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Application of nuclear analysis for bioaccumulation of microplastics with iodine-131 in marine organisms

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Abstract

Oceans are considered the endpoint of plastic fluxes. Microplastics (MPs) are well-known as a serious threat to the marine environment. One of the primary pathways is through absorption, which can directly impact marine organisms, such as mangrove ecosystems (*Rhizophora mucronata* Lam.). These ecosystems can capture suspended pollutants in water and soil, leading to the entry of pollutants into the food chain and their uptake by fish *Chanos* chanos. The presence of radionuclide activity can be utilized to track elements that can be harmful to the environment. This study investigates the accumulation of MPs in *R. mucronata* and *C. chanos* using polystyrene labeled Iodine-131 as an MP model measured through the nuclear analysis method. ^{131}I is used as a stable radiotracer measured with a Gamma spectrometer NaI(Tl) technology. The lowest accumulation of MP particles in *R. mucronata* was found in the apical bud, with values from 4.1×10^{-10} to 6.2×10^{-10} g, and the highest particles were observed in the roots, with values 2.4×10^{-9} to 4.8×10^{-9} g. Meanwhile, in *C. chanos* (2.118 g to 13.472 g) ranged from 0.036 to 0.398 mL.g^{-1} after the seventh day of exposure in his body tissue.

Keywords: Accumulation; Microplastic; Iodine-131; *R. mucronata*; *C. chanos*

1. Introduction

Global plastic pollution has emerged as a prominent environmental issue, due to the prolonged natural degradation of plastic in the environment. Such a condition is aggravated by the abundance of microplastics (MPs) in the environment, which has notably risen due to human activities. Recently, more than 5.2 trillion microplastic particles (ranging from 0.33 to 200 mm) with a total weight of 66,140 tons have been floating in the oceans [1]. The introduction of harmful substances, e.g. persistent, bioaccumulative, and toxic substances (PBTs), and persistent organic pollutants (POPs), potentially increase the microplastic hazard [2]. As microplastics continuously fragment into smaller particles, they can assimilate these toxic chemicals from their environment [3]. Micro or nanoplastics, possessing lower density than water, tend to float on the water's surface, facilitating easy absorption by marine organisms. Consequently, these pollutants can infiltrate and accumulate within coastal plant ecosystems and marine biota [4].

The marine environment, particularly in coastal regions, is acknowledged as the primary recipient of microplastic pollution, accounting for 47.9% of coastal zones worldwide face substantial human-induced stressors [5]. Tropical wetlands with mangrove forests and aquaculture, including *C. chanos* cultivation represent globally endangered socio-ecological systems due to plastic waste resulting from anthropogenic impacts and stressors, particularly their proximity to development areas. Mangrove ecosystems serve a vital ecological function and are recognized for their capacity to capture sediments and various particulate materials, including microplastics. Several studies that microplastics have the potential to impact the structure and functionality of mangroves [6], [7]. Contact between mangrove roots and

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plastic polymers, including the common polystyrene (PS) induces a shading effect, limiting energy and substance transfer, ultimately resulting in reduced nutrient and light uptake [8], [9].

According to the 2010 report by the Ministry of Marine Affairs and Fisheries, milkfish farming is the fourth-largest aquaculture commodity in the country. In 2022, trends over the previous decade showed milkfish production peaking at 875,592.01 tons in 2018. However, production declined by 3.97% from 2019 to 2021. Miklfish have a high tolerance to salinity, allowing them to thrive in various environments [10], [11]. This adaptability makes them susceptible to ingesting and bioaccumulating MP contaminants, which can pose ecological and health risks [12]. In general, the influence of microplastics on mangrove plants and milkfish (*C. chanos*) varies depending on factors such as their type, form, size, and concentration

The accumulation of microplastics has been investigated through both particle characterization and visual assessment. The development of radio-labeled microplastics using Iodine-131 (^{131}I) is successfully performed and further used as a radiotracer for examining the bioaccumulation and biodistribution of microplastics in organisms [13]. The advantages of using ^{131}I include its half-life (8.02 days), which is suitable for the accumulation of microplastics in plant tissue and aquatic biota, and its sufficiently high gamma energy (365 keV, 80%). Nuclear techniques are highly sensitive, accurate, and real-time tools for assessing the toxicodynamics of microplastics in authentic environmental conditions, as well as for investigating bioaccumulation and biotransfer within aquatic food chains [14]. The investigation of microplastic behavior in marine organisms using the radioanalytical method has not been reported. Therefore, this study aims to investigate the behavior of microplastics in *R. mucronata* Lam. tissues and *C. chanos* using Iodine-131 labeled polystyrene (PS) for a more comprehensive evaluation of the ecological risks associated with microplastics in the marine environment.

2. Material and Methods

The preparation method of ^{131}I labeled polystyrene (PS-^{131}I) has been developed at the Installation Radioisotope, Radiopharmaceuticals, and Biodosimetry Technology. Experiment process on marine organisms *R. mucronta* Lam. and *C. chanos* represented in Fig 1.

2.1. Bioaccumulation of Microplastic through the Sediment and Seawater Pathway

R. mucronata Lam. mangroves are transferred to muddy soil and microplastics labeled with ^{131}I are introduced to attain four concentrations, i.e. 0.078 ppm, 0.117 ppm, 0.156 ppm, and 0.195 ppm. The radioactivity of each ^{131}I-labeled microplastic content is assessed using a Gamma Ray Spectrometer with a NaI(Tl) detector connected to a multi-channel analyzer and a computer equipped with DppMCA software from Amptek on the seventh day of bioaccumulation. The kinetics of radiotracer absorption are quantified through concentration factors (CF showing the ratio between the weight of ^{131}I-labeled MP (MP (g)) and the concentration of MP in the soil (C_w). The kinetics were described utilizing a simple linear regression model [Eq. (2)].

$$\text{MP (g)} = \frac{\text{Activity of plant (Bq)}}{\text{Activity of MP 131I (Bq)}} \times \text{MP Concentration (g)} \text{..............(1)}$$

$$CF_t = \frac{MP}{Cw} \text{...........................(2)}$$

C. chanos of different weights/sizes (2.1 to 13.5 g; 4-7 cm of length, 1-2.5 cm of width; n = 4) were placed in a 20 L aquarium, exposed for the seventh day to dissolved radiotracers in 5.418 $Bq.mL^{-1}$ ^{131}I. The bioaccumulation of ^{131}I-labeled PS microplastics through the seawater pathway in milkfish was conducted in four 20 L aquariums, each filled with 12 L of seawater with different salinities, i.e. 28 ppt, 30 ppt, 32 ppt, and 34 ppt. To each aquarium, 20 mL of a ^{131}I solution was added, achieving a total concentration of 0.026 ppm (activity of 80.783 $Bq.mL^{-1}$).

Whole-body uptake kinetics of radiotracers were expressed in terms of changes in bioconcentration factor over time (CF was the ratio between the activity of the radiotracer in the whole organism or in a body compartment ($Bq.g^{-1}$ wet weight) and time-integrated activity of radiotracer in seawater ($Bq.g^{-1}$)). Radiotracer uptake kinetics were best described using a first-order simple linear regression model [Eq. (3)] which refers to [15], [16]:

$$CF_t = \frac{Ct}{Cw} \text{...............(3)}$$

Where CF_t (mL.g^{-1}) are the concentration factor at time t (d), uptake rate constant, and depuration rate constant, respectively [17], [18].

2.2. Bioaccumulation Model

The relative contribution of dissolved and seawater pathways was determined using a bioaccumulation model as modified by Hédouin et al., (2010); and Susetyo et al., (2023). The release rate constant (k_e) is derived from the graph's slope, illustrating the percentage (%) of retained contaminant activity across time. Subsequently, the calculation of the bioconcentration factor (BCF) is performed to ascertain the bioaccumulation concentration factor [Eq. (4)]:

$$BCF = \frac{k_u}{k_e} \text{..................} (4)$$

BCF is the fundamental measure of an organism's ability to absorb and eliminate heavy metals [19]. The biological half-life of Iodine in milkfish (*C. chanos*) was indicated by [Eq. (5)]:

$$t_{b1/2} = \frac{\ln 2}{k_e} \text{....................} (6)$$

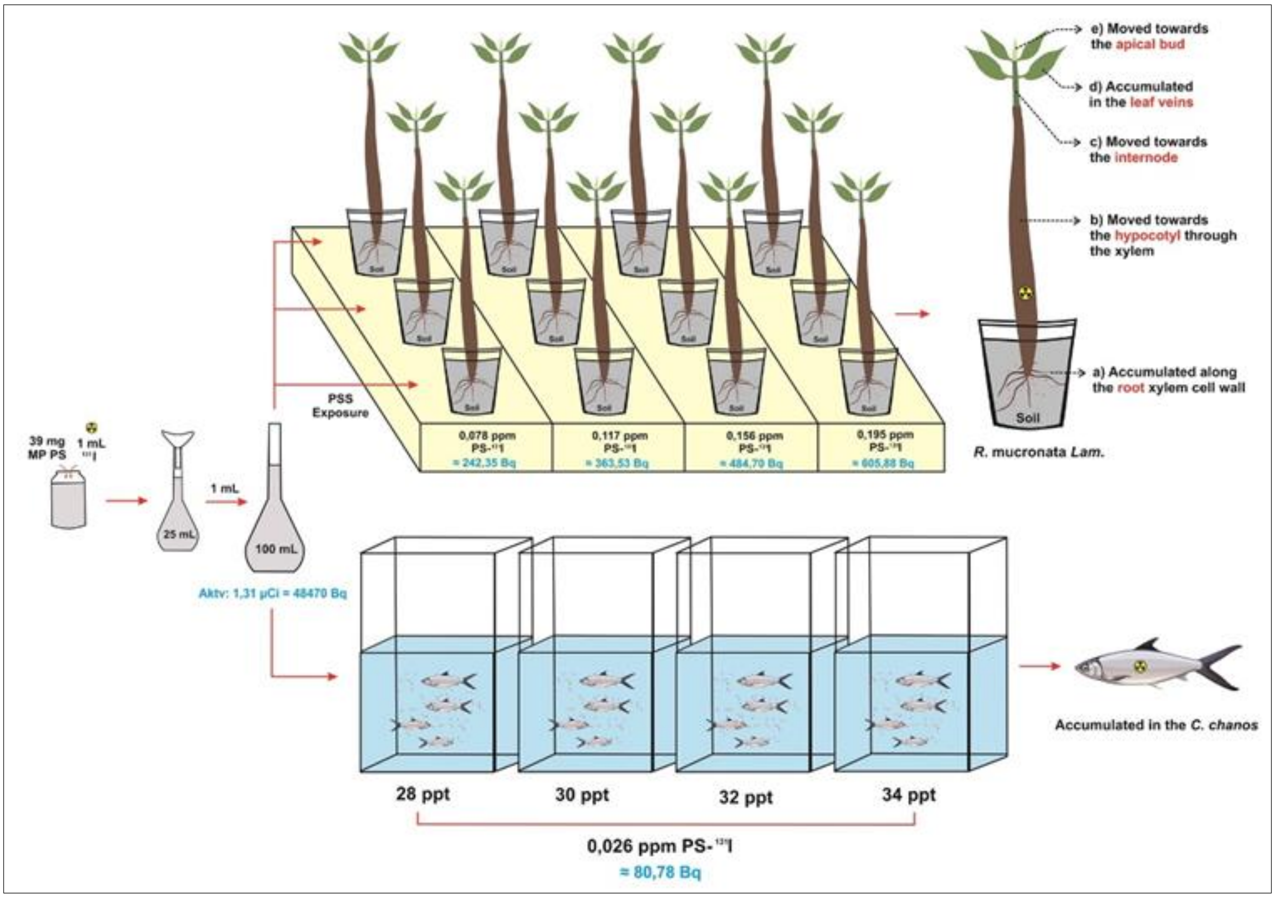


Figure 1 Illustration of experiments on marine organisms using nuclear analysis

2.3. Data Analysis

The data obtained from the laboratory experiment was analyzed quantitatively. The significance level for statistical analysis was consistently set at α = 0.05. Data analysis was performed using the Origin2024 software.

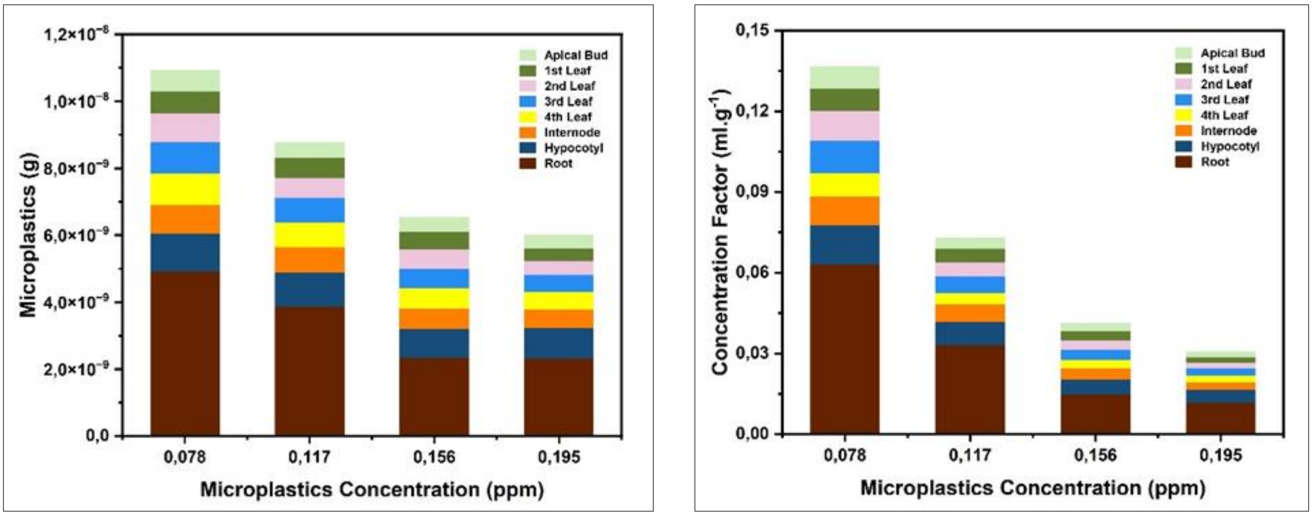
3. Result and Discussion

The uptake of MPs by plant species and marine biota is crucial to evaluate. Marine waters with high anthropogenic activity tend to contain significant amounts of MPs. The mangrove species *R. mucronata* Lam. absorbs MP pollutants from contaminated soil or sediment, as does the milkfish (*C. chanos*). bioaccumulation of MPs and associated chemicals is anticipated across all species at various levels. The absorption, accumulation, and translocation of MPs in mangroves and fish vary by species due to their differing physiological and anatomical characteristics. Mangroves represent pioneering halophytic plants with evergreen foliage and broad leaves. *C. chanos* has a high salinity tolerance, and the effects of MPs on *C. chanos* vary depending on the type, size, shape, and concentration accumulation. Mangrove forest ecosystems near fish farming areas have largely been transformed into absorbing large amounts of plastic waste from various sources.

Mangrove ecosystems play a vital role for coastal communities by supplying food and transporting carbon and nutrients to coastal areas or nearby seas [20]. They function not only as crucial habitats for numerous marine and terrestrial organisms [21] but also contribute to stabilizing the marine ecosystem, an essential component of the trophic chain [22], [23], [24]. Despite the increasing volume of scientific research on the occurrence, movement, and distribution of microplastics in the marine environment and their adverse effects on marine life [25], researchers have recently started exploring their potential impact on human health. This is because MP particles may have various negative effects when absorbed by plants and ingested by various organisms like fish, mollusks, crustaceans, and other biota living around mangroves [26], [27].

The detection of the uptake, translocation, and deposition of MP particles in various tissues of marine organisms is triggering their biological and physiological impacts. The uptake of MPs in marine organisms varies among different species due to differences in physiological and anatomical traits [28]. Our study shows that during bioaccumulation experiments, the species did not experience mortality. The experiments revealed the uptake rate of PS microplastics in *R. mucronata* Lam. and *C. chanos* through a nondestructive radiotracer technique. It was found that PS microplastics, with a weight composition ranging from 4.1×10^{-10} to 4.8×10^{-9} grams at concentrations of 0.078 ppm to 0.195 ppm, could penetrate various plant tissue structures, including roots, hypocotyls, internodes, leaves, and apical buds, facilitated by ^{131}I radioisotope labeling as a radiotracer.

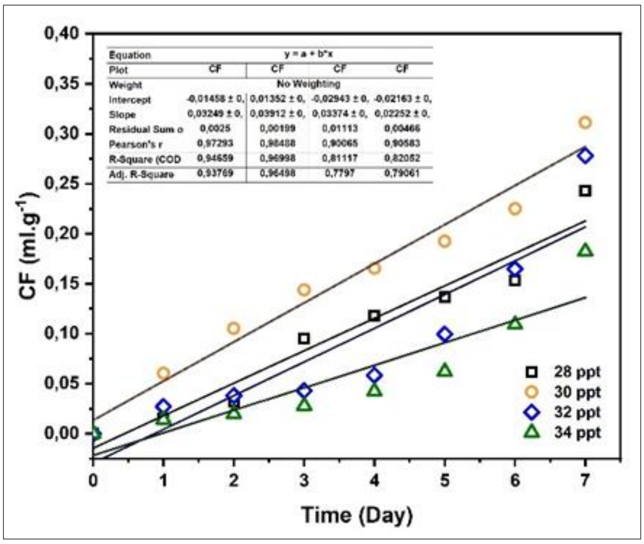
The study results (Fig 2a) reveal that the lowest ability to accumulate MP particles for each concentration variation was found in the apical bud, measuring 6.2×10^{-10} (a); 4.5×10^{-10} (b); 4.4×10^{-10} (c); 4.1×10^{-10} (d). in contrast, the highest accumulation of MP particles absorbed by *R. mucronata* Lam. is in the root section, measuring 4.8×10^{-9} (a); 3.8×10^{-9} (b); 2.4×10^{-9} (c); 2.4×10^{-9} (d). With an increase in MP concentration, the plant tissue's absorption capacity for the number of MP particles decreases. The ability of *R. mucronata* Lam. to accumulate ^{131}I-labeled PS microplastics is represented as the concentration factor (CF). The CF value represents the ratio of MP weight to the concentration of ^{131}I-labeled PSS microplastics in the soil. According to Fig 2b, an elevated concentration of ^{131}I-labeled PS microplastics in the soil medium can reduce the accumulation ability of *R. mucronata* Lam. for MP particles. The absorption kinetics of ^{131}I-labeled PS microplastic particles in marine organisms are shown in Table 1.



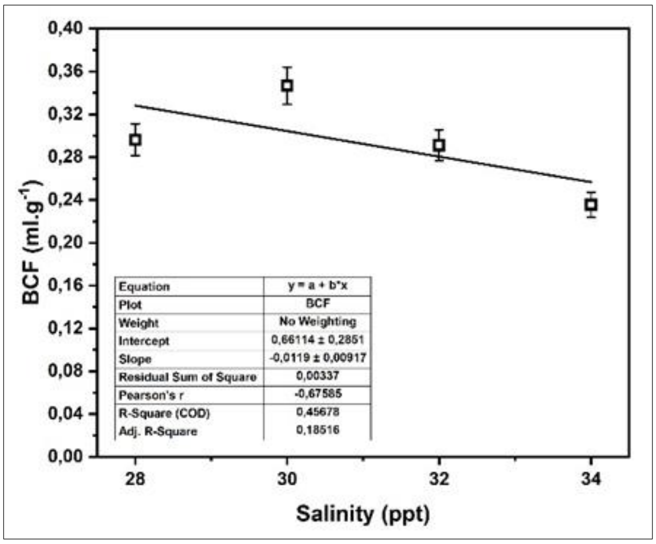
(a) (b)

Figure 2 (a) the weight of MPs at various concentrations, (b) bioaccumulation of *R. mucronata* Lam. at various concentrations

MPs pose a potential health risk to humans when seafood is contaminated with microplastics through bioaccumulation and biomagnification in the food chain. Coastal fisheries, including aquaculture and wild-caught species, are vital food sources and economic resources for the country [29]. The absorption of MPs by *C. chanos* occurs through the mouth and gills, flowing into the digestive system. The CF represents the biota's ability to accumulate contaminants (MPs). Fig 3 shows the different increases in PS-^{131}I values and BCF in seawater with various salinities.



(a)



(b)

Figure 3 (a) bioaccumulation PS-^{131}I of *C. chanos*, (b) BCF of *C. chanos* at various salinities

The estimated kinetic (Table 1) demonstrates that salinity variations in a medium affect the bioaccumulation ability of *C. chanos* with smaller fish potentially absorbing more MPs, possibly considering contaminants in water as food, aiding in the growth process, and allowing for greater contaminant absorption. The highest bioaccumulation capability of microplastics (MPs) up to the seventh day was observed in the aquarium with a salinity of 30 ppt, with a CF value of 0.172 mL.g^{-1}. This was followed by salinity 28 ppt with a CF value of 0.113 mL.g^{-1}, salinity 32 ppt with a CF value of 0.101 mL.g^{-1}, and the lowest capability was found in salinity 34 ppt with a CF value of 0.065 ml.g^{-1}. It can be concluded that a salinity of 30 ppt is the salinity condition for optimal MP uptake in seawater. The accumulation of radioactive substances by an organism occurs when the absorption rate exceeds the excretion rate.

Bioaccumulation of PS microplastics in milkfish shows an increase in accumulation until the seventh day. The side effects resulting from exposure to microplastic particles do not depend on their accumulation throughout the body. The impact of radiation on health depends on the radiation dose received by the biota, both externally and internally. High exposure to ^{131}I radiation in biota can lead to cell damage, genetic mutations, and death [30]. If the concentration of ^{131}I in the water is low and only bioaccumulated by milkfish, the radiation will remain low.

Table 1 Biokinetic parameters of marine organisms

Sample	Parameter	PS Microplastic (g)							
***R. mucronata* Lam.**	**Concentration (ppm)**	**Structure of *R. mucronata* Lam.**							
		Apical bud	**Leaf [1]**	**Leaf [2]**	**Leaf [3]**	**Leaf [4]**	**Internode**	**Hypocotyl**	**Root**
	0.078	6.2×10^{-10}	6.6×10^{-10}	8.7×10^{-10}	9.5×10^{-10}	9.5×10^{-10}	8.5×10^{-10}	1.2×10^{-9}	4.8×10^{-9}
	0.117	4.5×10^{-10}	5.8×10^{-10}	6.2×10^{-10}	7.4×10^{-10}	7.4×10^{-10}	7.7×10^{-10}	1.1×10^{-9}	3.8×10^{-9}
	0.156	4.4×10^{-10}	5.4×10^{-10}	5.8×10^{-10}	5.8×10^{-10}	5.8×10^{-10}	6.3×10^{-10}	8.7×10^{-10}	2.4×10^{-9}

	0.195	4.1×10^{-10}	3.8×10^{-10}	4.3×10^{-10}	5.2×10^{-10}	5.2×10^{-10}	5.8×10^{-10}	9.2×10^{-10}	2.4×10^{-9}
		CF ($mL.g^{-1}$)							
	0.078	0,0079	0.0084	0.0111	0.0122	0.0086	0.0108	0.0146	0.0633
	0.117	0.0039	0.0051	0.0052	0.0063	0.0043	0.0065	0.0088	0.0333
	0.156	0.0027	0.0034	0.0037	0.0038	0.0032	0.0038	0.0056	0.0151
	0.195	0.0022	0.0019	0.0022	0.0027	0.0025	0.0028	0.0045	0.0118
C. chanos		CF ($mL.g^{-1}$)							
	Salinity (ppt)	28 ppt		30 ppt		32 ppt		34 ppt	
		0.113		0.172		0.065		0.101	
		BCF ($mL.g^{-1}$)							
		0.296		0.347		0.245		0.291	

The translocation of microplastics from the roots to other parts of the mangrove takes place through transportation tissues like the xylem and phloem, while in fish, it occurs through the digestive tract. This process is assisted by ^{131}I radionuclides labeled on MP particles, facilitating translocation to upper mangrove plant structures such as shoots and leaves through the xylem vascular tissues and milkfish, MPs are ingested through the mouth and accumulate in the digestive tract. Detecting MPs in marine organisms presents a technical challenge due to their small size and elemental composition that resembles biomass [31], [32]. The presence of radionuclide activity can be utilized to trace specific MP elements with potential environmental harm, contributing to the assessment of plant and biota responses, exploration of mechanical aspects, and understanding of sub-microscopic interactions between marine organisms and MPs, along with other contaminants.

4. Conclusion

The biokinetics of uptake of ^{131}I-labeled PS microplastic in *R. mucronata* Lam. through the sediment pathway and *C. chanos* through the seawater pathway are crucial for determining the characteristics of their bioaccumulation and validating their role as biomonitoring agents, providing a comprehensive a temporal overview of environmental pollution. Gamma spectrometer NaI(Tl) detector technology demonstrated that PS-^{131}I can migrate from sediment to roots and accumulate in mangrove tips, while in milkfish, it accumulates through the digestive tract. Our study indicates that mangrove plants and milkfish can rapidly and efficiently accumulate ^{131}I-labeled PS microplastics. This suggests that MP contamination can be assessed through routine and periodic monitoring of contaminant concentrations in marine organisms.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors of this manuscript agreed and contributed meaningfully to ensure that the paper comes out successfully without any conflict of interest or grievances.

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