



## Research article

# Primary risk assessment of microplastic pollution in spineless cuttlefish (*Sepiella inermis*) from the North-East Bay of Bengal: A tissue-based analysis

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## ABSTRACT

Microplastic pollution has a significant threat to marine ecosystems, yet its impact on spineless cuttlefish (*Sepiella inermis*) remains under-researched. This study aims to address this gap by analysing microplastic contamination in *Sepiella inermis* from the North-East Bay of Bengal. This species is widely consumed and transported globally as food, thus holding significant health concerns. A total of 40 adult female cuttlefish were collected from two sampling sites (18°36'31.35"N 87°48'10.63"E and 15°43'35.37"N 88°12'07.01"E) in the Bay of Bengal. Tissue samples from tentacles, gut, and nidamental glands were analysed for microplastic content, alongside sediment and surface water samples. Parameters such as microplastic abundance, size, shape, and colour were recorded. The average abundance of microplastic particles was measured at 2.003 particles per gram in tentacle tissue, 2.31 particles per gram in gut tissue, and 0.99 particles per gram in nidamental gland tissue. The gut tissue exhibited the highest abundance of microplastics per gram. Chemical characterization using FT-IR and confocal Raman spectroscopy identified 11 types of microplastic polymers. Of the 11 types of plastic polymers identified, PVC was the most prevalent, accounting for 17.64 % of the microplastics found across all tissues. PVC microplastics can cause significant harm to marine life and human health by accumulating in the food chain and releasing harmful chemicals like phthalates, which can lead to endocrine disruption. ABS, PET, PP, PE, and PA microplastic polymers are highly persistent in environment, leading to long-term pollution in oceans. When ingested by marine organisms, they can disrupt entire ecosystems. In humans, the accumulation of these microplastics can impair the immune system and contribute to chronic diseases. The Pollution Load Index (PLI) was calculated for each tissue type, revealing that gut tissue is more prone to microplastic pollution compared to the nidamental gland and tentacles. The average PLI per gram of gut tissue was 2.26, which was significantly higher than 1, indicating substantial pollution. This research highlights the urgent need for comprehensive strategies to mitigate microplastic pollution, given the potential health risks associated with the consumption of contaminated marine species.

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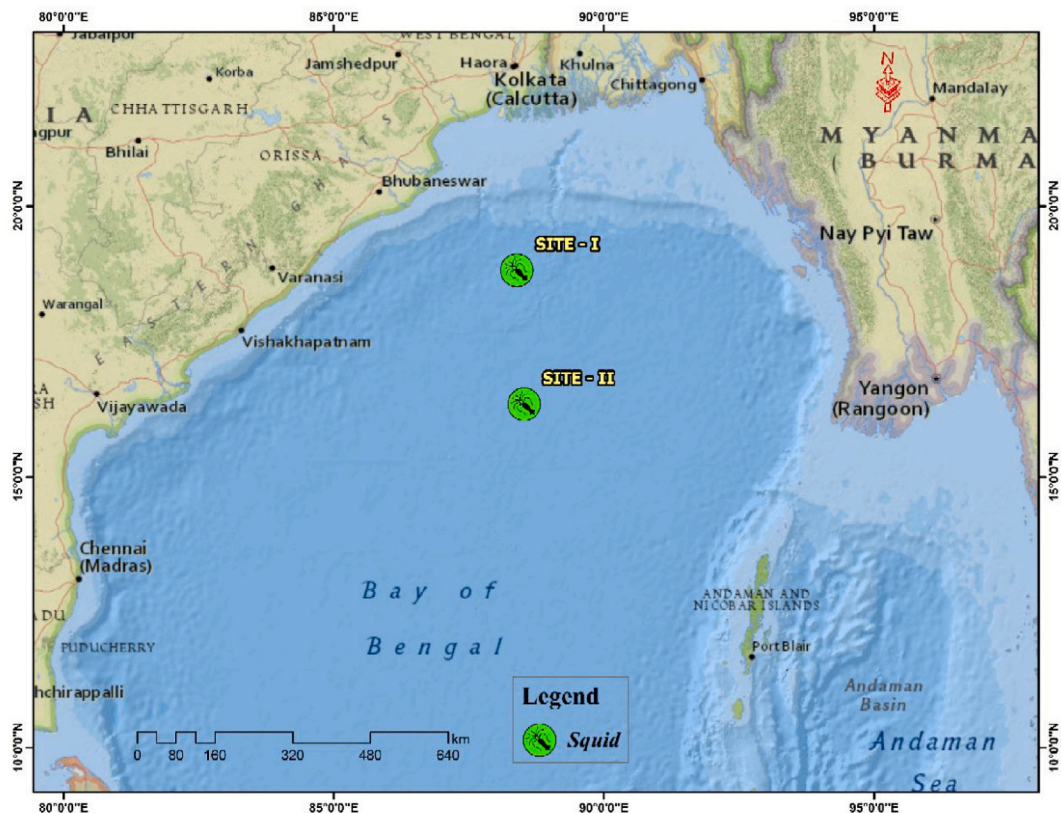
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## 1. Introduction

Due to its detrimental effects on marine ecosystems, microplastic pollution has become a major environmental problem on a worldwide scale, drawing the attention of both scientists and policymakers [10,26]. These tiny plastic particles, which are smaller than 5 mm, come from a variety of places, including industrial discharges, microbeads in personal care products, and the disintegration of bigger plastic objects [6]. The disposable face masks, especially N95 masks, release different amounts of microplastics, significantly contribute to the microplastic pollution [3]. Microplastics (MPs) can be found almost everywhere in marine ecosystems and frequently enter into the body of marine creatures, either directly or by consuming contaminated prey [30]. Research has demonstrated MP exposure across various aquatic species globally, including zooplankton [5], crustaceans [22], bivalves [35], cephalopods [9], and fish [31]. Additionally, the high hydrophobic nature of the plastic polymers helps to carry toxic chemicals like persistent organic pollutants (POPs) and heavy metals [14]. Thus, MPs are playing a major role in carrying the contaminants [18]. Because of their durability, propensity for ingestion, and capacity to absorb and transfer chemical pollutants, microplastics, despite their small size, represent serious hazards to marine organisms [32]. Microplastics that carry harmful bacteria can weaken the immune system in mussels, indicating a potential threat to both marine life and human health [24]. The adsorption of dye pollutants onto microplastics is influenced by various factors, including environmental conditions such as salinity, pH, and temperature, as well as the properties of the microplastics themselves, like particle size and surface area. For instance, polyethylene microplastics can adsorb up to 2874.4 mg/g of Cyan dye, while polystyrene microplastics exhibit the highest adsorption capacity for aniline, largely due to their surface area [13]. Tetracycline adsorption onto PVC microplastics is most effective under specific conditions, with the process being spontaneous and exothermic [34]. DZN, or diazinon, a non-systemic organophosphorus pesticide, can adsorb onto PE microplastics in a spontaneous, exothermic process. The adsorption efficiency varies with water type [33]. All these interaction may pose significant risks to human health and the environment. Because of their feeding behaviours and ecological significance, cephalopods within the different marine fauna are especially susceptible to microplastic pollution [29]. The spineless cuttlefish, or *Sepiella inermis* (Orbigny, 1848), is a commercially significant species of mollusc that inhabits in the North-East Bay of Bengal, a region that is affected by high levels of human pollution [7,25]. The magnitude and consequences of microplastic contamination in *S. inermis* populations in this region are little understood, despite its ecological and economic relevance.

Thus, the purpose of this work is to use a tissue-based method of analysis to perform a primary risk assessment of microplastic contamination in *S. inermis* from the North-East Bay of Bengal. Dissection of the specimens is followed by the collection and digestion



**Fig. 1.** Two sampling sites (site-I and site-II) of Northeast Bay of Bengal from where female adult samples of *Sepiella inermis*, sediment sample and surface water were collected.

of various tissue samples such as gut tissue, nidamental gland and tentacles, which confirms the existence and distribution of microplastics. In addition, this study aims to evaluate the consumption and accumulation of microplastic in the tissues of *S. inermis*, providing insight into the wider effects of microplastic pollution on human health and marine food webs.

## 2. Materials and methods

### 2.1. Sample collection and processing

To ensure accurate microplastic analysis in different tissue samples of *Sepiella inermis*, a standard method for sample collection and processing was employed. Twenty adult female specimens of *S. inermis* were collected from two selected sampling stations (18°36'31.35"N 87°48'10.63"E and 15°43'35.37"N 88°12'07.01"E) (Fig. 1) during the month of February (post-monsoon season) in 2024. The north-east part of the Bay of Bengal is frequently polluted by industrial waste from the Haldia industrial zone via the Haldi river [12]. The collected specimens of *S. inermis* were stored at  $-20^{\circ}\text{C}$  to preserve the integrity of the tissues. Prior to dissection, the total body weight, length, and width of each specimen were measured. The total body weights ranged from 50.51 g to 67.79 g, lengths from 7.8 cm to 10.5 cm, and widths from 3.2 cm to 5.5 cm. After specimen measurement, the gut tissue, tentacle tissues, and nidamental glands were isolated through careful dissection. The weights of the gut tissues ranged from 1 g to 2.36 g, tentacle tissues from 1.02 g to 2.1 g, and nidamental glands from 1.54 g to 4.62 g.

For the collection of environmental samples, triplicate samples of surface water and deep-sea sediments were collected from the two sampling stations. Seven liters of surface water were collected each time, ensuring a comprehensive representation of the water column. Sediments were carefully collected using grab sampler from the seabed at each station to capture any microplastic particles present. To prepare tissue samples for microplastic analysis, thorough rinsing with MilliQ water was conducted to remove any adhered particles, ensuring the accuracy of the subsequent analysis. The collected environmental samples were also processed according to established protocols to isolate and concentrate any microplastic particles present.

### 2.2. Isolation, observation and identification of MPs

Microplastic extraction, observation, and identification were conducted following established protocols with slight modifications. Tissue samples underwent extraction as per Gong et al. (2021) [11], where each type of tissue was treated with 10 % KOH (Merck) solution at a ratio of 20 mL per gram of wet tissue. The treated samples were then digested at  $70^{\circ}\text{C}$  to ensure complete breakdown. Subsequently, the digested tissue samples were vacuum filtered using Whatman GF/C (GE Healthcare Pvt. Ltd.) glass microfiber filter papers (0.1  $\mu\text{m}$  pore size, 47 mm diameter) to separate microplastics from the solution, with precautions taken to prevent airborne contamination by covering the beakers with aluminium foil.

For extraction from deep sea sediment, a modified approach based on Banik et al. (2022) [2] was employed. Dried sediment samples were treated with a 1:1 mixture of 30 %  $\text{H}_2\text{O}_2$  (Merck) and  $\text{FeSO}_4$  (Merck) solution, followed by heating at  $70^{\circ}\text{C}$  for 30 min. Density separation was achieved by adding  $\text{ZnCl}_2$  (Merck) ( $1.8\text{ g/cm}^3$ ) salt solution and allowing it to stand overnight. The supernatant was then filtered using Whatman GF/C (GE Healthcare Pvt. Ltd.) glass microfiber filter papers (0.1  $\mu\text{m}$  pore size, 47 mm diameter).

Surface water samples were processed according to the NOAA (2015) protocol [16]. Seawater samples were sieved through a 45  $\mu\text{m}$  sieve, followed by careful washing with MilliQ water. The washed materials were treated with a 1:1 mixture of 30 %  $\text{H}_2\text{O}_2$  (Merck) and  $\text{FeSO}_4$  (Merck) solution, heated at  $70^{\circ}\text{C}$  to evaporate excess water, and then mixed with 5M NaCl solution for density separation. After overnight settling, the supernatant was filtered using Whatman GF/C (GE Healthcare Pvt. Ltd.) glass microfiber filter papers (0.1  $\mu\text{m}$  pore size, 47 mm diameter).

Throughout the extraction processes, precautions were taken to prevent contamination, including the use of aluminium foil to cover samples. Finally, all filter papers containing extracted microplastics were placed into clean petri dishes for further analysis.

To observe and identify microplastics (MPs) effectively, a comprehensive method combining visual observation, image analysis, and spectroscopic techniques was employed. Initially, the size, shape, and colour of microplastics present on filter paper were examined using a stereomicroscope (MSZ-TR trinocular, Magnus). The size measurements were precisely recorded using Magvision image analysis software. The images of the microplastics were captured using a digital camera (MagCam DC-5, Magnus) attached to the stereomicroscope. Subsequently, visual analysis was conducted to identify the shape and colour of the microplastics based on their physical characteristics. This step helps in categorizing the microplastics into different groups according to their visual attributes, aiding in subsequent identification processes.

To confirm the chemical composition of the microplastics, Fourier Transform Infrared Spectroscopy in Attenuated Total Reflection mode (FTIR-ATR) (Bruker-Alpha, Bruker) and Confocal Raman Microscopy (LabRAM HR Evolution, HORIBA Scientific) were employed. Spectra were collected over a broad spectral range ( $500\text{--}4000\text{ cm}^{-1}$ ) using both techniques. These spectra were then compared with standard spectral libraries dedicated to plastic polymers for identification purposes. In the analysis, particular attention is paid to achieve a confidence level of at least 70 % match or reliable spectral match through visual inspection. This ensures robust identification of the plastic polymer composition present in the microplastics.

### 2.3. Quality control

During sample processing, several key steps were followed to minimize both internal and external sources of contamination [21]. Firstly, all chemical solutions utilized for microplastic extraction underwent filtration through glass microfiber filter paper (0.1  $\mu\text{m}$

pore size 47 mm diameter, Whatman GF/C). This step was critical for removing any potential contaminants present in the chemicals themselves, ensuring that the solutions used are free from microplastic particles. Moreover, the Milli-Q water, a common solvent in laboratory procedures, is also filtered using the same glass microfiber filter paper. This ensures that the water used in the extraction process is pristine and devoid of any microplastic contamination. Equipment used in sample processing underwent thorough rinsing with Milli-Q water two to three times. The petri dishes utilized for sample transfer were washed with Milli-Q water prior to transferring the filter paper into them. Throughout the sample processing, aluminium foil was utilized to cover the samples. This protective measure shielded the samples from potential external sources of contamination, such as airborne microplastic particles or dust, thus maintaining the integrity of the samples.

Furthermore, a blank sample was taken during each step of the processing as a negative control [21]. This serves as a baseline measurement to accurately assess the abundance of microplastics in the respective samples, allowing for the differentiation between genuine microplastic particles and any potential contaminants introduced during the processing steps. Only a minimal number of microplastics were detected in the blank samples, which served as negative controls. The average microplastic contamination in the blank samples was 0.3, and this value was subtracted from the results of our target samples.

#### 2.4. Risk assessment

To conduct a risk assessment of microplastic pollution in *Sepiella inermis*, the Pollution Load Index (PLI) methodology was utilized [27]. This approach allows to systematically evaluate the potential risk posed by microplastic contamination in different organs of this species, utilizing available data on microplastic abundance [30].

The Pollution Load Index (PLI) for each individual  $i$  was calculated using the formula:

$$CF_i = C_i / C_0$$

$$PLI_i = \sqrt{CF_i}$$

where  $C_i$  represents the microplastic abundance for individual  $i$ , and  $C_0$  is defined as the baseline microplastic abundance. In this case, due to the absence of a universally recognized reference value for microplastic pollution in *Sepiella inermis*,  $C_0$  was set to the lowest observed microplastic abundance value in this species [30].

It's important to note that while variations in the selected constant  $C_0$  may influence the absolute values of PLI, they do not affect the relative relationships among individuals. Therefore, our comparison results remain valid, enabling us to assess and compare the risk of microplastic pollution across different individuals of *Sepiella inermis*.

#### 2.5. Statistical analysis

A robust statistical analysis was conducted to evaluate the abundance of microplastics across various tissue samples of *Sepiella inermis*, as well as in deep sea sediment and surface water. The study encompassed three distinct tissue types: gut tissue, tentacle tissue, and nidamental gland. Due to the non-normal distribution and unequal variances among the samples, a Kruskal-Wallis test was employed for comparison. This non-parametric test is well-suited for situations where standard parametric assumptions are not met. Results were considered statistically significant if the p-value was less than 0.05, indicating substantial differences in microplastic abundance among the tissue types. The data were presented as mean values accompanied by their respective standard deviations (SD), providing a comprehensive overview of the variability within each tissue sample.

### 3. Result and discussion

The spineless cuttlefish, *Sepiella inermis*, is a valuable species in the Indian Ocean known for its economic importance. Unlike other cuttlefish species like *Sepia*, *S. inermis* exhibits a benthonektonic habit, remaining active while tolerating environmental fluctuations well [17]. It is found in numerous locations such as the Andaman Islands, Ceylon, Burma, Malaysia, Indonesia, Vietnam, the Red Sea, South Arabia, and the east and west coasts of India [28]. There is no work done till now on microplastic pollution in *Sepiella inermis*. As it is widely consumed as delicious food throughout the world [1], microplastic pollution holds significant concern for human health. Specially, the nidamental gland is known for its delicious taste and commonly consumed as part of this cuttlefish [20]. So, this is chosen as one of the target tissues for microplastic analysis. In this investigation, 20 female individuals of *S. inermis* were selected to test the hypothesis regarding the variability of microplastic contamination across different tissues. A notable variance in microplastic levels per gram of tissue sample was found among three distinct tissues (Kruskal-Wallis test,  $\chi^2 = 18.19$ ,  $p = 0.00011$ ), indicating significant differences.

#### 3.1. Microplastic abundance

Analysis revealed varying concentrations across tentacle, gut, and nidamental gland tissues, with tentacle and gut tissues exhibiting significantly higher levels compared to the nidamental gland (Fig. 2) (Kruskal-Wallis test,  $p < 0.05$ ). Specifically, the average abundance was recorded at 2.003 microplastic particles per gram for tentacle tissue, 2.31 microplastic particles per gram for gut tissue, and 0.99 microplastic particles per gram for nidamental gland tissue (Fig. 2). This discrepancy suggests a differential affinity for



microplastic uptake among the tissues, likely influenced by their respective functions and exposure routes. Moreover, comparison with sediment and surface water samples from the deep sea further emphasizes the widespread distribution of microplastics, with approximately 22 particles per 20 g of sediment and an average of 17.5 particles per 7 L of surface water.

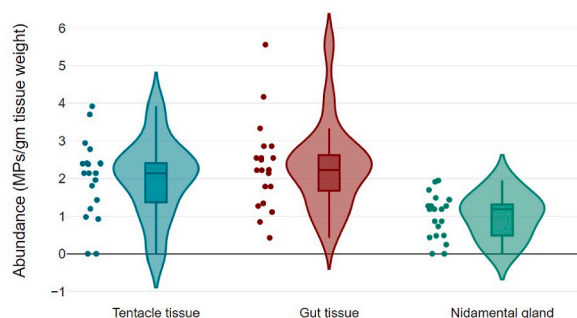
The disparity in microplastic abundance observed among the tissues may be attributed to several factors. Tentacles and gut tissues, being directly exposed to the external environment and involved in food intake and processing, are more prone to encountering and ingesting microplastic particles present in the surrounding water and prey items [8]. In contrast, the lower abundance in the nidamental gland, responsible for egg production, may reflect a reduced exposure pathway or a physiological barrier limiting microplastic uptake.

### 3.2. Size, shape and colour of microplastics

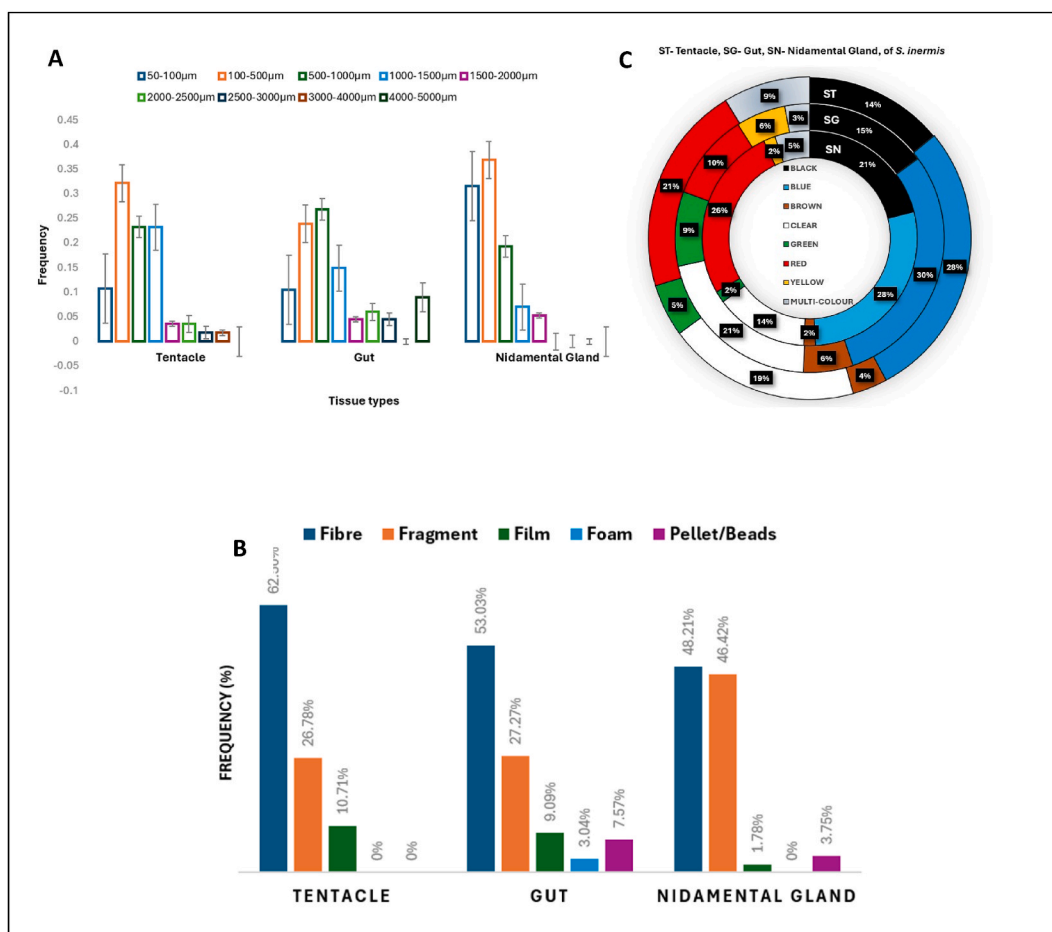
During the examination of microplastic size distribution within different tissues of *Sepiella inermis*, Microplastics were categorized into nine size ranges, ranging from 50–100  $\mu\text{m}$  to 4000–5000  $\mu\text{m}$  (Fig. 3A), and their abundance within tentacle, gut, and nidamental gland tissues was assessed. In 20 samples of tentacle tissue, the highest number of microplastics fall within the 100–500  $\mu\text{m}$  size range, followed by the 500–1500  $\mu\text{m}$  range, with the 50–100  $\mu\text{m}$  range ranking third (Fig. 3A). The least abundant range was observed for microplastics sized between 1500 and 4000  $\mu\text{m}$ . Conversely, in 20 samples of gut tissue, the majority of microplastics were found within the 500–1000  $\mu\text{m}$  range, with the 100–500  $\mu\text{m}$  range ranking second, and the 1000–1500  $\mu\text{m}$  range ranking third (Fig. 3A). A few numbers of tangled microplastic fibres were found in the gut. Due to their tangled structure, the overall size of the fibres ranged between 4000 and 5000  $\mu\text{m}$ . After untangling them with sharp needles, the fibres extended beyond 5000  $\mu\text{m}$ . However, because the fibres were originally tangled when discovered in the gut, they were classified as microplastics in the study. (Fig. 3A), although the least abundant size range was observed for microplastics sized between 1500–2000  $\mu\text{m}$  and 2500–3000  $\mu\text{m}$ . Within the 20 samples of nidamental gland tissue, microplastics primarily fall within the 100–500  $\mu\text{m}$  range, with the 50–100  $\mu\text{m}$  range ranking second (Fig. 3A). The third most abundant range was observed for microplastics sized between 500 and 1000  $\mu\text{m}$ , while the least abundant range was between 1000 and 2000  $\mu\text{m}$  (Fig. 3A). Despite the variation in size distribution among tissues, the average size of microplastics ranged from 50 to 1500  $\mu\text{m}$  across all tissue types (Fig. 3A). Statistical analysis using the Kruskal-Wallis test revealed no significant difference in the size distribution of microplastics among the three tissue samples ( $\chi^2 = 1.169$ ,  $p = 0.55$ ), indicating that the observed differences in size distribution were not statistically significant. In the sediment samples, an average of 63.63 % of microplastics within a 20-g sample fall within the size range of 100–500  $\mu\text{m}$ . Notably, larger microplastics were observed to be lost during the density separation process from sediment samples. Conversely, in surface water samples, 62.85 % of microplastics ranged from 100 to 1000  $\mu\text{m}$ , with 14.28 % falling within the 1000–2000  $\mu\text{m}$  range. Interestingly, only a small fraction (2.85 %) of tangled microplastics having 4000–5000  $\mu\text{m}$  size were observed in surface water, suggesting a predominance of smaller particles in this environment.

Shape analysis identified five main categories of microplastics: fibre (Fig. 4C and D), fragment, film (Fig. 4A), foam, and pellet or bead (Fig. 4B). Within tentacle tissue, fibres were the most prevalent microplastic type, with some fragments also present, while films were notably less abundant (Fig. 3B). Similarly, in gut tissue, fibres dominated, followed by significant amounts of fragments, and a minimal presence of films (Fig. 3B). Nidamental gland tissue also exhibited high proportions of fibres and fragments. In 20 samples of tentacle tissue the percentages of fibres, fragments and films were 62.5 %, 26.78 %, 10.71 % respectively. In 20 samples of gut tissue, the percentage of fibres, fragments, films, foams and pellets or beads were 53.03 %, 27.27 %, 9.09 %, 3.04 % and 7.57 % respectively. In 20 samples of nidamental gland, the percentage of fibres, fragments, films, and pellets or beads were 48.21 %, 46.42 %, 1.78 % and 3.75 % respectively. Foam was only detected in trace amounts within gut tissue (Fig. 3B). In deep sea surface water, a striking 85.71 % of microplastics were identified as fibres, indicating a prevalence of fibrous materials in this environment. In contrast, deep sea sediment samples exhibited a different composition, with 54.16 % of microplastics classified as fibres, 20.83 % as fragments, and 25 % as beads, suggesting a more diverse array of microplastic types within sedimentary deposits.

Eight colours of microplastics were observed: black, blue, brown, clear, green, red, yellow, and multi-colour (Fig. 3C). Blue emerged as the predominant colour across all tissue types (Fig. 3C), indicating a potentially common source or preferential degradation pathway for blue-coloured plastics. In tentacle, gut, and nidamental gland tissues, black microplastics comprised 14.03 %, 14.03 %, and 14.03 % respectively.



**Fig. 2.** Violin plot represents the abundance of microplastics per gram tissue samples (tentacle, gut and nidamental gland) of *Sepiella inermis*. The violin plot includes a standard box plot with minimum, interquartile range, mean, maximum and standard deviation values.



**Fig. 3.** (A) represents the frequency of different sized microplastics (50 µm–5000µm) occurred in tentacle, gut and nidamental gland of *Sepiella inermis*. (B) Represents different shapes of microplastics found in three different tissues of *S. inermis*. (C) Represents different colours of microplastics found in three different tissues of *S. inermis*.

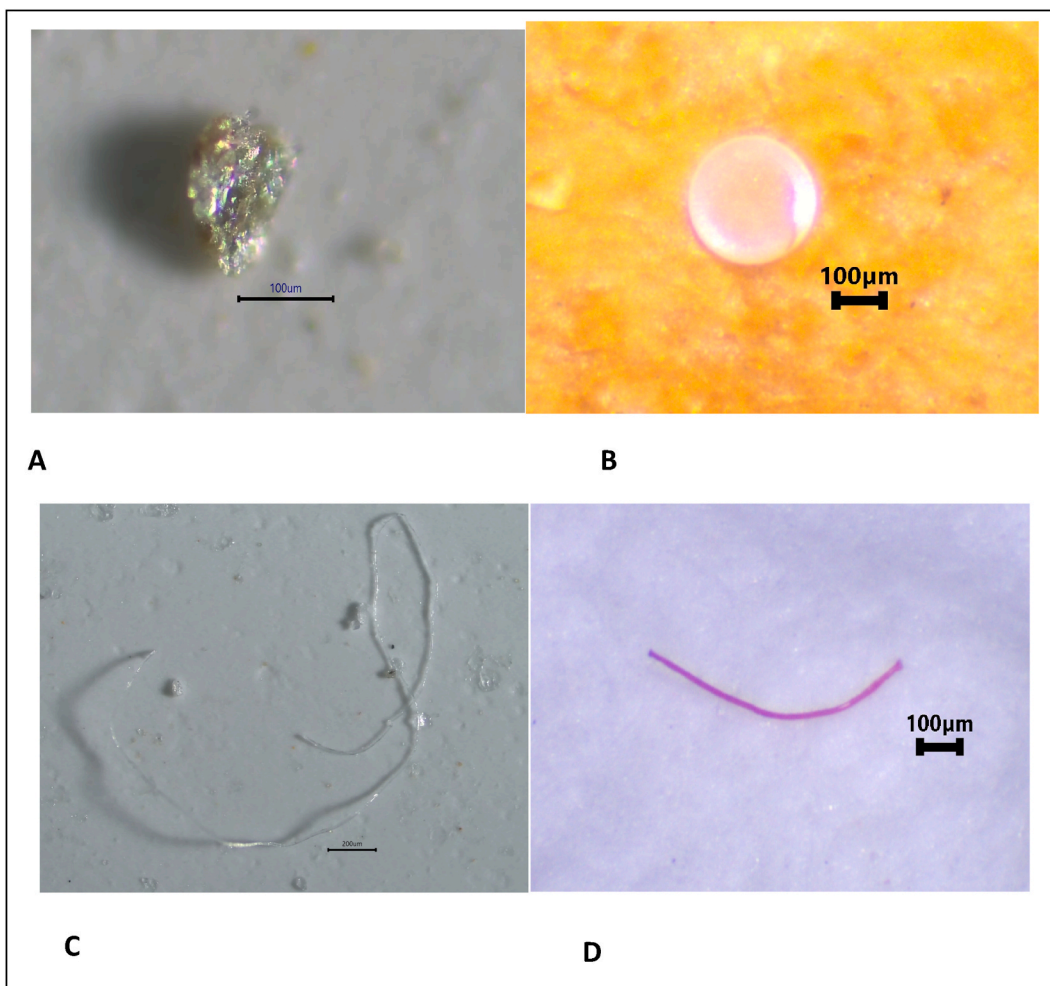
14.92 %, and 21.05 %, respectively, while blue microplastics accounted for 28.07 %, 29.85 %, and 28.07 %, respectively (Fig. 3C). Brown, clear, and red microplastics showed varying proportions across tissues, suggesting differences in sources or degradation processes. Multi-coloured microplastics were also present, though in lower percentages compared to single-coloured counterparts. In surface water, the majority of microplastics were observed to be blue, followed by clear and red. Conversely, in sediment samples, blue microplastics were predominant, followed by clear and brown varieties. These differences in colour distribution may reflect varying sources and degradation processes influencing microplastic composition within different marine environments [4].

### 3.3. Chemical nature of microplastics

Chemical characterization of microplastic samples (Fig. B–E) from *Sepiella inermis* tissue, deep sea sediment, and surface water from two different sampling stations was performed using FTIR-ATR (Fig. B) and confocal Raman spectroscopy (Fig. C, D, E), revealing the presence of 11 distinct plastic polymers. These polymers included acrylonitrile butadiene styrene (ABS), polyvinyl chloride (PVC), polyester (PES) (Fig. C), polyethylene terephthalate (PET), polypropylene (PP) (Fig. B), polyurea (PU), polyethylene (PE), polyamide (PA), polystyrene (PS), polytetrafluoroethylene (PTFE), and polycarbonate (PC) (Fig. 5A).

In tissue samples, PVC was the most prevalent polymer, constituting 17.64 % of the microplastics. ABS, PET, PP, PE, and PA each had a frequency of 11.76 %. In sediment samples, PVC also dominated with a frequency of 18.18 %, followed by PE at 15.9 %. ABS and PS each had a frequency of 13.63 %, while PET, PP, and PC were present at frequencies of 11.36 %, 6.81 %, and 2.27 %, respectively. PA and PTFE each accounted for 9.09 % of the sediment microplastics (Fig. 5A). In the marine environment, fishing nets are one of the largest sources of microplastics. These nets, often used in ghost fishing, release microplastics that are highly detrimental to both the environment and human health [15]. Additionally, microplastics from industrial waste, particularly those containing large amounts of PVC, pose severe ecotoxicological risks [19].

Surface water samples showed a different distribution, with PP being the most common polymer at 20.58 %, followed by PE at



**Fig. 4.** (A) represents Film, (B) represents pellet, (C) represents transparent (clear) fibre and (D) represents red fibre found in different tissues and sediment samples.

17.64 % and ABS at 14.7 %. PET and PS were both found at a frequency of 11.76 %, while PU and PA were less common, each with a frequency of 8.82 %. PVC was the least frequent, at 5.88 % (Fig. 5A).

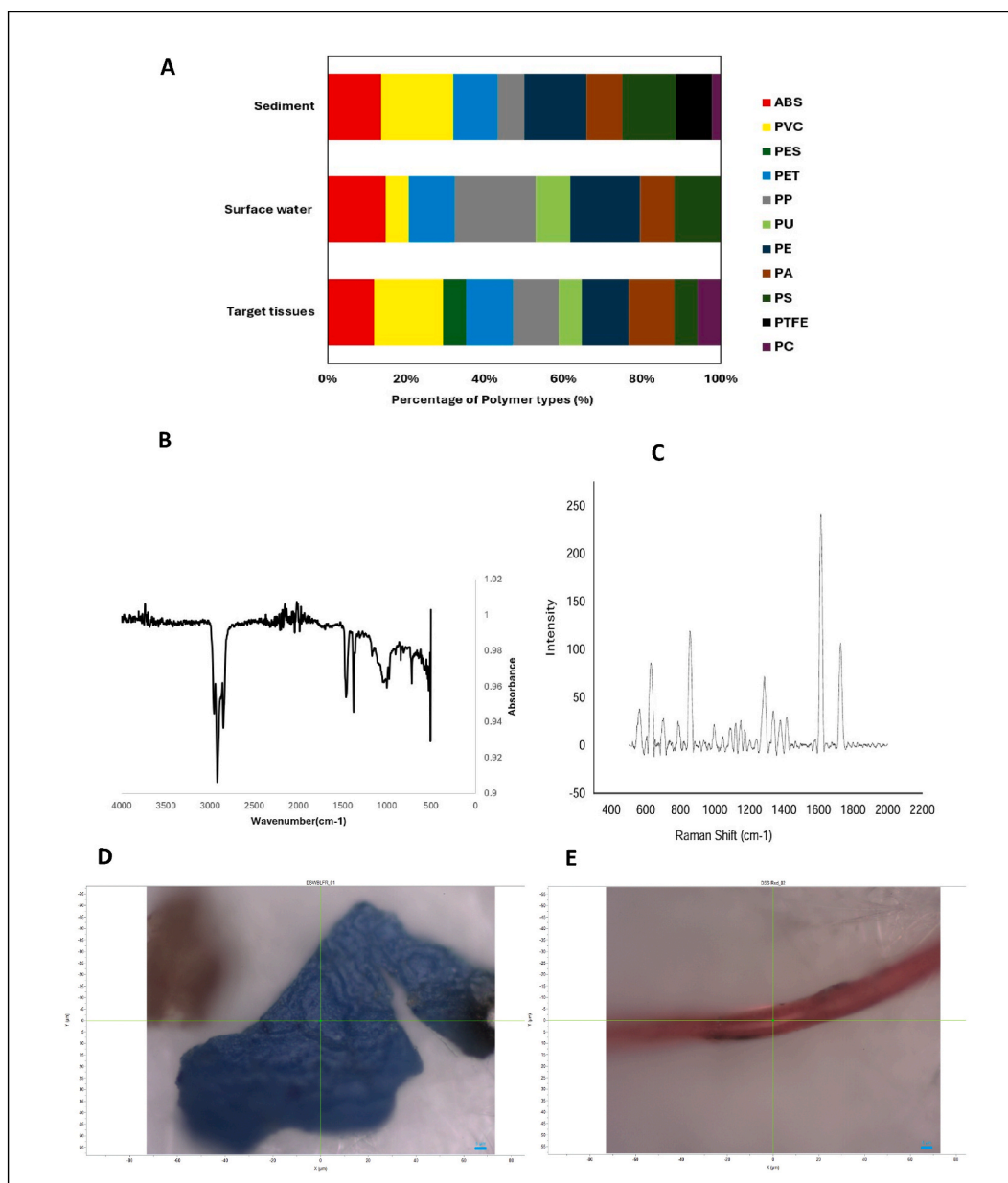
### 3.4. Risk assessment

The primary risk assessment of microplastics in *Sepiella inermis* was conducted by calculating the Pollution Load Index (PLI) for tentacle, gut, and nidamental gland tissues. The PLI was determined using the microplastic abundance per gram of tissue, with the lowest abundance taken as the baseline for calculation, as per the methodology outlined by Wang and Chen (2023) [30]. The results indicated an average PLI of 1.37 per gram for tentacle tissue, 2.26 per gram for gut tissue, and 1.86 per gram for nidamental gland tissue (Fig. 6).

The Kruskal-Wallis test was employed to assess whether there were significant differences in PLI among the three tissue types. The test yielded a p-value of 0.0001 and a chi-square statistic ( $\chi^2$ ) of 18.37, indicating a significant difference in PLI between the tissues. This significant difference suggests that the gut tissue accumulates microplastics at a higher rate than the tentacle and nidamental gland tissues. The higher PLI observed in the gut tissue (2.26) compared to the tentacle (1.37) and nidamental gland tissues (1.86) can be attributed to the gut's direct involvement in ingestion and processing of food, which may contain microplastics. A PLI greater than 1 indicates significant pollution. In the Bay of Bengal, various fish species were found to have a PLI greater than 1 [23]. Similarly, in this study, the PLI for different tissue types increased in the following order: tentacle, nidamental gland, and gut.

## 4. Conclusion

The presence of microplastics in various tissues of *Sepiella inermis* indicates the high-level of pollution in the North-East Bay of



**Fig. 5.** (A) represents the different types of microplastic polymers found in tissue samples, sediment sample and surface water sample. (B) Represents FT-IR spectra of Polypropylene (PP). (C) Represents spectra of Polyester (PES) using confocal Raman spectroscopy. (D)&(E) represent microplastic fragment and fibre under confocal Raman microscopy.

Bengal. On the other hand, higher PLI values ( $>1$ ) per gram in different tissues of *Sepiella inermis* are showing alarming level of pollution. The elevated levels of PVC microplastic particles found in this study suggest that industrial waste and fishing vessels may be significant contributors to microplastic pollution in the marine environment. PE, PET, and PA microplastics were also found in significant amounts in this study. These particles are major components of fishing nets, which frequently end up as plastic debris in the marine environment. The study found a significant number of blue microplastics, which can blend into the blue ocean environment and be accidentally ingested by marine organisms through water intake. The outcome of this study showcases the potential threat to marine ecosystem as well as human health. As this species is widely consumed in various part of the globe, this study holds significant health concerns. The high pollution in gut tissue represents the direct exposure of marine environment through digestive system. The presence of microplastic in nidamental gland can be the alarming sign for potential reproductive complications. The microplastic pollution is harmful for ecological and economic wellbeing of coast and coastal people respectively. Several necessary steps should be taken by policy makers to reduce the microplastic abundance in marine environment.



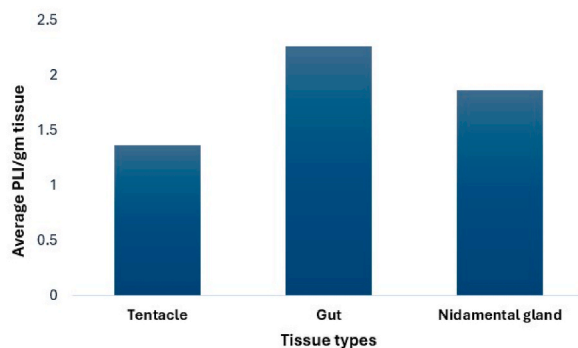


Fig. 6. The average Pollution Load Index in three different tissues (tentacle, gut, nidamental gland) of *Sepiella inermis*.

## Declaration

During the preparation of this work the author(s) used ChatGPT in order to arrange the sentences properly in the manuscript. After using ChatGPT, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

## Consent to publish

All the authors consented on the publication of any material or information used in this manuscript.

## Data availability statement

Supplementary data related to this article can be found, in the Mendeley Data, at <https://doi.org/10.17632/z47bhdhm2p.1>.

## CRediT authorship contribution statement

**Sourav Bar:** Writing – original draft, Methodology, Investigation, Conceptualization. **Soumik Dhara:** Visualization. **Jhumpa Majhi:** Conceptualization. **Dipak Bisai:** Software. **Edris Alam:** Funding acquisition. **Md Kamrul Islam:** Funding acquisition. **Uday Chatterjee:** Software. **Sudipta Kumar Ghorai:** Writing – review & editing, Supervision, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: SOURAV BAR reports financial support was provided by University Grants Commission. SOURAV BAR reports a relationship with University Grants Commission that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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