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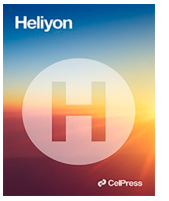

Research article

Low-level ionizing radiation-induced DNA responses in the Asian green mussel *Perna viridis*

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ABSTRACT

Cesium-137 (Cs-137) is a radioactive isotope present in marine environments due to the operation of nuclear power plants and weapons testing. Radiocesium poses a potential risk to marine life due to its long half-life and bioaccumulation. This study evaluated the genotoxicity of low doses of Cs-137 in the Asian green mussel *Perna viridis*, a sentinel species for marine pollution monitoring, by performing the comet assay and micronucleus test on hemolymph samples. Genotoxicity was assessed after exposing mussels to Cs-137 at dose rates of 0, 5, 10, and 15 μGy/h for 48 h. Cs-137's organ-specific distribution was also determined using HPGe gamma spectrometry. Even at low radiation doses, Cs-137 was found to exert genotoxic effects. Significant increases in DNA strand breaks (%Tail DNA) and micronucleus formation (MNF) were observed at all tested dose rates compared with the levels in controls, with dose-dependent responses. Cs-137 predominantly accumulated in the soft tissues, specifically the gills and digestive gland. The findings support the recommended safety level of 10 μGy/h for aquatic organisms, suggesting its appropriateness as a fundamental criterion for developing the national marine water quality standard for Cs-137 in Thailand.

1. Introduction

Cesium-137 (Cs-137) is an artificial radioactive isotope widely used in medical, industrial, and energy sectors. Despite its value in such applications, the marine environment is at risk of both intentional and accidental Cs-137 contamination from activities such as nuclear power generation and weapons testing. Such contamination has the potential to inflict harmful effects on marine organisms and ultimately on humans due to its long-lasting nature (with a half-life of 30.17 years), bioaccumulation, and emission of ionizing radiation. Gamma radiation, in particular, has been documented to have deleterious effects in aquatic organisms, ranging from DNA and cellular damage to physiological impairments [1,2]. Multiple studies have reported that radiation doses below 400 μGy/h had no observed effect on the population levels of aquatic animals [3,4]. It has also been reported that dose rates of less than 200 μGy/h conferred no risks to reproductive competency [5,6]. Elsewhere it was postulated that doses lower than 10 μGy/h have no harmful effects on terrestrial, freshwater, and marine organisms [6–10]. The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) proposed a safety level for ionizing radiation of 10 μGy/h [4]. Furthermore, 10 μGy/h has been chosen as the

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screening level in the Environmental Risk from Ionizing Contaminants Assessment and Management (ERICA) Tool, a simplified model designed and widely used to assess the radiological risk to various non-human biota in marine, freshwater, and terrestrial ecosystems [11–13].

In response to the Fukushima nuclear accident in 2011, regulatory authorities in Thailand have made efforts to establish a national marine water quality standard for Cs-137. Studies across a broad spectrum of marine species including mussels, cockles, squid, shrimp, and fishes in Thai waters have consistently shown Cs-137 radiation doses in the range from 3.1×10^{-6} to 4.5×10^{-2} μGy/h, well below the guideline level [14–19]. These low-dose levels align with the minimal genetic damage and chromosomal abnormalities seen in the Asian green mussel, *Perna viridis*, from shellfish farms in the Gulf of Thailand and the Andaman Sea [15,18]. This suggests that the current radiation levels pose no threat to Thai marine life. However, to substantiate the safety level for implementation as a national standard, there is a need for laboratory experiments to verify that 10 μGy/h is indeed an appropriate threshold for ensuring the safety of local marine species.

Marine mussels, sentinel organisms for monitoring pollutants including radionuclides, are particularly vulnerable to contamination due to their sessile and filter-feeding nature [20–26]. The accumulation of pollutants in these species might pose a threat to their populations, to consumer safety, and to mussel industry because of their economic importance. The Asian green mussel, with the scientific name *Perna viridis* and belonging to the Mytilidae family, is a commercially valuable species that is widely distributed in the Indo–Pacific region, including the coastal areas of the Gulf of Thailand and the Andaman Sea.

Single-cell gel electrophoresis, also known as the comet assay, and the micronucleus test are frequently employed to detect and quantify the potential effects of various environmental pollutants (e.g., pesticides, infectious pathogens, microplastics, and radionuclides) on aquatic animals [27–30]. These methods are valued for their simplicity, sensitivity, and reliability in assessing chromosomal and DNA damage across a variety of cell types [31]. The effectiveness of these techniques in detecting even low-level impacts of ionizing radiation on various marine invertebrates has been confirmed [21,22,24,25,30,32,33]. For example, it was revealed in the hemocytes of the blue mussel *Mytilus edulis* that all total doses of tritium of 12, 121, and 485 μGy/h induced DNA strand breaks (%Tail DNA) and chromosomal aberrations (micronucleus frequency, MNF) [21]. In parallel with this, genetic damage (%Tail DNA) was significantly increased in the gills and digestive gland of the Mediterranean mussel *M. galloprovincialis* and the freshwater mussel *Dreissena polymorpha* after exposure to phosphorus-32 at dose rates of 0.1, 1, and 10 mGy/day (equivalent to 4.17, 41.7, and 417 μGy/h, respectively) for 10 days. Notable increases in genetic damage were observed upon exposure above 1 mGy/day, with a clear dose-response relationship. A similar pattern was observed in the digestive gland of *M. galloprovincialis* regarding MNF across all radiation doses, except at the lowest dose of 0.1 mGy/day [30].

Hemocytes play crucial roles in bivalve physiology, such as in the transportation of oxygen and nutrients, removal of waste and toxicants, and immunity [34,35]. They have thus been widely used as proxy cells to assess radiotoxicity through comet assays and micronucleus tests [21,24]. Owing to their presence in the circulation, hemocytes are readily and uniformly exposed to ionizing radiation from both external and internal sources. In addition, hemocytes exist freely as single cells, making their collection easy and eliminating the need to dissociate the cells [36,37].

Marine organisms have a tendency to accumulate radiocesium in specific tissues [38]. In *Mytilus* mussels, it has been revealed that the radioactive isotopes Cs-134 and Cs-137 were overwhelmingly found in the soft tissue, rather than in the shell. For example, in *Mytilus edulis*, ~95 % of the isotopes were located in the soft tissue, while in *Perna viridis*, the proportion was ~98 % [39,40]. This was attributed to the similarity in chemical properties between cesium and potassium, the latter being a significant cation in living cells involved in the regulation of muscle contraction [41,42]. However, the ratio of radiocesium accumulation between soft tissue and shell can be influenced by factors such as species, exposure pathway, and shell morphology [43].

The aim of this study was to evaluate the effects of low doses of Cs-137, specifically at a screening level of 10 μGy/h, on DNA strand breaks and chromosomal aberrations in hemocytes of *Perna viridis*, a local marine mussel species in Thailand. These evaluations were performed using the comet assay and micronucleus test. We also sought to determine the organ-specific distribution of radiocesium in this species following its exposure. This work should help national regulators to establish an appropriate national standard for Cs-137 marine water quality and offer insights into the management of potential marine contamination, thus minimizing impacts on both seafood consumers and the seafood industry.

2. Materials and methods

2.1. Animal maintenance

Perna viridis, with a shell length of 7.28 ± 0.26 cm and a wet weight of 24.6 ± 2.6 g (n = 32), was collected from mussel rafts at the Fisheries Research Station, Faculty of Fisheries, Kasetsart University, Sriracha, Chonburi Province, Thailand. These bivalves were transported to the Radioecology Laboratory at the Office of Atoms for Peace in Bangkok, Thailand. Upon arrival, all epibionts were carefully removed from the mussel shells. The mussels underwent a 1-week acclimation period in glass aquariums, each containing 1.5 L of filtered (5 μm) seawater per mussel. Laboratory conditions were controlled at a temperature of 29.0 °C ± 1.0 °C, salinity of 32.0 ± 1.2 ppt, and pH of 8.0 ± 0.1, with a 12:12 h light-dark cycle. Mussels were fed *Chlorella* sp. daily, at a density of 5.0×10^4 cells/ml. The systems were continuously aerated, and seawater was replaced every other day.

2.2. Mussel exposure conditions and sampling

Eight mussels of a similar size were placed in each aquarium containing 12 L of seawater (three aquariums with different Cs-137

concentrations and one with no Cs-137, serving as a control). Cs-137 solution (as ^{137}CsCl; $T_{1/2} = 30$ y) was purchased from Eckert & Ziegler Isotope Products, USA. Cs-137 was dissolved in 0.1 M HCl in a stock solution. After several pilot studies, the volumes of Cs-137 spikes were 19.8, 39.8, and 59.8 μl targeting the Cs-137 concentrations in the spiked seawater at 6.3×10^3, 12.7×10^3, and 19.0×10^3 Bq/L, respectively. Such concentrations would establish the expected dose rates of 5, 10, and 15 μGy/h. During the 48-h exposure period, conditions were consistent with those applied during the acclimation phase. At 0, 24, and 48 h of exposure, 150 ml of seawater from each experimental setup was sampled to measure radioactivity and assess external doses. For genotoxicity assays, hemolymph was extracted from the posterior adductor muscle of each mussel using a 1 ml syringe with a 21G × 1½ inch needle. Each sample (500 μl) was then transferred into a microcentrifuge tube containing an equal volume of cold calcium- and magnesium-free phosphate-buffered saline (CMF-PBS). Measurements of radiocesium activity were performed on the entire bivalve for internal dose assessment.

2.3. Cs-137 activity measurement and dose rate assessment

The activity levels of Cs-137 in seawater and whole mussels under each exposure condition were quantified using gamma spectrometry, employing a high-purity germanium (HPGe) detector over a 600-s count at 661.6 keV (Canberra, USA). The data were obtained using GENIE 2000 data analysis software (Canberra, USA). To calibrate the detector, 150 ml of standard seawater and a standard dummy mussel spiked with Cs-137 solution (as ^{137}CsCl; $T_{1/2} = 30$ y) were used. Cs-137 radiotracer was purchased from Eckert & Ziegler Isotope Products. The mean activities in seawater were calculated based on data from three time points at 0, 24, and 48 h of exposure, followed by evaluation of the achieved dose rates using ERICA Tool version 1.3.

The 3-tiered ERICA model is capable of estimating activity concentration in environmental media and organisms, calculating absorbed dose rates in selected biota, and assessing radiological effects or risks from ionizing radiation to non-human biota living in terrestrial, freshwater, or marine ecosystems. Radiation weighting factors of 10 for alpha radiation, 3 for low-energy beta, and 1 for (high-energy) beta and gamma radiation are employed by the software when estimating the weighted total (internal and external summed) dose rates [44]. In this experiment, the Cs-137 concentrations in the exposed mussels and the spiked seawater were converted into internal and external absorbed dose rates, respectively (in μGy/h). The total weighted radiation doses from Cs-137 absorbed by the studied mussels were then estimated.

2.4. Cs-137 tissue distribution

To measure the Cs-137 tissue distribution, at the end of the exposure experiment, eight mussels exposed to each of the tested Cs-137 radiation doses were dissected and separated into various body compartments, including gills, digestive gland, shell, and remaining soft tissues. The count of Cs-137 in each organ was measured for 600 s at 661.6 keV using HPGe gamma spectrometry. They were then calculated into the gross count rate in terms of count per minute (gross cpm), which is one of the commonly used quantities. The background count rate (bg cpm) was measured before the tissue counting and was subtracted from the gross cpm to obtain the net count rate (net cpm). The accumulation of Cs-137 in different body parts was reported in the form of percentage of total body load based on the net cpm/unit mass (g) for subsequent comparison.

2.5. Genotoxicity assays

For genotoxicity assays, hemolymph samples collected as described in section 2.2 were first centrifuged at 300 g for 5 min, after which the supernatant was discarded and the hemocytes were then washed twice with 1000 μl of CMF-PBS. Total hemocyte count (THC) and cell viability were determined using the Trypan Blue exclusion test, mixing hemocyte suspensions with 0.4 % Trypan Blue Solution at a 1:1 ratio and assessing them under a light microscope (Olympus CH30, Japan) at 400 × magnification using a hemocytometer. Samples exhibiting a THC of approximately 1.0×10^6 cells/ml and cell viability exceeding 75 % underwent further genotoxicity analyses, including the comet assay and micronucleus test.

2.5.1. Comet assay

The single-cell gel electrophoresis (comet) assay was performed to evaluate DNA damage in individual hemocytes, using a slightly modified version of the protocol reported by Singh et al. [45]. For each sample, 300 μl of cell suspension was mixed with 900 μl of 1 % low-melting-point agarose (Invitrogen) in CMF-PBS at 37 °C. This mixture (125 μl) was spread onto a slide pre-coated with 1 % normal-melting-point agarose, forming three replicates per sample. To prevent UV-induced DNA damage, all subsequent procedures were performed under cold (4 °C) and dark conditions. The slides were submerged in a chilled lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-base, pH 10, with fresh 1 % Triton X-100 and 10 % DMSO added) for 1 h, followed by rinsing with cold distilled water. The slides were then exposed to pre-chilled electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH > 13) for 40 min, and electrophoresis was conducted at 300 mA and 0.8 V/cm for 30 min. The slides underwent three rounds of washing by immersion in cold neutralizing buffer (0.4 M Tris-base, pH 7.5) for 5 min each. Subsequently, the slides were fixed in cold methanol for 10 min, stained with DAPI (1 μg/ml) for 15 min, and again rinsed with cold distilled water.

Nucleoids forming comet-like shapes were visualized and photographed randomly using a Nikon Eclipse 80i fluorescent microscope at 400 × magnification with a Nikon DXM1200C digital camera and the Nikon ACT-1C program (Japan). Comets were scored as the percentage of tail DNA (%TDNA) using the LUCIA Comet Assay software, version 4.81 (Laboratory Imaging s.r.o., Czech Republic). The level of DNA damage was scored using the classification system described by Mitchelmore et al. [46] as follows: no or minimal damage (<10 %), low damage (10%–25 %), moderate damage (25%–50 %), high damage (50%–75 %), and extreme damage (>75 %).

2.5.2. Micronucleus test

For the micronucleus test, a 500 μl cell suspension was fixed in a cold fixative solution (methanol:acetic acid, 3:1) at a 1:1 ratio (v/v) at 4 °C for 15 min. The suspension was then centrifuged at 300 g for 5 min, after which the supernatant was discarded. The hemocytes were then stained with DAPI (1 μg/ml) in 1000 μl of solution at 4 °C in the dark for 15 min. After staining, the cells were centrifuged and washed twice with CMF-PBS. Next, 1000 μl of CMF-PBS was added to the hemocytes, a 200 μl aliquot of the hemocyte suspension was spread onto a glass slide, and three replicates were prepared for each sample. The frequency of micronucleus formation (MNF) was assessed in 1000 cells per sample using a fluorescence microscope (Axio Imager Z2; Zeiss, Germany) at 400 × magnification equipped with a Cool Cube 1 CCD camera (MetaSystems, Germany). Micronuclei were identified in accordance with the criteria reported by Barsiene et al. [47].

2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 22.0 software. Data were assessed for normality of distribution and homogeneity of variances using Shapiro–Wilk and Levene's tests, respectively. Since the data were normally distributed, one-way ANOVA followed by Duncan's multiple rank test was used to determine the significance of differences in %TDNA and MNF among different Cs-137 dose rates. The dose dependence of both genetic responses was analyzed using linear regression.

3. Results and discussion

3.1. Cs-137 activities, dose rates, and organ distribution

After the 48-h exposure period, the activities of Cs-137 in both seawater and *Perna viridis* were measured. The dose rates achieved for the mussels, calculated using the ERICA Tool, are presented in Table 1. The specific distribution of radiocesium is reported in the form of the percentage of total body load based on net cpm/unit mass (g) across various organs, as shown in Fig. 1. A consistent pattern of organ distribution was observed across all dose rates, with Cs-137 overwhelmingly accumulating in the soft tissues (97.2%–98.1 %), rather than in the shell (1.9%–2.8 %). Among the soft tissues, Cs-137 accumulated most highly in the gills, accounting for 36.3%–39.0 % of the total. The digestive gland also showed significant accumulation, containing 31.2%–35.1 % of the total radiocesium, which was higher than the level that accumulated in the remaining tissues (25.6%–27.0 %). At the end of the experiment, Cs-137 net cpm/unit mass (g) was in the following order: gills > digestive gland > remaining soft tissues > shell.

Previous studies examined the potential impact of ionizing radiation on mollusks using external gamma radiation sources, which allowed precise manipulation of the applied radiation dose [32,48,49]. However, radionuclides contaminating marine environments pose a threat to marine organisms not only through external exposure but also internally. This study aimed to replicate the natural exposure of the native mussel *Perna viridis* to Cs-137 in a laboratory experiment. Over 97 % of the radiocesium was found in the soft tissues across all dose rates, with very little accumulating in the shell, indicating a specific organ distribution of Cs-137. Cs-137 tends to accumulate in soft tissues due to its biochemical similarity to potassium, an essential mineral and electrolyte in animal tissues [41,50]. Meanwhile, the low concentration of Cs-137 in the shell could be attributable to the poor absorption of cesium from the exoskeleton of marine invertebrates. This is because cesium does not readily react with particles and surfaces [51]. Interestingly, a consistent pattern across all radiation doses revealed a specific organ distribution of Cs-137 in this mollusk, with activity levels in the following order being recorded: gills > digestive gland > other soft tissues. The gills of marine mussels perform vital roles, including in respiration, filter feeding, osmoregulation, excretion, and maintaining the acid–base balance. They primarily and effectively absorb pollutants due to them being the anatomical site that is initially and directly exposed to a large volume of seawater from the environment and their extensive surface area [52]. Indeed, the gills were identified as one of the primary biological sites in which Cs-134 and Cs-137 accumulated in *Pecten maximus* and *Cyclina sinesis*, respectively, [53,54]. The digestive gland in bivalves is a crucial and versatile organ responsible for digestion, absorption, nutrient storage, immune defense, and detoxification. Exogenous xenobiotics entering this gland either through blood plasma or food particles are endocytosed and phagocytosed by lysosomes in digestive cells for sequestration and detoxification [52,55,56]. Upon exposure of *Perna viridis* to Cs-137 at concentrations of 123 kBq/L for 12 h and 12.3 kBq/L for 7 days, bioaccumulation in the digestive gland accounted for ~28 % and ~27 % of the total, respectively. This represented the second highest proportion, with the highest being in the soft tissue not including the digestive gland at ~70 % and ~71 %, respectively [40].

Table 1
Cs-137 expected dose rates, achieved mean activities in seawater and *Perna viridis* and dose rates. (mean ± SD).

Expected dose rate (μGy/h)	Achieved mean activity		Achieved mean dose rate (μGy/h)
	Seawater (× 10^3) (Bq/L)	Mussel (× 10^3) (Bq/kg_{ww})	
0	0	0	0
5	7.92 ± 0.05	16.5 ± 4.9	5.09 ± 0.78
10	16.4 ± 0.4	37.3 ± 6.0	11.1 ± 1.0
15	24.6 ± 0.4	51.8 ± 8.3	15.9 ± 1.3

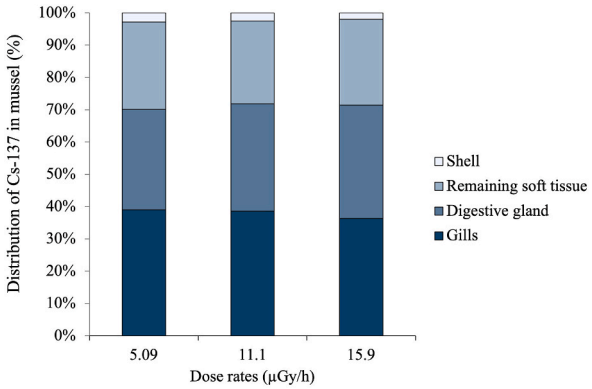

Fig. 1. Distribution of Cs-137 in specific organs (%) of *Perna viridis* under different dose rates.

3.2. DNA strand breaks and micronucleus formation

Throughout the period of exposure to Cs-137, survival was 100 % among all tested mussels. Additionally, 93.8 % of the specimens maintained optimal total hemocyte counts and cell viability, highlighting their suitability for assessing genotoxic responses. Meanwhile, the level of DNA damage, indicated by the proportion of tail DNA (%TDNA), ranged from 11.2 % ± 1.9 %–31.0 % ± 1.7 % across various Cs-137 dose rates (5.09–15.9 μGy/h). These values were significantly higher than those observed in the control group, for which %TDNA of 4.4 % ± 1.1 % ($p < 0.05$) was recorded, as illustrated in Fig. 2A. Similarly, the frequency of micronucleus formation (MNF) indicating chromosomal aberration was significantly elevated in mussels exposed to Cs-137, with values ranging from 5.4 ‰ ± 1.1 ‰–10.4 ‰ ± 1.7 ‰, compared with 2.0 ‰ ± 1.1 ‰ in the control group ($p < 0.05$) (Fig. 2B). Both genotoxic indicators demonstrated a dose-dependent increase, as evidenced by the fitted regression models ($p < 0.01$): for %TDNA, $Y = 4.09 + 1.68X$ with $R^2 = 0.953$, and for MNF, $Y = 2.34 + 0.50X$ with $R^2 = 0.843$. A significant positive correlation was observed between % TDNA and MNF, with a correlation coefficient (r) of 0.938 ($p < 0.05$).

Although cesium-137, a byproduct of nuclear fission, has advantages for various applications, it can be hazardous to marine organisms when it contaminates the ocean. Substantial evidence has shown that the gamma rays emitted from this radioisotope cause alteration in organisms. Ionizing radiation can directly break chemical bonds in DNA or indirectly induce the formation of reactive oxygen species, leading to DNA damage or chromosomal aberrations. Such genetic disruptions can have deleterious effects on cellular functions, potentially resulting in retardation of growth or reproductive ability, leading to illness and ultimately death [2,57–63]. The severity of these adverse effects can vary at the population or ecosystem level, in a manner dependent on the total dose, duration, and pathway of exposure, as well as the susceptibility of each individual species. The significantly elevated proportions of tail DNA and frequencies of micronuclei observed in *Perna viridis* following exposure to Cs-137 in the current study confirmed the genetic impact of ionizing radiation. These results are consistent with those in previous studies on *Mytilus edulis* [21,25], *M. galloprovincialis* [64], and *Crassostrea gigas* [33] exposed to tritium, as well as *M. galloprovincialis* and *Dreissena polymorpha* exposed to phosphorus-32 [30]. Our results also revealed dose-dependent effects of Cs-137 on both biomarkers which have been presented in the hemocytes of *Mytilus edulis* and in the gills and digestive gland of *M. galloprovincialis* in the case of exposure to HTO and P-32, respectively [21,30].

The strong positive correlation between micronucleus frequency and the proportion of tail DNA reflecting DNA damage as indicated in our results implies that MNF is strongly associated with DNA damage. Certain substances can induce MNF without causing

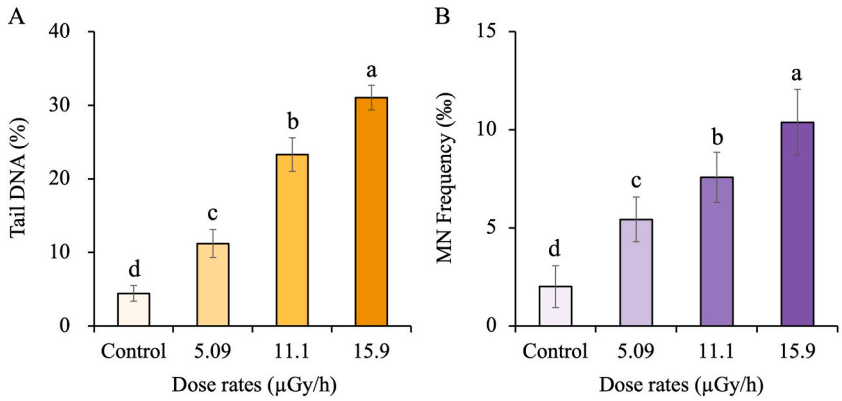

Fig. 2. Proportion of tail DNA (A) and micronucleus frequency (B) in hemocytes of *Perna viridis* exposed to different radiation doses of Cs-137 (mean ± SD, n = 7–8, $p < 0.05$).

detectable DNA damage in comet assays. This suggests that micronuclei could originate from whole-chromosome lagging after anaphase, potentially due to spindle fiber dysfunction caused by aneuploidogenic agents [65]. In addition, our findings confirm that Cs-137 can also induce micronuclei in the hemocytes of *P. viridis* following short-term exposure for 48 h. Similarly, ethyl methanesulfonate and benzo[a]pyrene have been identified as examples of genotoxic compounds that can cause MNF in *M. edulis* [66] and *M. galloprovincialis* [67,68], respectively, within the same timeframe. Based on our findings regarding the specific organ distribution of Cs-137, there is a need for future study investigating the relationship between dose rates from any other radionuclide and subsequent genetic responses in each tissue.

To establish national water quality standards, our genotoxicity results, along with previously published data and the criteria established by Mitchelmore et al. [46], have been carefully evaluated to determine whether the guideline levels of ionizing radiation for aquatic organisms, as proposed by UNSCEAR [4], are appropriate for Thai marine biota. The levels of DNA damage in *P. viridis* specimens taken from the Gulf of Thailand and Andaman Sea typically varied between 1.36 % and 2.49 % [15,16,18]. In contrast, congeners from the heavy metal-contaminated Ennore estuary in India exhibited %TDNA values of 12.44 % in the gills and 10.14 % in the hepatopancreas [69]. However, similar rates of DNA strand breaks were observed in both polluted and reference sites along the southwest coast of India [70] and in the coastal waters of Hong Kong [71]. Based on the chosen criteria [46], the %TDNA across various achieved dose rates in this study was classified as follows: no damage for the control group, low damage for dose rates of 5.09 and 11.1 μGy/h, and medium damage for a dose rate of 15.9 μGy/h.

In the absence of specific criteria for evaluating MNF, the threat of exposure to Cs-137 radiation is assessed in the current work using previous data. Frequencies of micronuclei in Mytilidae from unpolluted sites in Singapore, Brazil, Spain, and Italy as reported previously did not exceed 5.0 per 1000 cells [72–75]. The background MNF for *P. viridis* in Thai waters varied between 3.10 and 6.50 MN cells/1000 cells [16,18]. Elevated chromosomal aberrations were observed in mussels from a polluted coast in Hong Kong, with values ranging from 4.00 ‰ to 13.00 ‰ [71], and near an industrial estate in Thailand, with a mean of 20.50 ‰ [76]. The MNF results obtained in this study suggest that a dose rate of less than 11.1 μGy/h poses no threat to these mussels. Based on both genotoxic indicators, it is evident that the screening level of 10 μGy/h can be effectively applied as a safety criterion for the total dose of Cs-137 in Thai marine biota.

4. Conclusion

This laboratory experiment was a pioneering effort as, to the best of our knowledge, no study has evaluated the genotoxic effects of exposure of marine biota to low-dose cesium-137 (Cs-137), simulating natural exposure, neither in Thailand nor throughout the ASEAN region. Various doses consistently led to significant increases in DNA damage (%Tail DNA) and chromosomal aberrations (MNF) in the hemocytes of *Perna viridis*, a marine mussel species native to the Indo–Pacific region, compared with the levels in the control group. The results indicated dose-dependent increases in both of these genetic biomarkers. A positive correlation between % Tail DNA and MNF was also observed, suggesting that Cs-137 induces genetic and chromosomal alterations via clastogenicity. Our research confirms that *P. viridis* is susceptible to radiocesium exposure, which aligns with the findings of previous studies on the impact of ionizing radiation on marine invertebrates. Furthermore, soft tissues, particularly the gills and digestive gland, were identified as the primary sites of Cs-137 accumulation, reflecting the susceptibility of these tissues to radiocesium contamination.

Supporting previous studies and criteria for safe levels of DNA damage, our experimental data confirm that dose rates below 11.1 μGy/h are not harmful to the mussel. Consequently, a level of 10 μGy/h is validated as a safe threshold for local marine species in Thailand, reinforcing its adoption in national regulatory standards for Cs-137. These findings contribute to the global understanding of the impacts of radiocesium and support further implementation of safety measures.

To build on this study, future work should focus on (i) radiation dose (estimated using specific activity)-response (using a DNA-based approach) of particular compartments of the body under controlled experiments, and (ii) potential effects of Technologically Enhanced Naturally Occurring Radioactive Materials (TENORM) in Thai marine environments by field monitoring. The latter are often byproducts of various non-nuclear industrial activities, including oil and gas exploration, extraction of tantalum and niobium, coal mining, and chemical fertilizer production. The scientific data generated by such studies could then be used to further improve the national nuclear and radiation regulations to protection the marine environment from possible radiation hazards.

CRediT authorship contribution statement

Yuttanagon Sookjuntra: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Wanwiwa Tumnoi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Conceptualization. **Varalee Kongcharoen:** Investigation, Data curation. **Chitsanupong Khrautongkieo:** Investigation, Data curation. **Yutthana Tumnoi:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Ethical statement

All Animal care and experimental procedures performed in studies involving animals was approved by an Institutional Animal Care and Use Committee (IACUC), Silpakorn University, Thailand (Permit Number: 09/2563). All efforts were made to minimize animal suffering and to reduce the number of animals used. Radioactively contaminated animal carcasses were transferred to the Radioactive

Waste Management Center at the Thailand Institute of Nuclear Technology (Public Organization) to ensure safe and compliant disposal methods.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary material.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used the QuillBot paraphrasing tool and ChatGPT for grammar checking and language improvement. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Declaration of competing interest

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