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The impact of chronic and acute problems on sea turtles: The consequences of the oil spill and ingestion of anthropogenic debris on the tropical semi-arid coast of Ceará, Brazil

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ABSTRACT

Sea turtle mortality is often related to materials that reach the coast from different anthropic activities worldwide. This study aimed to investigate whether sea turtle mortality was related to older marine problems, such as solid waste, or one of the largest oil spill accidents on the Brazilian coast, that occurred in 2019. We posed three questions: 1) Are there solid residues in the digestive tract samples, and which typology is the most abundant? 2) Can meso- and macro-waste marine pollutants cause mortality? 3) Is the dark material found really oil? A total of 25 gastrointestinal content (GC) samples were obtained, of which 22 ingested waste of anthropogenic origin and 18 were necropsied. These 22 samples were obtained during or after the 2019 oil spill, of which 17 specimens were affected, making it possible to suggest oil ingestion with the cause of death in the animals that could be necropsied. Macroscopic data showed that the most abundant solid waste was plastic (76.05 %), followed by fabrics (12.18 %) and oil-like materials. However, chemical data confirmed only three specimens with oil levels ranging from remnants to high. It was possible to infer possible causes of death in 16 of the total 18 necropsied cases: Most deaths were due to respiratory arrest (62.5 %), followed by pulmonary edema (12.5 %), cachexia syndrome (12.5 %), circulatory shock (6.25 %), and head trauma (6.25 %), which may have been caused by contact with solid waste, oil, or both. The study showed that not all dark material found in the GCs of turtles killed in oiled areas is truly oil, and in this sense, a chemical analysis step to prove the evidence of oil must be added to international protocols.

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1. Introduction

Ingestion and entanglement of debris, mainly plastics, have been reported in a variety of organisms, from microscopic zooplankton to large whales (Kühn, 2015). Cases of plastic ingestion in marine environments have been reported in more than 700 species (Gall and Thompson, 2015), and the number of occurrences is constantly increasing. Many sea turtles cannot differentiate between litter and food (Shuyler et al., 2014; Matiddi et al., 2017), and their complex life cycle (e.g., late sexual maturity, reproductive behavior, and cosmopolitan feeding) makes them particularly vulnerable to marine pollution (Shuyler et al., 2014), especially contamination by microplastics, rendering this taxon as the most affected by this type of waste (Ugwu et al., 2021; Rodríguez et al., 2022). The direct consequences of ingestion can range from internal injuries, intestinal blockage, and interference with swimming behavior and buoyancy to the accumulation of plasticizers (Nelms et al., 2016), whereas indirect consequences involve the ingestion of waste contaminated with heavy metals and/or various toxins, as well as diverse persistent organic pollutants (POPs) which can cause various problems for marine life (e.g., chemical intoxication, decreased immunity) (Nelms et al., 2016).

Despite the impact of old and chronic problems, such as solid waste, in August 2019, more than 3000 km of the Brazilian coast was affected by one of the largest oil spills on the South American coast and in tropical areas (Soares et al., 2020; 2022; Reddy et al., 2022). The oil reached beaches, mangroves, reefs, and seagrass beds, compromising many marine and coastal ecosystems (Soares et al., 2020). In this event, hundreds of animals were affected, and green turtle (*Chelonia mydas)*, hawksbill turtle (*Eretmochelys imbricata)*, and olive ridley (*Lepidochelys olivacea)* were the most affected species of sea turtles found oiled (Soares et al., 2020; Disner and Torres, 2020).

Primary contact with oil is dangerous for sea turtles, and their nostrils can be easily blocked or covered by oil, resulting in continuous exposure and inhalation of gases present in the oil, thereby causing respiratory illness and reduced breathing of oxygen (Camacho et al., 2013). In addition, this contact with oil can cause inflammation, increase of skin temperature, chemical burns, skin peeling, necrotizing dermatitis, and epidermal dysplasia (Lutcavage et al., 1995; Camacho et al., 2013; Manire et al., 2017; Otten et al., 2022). When ingested, oil reduces the assimilation of food, obstructs the gastrointestinal tract (GIT), and severely affects the tissue of the GIT, causing diseases such as tubulonephrosis, necrotizing gastritis, necrotizing hepatitis, and necrotizing enteritis. In addition, positive buoyancy disorders are caused by intestinal blockage and gas accumulation due to fermentation in the GIT (Milton and Lutz, 2010; Manire et al., 2017). According to Ruberg et al. (2021), after the *Deepwater Horizon* (DWH) oil spill, several sea turtles were covered in oil, compromising their mobility and thermoregulation capacity. Upon rescue, these turtles were lethargic and their body temperatures were higher than normal; heavily coated individuals would have died if they had not been rescued.

Until now, there have been no studies on the coast of South America focusing on chronic problems, such as plastic waste, combined with recent problems, such as the oil spill in 2019 off the Brazilian coast. In this sense, it is essential to assess the threats to sea turtles caused by oil spillage in recent years to prove the veracity of oil ingestion. Therefore, the present study posed the following questions: (1) Are there solid residues in the gastrointestinal content (GC) samples of sea turtles, and what is the most abundant typology? (2) Can meso- and macro-waste pollutants cause mortality? (3) Is the dark material found really oil?

These data will certainly contribute to verifying the impacts on turtle mortality arising from the synergism of chronic problems with acute ones. In addition, this is the first study on oiled turtles in the region of the largest oil accident in South America, and this will certainly contribute to the assessment of environmental impacts.

2. Material and methods

2.1. Study area

The research was carried out along the coast of the state of Ceará (Fig. 1), which is 573 km long (Borzacchiello et al., 2007). In this region, there is a predominance of extensive sandy beaches intersected by mangroves and rocky outcrops positioned on the edges of dune fields and sedimentary cliffs (Borzacchiello et al., 2007). Ceará has a hot, semi-arid climate with large variations in rainfall between years. Two seasons are typically observed: a rainy season from December to May, and a dry season from June to November (INESP 2009). It is noteworthy that during the dry season and years, there is increased wind intensity of the winds, which significantly affects coastal and marine ecosystems (Santos and Manzi 2010).

Notably, the coast of Ceará is an important area in the South Atlantic for the feeding and development of sea turtle species, such as *C. mydas* (Chambault et al., 2015; ICMBIO, 2018). Moreover, the intense natural dynamics of the coastal zone of the state, especially regarding the flow of matter from the continent to the ocean, have been altered by the growth and densification of human populations (Soares et al., 2021), threatening the stability of ecosystems. In addition, the northeast region of Brazil is the second largest producer of waste, with Ceará being the second largest producer in this region (Abrelpe, 2020).

2.2. Data collection and sampling

The material analyzed was collected from February 2019 to August 2020, from turtles that were stranded dead on beaches along the beach strip of the State of Ceará or that died before being able to mobilize their rescue and rehabilitation (**Table A1**, Supplementary material). All occurrences were reported to the GTAR-Verdeluz project team (Instituto Verdeluz) by bathers, visitors, and workers on beaches where each animal ran aground.

Individuals were weighed to the nearest 0.01 kg, submitted to biometrics with millimeter graduation [curvilinear carapace length (CCL) and curvilinear carapace width (CCW)], identified to the species level, place of stranding (with and GPS, in decimal degree) and classified according to estimated age (young, adult, and indeterminate), body score (C1. cachectic, C2. thin, and C3. ideal), and sex (male, female, and indeterminate), following the protocols and classifications proposed by Wyneken (2001) and Santos and Manzi (2011). Other external observations were recorded (e.g., the occurrence of barnacles, tumors, and abrasions). If specimens were freshly dead, they were necropsied, following the method of Wyneken (2001), and the cause of death was identified.

After the removal of the GCs, ties were placed between the esophagus, stomach, intestine, and rectum, and each GIT sample was refrigerated (< 10 °C) until further analysis. The contents of each section of the GIT were filtered through 0.5 mm mesh sieves using clean running water and screened with the naked eye or a stereoscopic microscope. All debris founded on the GCs was allowed to dry in the open air, weighed on a scale to the nearest 0.001 g, and its volume was measured by the displacement of the water column (Motta-Junior et al., 1994) using graduated cylinders ranging between 10 and 1000 ml. Following the proposal by the United Nations Environment Programme (UNEP) in "Guidelines on marine litter monitoring" (Cheshire et al., 2009), each item was classified with respect to its base material as follows: (1) plastic, (2) fabric, (3) rubber, and (4) material suggestive of petroleum. Each item was also classified by color (e.g., colored, transparent, black, white), and their lengths were measured in centimeters. Items smaller than 20 mm were classified as "meso-waste" (Fendall and Sewell, 2009; Betts, 2008; Moore, 2008) and above 20 mm as "macro-waste" (Lippiatt et al., 2013).

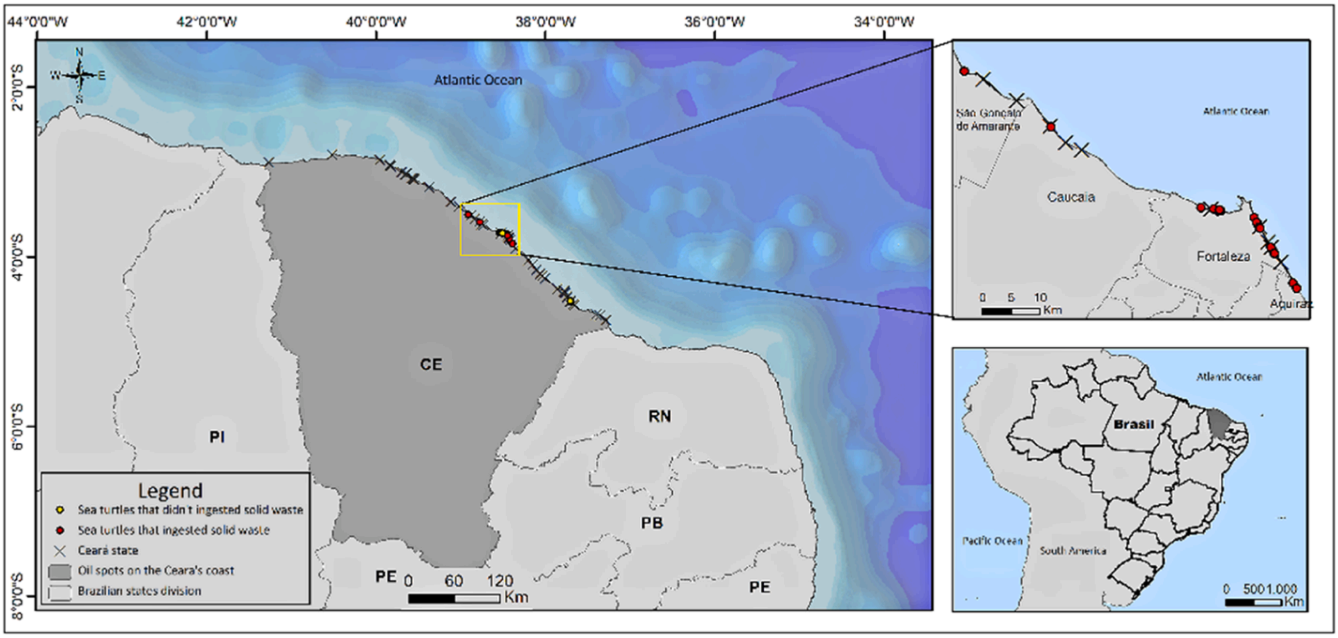

Fig. 1. Sampling area of sea turtles along the coast of Ceará from February 2019 to September 2021.

2.3. Analysis of petroleum hydrocarbons

This methodology can be seen in detail in recent studies involving the extraction of petroleum hydrocarbons from oiled materials that arrived on the Brazilian coast due to the oil spills (Carregosa et al., 2021; Azevedo et al., 2022). Briefly, around 0.4 g of lyophilized samples were extracted by stirring in 6 mL of dichloromethane for 3 min, followed by sonication for 10 min. The samples were then centrifuged for 5 min at 3000 rpm, followed by funnel filtration using glass wool. The extraction step was repeated four more times to ensure maximum extraction of organic compounds. The extract was concentrated to 1 mL and placed in a freezer until further fractionation.

The extract (5–40 mg) was fractionated on a silica gel 60 column (12 g of silica in *n*-hexane solution; 70–230 mesh; Merck) with a layer of anhydrous sodium sulfate (0.5 g). Then, 30 mL of *n*-hexane was used to elute the saturated fraction, which was then dried using nitrogen flow, weighed, and placed in a freezer until further analysis using GC-FID (modified from Martins et al., 2014).

Petroleum hydrocarbons were identified and quantified using a gas chromatography with Flame Ionization Detector (GC-FID, Shimadzu). The injector temperature was 260 °C, and the injection volume was 2 μL in splitless mode. Separation was performed using a capillary column DB-5 Agilent J&W (5 % diphenyl-95 % dimethyl polysiloxane) of 30 $m \times$ 0.25 mm i.d. and 0.25 μm film. All chromatographic conditions and details for determination can be found in Azevedo et al. (2022).

All data were subjected to rigorous quality control procedures. Analysis of the reagent blanks demonstrated that the analytical system used (glassware, solvents, and materials) was free of contamination. The identity and quantity of the analytes of interest were confirmed by chromatographic validation using the Laboratory Guide to Method Validation and Related Topics (www.eurachem.org), and all figures of analytical merit are provided in **Table A2** (Supplementary material).

2.4. Data analysis

To analyze the data on the occurrence of solid waste in the GIT, their frequencies were calculated in the different compartments. The abundance of each residue relative to the total content, obtained by the number of pieces ingested, classified by the type of material, color, and gastrointestinal tract registration sector (esophagus, stomach or intestine) were analyzed.

The relative importance index (RII) was applied (adapted from Kawakami and Vazzoler, 1980), which considered the item weight as well as its frequency of occurrence using the following equation:

$$< \mathbf{RII} = \mathbf{FixPi} \Big/ \sum \mathbf{ni} = \mathbf{1} \Big(\mathbf{FixPi} \Big) >$$

where RII = relative importance index; $i = 1, 2, \ldots$; $n =$ certain food item; Fi = Frequency of occurrence (%) of the given item; Pi = weight (%) of the given item.

3. Results

3.1. Ingestion of solid waste

Of the 25 turtles collected, 22 (88 %) ingested waste of anthropogenic origin, thus the sample size considered in this section is 22 specimens. Of these, 14 were juveniles (seven females and seven of undetermined sex) and eight were adults (seven females and one male). All specimens of L. *olivacea* ($n = 2$; CCC = 59 and 63 cm; weight = 17.85 and 24 kg) and *E. imbricata* ($n = 3$; CCC = 41–98 cm; weight = 21.85–82.40 kg) and 75 % of *C. mydas* ($n = 16$; CCC = 32–129.4 cm; weight = 3–80 kg) ingested residues of anthropogenic origin. Additionally, 20 individuals were collected during and after the oil spill, three specimens were found externally oiled, and another fourteen had internal characteristics of interaction with oil.

A total of 238 items of anthropogenic origin were recorded, with each ingesting from 0 to 81 items (**Fig. B1**, Supplementary material). Each specimen ingested an average of 4276 g (standard deviation 27.67) of waste. An ingestion of more than 15 items was recorded in three specimens (12 %), while another 4 (16 %) ingested more than 30 g. Considering the location of the residues, the highest number was observed in the intestinal compartment (294.425 g; $n = 211$), followed by the esophagus (12.582 g; $n = 20$) and stomach (4.942 g; $n = 8$).

Items of anthropogenic origin were classified into 16 types, which were grouped into five categories according to the nature of the material (plastic, fabric, material suggestive of petroleum, hair and rubber) (Table 1). Of these, three stood out for their origin or type of material rarely found in other works: silica, a plastic fragment with Korean writing, and cassette tape (**Figure B2**, Supplementary material).

Table 1
Typology and index of solid waste in sea turtles.

	C. mydas				*Eretmochelys imbricata*				*Lepidochelys olivacea*				Total			
Waste Type	Vol (ml)	Weight (g)	FO (%)	IIR	Vol (ml)	Weight (g)	FO %	IIR	Vol (ml)	Weight (g)	FO (%)	IIR	Vol. (ml)	Weight (g)	FO (%)	IIR
Rubber, Bladder and Fragments	0.2	0.8	11.1	3.1E-4	0	0	0	–	0	0	0	–	0.2	0.8	9.1	3.7E-4
Plastics *	2	36.9	66.7	1.5E-1	13.8	1.1	66.7	5.4E-2	5.4	5.7	100	1.4	21.2	43.7	64	1.4E-1
Tissue (Fragments)	3.4	3.1	27.8	5.2E-3	0	0	0	0	0	0	0	0	3.4	3.0	20	3.2E-3
Suggestive oil material (Solid pasty)	258.9	255.8	66.7	1.0	14.7	13.2	100	9.6E-1	0.2	0.1	100	1.5E-2	288.6	287.5	68	9.5E-1
Hair	0.3	0.023	16.7	2.3E-5	0	0	0	–	0	0	0	–	0.3	0.02	16.7	2.3E-5
Vol. Total	355.4	296.6			28.5	14.2			5.6	5.7			389.2	335,1		

Vol=Volume; FO%= frequency of occurrence in percent; RII= relative importance index. *Types of plastics (Styrofoam; silica, plastic spoon, plastic cup, plastic rope, nylon wire, frag. p inflex, flex plastic, raffia and plastic bag).

The most frequent categories, considering all turtles collected regardless of species, were plastics ($n = 138$; 43.7 g), followed by fabrics ($n = 29$; 3.594 g), residues suggestive of petroleum ("oil," $n = 24$; 288.665 g), human hair ($n = 7$; 0.023 g), and rubber ($n = 3$; 0.781 g). When comparing the weight (%), abundance (%), and frequency of occurrence of the items, it is evident the predominance of plastic in these three parameters as well as the high frequency and weight, with a low abundance of "oil" (due to its liquidity preventing a higher item count) (Fig. 2). However, according to the RII, the order of importance of the items is different, from the highest to the lowest value: residues suggestive of oil (RII = 0.94762602), plastics (RII = 0.144859), fabrics (RII = 0.00319366), rubber (RII = 0.00036774), and hair (RII = 0.000023). It is noteworthy that sixteen individuals ingested nylon threads, which are likely items from fishing gear (**Fig. B2–D**, Supplementary material).

Regarding the sizes of anthropogenic solid waste, their lengths varied, on average, from 22.5 cm (0.1–844.0 cm). Most of the ingested residues ($n = 182$; 83.9 %) were classified as macro-waste, and 16.1 % ($n = 35$) as meso-waste. In addition, the most abundant residues were colored ($n = 86$; 37.73 %) and white ($n = 62$; 27.19 %), followed by transparent ($n = 40$; 17.54 %) and black ($n = 40$; 17.54 %) (**Fig. B3**, Supplementary material).

Of the 17 samples in which oil-like material was recorded, 12 also had solid residues in the GTI. According to visual analysis, the presence of material suggestive of petroleum in sea turtle tissues sampled and necrotic or stenosed portions in the intestinal tissues were observed, indicating a possible inflammatory process, in addition to changes in other organs, such as the liver and pancreas (Fig. 3). These findings may be a possible reason for the death of these animals.

3.2. Necroscopic inferences

Table 2 is synthesized the number of specimens of sea turtles sampled along Ceará coast and separated by age group, ingestion of solid waste, suggestive oil, presence of parasites and fishery interactions. The majority ($n = 18$) of the specimens had an excellent body score, even those that ingested waste, with a low number of individuals with fibropapilloma ($n = 6$), parasites ($n = 6$), and interaction with fishing ($n = 5$) (Table 2).

Table 3 provides information regarding the record of solid residues and oil found in the gastrointestinal tract of sea turtles sampled off the coast of Ceará. These findings were associated with necropsy results and ultimately led to the death of the animals. From the results of the 18 necropsies, it was possible to infer the factors that led to the death of each animal (**Table 3**). Notably, 50 % of the necropsied animals showed strong evidence of death related to physiological alterations due to ingestion of material suggestive of petroleum and 22.22 % to the ingestion of other types of anthropogenic residues.

Among the macroscopic inferences made by the residues in the organisms of the necropsied animals, it was possible to notice stenosis, suggestive of interaction with topical oil, impediment of food passage in one of the individuals who ingested oil and garbage, and two cachectic individuals, one of them with fecalomas and garbage, causing the GIT to

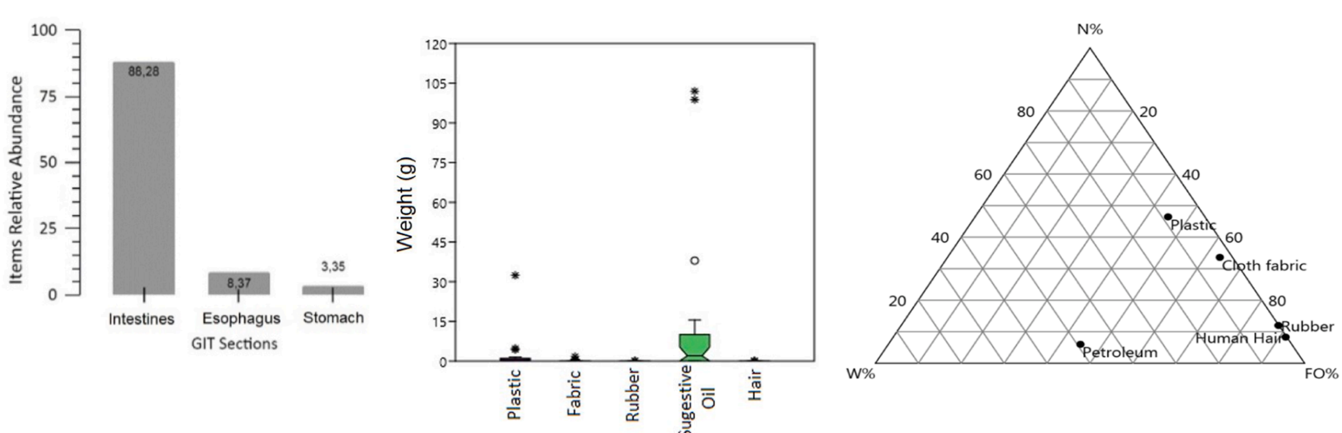

Fig. 2. Types of waste found in sea turtles (L. *olivacea, E. imbricata* and *C. mydas*): A) Relative abundance (%), B) Weight and C) Relative abundance (N,%); Relative weight (W,%); Frequency of occurrence (FO,%) (*Rubber and hair are superimposed).

Fig. 3. Visual verification: suggestive of contamination by ingestion of oil (A and B) and external tissues contaminated with oil (C and D).

Table 2
Characteristics and specimens of sea turtles.

	N°	(A)	(J)	F	M	Und.	Waste (A)	Waste (J)	Pet. (A)	Pet. (J)	Fish (A)	Fish (J)	Par.s (A)	Par. (J)	W/ Pap	WO Pap	C.3	C.2	C.1
Total	25	8	17	15	2	9	8	20	6	11	1	4	4	0	6	19	18	6	1
Partial Total (*J* + *A*)	25	25		25			22		17		5		4		25		25		

J/A: Juvenile and adult age group, respectively; F: female; M: male; Und.: Undetermined; Waste: GIT solid waste presence; Pet.: Suggestive interaction with petroleum; Fish: Fishery interaction; Paras: turtles with parasites; Pap: Fibropapillomatosis; C.1, C.2, C.3: Body condition (Cachetic, Slim and Ideal, respectively).

be obliterated.

3.3. Chemical confirmation

Fig. 4 presents the chromatograms obtained from GC-FID analysis of the saturated fraction of the organic extracts obtained from the GC of the investigated turtles. Although the microscopic study revealed eight animals showing strong evidence of oil ingestion, chemical data supported this evidence in three animals (18.7 %). The whole series of the *n*-alkanes (*n*-C_{14} to *n*-C_{31}), pristane, and phytane were detected for the samples T05, T18, and T17, profiles characteristic of petroleum. The presence of pristane and phytane, which are not primary constituents of biota, corroborate the petrogenic origin of the detected hydrocarbons. In addition, chromatograms present an unresolved complex mixture (UCM), which is a common feature of weathered petroleum (Stout and Wang, 2007). Moreover, in samples T14 and T23, a limited number of *n*-alkanes (*n*-C_{16} to *n*-C_{23} for sample T14; *n*-C_{15} to *n*-C_{20} for sample T23) and phytane were detected in low abundance; no UCMs were observed for these two samples.

Table 4 shows parameters calculated from the data obtained in GC-FID analysis, using the *n*-alkanes and isoprenoids. The concentration of *n*-alkanes is high for samples T05 and T18, as already observed in their gas chromatograms. Samples T05, T18, and T17 show high Res/UCM, which can be explained by the higher absorption of the *n*-alkanes and isoprenoids than the more complex compounds present in the UCM. The ratios Pr/Ph, Pr/*n*-C_{17}, and Ph/*n*-C_{18} present values in agreement with the petrogenic source for the isoprenoids. In addition, the odd-to-even preference index (OEP) also has values consistent with the petrogenic origin, varying from 0.4 to 1.6 for samples T05, T18, T17, and T14, thereby showing no significant preference for even *n*-alkanes from biogenic sources.

4. Discussion

This study presents relevant results, since the ingestion of solid wastes by sea turtles is still a subject that has been studied little on the coast of South America, and the expansion of data is of paramount importance since these animals occur throughout the Brazilian coast (Santos, 2011). Despite the significant length range of the *C. mydas* species, no species was collected in its initial years of life (< 30 cm) when they tend to have a pelagic lifestyle (Balazs, 1995). This result is due to the greater abundance of strandings of this species of sea turtle in Brazil (Santos and Manzi, 2011), especially on the coast of Ceará, where populations of young adults and adults occur (Santos, 2011), in addition to presenting the highest number of strandings for this taxon (Feitosa et al., 2022). *C. mydas* has coastal habits and frequently feeds on seaweed banks (Márquez, 1990), and they may have more contact with anthropogenic discards because they are closer to the coast. The number of juveniles was substantially higher than that of adults because, in the juvenile stage, these animals tend to remain in a neritic environment, mainly due to the greater availability of food (Reis and Goldberg, 2017), being more subject to human interference, either by fishing or coastal pollution (Macedo et al., 2011). The same occurred for the number of females because males, mainly adults, do not stay very close to the coast, migrating only between mating and feeding areas (Lohmann et al., 1997; Chan and Liew, 1999; Hays et al., 2010).

The fact that 88 % of the turtles ingested some amount of waste was expected and is corroborated by previous research on turtles in the South Atlantic (Bugoni et al., 2001; Monteiro et al., 2016; Machovsky-Capuska, 2020). It was demonstrated that this ingestion was not directly related to the age or size of the specimen and possibly was not the cause of death (results of necropsy). Another important finding was the great amount of solid waste in the intestinal compartment. In fact, the ingestion of residues by these species, mainly in smaller amounts, is common (Balazs, 1985; Plotkin and Amos, 1990; Bjorndal et al., 1994; Bugoni et al., 2001; Mascarenhas et al., 2004; P.S. Tourinho et al., 2010), where both juvenile species and adults ingest it (Mrosovsky et al., 2009; Witherington et al., 2012; Campani et al., 2013) and tends to accumulate in the intestine (P.S. Tourinho et al., 2010). This is because complete digestion by sea turtles can take twenty-three days or more, depending on the diet, species, and size (Valente et al., 2008). In addition, since the solid waste is not absorbed and difficult to excrete intestinal disorders, such as obstruction or entanglement are expected (Bjorndal et al., 1994 Derraik, 2002). In addition, other studies did not report frequent mortality of turtles directly associated with detritus consumption; therefore, mortality is not the direct primary consequence, with more sublethal effects (e.g., malnutrition, intestinal obstruction, and fecaloma formation) (Bjorndal et al., 1994; Bjorndal,

Table 3
Record of solid residues and oil in the gastrointestinal tract associated with necropsy.

Sample n°	Specie	Sex	Phase	SW (g)	Oil (g)	Hair (g)	Para.	DTI	FB	Main death cause	Secundary death cause
1	CM	F	Juvenile	0.39					X	NE	
2	CM	F	Adult	0.17						Head trauma	The head trauma may have affected the animal's physiological functions.
4	CM	F	Juvenile	1.46	4.08		X	X		Undefined	The presence of material suggestive of oil may have intoxicated the animal
5	EI	F	Juvenile	1.05	0.41		X	X		Pulmonary edema	Presence of sticky dark material on both carapace and plastron. Oral cavity filled with oil.
6	CM	U	Juvenile	0.06	0.10			X		Cardio. arrest	Alterations suggestive of an acute infectious condition.
7	CM	F	Adult	0.07				X		NE	
8	CM	U	Juvenile	30.49			X	X		Cachexia syndrome	Acute inflammation of the intestine caused by fecaloma.
10	LO	U	Adult	4.68	0.06					Cardio. arrest	Presence of oil in the respiratory tract that culminated in respiratory distress, pulmonary edema and death due to respiratory arrest. Other alterations were difficult to identify due to the severe autolysis of the animal.
11	CM	F	Juvenile	0.00	10.00		X	X		Circulatory shock	Presence of dark material (oil) in the intestines, with stenosis, suggestive of intoxication, culminating in circulatory shock.
13	CM	F	Juvenile	0.00	6.43			X		NE	
14	CM	U	Juvenile	0.00					X	Cardio. arrest	Due to the presence of severe autolysis of the organs, it is difficult to determine the cause of death, however, interaction with anthropogenic residues wrapped around the neck and fin of the animal can be observed, which may be suggested to have hindered the animal's locomotion, as well as its breathing.
14	EI	F	Adult	0.01	15.54			X		NE	
15	EI	F	Juvenile	0.00	4.00		X	X		Cardio. arrest	Observe sticky dark material within the entire gastrointestinal tract of the animal suggestive of crude oil, indicating ingestion and possible intoxication Leading to circulatory shock
16	CM	U	Juvenile	0.25				X		Cardio. arrest	Lacerative lesion on the fin and Interaction with waste, where garbage can be observed throughout the gastrointestinal tract of the animal, with this animal presented starvation, loss of buoyancy, respiratory distress and death from respiratory arrest.
17	CM	U	Juvenile	0.00	10.00			X		Cardio. arrest	Observe sticky dark material inside the intestines of the animal suggestive of crude oil, indicating ingestion and possible intoxication, hyperemic organs suggesting multicystemic inflammatory process leading to circulatory shock
18	CM	U	Juvenile	0.85	6.00	0.021			X	Cardio. arrest	Presence of Garbage and Oil in the GIT, contributing to the formation of strictures and starvation, and possible immunosuppression and locomotive difficulty, being subject to infectious agents
19	CM	U	Juvenile	0.03	102.00	0.001		X	X	Cardio. arrest	Presence of large food matter in the stomach, suggesting impossibility of gastric passage, in addition to the Presence of Garbage and Oil in the GIT, contributing to the formation of strictures and starvation, and possible immunosuppression, being subject to infectious agents
21	CM	F	Adult	0.00	98.81			X		NE	
22	LO	F	Adult	1.00	0.10			X		NE	
23	CM	F	Adult	1.34	35.00			X		NE	
24	CM	M	Adult	0.06	2.00			X		Undefined	Hyperemic intestinal mucosa, indicative of an acute inflammatory condition leading to animal death. Large amount of food in the intestines and presence of oil, which can cause inflammation in the intestines
25	CM	F	Juvenile	6.72	2.00	0.001	X		X	Pulmonary edema	Due to anthropogenic interaction with fishing lines on the animal's anterior fin, leading to difficulty in the animal's locomotion, as well as breathing. Thus, the animal had pulmonary edema and consequent cardiorespiratory arrest.

SN: sample number; CM: *C. mydas*; EI: *Eretmochelys imbricata*; LO: *Lepidochelys olivacea*; SW: weight of the solid waste; Para.: presence of parasites; Oil: petroleum suggestive material; DTI: Digestive tissue inflamation; FB: presence of fibropapilomas; NE: not evaluated. The "x" letters indicates the presence of characteristic data indicated in the header.

1997; Mccauley and BJorndal, 1999). Regarding the length of the residues, most did not exceed 87 cm, corroborating the hypothesis that the animal involuntarily ingests the artifact, along with the food item, in similar size proportions to the food it usually looks for (Gramentz, 1988).

Plastic was the most ingested item, although various residues have been reported in several studies (Tomás et al., 2002; Tourinho, 2010). Plastics are known to constitute the majority of residues in marine and coastal ecosystems and are present in different forms and compositions (Campani et al., 2013; Ryan, 2014). The high rates of plastic ingestion may be associated with greater availability of these materials in the environment, mainly because of their high durability, large production, and inappropriate disposal on land or at sea, and may also be related to their varied shapes, materials, or colors. (Schuyler et al., 2012; Campani et al., 2013). Krishnan et al., 1993 proposed that plastic items, for example, work as endocrine disruptors, as has been attested in seabirds (Van-Franeker, 2011), in addition to being possible accumulators of toxic agents (Oehlmann et al., 2009; Koch and Calafat, 2009).

The number of colored items prevailed over the other colors; however, as observed in studies carried out by Carvalho et al. (2015), Poli et al. (2015), and Rizzi et al. (2019), the set of white and transparent items had a greater proportion, reinforcing the hypothesis that these animals confuse wastes of this color with gelatinous food items (Carr, 1987; Gramentz, 1988; Bugoni et al., 2001; Matiddi et al., 2017). The

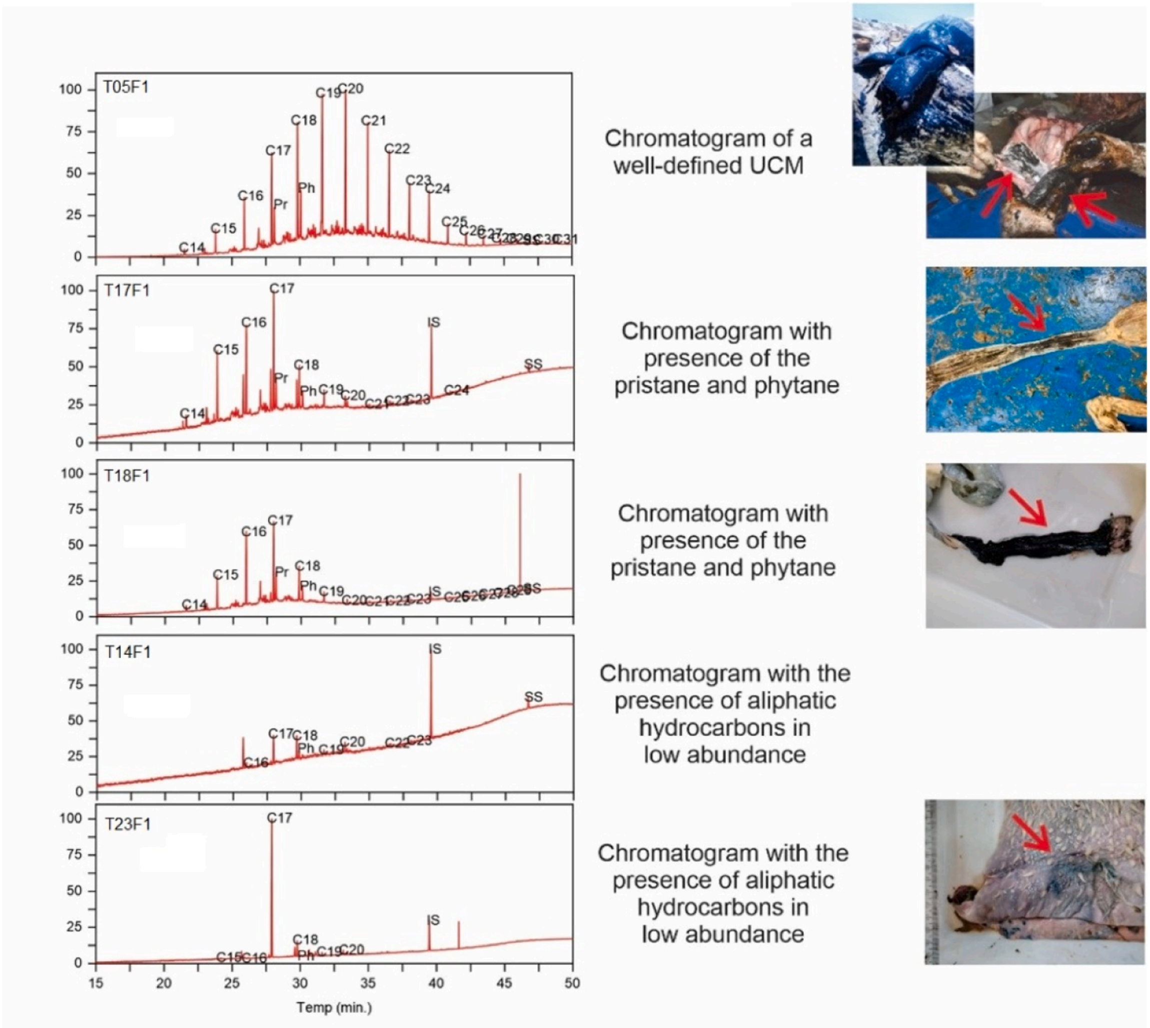

Fig. 4. GC-FID chromatograms of the extracts of the turtles found dead in the beaches after the oil spills in 2019.

Table 4
Geochemical parameters based on the *n*-alkanes and isoprenoids (pristane and phytane) content in the oil samples extracted from the turtles found dead.

PARAMETERS	T05F1	T18F1	T17F1	T14F1	T23F1
$\sum C_{14}–C_{31}$ (ppm)[1]	103.8	109.7	48.9	44.2	23.1
Res/UCM (%)[2]	26.6	23.8	26.2	n.d.	n.d.
Pr/Ph[3]	0.7	1.7	1.5	n.d.	n.d.
Pr/*n*-C_{17}[4]	0.6	0.4	0.3	n.d.	n.d.
Ph/*n*-C_{18}[5]	0.6	0.5	0.5	0.4	n.d.
OEP[6]	0.9	1.6	1.6	0.4	n.d.

1 $\sum C_{14}–C_{31}$ *n*- alkanes.
2 $\sum C_{14}–C_{31}$ *n*- alkanes + Pristane + Phytane/UCM.
3 Pristane/Phytane.
4 Pristane/*n*-C_{17}.
5 Phytane/*n*-C_{18}.
6 OEP: (*n*-C_{21} + 6 *n*-C_{23} + *n*-C_{25})/(4 *n*-C_{22} + 4 *n*-C_{24}).

more vibrantly colored fragments (colorful) were probably ingested because they were entangled with food resources (Schuyler et al., 2012), which is a palpable explanation, especially for juveniles, who tend to be less selective in their diet (Frick et al., 2010; Schuyler et al., 2012). The material found in the GCs of the turtles studied follows the typological pattern verified in studies of waste on beaches in the region, which is a strong indication that the inefficient management of solid waste is influencing the impact on the turtles (Cavalcante et al., 2020; Nolasco et al., 2022). According to Cavalcante et al. (2020), the coast of the region is subject to the deposition of debris resulting from various human activities such as recreational pursuits, navigation and fishing, as well as domestic and industrial/port operations.

It was also noted that the majority ($n = 18$) of the specimens had an excellent body score, even those that ingested waste, suggesting that some of the deaths were caused by sudden events or nonchronic situations. A series of authors have discussed this type of result, proving that these animals are very resistant to long-standing impacts and diseases (Bugoni et al., 2001; ; Works and Balazs, 2010; Campani et al., 2013; Monteiro et al., 2016; Cluckey et al., 2017; Matiddi et al., 2017).

The ingestion of petroleum and the topical effects observed from the necropsies, possibly caused by its toxicity, were the causes of death in most animals. The ingestion of any type of oil reduces the nutritional assimilation of food and increases inflammation in internal tissues, which can cause necrosis and infections in other organs in addition to the intestine (Mignucci-Giannoni, 1999; Camacho et al., 2013). Gramentz (1988) and Manire et al. (2017) stated that not only is the ingestion of oil harmful and suscepts the animal to intoxication, but harm can also come from topical contact, the inhalation of vapors on the surface of the water, and even trophic ingestion when feeding on organisms previously intoxicated with oil. The harmful effects of this type of anthropogenic impact include compromised physiology, chronic stress, impaired immune function, and increased susceptibility to diseases (Aguirre et al., 1995; Lutz, 1998).

The observation that there were a significant number of individuals showing evidence of oily material and solid waste ($n = 11$) results in the hypothesis that the consumption of waste is indirectly linked to the consumption of oil, which causes the death of the animal. This can be explained by the drift of residues according to coastal currents (Derraik, 2002), as well as other waste, resulting in places where oilier material and anthropogenic debris overlap.

The occurrence of fishery interaction, hair ingestion, and the presence of fibropapillomatosis can be explained by the proximity between feeding areas, coastal fishing, stress areas (e.g., presence of predators and food competition), marine pollution by fishing gear (ghost fishing) (Lima et al., 2020), and areas close to submarine outfalls. These factors are known to trigger fibropapillomatosis (Herbst, 1994) and poor health of the animal, making parasitic infections favorable. This was observed since samples with parasites and containing waste in the GIT were recorded, and previous research stated that they are usually present in weakened organisms (Alfaro et al., 2008; Mader, 2006). Furthermore, it can be hypothesized that when these animals find a fishing net colonized by algae, they try to feed on the net itself, making it easy to entangle them.

The hydrocarbon distributions of samples T05, T18, and T17 presented in Table 3 and Fig. 4 confirm the petroleum contamination in sea turtles at a considerably higher level than other specimens, as shown by the higher concentration of *n*-alkanes. Additionally, the gas chromatogram for sample T05 is very similar to the 2019 spilled oils, showing a well-resolved series of *n*-alkanes (maximum in n-C_{19} to n-$_{C20}$) upon a smooth UCM, in a unimodal distribution (Reddy et al., 2022; Azevedo et al., 2022). The Pr/n-C_{17} and Ph/n-C_{18} ratios were higher for sample T05 (Table 2) than for the 2019 spilled oils (Azevedo et al., 2022) likely because of the greater degradation susceptibility of n-C_{17} and n-C_{18} in comparison with Pr and Ph, respectively.

There are several clinical analyzes, such as physical and neurological examinations, X-ray exploration, blood collection, hematological and biochemical evaluation, endoscopy, skin and shell biopsies, microbiological cultures, as well as histopathology, however, it is still difficult to attribute the death of the turtles exclusively to the effect of the oil (Mignucci-Giannoni, 1999; Camacho et al., 2013; Manire et al., 2017). According to Wallace et al. (2020) there is much clarity in the demonstration of the physical effects of oil on sea turtles, which cannot be attested with as much frequency or clarity for the chemical effects of exposure.

Unfortunately, there are scarce studies proving that the material found in the digestive tract is oil and its consequence in the death of sea turtles in oiled areas is not so easy to prove (Wallace et al., 2020; NOAA, 2021). This is due to several factors, and one of the main ones is the fact that most studies use guides based on visual analysis, which classify any shiny dark material found during necropsy as oil without demonstrable chemical analysis (NOAA, 2021). Scarce studies that have investigated tissue, gastroenteric, or biliary samples from visibly oiled and non-oiled turtles that died during and after the DWA spill and other events have attributed high levels of petroleum hydrocarbons in the oiled turtles from accidents (Ylitalo et al., 2017; Ruberg et al., 2021; Liu et al., 2022).

Advances towards precisely verifying the cause of death, especially in events involving oil spills, are a priority because few laboratory experiments have reported skin lesions, decreased salt gland function, and alterations in some blood cell parameters (Lutcavage et al., 1995). On the other hand, studies examining the effects of ingested DWH oil detected sublethal effects, including oxidative stress, dehydration, and potential alteration of gastrointestinal function, although no exposures resulted in mortality (Mitchelmore and Rowe, 2015).

5. Conclusions

For the first time, on the oiled zone in South America coast, it was chemically proven that in three turtles found in oiled areas, the material was oil, and this may have contributed to their death. Our results prove that most deaths were due to respiratory arrest, followed by pulmonary edema, cachexia syndrome, circulatory shock, and head trauma, which may have been caused by contact with solid waste, oil, or both.

The study also showed that not all dark material found in the GCs of turtles killed in oiled areas is truly oil, and in this sense, a chemical analysis step to prove the evidence of oil must be added to international protocols.

CRediT authorship contribution statement

Alice F. Feitosa: Conceptualization, Formal analysis, Methodology. **Ícaro B.H.M.P. Menezes:** Conceptualization, Data curation, Methodology. **Oscar S. Duarte:** Conceptualization, Data curation, Formal analysis, Methodology. **Carminda S . B . Salmito-Vanderley:** Conceptualization, Formal analysis, Methodology. **Pedro B.M. Carneiro:** Conceptualization, Formal analysis, Methodology. **Rufino N.A. Azevedo:** Conceptualization, Data curation, Methodology. **André H.B. Oliveira:** Conceptualization, Formal analysis, Methodology. **Ana C.S. Luz:** Conceptualization, Formal analysis, Methodology. **Adriana P. Nascimento:** Conceptualization, Formal analysis, Methodology. **Ronaldo F. Nascimento:** Conceptualization, Formal analysis, Methodology. **Laercio L. Martins:** Methodology, Writing – original draft, Writing – review & editing. **Rivelino M. Cavalcante:** Resources, Writing – original draft, Writing – review & editing. **Caroline V. Feitosa:** Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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