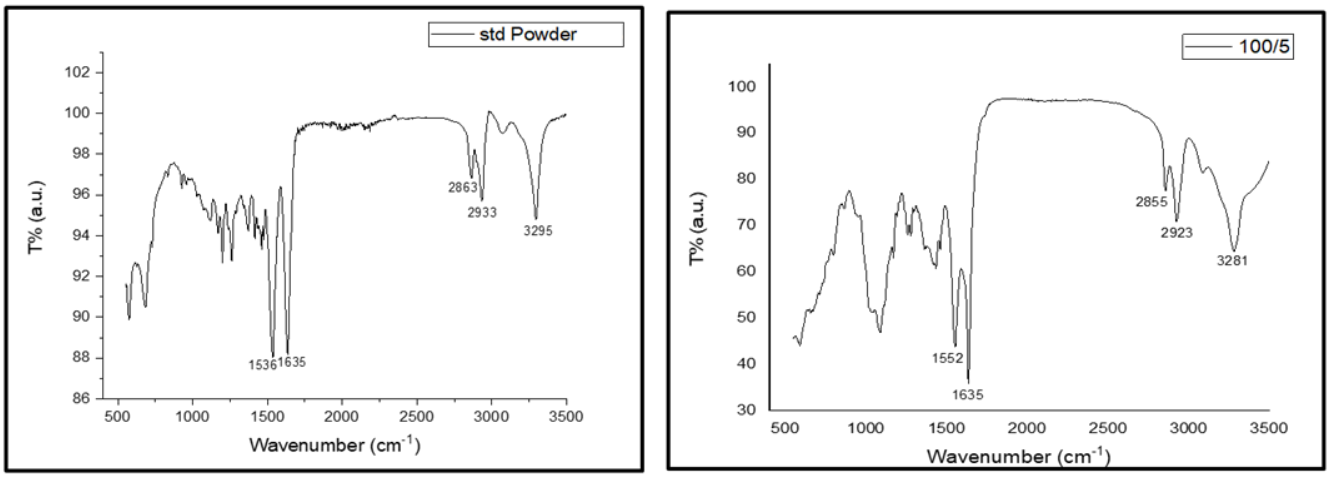
9.2 Methodology and Results of Objective 1 and objective 2

Microplastics are fragment of any type of plastic less than 5 mm in length, according to the U.S. National oceanic and atmospheric administration (NOAA) and the European chemicals agency. Their presence in environment is caused by pollution from various sectors, causing adverse health effect and ecological imbalance. Thus, in our current work, we are focusing on devising the methodologies for detection and quantification of these microplastic, in order to monitor their presence in different matrices.

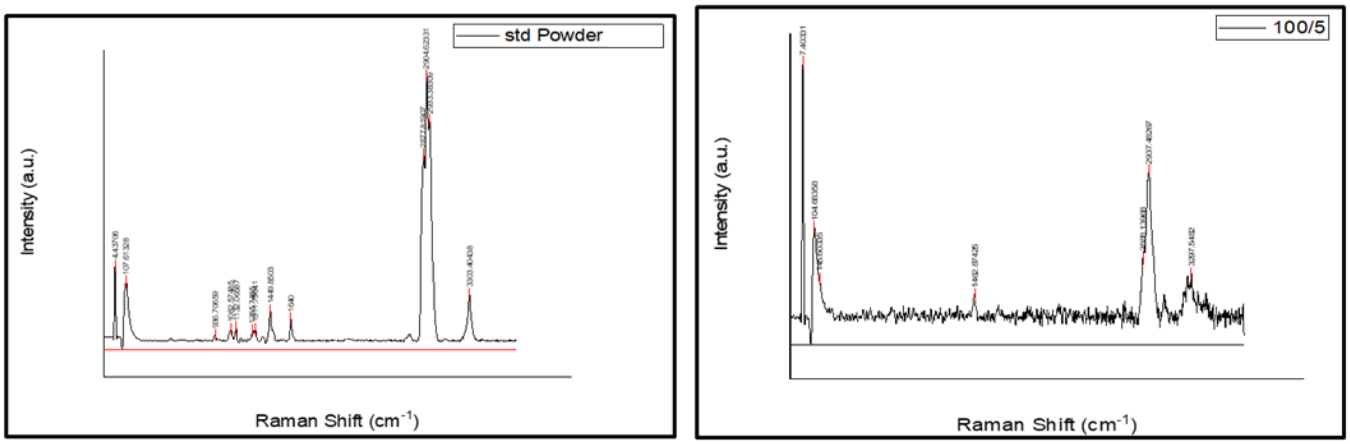
The development of a standardized Nile red staining protocol marked the initial step in the identification of plastics. This study introduces a rapid, reliable, and cost-efficient approach for detecting and sizing microplastics, specifically polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and nylon-6. The method involves labeling microplastics with the lipophilic dye Nile red at a concentration of 10 µg/mL, followed by fluorescence microscopy to capture images at varying magnifications. Validation using FT-IR and Raman spectroscopy confirmed that all fluorescing particles were accurately identified as plastics. Currently, this standardized technique is under review for its application in assessing microplastics in food samples. We validated the above-mentioned protocol in leachates obtained from tea bags by heating at 100^{o}C for 5 minutes, wherein we detected the presence of nylon-6 in the form of small fragments using FT-IR and Raman spectroscopy.



Fluorescence microscopic images of Nylon standard and tea bag leachate (100^{o}C, 5min) at 4 X magnification.



FTIR Analysis of Nylon-6 powder and Tea-bag leachate

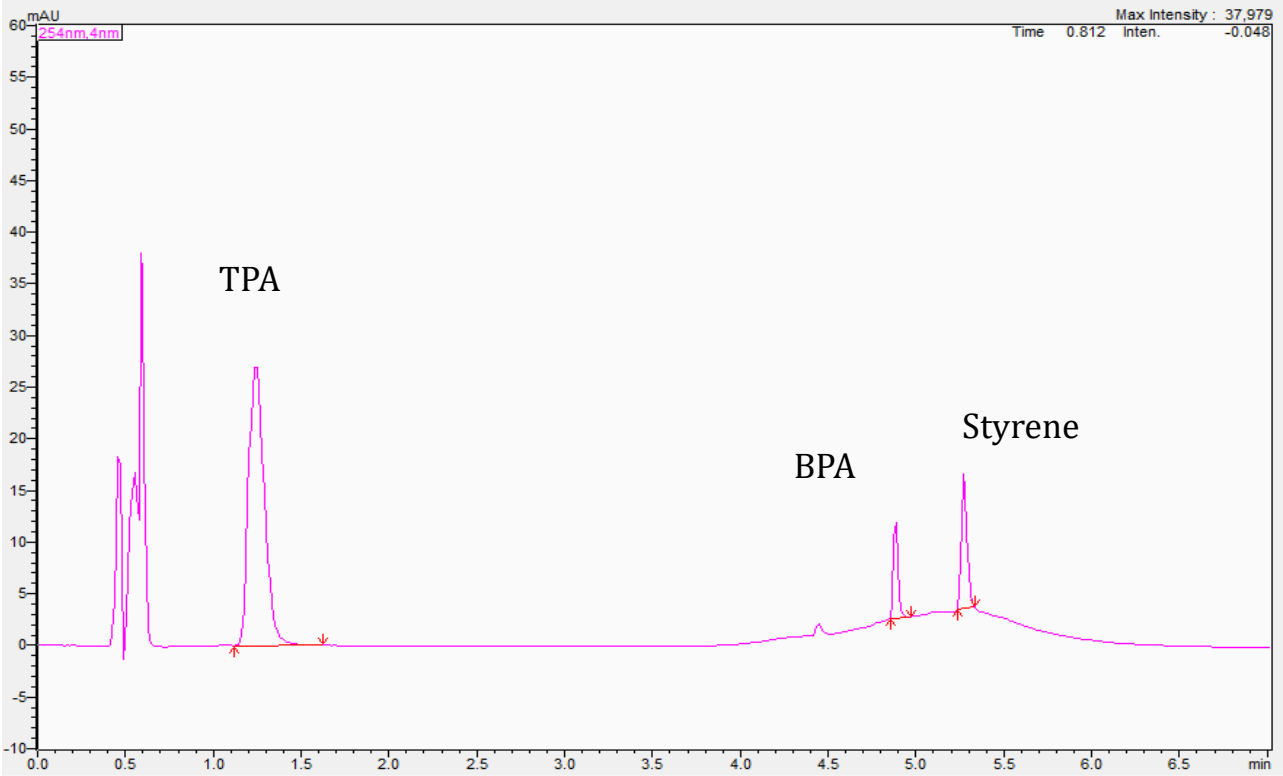


Raman Analysis of Nylon-6 powder and Tea-bag leachate

Presently, three different plastics (Polystyrene (PS), polycarbonate (PC) and polyethylene terephthalate) (PET) are targeted for purpose of method development and validation. Plastic standards and their monomers are procured for the purpose of method development. Depolymerization procedure of plastic were optimized at first. Method comprising the depolymerization procedure for PC and PET is same and a separate method was developed for depolymerization for PS. The depolymerized extracts of respective plastic polymer were subjected to preliminary examination via FTIR and Raman spectrometry. Observed results were compared with plastic standards and available literature. Preliminary instrumental analysis has shown positive identity for targeted analytes. Further, we proceeded for confirmatory analysis through UHPLC (ultra-high performance liquid chromatography) and direct mass analysis. All three targeted analytes were identified on the basis of their mass, retention time and UV absorbance range. Subsequently, we developed another method for simultaneous detection of all targeted via UHPLC.

Currently, we are optimizing extraction procedure for analytes in targeted matrices i.e. packed meat and packed beverages and designing experiments on method validation parameters i.e., Limit of detection (LOD) and limit of quantification (LOQ). In addition, compound databases for the monomers of PET, Nylon-6 (PA-6) and PC have been created and validated with published literature. We also

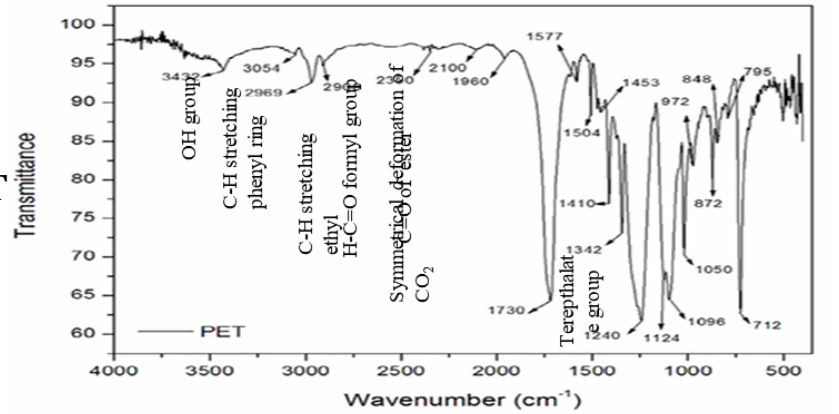
conducted pyrolysis of PET and monomers (Benzoic acid and p-terephthalic acid) were identified on LC-MS/MS. LC-MS/MS based method has been developed for simultaneous analysis of 5 Bisphenols.



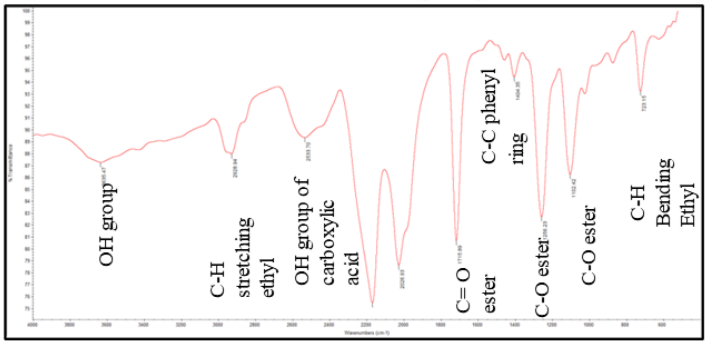
Method development for simultaneous analysis of targeted analytes

UHPLC PARAMETERS	
COLUMN	Reverse phase C18 (100 x 2.1mm 2.6µm) column
MOBILE PHASE	Milli-Q and ACN (modified with 0.1 TFA)
FLOW RATE	0.5 ml/min
INJECTION VOLUME	5µl
RUN TIME	9 min
COLUMN TEMPERATURE	30°C
UV detection	PC (bisphenol)- 226 nm PET(terephthalic acid)- 240 nm Polystyrene (styrene)- 247 nm

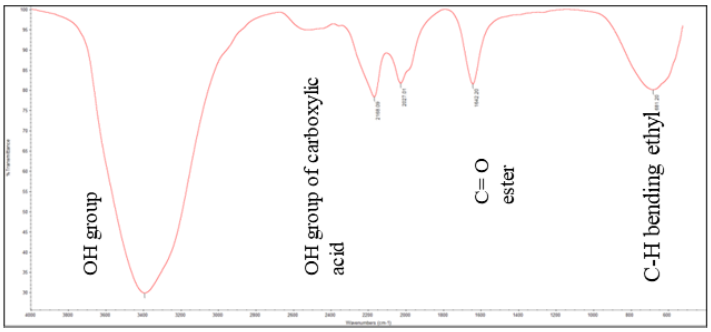
Standard PET



PET bead

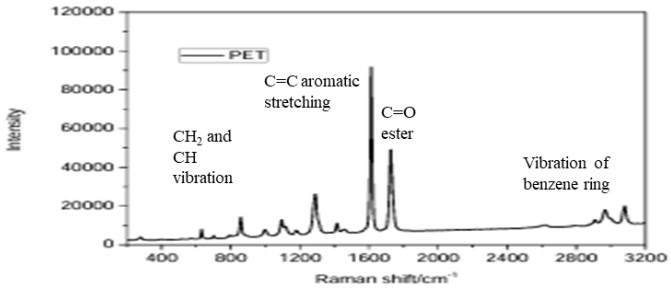


Depolymerized PET

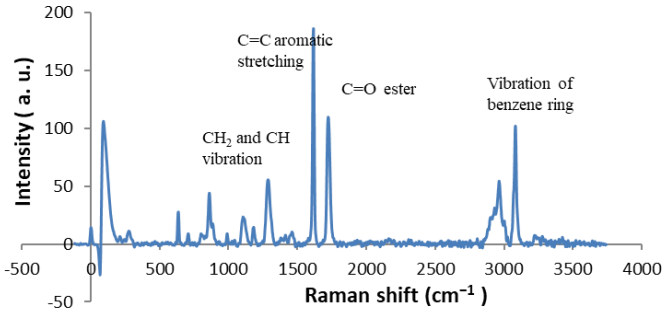


FTIR Analysis of depolymerized PET

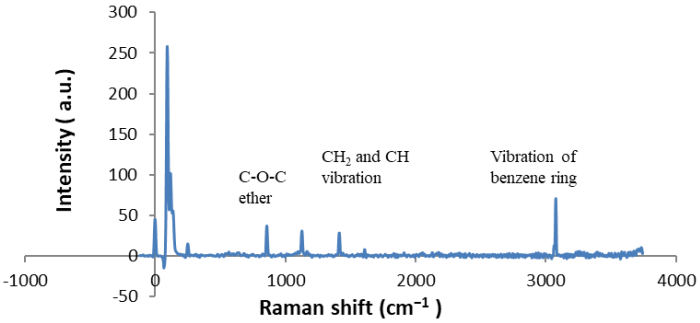
Standard PET



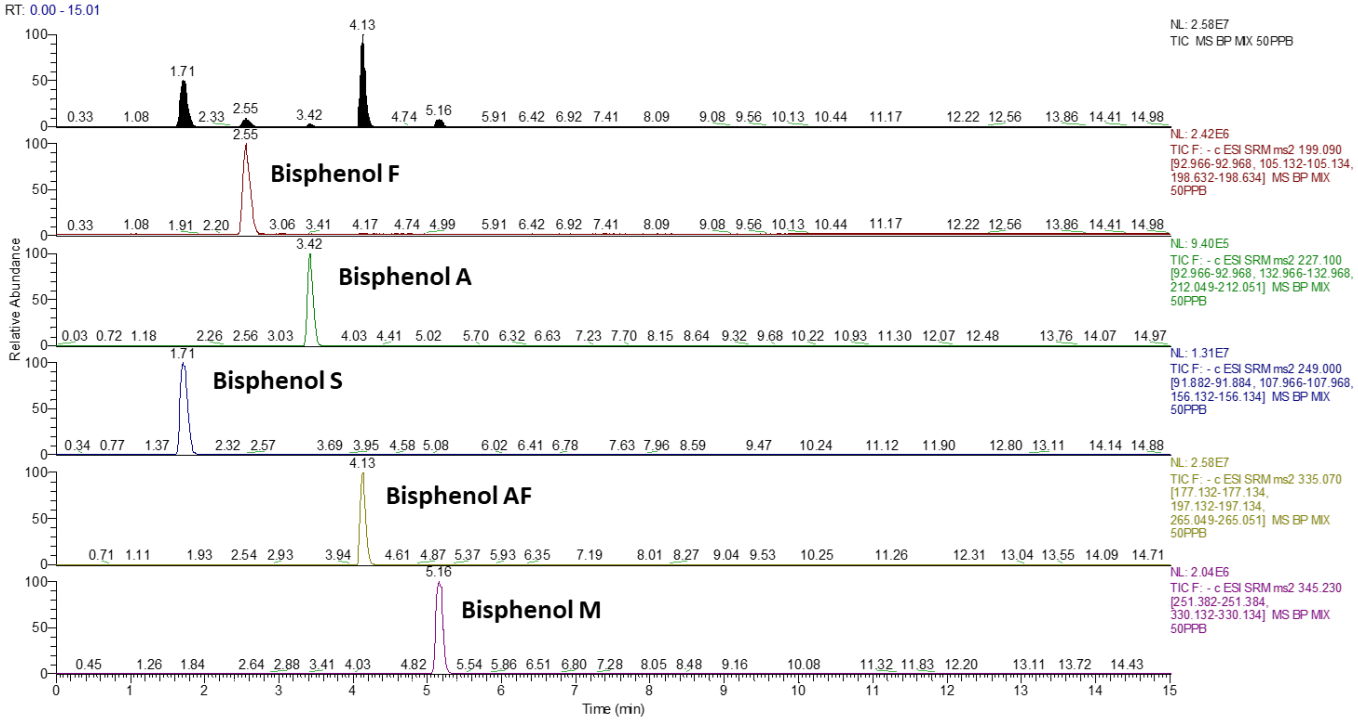
PET bead



Depolymerized PET



Raman Spectrometry analysis of PET



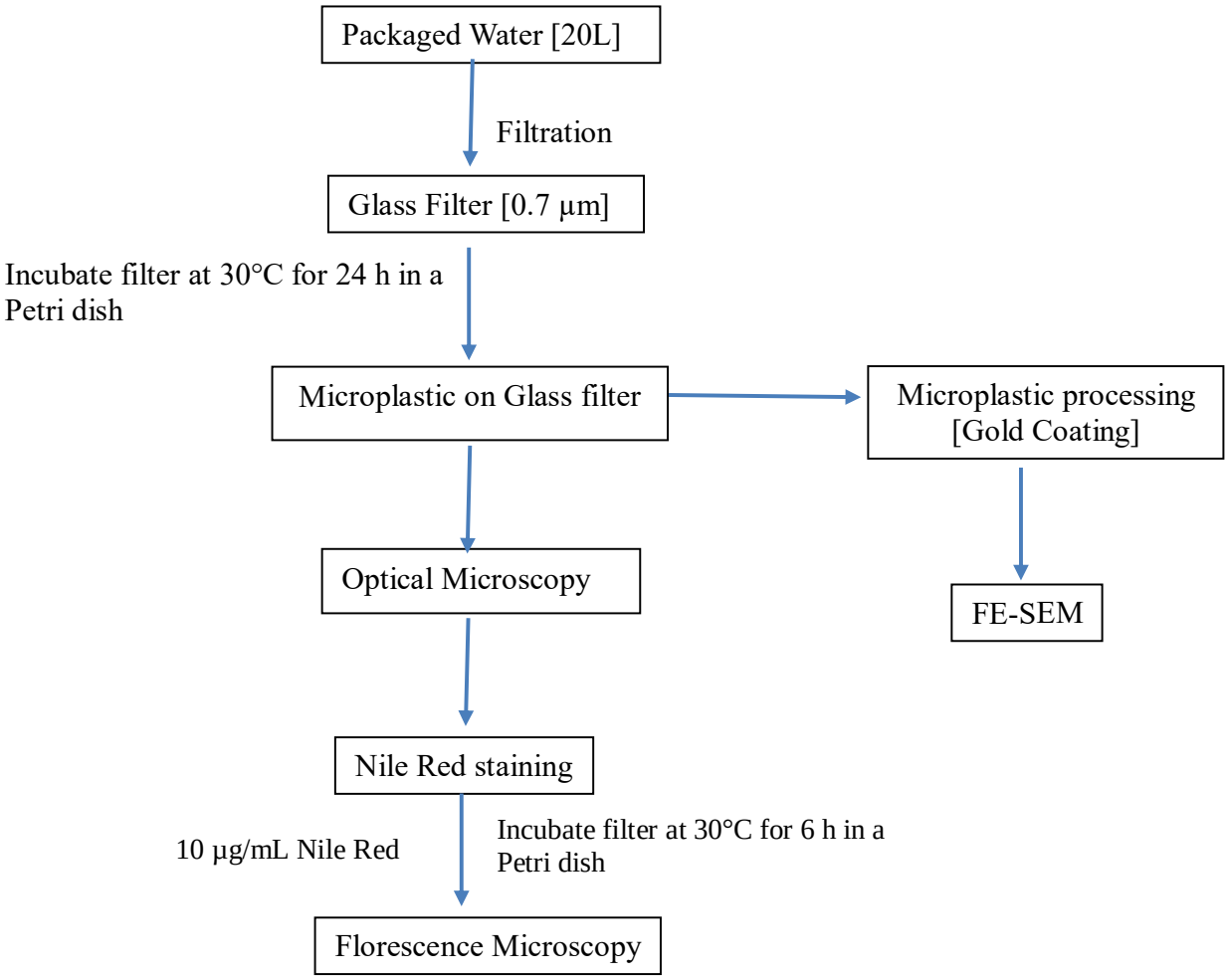
LC – MS/MS Analysis of Bisphenols (PC monomers)

LC PARAMETERS		
Time	Flow (mL/min)	%B
0-1	0.250	40
1-1.5	0.250	50
1.5-2.5	0.250	65
2.5-4	0.250	75
4-7.5	0.250	85
7.5-8.5	0.250	85
8.5-11.5	0.250	40
11.5-15	0.250	40

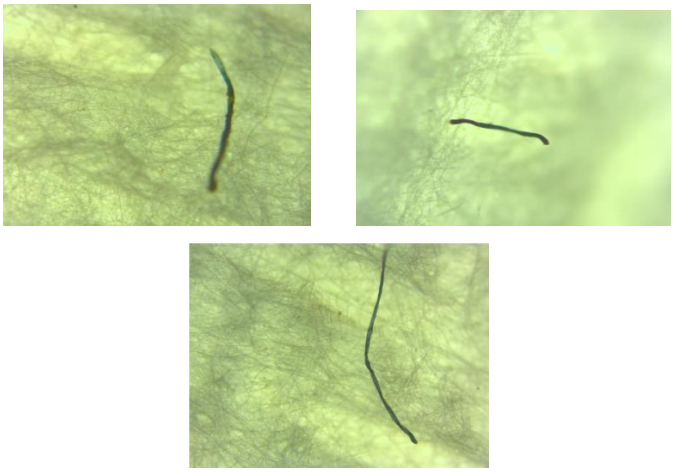
MS PARAMETERS		
Compound	Precursor	Product (m/z)
Bisphenol A	227.100	132.967, 212.050, 92.967
Bisphenol S	249.000	107.967, 91.883, 156.133
Bisphenol F	199.090	105.133, 92.967, 198.633
Bisphenol AF	335.070	265.050, 197.13, 177.13
Bisphenol M	345.230	251.383, 330.133

Chromatographic Conditions	**Mass Conditions**
Column- C-18 (100mm X 2.1mm, 1.9 µm, Hypersil Gold, thermo scientific)	**Ion Source:** H – ESI
Solvent A- Water	**Polarity:** Negative
Solvent B- Acetonitrile	**Sheath Gas (Arb):** 50
	Aux Gas (Arb): 10
	Sweep Gas (Arb): 0
	Ion Transfer Tube Temp: 275°C
	Vaporizer Temp: 250°C
	CID Gas (Torr): 1.5

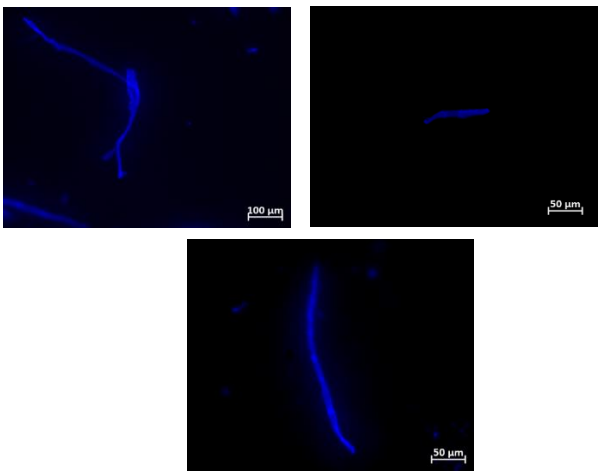
Microplastic analysis of Packaged water



Optical Microscopy of Microplastics

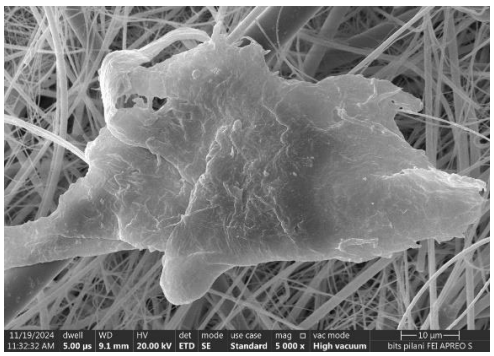


Nile red staining of Microplastics



Optical microscopy was carried out at 10 X magnification. Morphology of particles, fibers and fragments was noted . Additionally, color was also noted. DAPI (4′,6-diamidino-2-phenylindole; excitation 325–375 nm, emission 435–485 nm; blue fluorescence) was used for fluorescence microscopy.

FESEM images of Microplastics



FEI Apreo LoVac scanning electron microscopy (FE-SEM) was used for morphological characterization. A polymer image was detected and marked by a binocular microscope. Glass filter was cut with metal scissors. The gold coating was done using 5 mA sputting current to avoid excessive heating or damage of microplastics. The time duration for coating was 50 sec. After coating, samples were transferred using metal tweezers onto an aluminum SEM sample holder. The Dwell time was set to 5.00 µs with a working distance of 9.1mm. FE-SEM was operated at High voltage (HV) mode. A 20 kV was applied to generate the electron beam and the microscope operated at a high vacuum mode for precise imaging.

Microplastic analysis in fish tissue, fish meal, dry fish

A rapid method for extraction of microplastic from fish tissue, fish meal, and dry fish was developed using a microwave-assisted extraction method. The extraction time was only 30 min as compared to 72 h extraction in traditional methods.

Six different extraction conditions were initially evaluated for the extraction of polypropylene (PP), and high-density polypropylene (HDPE) from fish tissue. For this purpose, HDPE and PP microparticles were prepared in the laboratory by freezing using liquid nitrogen, followed by grinding in a high-speed homogenizer. The known weight of these microplastics was spiked in homogenized fish tissue mixed well and extracted using different experimental conditions mentioned in the following table. Digestion efficiency and plastic recovery were calculated gravimetrically using standard protocols. It was observed that microwave-assisted extraction at 120 °C for 30 min using 2.5 % KOH, 5 % H_2O_2, and 2.7 % methanol provides more than 90 % digestion efficiency and recovery. Hence, this method was further evaluated for other plastic types, such as PVC, LDPE, Polystyrene, and PET, and in all cases, the plastic recovery was greater than 78 to 80 %. The recovered microplastics were observed under a microscope with and without nile red staining to count the number of microplastics.

Hence, a rapid method was developed for extraction of six different microplastic types from fish tissue, fish meal, dry fish using microwave assisted extraction method that reduced the extraction time significantly and reduced the chances of contamination with microplastics from the laboratory environment.

	Plastic	Method 1 2.5% KOH + 5% H2O2 + 2.7% MeOH 60°C, 3Hrs	Method 2 0.50Mol/L HNO3 170°C, 5Min	Method 3 6.0M HCL 120°C, 30 Min	Method 4 4.0M NaOH 120°C, 30 Min	Method 5 2.0M HNO3 120°C, 30 Min	Method 6 2.5% KOH + 5% H2O2 + 2.7% MeOH 120°C, 30 Min
Digestion Efficiency (%)	PP	99.51	97.85	96.25	98.57	95.37	98.95
	HDPE	99.56	97.56	96.39	98.53	94.24	98.96
Plastic Recovery (%)	PP	74.79 ± 4.03	58.63 ± 141.45	-15.94 ± 48.29	78.92 ± 33.88	-193.65 ± 102.69	91.85 ± 5.05
	HDPE	63.84 ± 3.57	116.57 ± 172.73	-29.62 ± 41.97	82.59 ± 42.38	-79.62 ± 165.23	90.83 ± 9.72

We have geographically demarcated the sampling areas from different states into various zones on the basis of climate conditions. The samples that will be collected for analysis include bottled water, packed juices, beverages, fresh fish, dry fish, and fish meals.

Water, Cold drink and Tea SAMPLES

Zone	AREA	Sample name	Brand	Batch No	Mfg date	Exp date	Date of sampling	Weight (gm/ mL)	Regulatory Authority	Location	No. of samples	Temp /Humidity	Packaging material
Zone 1 West (GOA, GUJ, MH, RAJ, D&N, D&D	RAJ	Bottle water	Brand A	GRTL 52401555KG	11/10/24	10/10/24	23/10/24	1 L	FSSAI	28.362572829139367,75.5928325461697	20	33/38	PET bottle
	RAJ	Bottle water	Brand B	JB25100:59	07/09/24	06/03/24	25/10/24	1L	FSSAI	28.362572829139367,75.5928325461697	20	33/38	PET bottle
	RAJ	Tea Bag	Brand A	W3A	12/08/24	11/05/25	25/10/24	190 gm	FSSAI	28.362572829139367,75.5928325461697	300	33/38	Nylon
	RAJ	Cold drink	Brand A	08103/09	04/06/24	03/12/24	26/10/24	2 L	FSSAI	28.362572829139367,75.5928325461697	10	33/38	PET bottle
	RAJ	Cold drink	Brand B	161 KG 1809	19/06/24	18/12/24	04/11/24	2 L	FSSAI	28.362572829139367,75.5928325461697	10	34/22	PET bottle

Fresh fish SAMPLES

Zone	AREA	Sample name	Brand	Batch No	Mfg date	Exp date	Date of sampling	Weight (gm/mL)	Regulatory Authority	Location	No.of samples	Temp /Humidity	Packaging material
Zone 2 South (AP, KAR, KER TN, TEL, PUD, LAKSH)	Kochi	Mackeral Flesh	NA	NA	NA	NA	5.11.24	100g		Kochi, Kerala	03	-20°C	Glass
		Mackeral Gills	NA	NA	NA	NA	5.11.24	13.59g			03	-20°C	Glass
		Mackeral Gut	NA	NA	NA	NA	5.11.24	14.42g			03	-20°C	Glass
		Mackeral Liver	NA	NA	NA	NA	5.11.24	3.38g			03	-20°C	Glass
	Kochi	Tuna Flesh	NA	NA	NA	NA	5.11.24	100g		Kochi, Kerala	03	-20°C	Glass
		Tuna Gills	NA	NA	NA	NA	5.11.24	11.25g			03	-20°C	Glass
		Tuna Gut	NA	NA	NA	NA	5.11.24	20.53g			03	-20°C	Glass
		Tuna Liver	NA	NA	NA	NA	5.11.24	1.67g			03	-20°C	Glass

DRY FISH SAMPLES

Zone	AREA	Sample name	Brand	Batch No	Mfg date	Exp date	Date of sampling	Weight (gm/mL)	Regulatory Authority	Location	No.of samples	Temp /Humidity	Packaging material
Zone 1 West (GOA, GUJ, MH, RAJ, D&N, D&D	Veraval	Ribbon Fish	NA	NA	NA	NA		10g		Veraval, Gujrat	03	Room Temperature	Glass, Aluminium foil
		Anchovy	NA	NA	NA	NA		2.45g		Veraval, Gujrat	03	Room Temperature	Glass, Aluminium foil
		Shark	NA	NA	NA	NA		10g		Veraval, Gujrat	03	Room Temperature	Glass, Aluminium foil
		Solefish	NA	NA	NA	NA		10g		Veraval, Gujrat	03	Room Temperature	Glass, Aluminium foil
	Mumbai	Bombay Duck	NA	NA	NA	NA		3.10g		Mumbai, Maharashtra	03	Room Temperature	Glass, Aluminium foil
Zone 2 South (AP, KAR, KER TN, TEL, PUD, LAKSH)	Munambam	Mackeral, Brined	NA	NA	NA	NA		10g		Munambam, Kerala	03	Room Temperature	Glass Aluminium foil
		Mackeral,Salted	NA	NA	NA	NA		10g		Munambam, Kerala	03	Room Temperature	Glass, Aluminium foil

Fish Meal SAMPLES

Zone	AREA	Sample name	Brand	Batch No	Mfg date	Exp date	Date of sampling	Weight (gm/mL)	Regulatory Authority	Location	No.of samples	Temp /Humidity	Packaging material
Zone 2 South (AP, KAR, KER TN, TEL, PUD, LAKSH)	Andra pradesh	No.87	BMR Spectra	NA	NA	NA		10g		Adavuladeevi	03	Room Temperature	Glass
		No.83	Falcon	NA	NA	NA		10g		Adavuladeevi	03	Room Temperature	Glass
		No.37	BMR Spectra	NA	NA	NA		10g		Dindi	03	Room Temperature	Glass
		No.124	Groco best	NA	NA	NA		10g		Bandarulanka	03	Room Temperature	Glass
		No.163	Shenglong	NA	NA	NA		10g		Pedhakapavarum	03	Room Temperature	Glass
		No.174	Avanti	NA	NA	NA		10g		Vatsavalasa	03	Room Temperature	Glass
		No.69	BMR Spectra	NA	NA	NA		10g		Dindi	03	Room Temperature	Glass
		No.188	Growell	NA	NA	NA		10g		Vatsavalasa	03	Room Temperature	Glass
		No.130	Ifeed	NA	NA	NA		10g		Bandarulanka	03	Room Temperature	Glass
		No.169	Avanti	NA	NA	NA		10g		Kupanapuda	03	Room Temperature	Glass

10. Abstract

This study focuses on developing methodologies for detecting and quantifying microplastics in various matrices. A standardized Nile red staining protocol was established for identifying microplastics, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and nylon-6. Fluorescence microscopy combined with Nile red staining at 10 µg/mL allowed rapid and cost-effective detection. Validation through FT-IR and Raman spectroscopy confirmed the accuracy of this method. This protocol was applied to tea bag leachates, where nylon-6 fragments were identified after heating at 100°C for 5 minutes.

Further efforts included developing and optimizing depolymerization methods for PET, PC, and PS, validated via FT-IR and Raman spectroscopy. Confirmatory analyses using UHPLC and direct mass spectrometry identified targeted analytes based on retention times, UV absorbance, and mass spectra. Additionally, a simultaneous detection method for these analytes was established using UHPLC.

In another approach, pyrolysis of PET followed by LC-MS/MS identified its monomers, benzoic acid, and p-terephthalic acid. At the same time, a novel LC-MS/MS method was created to analyze five bisphenols simultaneously. For biological matrices like fish tissue, a microwave-assisted extraction method was developed, significantly reducing extraction time to 30 minutes. This method, involving optimized conditions with KOH, H2O2, and methanol, achieved over 90% digestion efficiency and recovery for PP and HDPE and was extended to other plastic types, yielding recoveries above 78%.

Geographically zoned sampling strategies have been outlined for collecting and analyzing diverse matrices, including bottled water, packaged beverages, fresh and dried fish, and fish meals. This research provides a robust framework for the detection and quantification of microplastics, contributing to environmental and food safety monitoring.