ZIBELINE INTERNATIONAL™ PUBLISHING

Water Conservation & Management (WCM)

DOI: http://doi.org/10.26480/wcm.04.2024.495.500

ISSN: 2523-5664 (Print)
ISSN: 2523-5672 (Online)
CODEN: WCMABD

RESEARCH ARTICLE

EXPLORING WATER QUALITY AS A DETERMINANT OF THE EXISTENCE OF SOFT SHELL CRAB (*SCYLLA OLIVACEA*) IN DIFFERENT HABITATS OF THE LARGE MARINE ECOSYSTEM IN THE BAY OF BENGAL.

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ARTICLE DETAILS

Article History:

Received 11 September 2024
Revised 13 October 2024
Accepted 26 November 2024
Available online 2 December 2024

ABSTRACT

The present study determined the concentration of semicarbazide (SEM) in water and soil samples from diverse habitats of mud crabs encompassing natural breeding grounds, mangrove-associated rivers, different commercial farms and the tissue samples in soft-shell crabs. Semicarbazide is a residue of banned veterinary drug nitrofurazone that can be found in some natural crustaceans that have never been exposed to nitrofurazone. Analysis of water and soil sediment confirmed the presence of SEM in natural habitat, however the concentrations was very low as <0.1 ng/g throughout the study. The extraction and analysis of nitrofuran metabolites was conducted by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. The commercial farms of mud crabs were also exhibited the lowest levels of SEM in both water (0.0003 ng/g) and soil sediment (0.0005 ng/g). Tissue-specific SEM analyses encompassing muscle, shell-muscle composite, and shell revealed a distinct spatial gradient in which shell tissues exhibiting the highest concentration of SEM (3.51±0.03 ng/g) in commercial farms, surpassing those observed in muscle tissues (0.33±0.01 ng/g). Remarkably, crabs from commercial farms exhibited higher SEM concentrations across all tissue types compared to those from natural breeding grounds. However, no SEM was detected in crab feed snail and tilapia fish in commercial farms, suggesting feed composition may not be a major contributor. The lowest concentrations of SEM in water and soil sediments towards physiological processes rather than environmental contamination as the source. This study highlights limitations of SEM as a sole nitrofuran abuse marker, advocating for broader regulatory frameworks and calls for reevaluating regulations to ensure public health and responsible aquaculture.

KEYWORDS

Semicarbazide, Soft-Shell Crabs, Orange mud crab, Aquaculture, Nitrofuran Metabolite

1. INTRODUCTION

Mud crabs, also known as mangrove crabs, belong to the family Portunidae under *Scylla* genus (1). They are one of the prospective and high value crustaceans that are numerous in the muddy bottoms of brackish water near the coast, mangrove areas, and river mouths (Soegianto et al., 2022). Crustacean and several marine decapods were captured from wild habitat were reported to contain Semicarbazide (SEM) in their tissues (Zhang et al., 2016). The SEM can be occurred naturally in wild crustaceans even that have never been exposed to nitrofurans metabolites (Rairat et al., 2024). The SEM can also be detected in river water and soill sediments of the mangrove ecosystem. Nitrofurans are broad-spectrum antibacterial drugs that were widely used as aquaculture and veterinary medicine (Points, J., Burns, D.T., Walker, M.J., 2015). The uses of SEM is currently prohibited worldwide in farmed animals, including aquaculture species because of their possible carcinogenicity properties (Stadler et al., 2004). Semicarbazide has been identified as a natural component in some wild crustaceans has garnered attention due to its presence as a protein-bound metabolite associated with the banned nitrofuran nitrofurazone (Rairat et al., 2024). SEM encompasses its status as a thermal decomposition product of azodicarbonamide, previously employed as a blowing agent in plastic sealing gaskets and as a flour additive to enhance flour quality (Stadler et al., 2004). This compound has been identified as a reliable marker for illicit practices in aquaculture and poses potential risks to both environmental ecosystems and human health (Pipoyan et al., 2023). The historical application of nitrofurans as broad-spectrum antimicrobial agents represented a transformative chapter in the domains of human and veterinary medicine, offering effective control against infectious diseases (Zuma et al., 2019). However, the promising trajectory of these compounds was abruptly altered by concerns regarding potential carcinogenicity. The global response to the potential health risks associated with nitrofurans prompted their formal prohibition in food-producing animals across the European Union, instigating a pivotal shift in global agricultural practices. As early as July 1, 1993, the European Union initiated a formal ban on nitrofurans in all food-producing animals, an action that predated the official establishment of the European Union in November 1993 (Benford et al., 2015) .The global prohibition extended across major agricultural nations, such as the United States, China, Korea, Japan, and Thailand, reflecting a shared commitment to safeguarding public health and

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	Website: www.watconman.org	**DOI:** 10.26480/wcm.04.2024.495.500

Cite The Article: Md. Jahidul Hasan, Mohammad Bodrul Munir, Adriyana Syazwani Ayuni Supian, Roslianah Asdari, Md. Shoebul Islam, Rabina Akther Lima, Sheikh Tanvir Akhter (2024). Exploring Water Quality as a Determinant of the Existence of Soft Shell Crab (*Scylla Olivacea*) in Different Habitats of the Large Marine Ecosystem in the Bay of Bengal. *Water Conservation & Management, 8(4): 495-500.*

ensuring food safety integrity (Rairat et al., 2024).

As nitrofurans, including SEM, are classified as prohibited substances, the establishment of maximum residue limits (MRL) becomes a challenge (Valera-Tarifa et al., 2013). The European Union, in response, introduced minimum required performance limits (MRPL) as reference points for action (RPA) in 2003, with subsequent adjustments, including a new RPA of 0.5 ng/g starting on November 28, 2022 (Commission Regulation, 2019). However, recognizing the potential natural occurrence of SEM in crayfish, the European Union stipulates that the RPA for SEM in crayfish will be enforced only when the illegal use of nitrofurazone (NFZ) or SEM has been established. This necessitates the confirmation of at least one of the other nitrofuran metabolites, adding layers of complexity to the regulatory framework.

Various wild crustaceans have shown evidence of SEM's natural occurrence in prior research. Notable instances include crayfish giant river prawn (*M. rosenbergii*) (McCracken, 2013) , and oriental river prawn (*M. nipponense*) and several marine decapods (Zhang et al., 2015; Huizhen et al., 2023; Li et al., 2021; Hui-Juan et al., 2012). Within this context, crabs (*S. olivacea*) serve as a noteworthy subject for investigation, given their role as a vital link in the aquatic food chain, their ecological importance, and their economic significance in regions such as Bangladesh (Saari and Peltonen, 2004). Investigations into mud crab (*Scylla* sp.) shells have reported SEM levels in diverse geographic locations and capture contexts. Specifically, wild-caught specimens in Belgium exhibited concentrations ranging from 94.12 to 12.6 ng/g (Van Poucke et al., 2011). The analysis commercial samples in China reported 81.8 ng/g and wild-caught specimens in Thailand showcased levels between 1.65 and 22.75 ng/g (Rairat, 2024; Van Poucke et al., 2011; Hui-Juan, 2012). Furthermore, a study revealed SEM concentrations in wild caught crab muscle ranging from 0.13 to 0.40 ng/g in Thailand (Rairat et al., 2024). Despite this breadth of research, there remains a conspicuous gap in the literature pertaining to SEM levels in soft shell crabs sourced from either natural habitats or commercial farms, thereby necessitating further investigation to discern the origins of SEM contamination in this specific crustacean species. Despite the widespread concern over the potential health risks associated with nitrofurans and their metabolites, a comprehensive examination of SEM in the context of soft-shell crabs in Bangladesh is notably absent from the existing body of literature. The study aimed to unfolds against the backdrop of the distinct spatial variations of shaping SEM concentrations in soft-shell crabs (*S. olivacea*). By offering a holistic perspective, this research seeks to contribute significantly to understanding SEM dynamics in water and soil sediments of mud crabs breeding grounds, and mangrove-associated rivers of mangrove ecosystem including the shell and tissue of mud crab populations.

2. Materials and Methods

2.1 Collection of Water and Soil Sediment Samples

Samples were meticulously gathered from natural breeding grounds in the Bay of Bengal, different rivers in mangrove areas, and three soft shell crab farming companies in the Khulna and Satkhira districts (Figure 1). Water and soil sediment samples were randomly collected from three different locations at 0.5 km distance of each site. The samples were then mixed homogenously and prepared a composite sample (Santos-Fernández et al., 2015. Water samples were collected from 2–3 feet below the surface level and sediment samples were collected from up to 20 cm depth of soil sediments by using sampling tubes (Boncompagni et al., 2003; Boncompagni et al., 2003; Hannan et al., 2024). The selected samples were intended to represent the diverse habitats and aquaculture practices associated with wild habitat and soft shell crab production within the specified regions. All collected samples were sent to an accredited Quality Control Laboratory in Khulna, where liquid chromatography coupled with mass spectrometry (MS) was utilized to detect and quantify semicarbazides. Ethical guidelines and regulations were strictly followed to ensure compliance with standards.

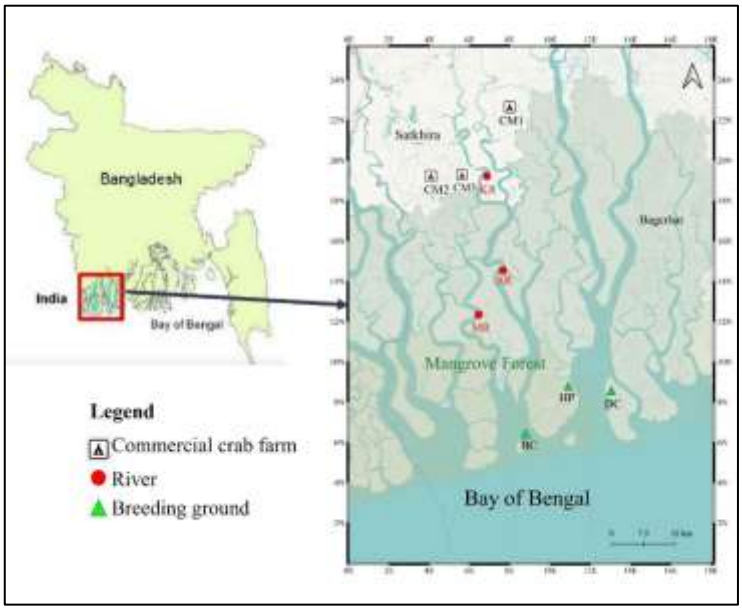

Figure 1: Experiment site (HP=Hiron point, BC=Bangabandhu Char and DC=Dublar Char, AR=Arpanagsia River, KR=Kholpetua River, MR=Maloncho River, CM=Commercial Crab farms-CM1, CM2, CM3)

2.2 Water Quality Assessment

Dissolved oxygen (DO), temperature, pH, salinity, total alkalinity, and ammonia contents of the pond water were measured between 9.00 and 10:00 am after seven-day intervals. The water quality parameters of the breeding ground and different location of rivers at Sundarbans mangrove region were conducted by using potable instruments. Salinity was measured using a transportable refractometer (ATAGO). A common centigrade thermometer was used to measure the surface water's temperature. A digital multimeter (HQ 40d digital multimeter, HACH) was used to record the water's pH and dissolved oxygen levels. Titrimetric analysis was utilized to calculate the total alkalinity (APHA, 2000). An ammonia test kit (HANNA) was used to determine the ammonia nitrogen level.

2.3 Collection of Mud Crab Samples

Mud crab samples were collected from all sampling sites by maintaining hygienic conditions. All samples of mud crabs (*S. olivacea*) were transported to the laboratory in a frozen state and subsequently stored at -20 °C prior to analysis. Individual crabs were thawed, and the exoskeleton was carefully removed. The tail meat of each crab was then minced using a food blender before being subjected to analysis. Simultaneously, the corresponding shells of each crab were separated, dried overnight at 60 °C, and subsequently cooled to room temperature. The dried shells were finely ground using a pestle and mortar to obtain a homogeneous sample for further analysis. This meticulous sample treatment process ensured the preparation of both muscle and exoskeleton samples for subsequent assessments of semicarbazide content (Zhang et al., 2015). The choice of these sample treatment steps aimed to provide accurate and representative data on the presence of contaminants in soft shell crab samples.

2.4 Extraction and Analysis of SEM from Water, Soil Sediments and Crabs

SEM analysis was done according to the procedure of (Cooper et al., 2005). The extraction procedure for analyzing nitrofuran metabolites via liquid

chromatography-tandem mass spectrometry (LC-MS/MS) involved weighing 1.00 ± 0.05 g portions for soil and soft-shell crab (*S. olivacea*) meat and 1.00 ± 0.05 ml for water. Samples and known negative tissue were taken into 50 ml centrifuge tubes for subsequent matrix blank and spiked recovery samples. Protein precipitation was achieved by adding 8 ml cold methanol, vortexing for 1 minute, and centrifuging at 4000 rpm for 4 minutes, with subsequent methanol discarding and a repeat using 4 ml methanol. Chemical treatment follows, incorporating 5 ml of 0.2 M HCl and 50 µl of nitrobenzaldehyde, along with the addition of 200 µl of 10 ng/ml d5-AMOZ and 100 µl of the 10 ng/ml working spiking standard to recovery tubes. Incubation occurred at 37 ± 2 °C for 16 ± 2 hours, emphasizing light avoidance. Neutralization was accomplished by adding 500 µl of 0.3 M KH_2PO_4 to each tube and adjusting to pH 7.0 ± 0.5 with 1 M NaOH solution. Extraction involved the addition of 4 ml ethyl acetate, vortexing, centrifugation at 4000 rpm for 8 minutes, and combining organic layers through repeated extractions. Evaporation to near dryness under nitrogen at 45°C was followed by reconstitution with 1 ml 50% methanol, vortexing, filtration through a 0.45 µm syringe filter, and collection in a vial. The resulting derivatives were subjected to LC–MS/MS analysis using an Acquity UPLC (R) BEH C18 column (1.7 µm, 2.1x100 mm) on an A10UPH2878 LC system (Waters Singapore) coupled with a QBB 933 triple quadrupole mass spectrometer (Waters UK) operating in positive electrospray mode. The chromatographic separation and mass spectrometric analysis facilitated the identification and quantification of SEM. AMOZ-d5 is carried through the analytical procedure to compensate for any analyte loss and for ion suppression during the MS stage. Results were calculated against standard curves, and concentrations were expressed as nanograms per gram (ng/g) wet weight of soft-shell crab meat and shell, ensuring a comprehensive assessment of SEM content using advanced analytical techniques.

2.5 Statistical Analysis

The Statistical Package for the Social Sciences, Version 25 (SPSS, Chicago, IL, USA) was used to compute basic descriptive statistics, such as minimum, maximum, mean, and standard error for every location and treatment. Boxplot analysis was performed using R version 4.2.2. The statistical significance between the experimental groups by one-way ANOVA (Mann Whitney U test) was also performed using SPSS, and a 95% significance level was considered**.** The Duncans Multiple Range Test (DMRT) was employed to examine the disparity in means among different location with respect to the standard error of the mean (SEM) within both water and bottom soil environment.

3. RESULTS

3.1 Water Quality Parameters

Water quality was evaluated during the sampling process in the wild to elucidate the natural aquatic conditions pertinent to crab habitats. The resultant water quality metrics are presented in Table 1. Salinity levels ranged from 16 to 23 ppt, indicative of an estuarine water environment. The pH measurements fell within the range of 7.5 to 8.10, denoting a slightly alkaline condition. Furthermore, water temperatures were recorded between 18 to 22 °C, while dissolved oxygen concentrations exceeded 6 mgL^{-1}. The total dissolved solids (TDS) exhibited a range between 3919 to 4554 mg/L, while conductivity ranged from 7034 to 9324 µS/cm. Hardness measurements fell within the range of 2978 to 5650 mg/L.

Table 1: The average (mean±SE) value and range of water quality parameters in different sampling locations.

Location	Salinity (ppt)	pH	DO (mg/L)	TDS (mg/L)	Conductivity (µScm^{-1})	Temp (□)	Hardness (mg/L)
BG	20.67±1.20 16-17	8.07±0.03 7.5-8.0	7.70±0.21 6-7.7	4046.33±68.76 4351-4554	7917±43.92 7034-9324	19.33±0.67 20-22	4050±18.91 2978-5650
RV	16.33±0.33 19-23	7.70±0.15 8-8.10	6.57±0.56 7.4-8.10	4445.33±59.04 3919-4155	7827±748.94 7839-7991	21±0.58 18-20	3876±887.02 3987-4050
Total	18.50±1.12 16-23	7.88±0.11 7.5-8.10	7.14±0.37 6-8.10	4245.83±97.99 3919-4554	7872±336.12 7034-9324	20.17±0.54 18-22	3944.67±397.97 2978-5650

Note: BG=Breeding ground, RV=River; Values in the parenthesis indicate the range of the parameters.

3.2 Spatial Occurrence of SEM in Mud Crabs

The spatial occurrence and concentration of crab muscle, muscle-shell combined and shell semicarbazide (SEM) are presented in Table 2. Semicarbazide was found in all samples investigated at different degrees. The study found significant (ANOVA, $P < 0.05$) spatial variation among the samples (Table 2). The mean concentration of SEM was highest for muscle (0.33±0.01 ng/g), shell-muscle combined 3.13±0.11 ng/g, and shell 3.51±0.03 ng/g respectively in commercial farming areas (FR) (Figure 2). On the other hand, SEM levels were in the lowest concentration in muscle (0.29±0.01 ng/g), shell-muscle combined 1.55±0.33 ng/g and shell 3.21±0.05 ng/g respectively from breeding ground areas (BR) (Figure 3). Moreover, the SEM concentration showed a significant difference (ANOVA, $P < 0.01$) among tissue samples (Figure 2), and the highest was recorded in shell (3.45±0.03 ng/g) compared to muscle tissue (0.33±0.01 ng/g) respectively (Table 2). The reference point for action level was within the limit (≤ 0.50 ng/g) for muscle and over the limit (> 0.50 ng/g) for shell and shell-muscle combined samples (Figure 3).

Table 2: The average (mean±SE) value and range of semicarbazide (ng/g) occurrence in mud crabs (*Scylla olivacea*) from different locations.

Location	Muscle		Shell-muscle combined		Shell		F value	P Value
	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range		
BG	0.29±0.01	0.26-0.32	1.55±0.33	1.11-2.54	3.21±0.05	3.06-3.35	3.89	0.03
FR	0.33±0.01	0.27-0.39	3.13±0.11	2.15-4.12	3.51±0.03	3.28-3.74	10.96	0.00
RV	0.33±0.02	0.31-0.39	2.77±0.64	2.01-4.07	3.37±0.08	3.27-3.55	9.32	0.00
Total	0.33±0.01	0.27-0.39	2.85±0.15	1.11-4.12	3.45±0.03	3.06-3.74		

Note: BG=Breeding ground, FR=Commercial farming, RV=River, RPA =Reference point for action.

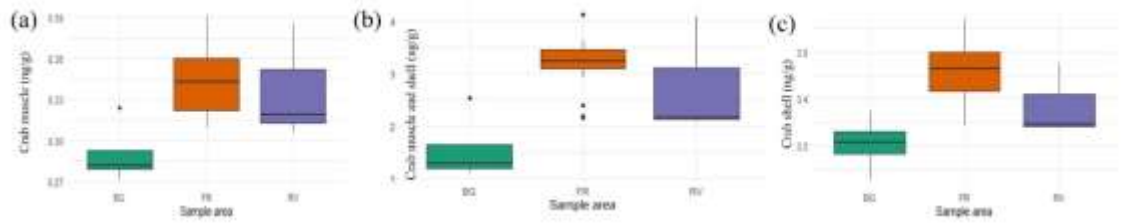

Figure 2: Spatial difference of semicarbazide in mud crabs (*S. olivacea*) from different locations (BG=Breeding ground, FR=Commercial farming, RV=River). The box plot's three horizontal lines from top to bottom represent the 75 %, 50 %, and 25 % quartiles, respectively. Blocks of different colors indicate the average value; the vertical bar above the box represents values from the 75 % quartile to the maximum, and the vertical bar below the box represents values from the 25 % quartile to the minimum value. The points represent the 5th and 95th percentiles.

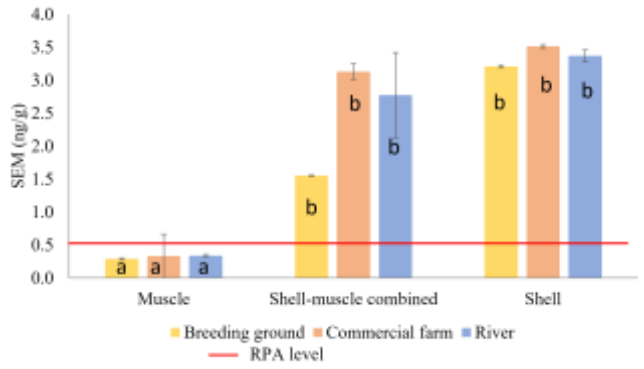

Figure 3: Semicarbazide concentration level in crab tissue from different sources and reference point for action (RPA) level .

3.3 Spatial Occurrence of SEM in Water and Soil Sediments

The spatial distribution of standard error of the mean (SEM) is delineated in Table 3. The SEM values demonstrated a degree of uniformity between between bottom soils in breeding grounds and commercial farms. However, a pronounced disparity was observed in river bottom soil, where SEM values surpassed those of the former environments. Conversely, water SEM levels exhibited significant heterogeneity, as detailed in Table 3. Across all instances, SEM values remained below 0.1 ng/g.

Table 3: The mean (mean±SE) value of semicarbazide (ng/g) in soil and water from different location.

Sample	BG	FR	RV
Bottom soil	0.0007±0.0003[a]	0.00057±0.0001[a]	0.087±0.005[b]
Water	0.004±0.0001[a]	0.0003±0.0001[b]	0.006±0.001[c]

Note: Values in each row with different superscripts are significantly different ($P < 0.05$). (BG=Breeding ground, FR=Commercial farming and RV=River).

3.4 Quantification of SEM in Snail and Tilapia Fed to Commercial Mud Crab Farms

The study investigated the effect of other feeds on SEM concentration in soft-shell crab muscle, shell-muscle combined, and shell. Commercial feed showed no significant variation (ANOVA, $P > 0.05$) between snail and tilapia feed (Table 4).

Table 4: The mean (mean±SE) value of semicarbazide (ng/g) of softshell crab fed with snail and tilapia feed in commercial farms.

Treatment	Muscle		Shell and muscle combined		Shell		F value	P Value
	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range		
Snail feed	0.34±0.01	0.31-.37	3.08±0.16	2.15-3.54	3.52±0.04	3.31-3.67	0.262	0.772
Tilapia feed	0.32±0.01	0.27-.35	3.33±0.11	3.16-3.54	3.58±0.03	3.51-3.65	0.521	0.603

The SEM concentration for muscle ranges (0.31-.37 ng/g), shell-muscle combined 2.15-3.54 ng/g and shell 3.31-3.67 ng/g respectively. In the case of tilapia feed, the SEM concentration for muscle ranges (0.31-.37 ng/g), shell-muscle combined 3.16-3.54 ng/g and shell 33.51-3.65 ng/g respectively (Figure 4).

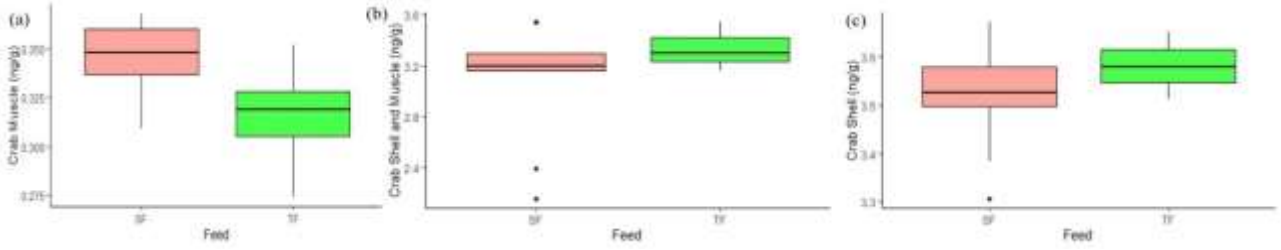

Figure 4: Semicarbazide of softshell crab (*S. olivacea*) fed with snail and tilapia feed in commercial farms. The box plot's three horizontal lines from top to bottom represent the 75 %, 50 % and 25 % quartiles, respectively. Block of different colors indicate the average value; the vertical bar above the box represents values from the 75 % quartile to the maximum and the vertical bar below the box represents values from the 25 % quartile to the minimum value and the points represents the 5th and 95th percentiles.

4. Discussion

The commercial raising of mud crabs is a substantial industry throughout Southeast Asia (Pavasovic et al., 2004). however the mud crabs are associated with the presence of Semicarbazide (SEM) contaminat. The health and survival of marine creatures are at risk due to the SEM, a common contaminant in marine ecosystems that can result in cancer and cell mutation (Zhou et al., 2022). SEM has emerged as an significant environmental and food contaminant because of its bio-toxicity and transmission through the food chain. SEM generated in aquatic environment in a variety of ways forming a new contaminant for marine ecosystem that can be accumulated in the water, soil sediments and muscle tissue of crustaceans that is the concern for human health because it has chances of bioaccumulation (Tian et al., 2017). As a result, SEM contamination in seafood-producing regions could pose a significant risk to human health (Tian et al., 2017). The present study findings revealed the spatial variation of the SEM occurrence and concentration at different levels in water, soil sediments and mud crab tissues. Analysis of water quality data provides insight into the ideal natural environment for mud crabs (*S. olivacea*). According to a review optimal water conditions for growth and acclimatization include a temperature range of 27–30 °C and salinity of 15-34 ppt by (Pati et al., 2023). Additionally, a pH of 7.8–8.2 is considered ideal for normal growth and physiological functions of mud crabs. SEM can be occurred in crustaceans naturally through its normal physiological conditions (Rairat et al., 2024). This is likely due to SEM absorption in marine environments (Tian et al., 2017). The crab muscle was recorded with the lowest and highest concentrations in the shell. Previous studies on the SEM concentration of crabs also recorded higher concentration in the shell than in the muscle (Table 5), which signifies that SEM originates in the shell and is then distributed to the muscle (McCracken et al., 2013; Rairat et al., 2024).

Table 5: Comparative review on semicarbazide (SEM) from a natural source and commercial farm (presumably had no use of NFZ usually).

Source	Country	Tissue	SEM (ng/g)	References
(Wild caught, imported)	Belgium	Shell	4.1-12.6	Santos-Fernández, et al., 2003.
Commercial sample	China	Shell	81.8	Hannan et al., 2024.
Wild	Eastern Thailand	Shell	3.52-22.75	Santos-Fernández et al., 2015
		Muscle	0.13-0.40	
Wild	Western Thailand	Shell	1.65-17.07	
		Muscle	<0.10	
Commercial Farm	Bangladesh	Shell	3.28-3.74	Present study
		Muscle	0.27-0.39	
Wild (Mangrove rivers and the Bay of Bengal)	Bangladesh	Shell	3.06-3.55	
		Muscle	0.27-0.39	

The measured SEM concentrations aligned with those reported for wild crabs (Table 5). The concentration of SEM in mussels, shrimps, and sea cucumbers was detected as low level such as 0.5 µg/kg to 1.0 µg/kg without utilizing NFZ reported by the institute in Shandong Province (Tian et al., 2017). Furthermore, it was reported that the tissue-bound SEM in mud crab muscle ranged 0-0.40 ng/g, and 1.65-22.75 ng/g in mud crab shell that was similar to the present study (Rairat et al., 2024). Besides SEM was not detected in blue swimming crab muscle but present at the shell ranged 0-2.92 ng/g however the tissue-bound SEM was detected in 33% of the muscle samples of the giant river prawn (McCracken et al., 2013). It is reported that SEM is commonly found in wild crustaceans that have never been exposed to NFZ meaning it occurs spontaneously however, the biosynthetic mechanisms and physiological actions in crustaceans are still unknown (Rairat et al., 2024). The analysis reported that SEM can be biosynthesized through a variety of processes from precursors including as urea, arginine, lysine, histidine, glutamine, citrulline, and hydrazine (Zhang et al., 2016). Furthermore, whether the SEM is created by the animal or symbiotic microbes, and how the SEM biosynthesis is related to the molting cycle, age, body size, nutritional state, and other physiological parameters, remain unresolved and warrant further investigation (Rairat et al., 2024).

On the other hand, analysis of crab feed revealed no significant discrepancies between snail-based and tilapia-based feeds (Table 4). This finding suggests that dietary sources were unlikely to be responsible for the elevated SEM. Moreover, SEM concentrations in the culture farm's water and bottom sediment were lower compared to the natural habitat (Table 3). The overall higher SEM levels in the crabs from the commercial farm compared to the wild population suggest that a combination of cultural practices and the crabs' physiological responses within that environment may have contributed to the observed accumulation.

Interestingly, spatial variations in water and bottom sediment SEM were observed, albeit at very low levels (<0.1 ng/g). The study demonstrated a temperature-dependent uptake and elimination of drugs in crab hemolymph at 26 °C, suggesting a potential link between lower habitat temperatures and SEM accumulation (Fang et al., 2008). Consequently, deviations from the optimal habitat temperature could potentially influence SEM deposition. Given the known biosynthesis of SEM from metabolic byproducts and fertilizers the observed low concentrations likely represent physiological processes rather than environmental contamination (Rairat et al., 2024; Ec Commission Regulation (EU), 2019/1871). However, additional expansion is necessary for this research because there are few sampling sites, geological data, in-depth investigations on other farming practices, and sufficient literature data on that species. The study can serve as a springboard for further research into SEM occurrences devoid of NFZ-implemented products.

The natural occurrence of SEM at different tissue levels, which is very high from the RPA level, is proven. As a result, this crustacean requires immediate attention to the RPA level of SEM. Thorough research into the SEM biosynthesis pathway is necessary to determine the valid reasons behind this phenomenon. In addition, the use of SEM alone to detect NFZ abuse raises doubts; alternate methods or the inclusion of other chemical remnants of nitrofuran abuse, such as metabolites like AOZ, AMOZ, and AHD, need to be considered (Rairat et al., 2024; Ec Commission Regulation (EU), 2019/1871).

5. Conclusion

Mud crab (*S. olivacea*) tissues and their surrounding environment exhibited spatial variability in SEM concentration. Shells contained significantly higher SEM levels compared to muscle tissue. Interestingly, commercially farmed crabs displayed the highest tissue concentrations of SEM, even though the water and bottom sediment surrounding these crabs had the lowest SEM concentrations of all sampling locations. This suggests that factors specific to commercial aquaculture practices, rather than dietary sources, may be driving the observed SEM accumulation in these crabs. Further research is needed to investigate these potential contributing factors, which could include elements of water management, pond substrate composition, or interactions with the crabs' natural physiology during the molting process. Additionally, the role of SEM in crustacean molting chemistry warrants further exploration. The current SEM guidelines should be reevaluated, as SEM occurs naturally exceed established thresholds, questioning SEM as a sole marker for nitrofuran abuse in crustaceans. A broader approach incorporating additional markers and a more in-depth analysis of nitrofuran metabolites is likely necessary.

Acknowledgement

This study was funded by United Nations Development Programme (UNDP), Bangladesh office. The authors are grateful to the management of Japan Fast Trade Ltd. (JFTL) for providing research facilities.

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