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INVITED REVIEW

Crustaceans (shrimp, crab, and lobster): A comprehensive review of their potential health hazards and detection methods to assure their biosafety

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Abstract

Crustaceans are popular seafood items worldwide owing to their rich nutritional value, unique tastes, and their incorporation in a variety of cuisines. There has been a great concern about the safety of crustaceans for human consumption being more prone to hazardous contaminants due to their exposure to diverse habitats and unhealthy farming and handling practices. These hazards can arise from chemical contaminants such as heavy metals, environmental pollutants, and biotoxins or biological sources, *that is*, pathogenic microbes and parasites. The different types of chemical contamination of crustaceans as well as biological hazards are reviewed as major part of this review. Although there are many reviews on contaminants in fisheries, nothing is traces to crustaceans. The current review compiles the food safety hazards of crustaceans arising from both chemical and biological origins and their impact on human health in farmed *versus* wild origins. The different methods of contaminants detection, *viz.* microbiological, molecular, and analytical methods, as well as control measures *viz.* cooking and processing methods that can be implemented to safeguard consumer safety are also reviewed. Future perspectives have been raised toward HACCP protocol implementation during handling, processing, and storage of crustaceans and posing real-time freshness monitoring tools such as intelligent packaging.

1 | INTRODUCTION

Crustaceans are invertebrates with segmented bodies protected by chitinous shells including shrimp, lobster, crayfish, crab, and krill. They form a crucial part in different seafood cuisines owing to their rich food value and acceptable sensory characters (Venugopal & Gopakumar, 2017). Major crustaceans are reviewed in this article, *that is*, shrimps, crabs, and lobsters. Among the most studied species are brown crab (*Cancer pagurus*), swimming crab (*Polybius henslowii*), warty crab (*Eriphia verrucosa*), blue swimmer crab (*Portunus pelagicus*), Chinese mitten crab (*Eriocheir sinenesis*), longlegged spiny lobster (*Panulirus longipes*), red rock lobster (*Jasus edwardsii*), sipper lobster (*Panulirus versicolor*), king prawn (*Litopenaeus vannamei*), speckled shrimp (*Metapenaeus monoceros*), pink shrimp (*Farfantepenaeus brevirostris*), rainbow Shrimp (*Parapenaeopsis sculptilis*), and Mantis shrimp (*Oratosquilla oratoria*) which are depicted in Figure 1, and discussed in text. Crustaceans are high-valued marine animals owing to their nutritional value being rich in proteins, essential elements, fatty acids and amino acids, vitamins and polyunsaturated fatty acids, such as omega-3 and omega-6 (Venugopal & Gopakumar, 2017).

In 2018, crustaceans were produced at 9.4 million tons in the world aquaculture, accounting for *ca.* 69.3 billion USD. They have become economically important seafood ingredients, with some of them being farmed and others are being harvested from wild stocks. However, substantial economic losses occur due to crustacean diseases which need rapid detection and control measures to be adopted (Tong et al., 2022).

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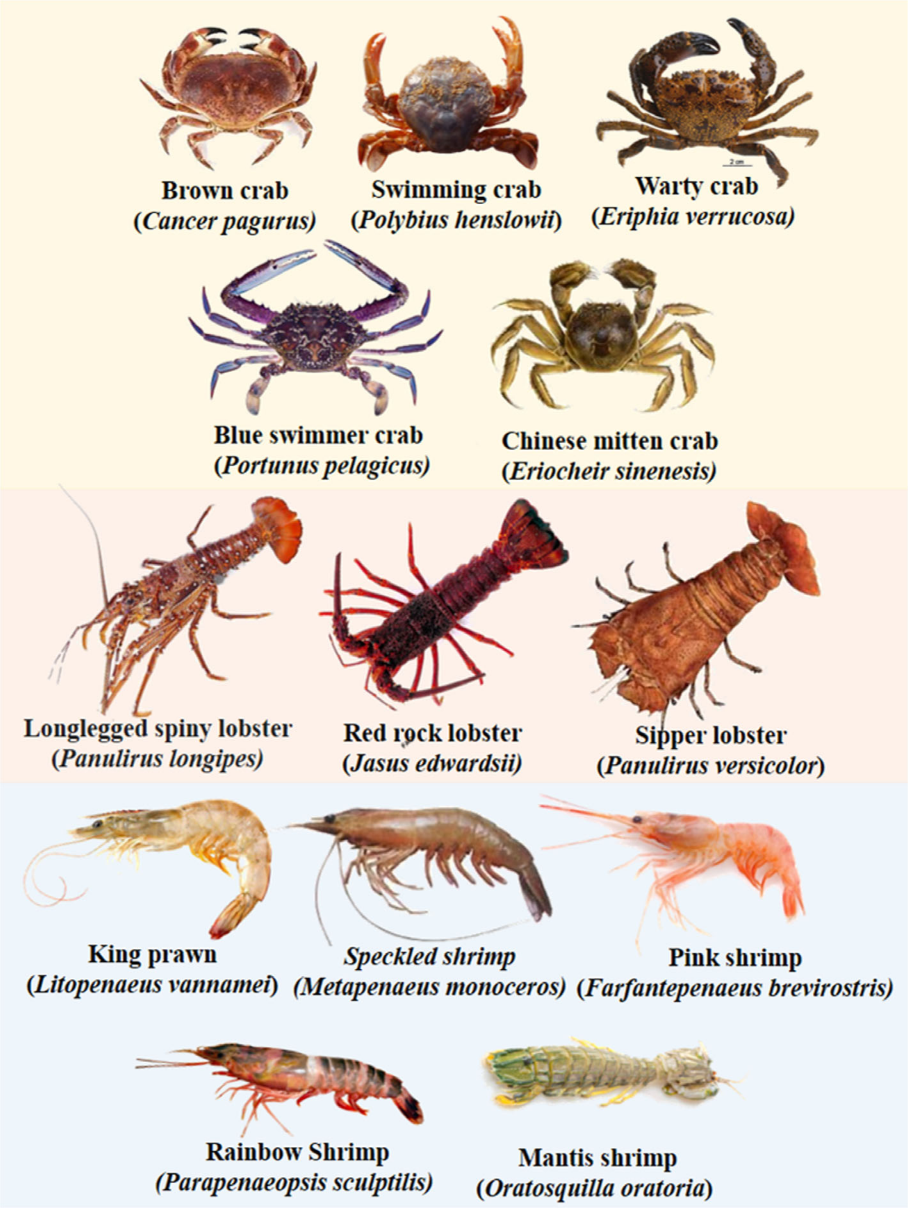

FIGURE 1 Different types of crustaceans, *that is*, crab, lobster, and shrimp discussed in this review

Regarding the world seafood production, crabs are ranked the third seafood item after shrimps and lobsters (Narayanasamy et al., 2020). Nevertheless, the consumption of these crustaceans is still vulnerable to some health risks arising from contamination with different chemicals such as heavy metals that accumulate in their flesh from the surrounding environment contaminated with industrial and agricultural wastes, untreated sewage effluents, and mining activities (Abd-Elghany et al., 2020). In that regard, farmed crustaceans are found to be less prone to chemical hazards than their wild-caught counterparts due to the precautionary measures applied in farming aquaculture production sites (Mostafiz et al., 2020).

Furthermore, the increasing globalization of seafood cuisines and the growing trend of consuming raw or minimally processed crustaceans have posed many biological hazards arising from crustaceans infestation with pathogenic microbial species, *that is*, bacteria, parasites, viruses, and fungi which cause food-borne illness to humans upon their consumption to their consumers with different toxicity symptoms ranging from mild to life-threatening and chronic adverse reactions (Venugopal & Gopakumar, 2017).

Crabs and shrimps could accumulate more metals than any other fish species as they stir up while feeding and hence to collect organic matter containing high amounts of heavy metals. These heavy metals are not readily degraded, and continuously bio-accumulate to levels causing undesirable toxic effects on human beings such as acute and chronic degenerative disorders to central nervous system (CNS), liver, and kidney. Dioxins are also hazardous chemicals that are nonvolatile, and resistant against degradation due to their long shelf-life of 7–10 years. Even a short-term exposure to high dioxins levels can result in altered liver functions whereas, exposure for long-term can impair the immune system, nervous system, and the endocrine system (Sinkkonen & Paasivirta, 2000). Biotoxins belong to chemical hazards although they are produced from microorganisms. Harmful algal bloom may occur in the coastal waters which is hazardous due to the proliferation of poisonous algae, *that is*, dinoflagellates and diatoms which can be fed by crustaceans and hence to accumulate toxins in their bodies. After consumption of biotoxin contaminated crustaceans, various poisoning symptoms appears ranging from acute gastrointestinal and neurological manifestations to allergic reactions which can be attributed to their effects on sodium and calcium channels, enzymes, sodium–potassium ATPase and protein phosphatases (Garthwaite, 2000).

Since pesticides are widely used to protect organisms against different infections, their residues can accumulate inside the tissues of marine creatures without being degraded due to their lipophilic nature. Therefore, excessive consumption of these seafood products might place regular consumers at risks of bioaccumulation of many hazardous chemicals, especially if consumers do not use efficient cooking practices that could reduce the bio-accessibility of these hazardous chemicals in foods (Abd-Elghany et al., 2020). This necessitates special considerations to be taken during processing, packaging and storage processes, in addition during cooking (Venugopal & Gopakumar, 2017).

Even though chemical and biological contaminants in crustaceans pose serious health hazards, information on their content, distribution, and their detection methods and control measures are not comprehensively compiled in literature. Accordingly, a compilation of the available knowledge with analysis on the different chemical and biological hazards to occur from the consumption of crustaceans, *that is*, shrimp, crab, and lobster, is presented for the first time in this review. Furthermore, analytical and molecular tools employed for monitoring crustacean biosafety are summarized along with the control measures of the different hazards. A layout summarizing the covered topics in this review is outlined in Figure 2.

2 | SEARCH STRATEGY METHOD

A comprehensive review was conducted through a rationalized search of the published literature with a focus on the effect of the chemical and biological hazards resulting from crustaceans' consumption. We comprehensively searched PubMed and Web of Science (up to November 2021) for published literature.

This comprehensive review included all articles reporting for animals and human studies and clinical trials, written in English and excluding other languages. We excluded the articles that were not published as full text or published in summary or as conference abstracts. The following search terms were used (“Crustacean” AND “Lobster” AND “Crab” AND “Shrimp” AND “Bacterial infection” AND

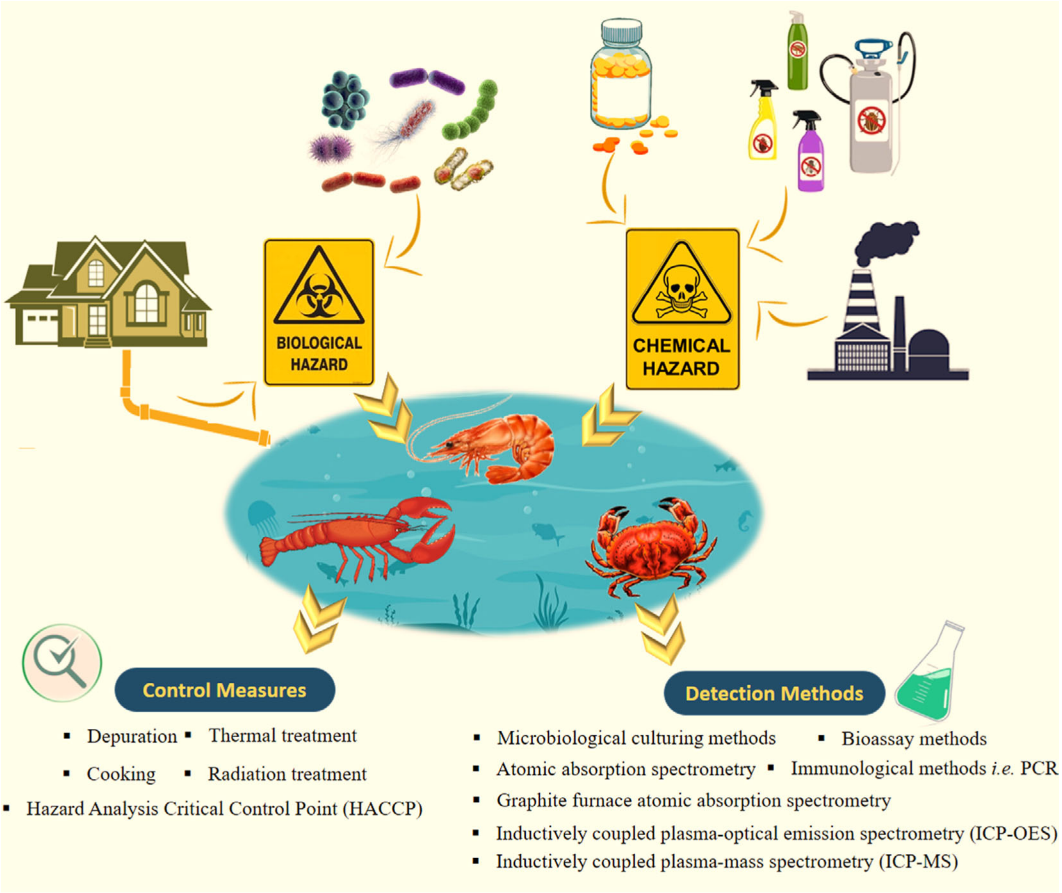

FIGURE 2 Overview of the topics covered in this review; chemical and biological hazards in crustaceans, *that is*, crab, lobster, and shrimp, detection methods and control measures

"Parasites" AND "Viruses" AND "Microbial hazards" AND "Chemical hazards" AND "Heavy metals" AND "Biotoxins" AND "Pesticides" AND "Food safety" AND "Control measures" AND "Detection methods"). The search yielded over 200 articles published between 2000 and 2021 from Web of science, Scopus, and Pubmed DATABASES. We screened the titles and abstracts of the search results according to our inclusion/exclusion criteria. About 140 articles that met these criteria were cited in this review.

3 | BIOLOGICAL HAZARDS: RISK ASSOCIATED WITH CRUSTACEANS AND THEIR CONTROL METHODS

3.1 | Microbial pathogens

Pathogenic bacteria, parasites, viruses, and fungi which live in the animal produces what is so called "zoonotic infection" leading to biological hazards that may cause significant harm to humans. The zoonotic infection can be spread directly or indirectly by eating undercooked meat or getting into close contact with an infected animal. These zoonotic diseases can cause mild to life-threatening cases. Microbial pathogens present the major class of biological hazards in crustaceans (Lee et al., 2006). This section will present upon the various types of pathogenic microbial species, *that is*, bacteria, parasites, viruses, and fungi reported to infest crustaceans causing food-borne illness to humans upon their consumption.

3.1.1 | Bacterial pathogens

The most common of the food-borne diseases are those of bacterial origin, which is considered the most difficult to control because of their rapid progression. The major pathogenic organisms implicated in seafood-borne diseases are *Salmonella* spp. such as *Salmonella paratyphi*, *S. enteritidis*, and *S. typhimurium*, enterohemorrhagic and cytoxin-producing strain of *Escherichia coli*, *Vibrio* spp., *Shigella* spp., *Campylobacter* spp., *Aeromonas* spp., *Plesiomonas* spp., *Yersinia enterocolitica*, *Clostridium botulinum*, and *Listeria monocytogenes*. Bacteria which can cause disease *via* the ingestion of seafood are classified into three groups. The first group includes bacteria that are typically found in crustaceans as their natural habitats, *that is*, *Vibrio* spp., non-proteolytic *Clostridium botulinum*, and *Plesiomonas*. The second group includes bacteria that are widespread in the water such as *Listeria* and *Bacillus* species. The third group is the bacteria which have two habitats in both animals and humans like *Salmonella* and *Shigella* (Ryder et al., 2014). Bacteria are the primary cause of spoilage in crustaceans and their products (Daschner, 2016).

On the other hand, the coastal water itself and its microbial count plays a key role in the spoilage of freshly hunted crustaceans. The potential of spoilage bacteria to cause illness typically depends on the amount of ingested bacteria as in terrestrial food. Only a high concentration of the bacteria can cause self-limiting gastrointestinal symptoms (Lehel et al., 2021). In contrast with spoilage bacteria, pathogenic bacteria will be able to cause diseases and infection. However, they are not detected easily as compared to spoilage bacteria because no change in the color, odor, and structure is observed in food. Food intoxication means the presence of bacterial toxin in the food, however, food infection is caused by the presence of the bacteria itself in the crustaceans which will later secrete toxins in gastrointestinal tract. A list of the various pathogenic bacteria reported to infect different crustaceans is presented in Table 1.

Vibrio species

Vibrio species is a gram-negative bacteria that have been identified as major causative organism of human diseases associated with crustacean's consumption. All aquatic crustaceans are susceptible to infection by many Vibrio spp. including *Vibrio parahaemolyticus*, *V. fuvialis*, *V. vulnifcus*, *V. hollisae*, *V. mimicus*, *V. alginolyticus*, and *V. cholera* causing substantial production losses. Among which, *V. parahaemolyticus* is reported to frequently contaminate crustaceans in their marine habitats, particularly in warm waters (Venugopal & Gopakumar, 2017). *Vibrios* are among the fastest growing bacteria in nature if they are

TABLE 1 List of pathogenic bacteria reported to infect different crustaceans

Crustaceans species	Bacterial strain/type	References
Mullet, shrimp, and canned lobster	*Clostridium botulinum* (Anaerobe)	Lalitha and Gopakumar (2001)
Shrimp and crab	*Clostridium perfringens* (Anaerobe)	La Sala et al. (2015)
Shrimp	*Listeria monocytogenes* (Anaerobic)	Gambarin et al. (2012)
Shrimp, crabs, and rock lobster	*Escherichia coli* (Facultative anaerobe)	Costa (2013)
Shrimp and crab	*Campylobacter jejuni* (Microaerophilic)	Wang et al. (2011)
Shrimp	*Shigella spp.* (Facultative anaerobe)	Iwamoto et al. (2010)
Shrimp	*Staphylococcus aureus* (Facultative aerobe)	Arfatahery et al. (2015)
Shrimp and crab	*Vibrio cholera* (Facultative anaerobe)	Ahmed et al. (2018)
Shrimp	*Carnobacterium maltaromaticum* (Facultative anaerobe)	Noonin et al. (2010)
Shrimp	*Vibrio vulnificus* (Facultative anaerobe)	Do Nascimento et al. (2001)
Shrimp and crab	*Yersinia enterocolitica* (Aerobe)	Li et al. (2018)
Crab and shrimp	*Pseudomonas aeruginosa* (Facultative aerobe)	Algammal et al. (2020)
Crab, lobster, and shrimp	*Aeromonas hydrophila* (Heterotrophic)	Noonin et al. (2010)

not cooled immediately post-harvest. Many vibriosis outbreaks occurred and threatened the farmed shrimps *that is*, *Penaeus vannamei* and *P. monodon*, resulting in acute hepatopancreatic necrosis disease. Hatchery and luminescent vibriosis, shell disease syndrome, tail necrosis, limp lobster disease, red body disease, Vibrio-caused bacteremia, and summer syndrome are examples of other vibriosis diseases that have been reported in crustaceans (de Souza Valente & Wan, 2021). Outbreaks due to *V. harveyi* infection in the gazami crab (*Portunus trituberculatus*) has led to high mortalities rate reaching to 100% at a concentration of 10^6 CFU/ml, after 18 h post inoculation (Zhang et al., 2014). Further, the resistance of these pathogenic bacteria to antibiotics can further add to their risk as previously reported in a study in China, where *ca*. 78% to 93% of *V. parahaemolyticus* strains isolated from commonly consumed crustaceans were found resistant to streptomycin, rifampin and ampicillin (Hu & Chen, 2016).

V. parahaemolyticus produces thermostable compounds which are known as thermostable direct hemolysin (TDH) and thermostable related hemolysin (TRH) which causes hemodialysis and RBCs rupture. After the consumption of raw or undercooked infected crustaceans, *V. parahaemolyticus* infection starts immediately which is manifested by fever, diarrhea, vomiting, and abdominal cramps (Xu et al., 2016). *V. parahaemolyticus* can also cause extreme hypotension which might result in shock (Broberg et al., 2011). The treatment protocol used to eradicate *V. parahaemolyticus* is mainly composed of antibiotics and rehydration (Nair et al., 2007).

V. parahaemolyticus can be killed by heat processing, though even after being cooked it can be recontaminated by several factors to include: crossover from other contaminated raw seafood, workers who previously touched raw seafood, equipment that was previously used to process raw seafood, or being rinsed with sea water. Such recontaminated seafood has to be thus reheated again (Su & Liu, 2007). One strategy for preventing *V. parahaemolyticus* infection is to cook crustaceans at high temperature and avoid eating raw or undercooked crustaceans.

The use of quorum sensing disruption, probiotics, prebiotics, green water, bacteriophages, and immune priming were reported as preventive and controlling measures for vibriosis outbreaks in farmed crustaceans (*i.e*., shrimp, lobster, and crab) (Zhang et al., 2014).

Salmonella species

Salmonella spp., gram-negative, rod-shaped enteric bacteria, is considered one of the primary causes of food transmitted disease (FTD) worldwide, which mostly affect the human gastrointestinal tract. Salmonella is transmitted to shellfish through sewage waste in the harvest area resulting in an infection characterized by fever, nausea, abdominal pain and vomiting (Vieira et al., 2004). High mortality rates were recorded due to the ingestion of food contaminated with *Salmonella typhi* and *S. paratyphi* (Amagliani et al., 2012). Salmonella was suggested to be inherently associated with shrimp culturing environment by a study of Bhaskar *et al*. who reported *Salmonella* isolation from all the 18 studied cultured shrimps collected from shrimp farm in the southern India coast (Bhaskar et al., 1995). In another study, *Salmonella* was concluded to be a part of the natural flora of 131 brackish water ponds in Southeast Asia farming cultured prawns where *Salmonella* was isolated from 16% of the examined prawns (Reilly & Twiddy, 1992).

Salmonella can be eradicated by cooking and irradiation, in addition the intake of raw or undercooked crustaceans should be reduced as the microbial pathogens remain viable in chilled products and the consumption of undercooked shrimp, lobster, and crabs cause bacterial infections in humans (Hsin-I Feng, 2012).

Bacillus cereus

Bacillus cereus is a gram-positive bacterium which can survive both aerobically and anaerobically. *B. cereus* form spores under unfavorable conditions which can cause intestinal and non-intestinal symptoms (Jensen et al., 2003). There are a group of virulence factors that enable *B. cereus* to cause an infection like protein and non-ribosomal peptides (Sineva et al., 2009). Two forms of toxins are produced *that is*, emetic toxins and enterotoxins (Nguyen & Tallent, 2019). The enterotoxin is a diarrheal toxin that is secreted in the small intestine, whereas emetic toxin is secreted by the bacterial cells. *B. cereus* infection is challenging to manage because it can withstand high temperatures by developing spores as it can survive at high temperatures and return to average growth when the temperature drops below 48°C (Omer et al., 2018). Infections with *B. cereus* may be caused by a variety of foods including crustaceans. *Bacillus* species have been identified as one of the organisms that cause outbreaks of food poisoning (European Parliament and Council, 2003). Crustaceans are thought to be a source of *B. cereus*, as many outbreaks have been linked to crustacean consumption in Europe(EFSA Panel on Biological Hazards, 2016).

3.1.2 | Parasites

Consumption of raw or not properly cooked crustaceans is one of the common transmission methods of zoonotic parasites. Shrimp, lobster, and crab meats are prone to infection by different types of parasites including *Ascaris* spp., *Trichuris* spp., and *Trichinella* spp, tapeworms such as *Diphyllobothrium* spp. and trematodes (*Chlonorchis* spp., particularly *C. sinensis*, *Opisthorchis* spp., *Heterophyes* spp., *Metagonimus* spp., *Nanophyetes* spp., and *Paragonimus* spp.) and nematodes (Anisakids) (Nollet & Toldrá, 2010).

Anisakis simplex is a parasitic nematode known as herring worm with one of its accidental intermediate hosts are crustaceans *via* consuming the third *Anisakis* larval stage. Only raw or undercooked infected crustaceans and marinated fish can cause *Anisakis* infection in humans (Nieuwenhuizen & Lopata, 2013). The clinical manifestations of *Anisakis* infection include nausea, vomiting and acute abdominal cramps similar to those of a peptic ulcer, appendicitis, or peritonitis. An allergic reaction ranging from urticaria to anaphylactic shock is considered the extremely dangerous symptoms of that parasite (Villazanakretzer et al., 2016).

Paragonimus westermani is a form of a fluke that infects the lungs which is mostly found in Asia, and it is now spreading throughout the world due to globalization and increased dietary diversity. *Paragonimus* is one of the most common parasites that infect crustaceans, especially crabs. According to the World Health Organization, *ca.* 22 million people were infected with *P. westermani* worldwide (WHO, 2002), mostly by ingesting fresh or undercooked crabs or crayfish contaminated with that parasite. These flukes live in the lung of infected humans and cause pulmonary paragonimiasis. *P. westermani* can migrate to the abdominal organs, eyes, and brain to cause ectopic paragonimiasis (Kim et al., 2017). The symptoms of acute infection with paragonimiasis involve fever, cough, bloody sputum with the presence of the fluke worms in certain parts of the body such as the brain and heart (Raatz & Bibus, 2016).

Diphyllobothrium latum is a type of intestinal cestodes known as broad tapeworms that is transmitted to humans after ingestion of raw or undercooked crustaceans causing a variety of diseases(Ito & Budke, 2014). Raw fish is the primary host for *Diphyllobothrium* species, however, crustaceans acts as their intermediary reservoirs (Arizono et al., 2009).

Candling and manual inspection are the most popular methods traditionally used for detecting parasites in crustaceans. Parasites embedded to a depth of 6 mm in the fillet can be manually spotted and removed using a white light table. The productivity of manual operators is estimated to detect 75% of parasites in fillets. Visual inspection cannot detect parasites though deeply embedded in the fillet, so even a comprehensive manual inspection would not guarantee a parasite-free product (Heia et al., 2007).

Deep-freezing was also reported to kill certain parasites in crustaceans, however, handling crustaceans with close hand contact during preparation and cooking puts humans at an increased risk (European Food Safety Authority, 2009).

Poor handling with close hand contact and processing of crustaceans result in parasite's transmission from crustaceans to humans. There are many approaches that can reduce the ability of parasite to survive such as cooking, freezing at -20°C and salting at concentration from 0.9% to 30% for 10 days. The Food and Drug Administration (FDA) recommended that crustaceans and shellfish tobe stored at -35°C for 15 h or at -20°C for at least 7 days before ingestion as the freezing kills and eliminates the majority of the parasites (Podolska et al., 2019). On the other hand, utilization of infected material from fresh or marinated crustaceans and uncooked crustacean parts may produce some clinical signs of parasitic infection (Köse, 2010).

Parasites can also be inactivated by heating at 55°C for 1 min, freezing at -20°C for 24 h, candling, trimming belly flaps, and physically removing cysts (Venugopal & Gopakumar, 2017). Deep freezing at least 10°C for 24 h can also eradicate *D. latum* (Scholz et al., 2009). According to the Centers for Disease Control and Prevention, *D. latum* can be eradicated using three different freezing procedures. It can be frozen for 7 days at $-4\,^{\circ}$F (-20°C) or below (total time). Second, it can be frozen until rigid at $-31\,^{\circ}$F (-35°C), then stored for 15 h at $-31\,^{\circ}$F (-35°C). Third, it can be frozen until rigid at $-31\,^{\circ}$F (-35°C), then kept at $-4\,^{\circ}$F (-20°C) for 24 h (Salminen, 1970).

3.1.3 | Viruses

Viruses spread from one person to another during food preparation or by coughing from an infected person to food, or through vomits. Human pathogenic viruses enter the marine environment by direct discharge of treated or untreated sewage wastewater. They can persist for extensive periods in the marine environment (Lehel et al., 2021). Viruses, unlike bacteria, can survive better than bacteria in sanitation procedures. Most of the shellfish-borne viral outbreaks were reported to be caused by Norwalk and hepatitis A viruses, which are the most serious viral infection associated with crustaceans consumption (Bosch et al., 2011).

Norwalk virus is a common cause of nonbacterial gastrointestinal illness (gastroenteritis). Consumption of raw and steamed crustaceans has been linked to Norwalk virus infection. In humans, the virus affects the small intestinal lining, resulting in nausea, vomiting, diarrhea, abdominal cramps and fever on rare occasions (Hsin-I Feng, 2012).

Hepatitis A is another virus that might be present in crustaceans which causes the most prevalent type of hepatitis with distinct symptoms. Viruses can persist in seawater for long periods and to survive in marine sediments. Hepatitis A outbreaks have been linked to consumption of raw and steamed crustaceans showing symptoms including fatigue, fever and abdominal pain, and eventually becomes jaundiced as the illness progresses (Bosch et al., 2011).

White spot syndrome virus (WSSV) is a highly contagious virus that causes white spot disease (WSD) characterized by white spots on the carapace of shrimp sand many other crustaceans. WSD leads to outbreak with a 100% mortality within 3–10 days after the disease onset. Survivors may transfer the virus to their progeny as they will remain carriers of the virus for long time (Dey et al., 2020).

Norwalk virus hazards can be minimized by extensively cooking crustaceans and avoiding against cross-contamination with cooked seafood. Furthermore, a recent outbreak showed that preventing untreated waste of shellfish processing vessels from being discharged overboard would minimize the number of Norwalk virus-related illnesses (Liu et al., 2009). Depuration alone has a limited effect though on reducing the level of viruses in crustaceans and is not suitable if harvested from more heavily contaminated areas.

Commercial processing methods were likewise revealed to be insufficient for the effective inactivation of some viruses such as hepatitis A virus, and pathogenic bacteria such as *L. monocytogenes*, *C. botulinum*, *Vibrio* spp. *and Salmonella* spp. Accordingly, physical, chemical and biological intervention strategies are needed for the effective safeguard against most microbial and parasitic hazards including: the use of preservatives, salting, drying, time/temperature control, fermentation *and the* exposure to ionizing radiations *that is*, gamma and X-rays(Fernandes, 2009). The use of ionizing radiation has been implemented by the U.S. FDA in its recent food additive regulations to inactivate foodborne pathogens in crustaceans and hence extend their shelf-life. Irradiation at a maximum permitted dose of 6.0 kGy was reported to reduce hepatitis A virus, *Vibrio* spp. and parasites (Shumway & Rodrick, 2009).

3.1.4 | Fungi

The majority of infections and risks that affect crustaceans are produced by organisms that are naturally present in their environment's biota (Couch 1935, Sparrow 1960). As it targets various aquatic creatures, fungi and fungal-like infections (straminipilous organisms) play an essential role in disease outbreaks. Marine fungal species that can affect crustaceans are depicted in Table 2. Straminipilous organisms are the most common cause of mycotic infections in freshwater crustaceans. Mechanical injury from handling, exposure to severe pH levels, extended exposure to cold water temperatures, a lack of food, and the presence of other microbial infections make crustaceans more susceptible to fungal infections (Baticados, Lio-Po *et al.* 1978).

Lagenidium spp

Lagenidium species have been identified on a variety of hosts in both freshwater and marine environments, including crustaceans. Certain marine algae have been found to have *Lagenidium* Callinectes (Armstrong, Buchanan *et al.* 1976). It has been discovered that *Lagenidium* species cause progressive systemic mycosis with little or no host inflammatory response. Infection appears to be fatal, with mortality rates ranging from 20% to 100% within 48 to 72 h of infection. When standard farming methods (such as washing eggs or nauplii in clean seawater) are not followed, *Lagenidium* species have been considered a severe disease (Hatai, 2012). *Lagenidium* spp is the cause of larval mycosis. Larval mycosis has been linked to a high rate of egg or early larval stage mortality in marine crabs. Fungal infection was found to be located in hatchery reared eggs and larvae of *P. monodon* causing a 100% mortality of the larvae. Poor quality of hatchery reared larvae and post larvae was attributed to the most of mycosis outbreaks (Aftab Uddin et al., 2013) *Lagenidium myophilum* was pathogenic towards shrimps of the genus *Pandalus* and larvae of the coonstripe shrimp. The fungus could infect shrimps at various stages, from larva to adult. Microscopy or isolation and identification of the fungus on mycological medium are used to diagnose larval mycosis. Outbreaks can be avoided by disinfecting contaminated larval rearing tanks and chlorinating and filtering the entering water (Nakamura et al., 1994).

Fusarium

Several *Fusarium* species *viz. F. oxysporum*, *F. solani* and *F. moniliforme* were reported to be the causative pathogen for causing black gill disease in prawns (Hatai, 2012). In a study by Van Khoa et al. (2005), *F. oxysporum* was reported to infect the pond-cultured kuruma prawn (*Penaeus japonicus*) in Japan (Van Khoa et al., 2005).

Atkinsiella

Another fungus *"Atkinsiella dubia"*, isolated from the swimming crab (*Portunus trituberculatus*) gills in Japan, was reported to cause mass mortality in crustaceans. The fungus produces crystalline, tuberculate and moist colonies, dimorphic and diplanetic zoospores (Hatai, 2012).

Pythium

Pythium myophilum (*Lagenidium myophilum*) is a pathogenic fungus infecting the abdominal muscles and swimmerets of adult northern shrimp (*Pandalus borealis*), larval and juvenile coonstripe shrimps and Hokkai shrimp (*Pandalus kessleri*) (Hatai, 2012).

4 | CHEMICAL HAZARDS

Contamination of seafood by heavy metals is becoming a global crisis due to the growing discharges of pollutants arising from industrial, agricultural, pharmaceutical and technological applications. Chemical hazards are classified into three types: incidental *that is*, heavy metals & dioxins, natural *that is*, biotoxins, and intentional *that is*, pesticides and veterinary drug residues. The differential accumulation of heavy metals in the reported crustaceans is outlined in Figure 3.

4.1 | Heavy metals

Heavy metals are mostly deposited in the sediment, followed by water dissolution, potentially contaminating resident biota. Crustaceans are considered excellent bioindicators, as they live in close contact with contaminated sediment. The accumulation of metals which are either essential elements present in excess amount or non-essential poses risk to the health of marine animals and human upon consumption of contaminated crustaceans (de Almeida Rodrigues et al., 2021).

Because of their persistence, long biological half-life and potential toxicity, heavy metals may pose a serious risk to humans through exposition to periodic food ingestion. Actually when accumulated in organisms, they can affect the digestive, cardiovascular and/or central nervous systems (Bonsignore et al., 2018). Among the most hazardous heavy metals to animal and human are arsenic, cadmium, chromium, lead and mercury.

TABLE 2 List of pathogenic fungi reported to infect different crustaceans and their worldwide distribution

Crustaceans species	Pathogenic fungi	Geographical distribution	References
Pacific white shrimp	*Aspergillus awamori*	Widely Distributed	Karthikeyan et al. (2015)
Various crustaceans	*Atkinsiella dubia*	Japan, United States of America	Eshraghi et al. (2005)
Shrimp and Crab	*Fusarium* species	France, Japan, Malaysia, Mexico, Philippines, United Kingdom, United States of America	Hatai (2012)
Decapod crustaceans	Microsporidian species	Widely distributed	McGladdery (2011)
Marine crustaceans	*Pythium* species	Widely distributed	Hatai (2012)

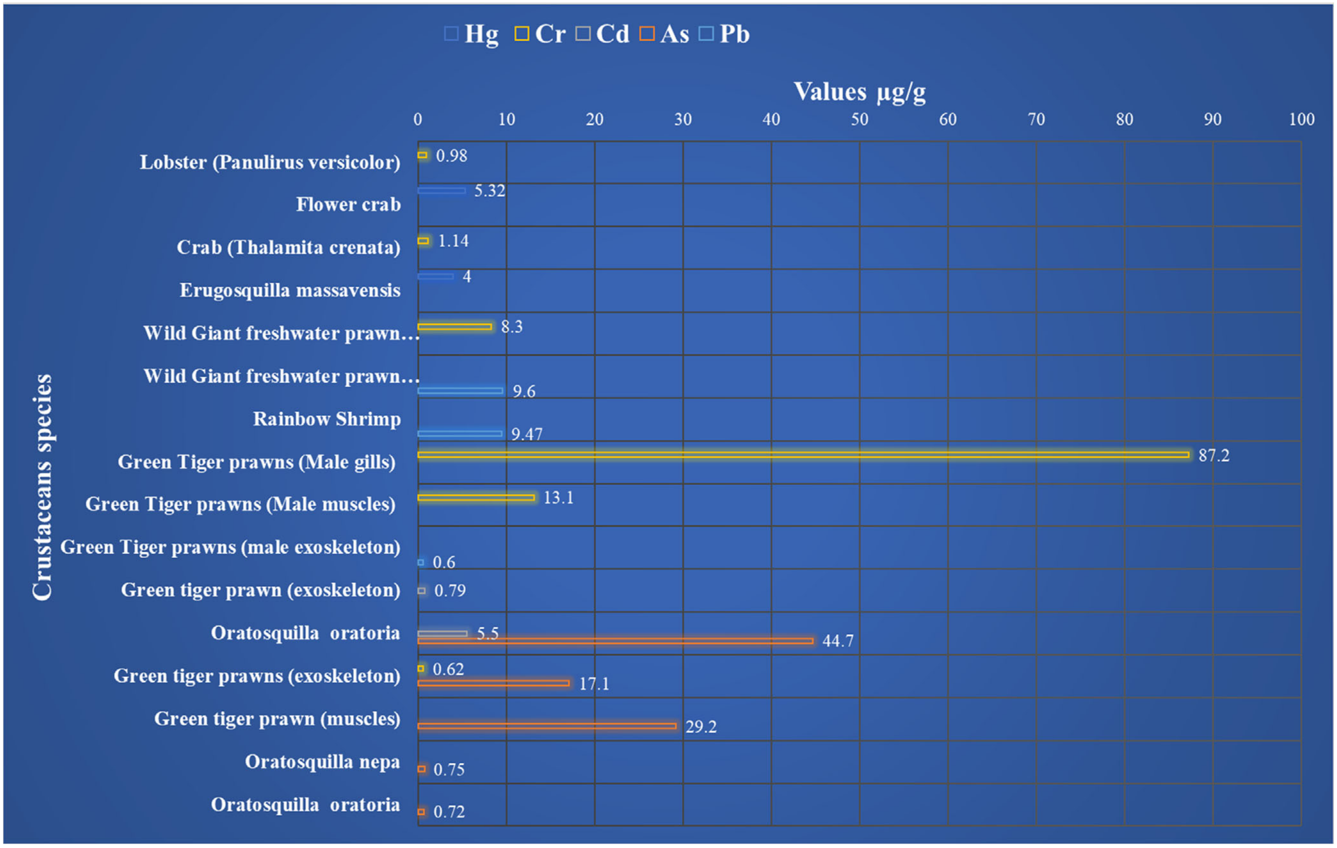

FIGURE 3 Differential heavy metals accumulation in the reported crustaceans

4.1.1 | Arsenic (As)

Arsenic is the most toxic element for humans which can interfere with several enzymes and cause oxidative stress. It occurs naturally in marine aquatic system due to anthropogenic action and is present in three different forms; elemental As (As0), inorganic As (iAs), such as AsIII and AsV salts (arsenides, arsenosulfides, arsenites, and arsenates), and organic As (methylarsine and dimethyarsine). The arsenite anion is the most toxic inorganic species which is responsible for carcinogenic activity. Organic arsenic is less toxic as it can be excreted rapidly from the human body. Methylation process changes iAs to its less toxic organic form (EFSA Panel on Contaminants in the Food Chain, 2009) However, arsenic elemental content was reported to exceed the maximum permissible limit in fish and shellfish which is 0.5 µg/g according to the Chinese Food Codex (MPHC) (Gu et al., 2016). Arsenic level was recorded at *ca*. 0.72 and 0.75 µg/g wet weight in two species of shrimp (*Oratosquilla oratoria* and *O. nepa*), respectively in a study conducted to estimate trace metals in wild marine species collected from Daya bay in the South China Sea (Gu et al., 2016). Likewise, another study showed that arsenic level ranged from 2.26 to 44.7 µg/g in shrimp (*O. oratoria*) collected from Shandong Province in China (Jiao et al., 2021).

Arsenic has different accumulation behavior within crustaceans found to be most concentrated in the exoskeleton and the muscles at 17.1 and 29.2 mg/kg as in shrimp (*P. semisulcatus*), respectively (Kaya & Turkoglu, 2017).

4.1.2 | Cadmium (Cd)

High consumption of cadmium-containing food causes chronic cadmium toxicity leading to kidney dysfunction and bone disease. Cadmium is considered a toxic element, when it exceeds an EU threshold limit of 0.5 µg/g wet weight in crustaceans (Järup & Åkesson, 2009). Previous studies reported that a differential cadmium accumulation in muscles and exoskeleton of shrimp (*P. semisulcatus*) which was detected at 0.1 to 0.79 µg/g in muscles, and at slightly higher level (*ca*.1.016 µg/g) in the exoskeleton (Pourang et al., 2005). A study conducted in Daya, China bay reported for cadmium distribution among crustaceans showed that Cd level in shrimps (*Oratosquilla oratoria*) exceeded its regulatory limit detected at 0.9 µg/g. In comparison, crab (*Portunus pelagicus*) showed a lower detectable value of 0.01 µg/g (Gu et al., 2016). Whether crabs accumulate less minerals than shrimp as in case of Cd has yet to be determined. In case of farmed shrimps, Cd was detected higher in the cephalothorax of farmed shrimps (0.51 µg/g) than wild types of similar sizes (0.28 µg/g) (Mostafiz et al., 2020).

4.1.3 | Chromium (Cr)

Chromium is an essential element, although harmful at high levels exceeding its tolerable limit of chromium in fish and seafood (0.5 mg/kg) (Commission of the European Communities, 2001). According to the EU, a study was conducted to estimate Cr level in green tiger shrimp (*P. semisulcatus*) males and females, which detected that male muscles have a higher tendency to accumulate Cr than females, which was found to be 13.1 and 9.3 μg/g, respectively compared with the rate of accumulation in gills that were up to 87.2 and 61.1 μg/g for males and females, respectively (Ayse Bahar Yilmaz & Yilmaz, 2007). Chromium concentration values may vary according to tissue type. Cr level in the exoskeleton of tiger shrimp reached 0.62 mg/kg, while muscles contained *ca*. 0.215 mg/kg of Cr traces (Kaya & Turkoglu, 2017). These results suggest that among muscles of most crustaceans accumulate less level of toxic elements than exoskeleton.

Studies carried out on mantis shrimp (*Oratosquilla oratoria*) collected from Shandong Province, China, showed a high level of Cd up to 5.5 mg/Kg(Jiao et al., 2021). Various distribution patterns were observed for chromium (Cr) in crab and lobster. For instance, in Bangladesh, about 1.14 μg/g of Cr was detected in crab (*T. crenata*) and *ca*. 0.98 μg/g was detected in lobster (*P. versicolor*), however, trace levels at less than 0.08 μg/g was observed in shrimp (*P. sculptilis*) (Mostafiz et al., 2020). Since the permitted daily intake of the hexavalent chromium is 0.9 μg/kg b.w/day (EFSA Panel on Contaminants in the Food Chain, 2014), crab was found to pose the highest risk.

4.1.4 | Lead (Pb)

It is a non-essential element that exists in an inorganic form which can lead to developmental neurotoxicity in children and cardiovascular effects in adults when exposed to lead in food or water. The maximum tolerable level of lead was reported to be 0.5 mg/kg w/w in shellfish and fish. Many studies were conducted to measure lead levels in seafood. A study conducted in Saint Martin, Bangladesh, revealed that lead level in lobster and crab species was lower than detectable limit less than 0.3 and 0.006 μg/g, respectively. In contrast, shrimp (*P. sculptilis*) contained the highest lead level (9.47 μg/g) among the examined species (Baki et al., 2018). For example, Pb level in muscles of shrimp (*P. semisulcatus*) caught from Iskenderun bay reached a level up to 0.6 μg/g exceeding the permissible lead level (Ayse Bahar Yilmaz & Yilmaz, 2007). Such high Pb level could be attributed to the situation of Iskenderun Bay in one of the most polluted coastal waters of Turkey where there is a harbor of heavy agricultural and industrial activities exist including iron and steel works, oil pipeline Installation and some other small (Ayşe Bahar Yilmaz, 2005).

4.1.5 | Mercury (Hg)

Mercury is ubiquitous in the environment which contaminate the air during industrial activities such as mining, solid-waste incineration and fuel combustion. It is introduced in the environment in many forms. The inorganic forms, particularly methyl mercury, is the most toxic and is widely presented in the aquatic system with high potential to accumulate in marine animal tissues. The toxicity of methylmercury is due to its lipophilic properties which enable it to cross the blood–brain barriers causing brain dysfunctions (Silbernagel et al., 2011). Methyl mercury toxicity develops from its binding with sulfhydryl-containing molecules that is, albumin, cysteine and homocysteine metallothionein. High mercury was found in crabs hepatopancreas, where metallothionein exists at higher level in muscles and gills (Barath Kumar et al., 2019). A study was conducted in the Persian Gulf, Iran, revealed a high accumulative content of Hg (5.32 mg/kg) in crabs (*P. pelagicus*) (Khoei & Bastami, 2013). Although, the maximum level of Hg in fish was set by the EU to be 0.50 mg/kg (No, 1881). Another study conducted in Ismailia, Egypt, on shrimp (*Erugosquilla massavensis*) muscles reported a higher level of mercury of 4 mg/kg in the tissues (Abdel-Salam, 2014). Table 3 lists crustacean species that were reported to contain trace metals above their maximum provisional allowed limit (MPAL) of trace metals as set by the European commission.

There is a limited number of studies reported for a comparative analysis of trace metal accumulation in famed and wild prawns. A study conducted to measure trace metals in wild and farmed prawns showed that wild species are more contaminated in Pb and Cr, however, farmed species are more susceptible to trace cadmium accumulation. Wild shrimps are more contaminated with heavy metals than farmed counterparts (Mostafiz et al., 2020). For examples, Pb level in wild shrimp abdomen reached up to 9.66 μg/g, whereas Cr level was higher in cephalothorax of wild shrimps reaching *ca*. 8.3 μg/g (Mostafiz et al., 2020).

Wild-caught prawns were reported to accumulate elevated levels of Pb, Cr, Fe, Cu, Zn, and Mn; however, Ni and Cd were higher in farmed prawns. A higher trace metal contamination was recorded from the cephalothorax part than the abdomen of prawns (Mostafiz et al., 2020).

However, there is still some gap in differential analysis of accumulation levels of heavy metals in both farmed *versus* wild shrimps. Silva *et al*. (2016) reported a similar pattern of trace metal accumulation in tissues of both wild and farmed shrimps (*Litopenaeus vannamei*) collected from Brazil. Higher levels of Cd, Mn, Zn, Se, and Al were observed in wild shrimp than in farmed shrimp samples. Higher Cd accumulation of in farmed prawn samples was proved to be associated with farm water and feeds (Fallah et al., 2011).

The levels of heavy metals can be significantly reduced by some processing treatments *viz*. high hydrostatic pressure, washing and cooking (Rasmussen & Morrissey, 2007). For example, selenium (Se) was reported to protect against the toxic effects of mercury, particularly organic methylmercury, and hence Hg:Se ratio is posed to serve as a useful indicator for the risk linked with fish intake (Olmedo et al., 2013).

According to some studies, cooking methods such as grilling and boiling, has a considerable role in reducing trace metals in some crustacean species. For instance, a study was conducted on the Mediterranean coast, Egypt proved the effect of boiling in reducing trace metals accumulated within crab (*Portunus pelagicus*) and shrimp (*Metapenaeus monoceros*) collected from local Damietta coast in which boiling reduced metals *that is*, As, Hg, Pb, and Cd nearly by 10.8, 17, 21.5, and 33%, respectively in crab, *versus ca*. 14.3%, 23%, 58%, and 60% for As, Hg, Cd, and Pb, respectively in shrimp. These results suggested

TABLE 3 Crustacean species reported to contain heavy metals at level exceeding the MPAL (μg/g) set by EU Commission

		Heavy metals (μg/g)					
Crustacean species	**Locations**	**Pb**	**As**	**Cd**	**Cr**	**Hg**	**References**
Shrimp (*Oratosquilla oratoria*) (*Oratosquilla nepa*)	Daya bay in South China Sea		0.72 0.75				Gu et al. (2016)
Green tiger prawn *Penaeus semisulcatus* (muscles) *P. semisulcatus* (exoskeleton)	Turkey		29.2 17. 1		0.62		Kaya and Turkoglu (2017)
O. oratoria	Shandong Province		2.24–44.7	5.5			Jiao et al. (2021)
P. semisulcatus	Persian Gulf			0.79			Pourang et al. (2005)
Green tiger prawns (male exoskeleton) Green tiger prawns (male muscles) Green tiger prawns (male gills)	Northern East Mediterranean Sea, Turkey	0.6			13.1		Ayse Bahar Yilmaz & Yilmaz (2007)
P. sculptilis the Rainbow Shrimp	Saint martin island Bangladesh	9.47					Baki et al. (2018)
Wild shrimp prawns Abdomen Exoskeleton	Bangladesh	9.6			8.3		Mostafiz et al. (2020)
Erugosquilla massavensis	Ismailia, Egypt					4	Abdel-Salam (2014)
Crab (*Thalamita crenata*) Flower crab (*P. pelagicus*)	Bangladesh Saint Martin Island Northern Persian Gulf				1.14	5.32	Baki et al. (2018) Khoei and Bastami (2013)
Lobster (*P. versicolor*)	Bangladash, Saint Martin Island				0.98		Kaya and Turkoglu (2017)
MPAL (μg/g)		0.50	0.015[a]	0.50	0.50	0.50	Commission of the European Communities (2001)

[a] For As, provisional tolerable weekly intake (PTWI) value, which is 0.015 μg/g weekly per body mass (Kaya & Turkoglu, 2017).

that boiling is more efficient in shrimp matrix *versus* crab for reducing trace toxic metals. However, grilling was not efficient to reduce metals in shrimp samples, though to show a significant reduction in crab samples. This can be explained by the effect of high cooking temperature which reduces protein and water content within the tissues since heavy metals are linked to soluble amino acids, and therefore decreasing the proteins will release the trace metals from the cooked tissues (Ganjavi et al., 2010).

4.2 | Dioxins

Dioxins have *ca*. 23 congeners that are called persistent organic pollutants (POPs), used in manufacturing pesticides, bleaching materials, electrical supplies, but mainly polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are the most toxic widely distributed compounds whose bioaccumulation in the living organisms is considered a significant health risk (Fiedler et al., 2013). Dioxins undergo a biomagnification process within the aquatic animal tissues because of their lipophilic nature (Marinković et al., 2010). That is why these compounds are more likely to accumulate in rich lipid foods rather than other ones. The more fat contents in the food, the more POP accumulative level is found (Van der Oost et al., 2003). Consumption of contaminated food with dioxins and dioxins-like substances would expose the human body to critical health hazards including cancer, according to the International Agency for Research on Cancer (IARC) (Polychlorinated Biphenyls and Polybrominated Biphenyls, 2016). The European Union has established a tolerable level for human consumption of contaminated food with PCDDs/Furans (Fs) and PCDDs/Fs with PCBs, 4 and 8 TEQ/kg/day. The total average of dioxins and dl-PCBs in crab were estimated at 31.9 pg TEQ g^{-1} in the edible parts of Chinese mitten crab meat (*Eriocheir sinenesis*) (Hoogenboom et al., 2015). Dioxin toxicity is measured by Toxin Equivalency (TEQ) (Ceu, 2006).

Another study was conducted to evaluate PCB toxicity and its derivatives found at higher level in the seabream fish (23,787 pg/g) *versus* shrimp which showed the lowest level of contamination among the analyzed species as it contained only 861 pg/g (Bordajandi et al., 2006).

The more effective way in decreasing the level of fat-soluble organic contaminants as dioxins is the cooking procedures that release fat from seafood products (Domingo, 2016).

4.3 | Biotoxins

The Codex Alimentarius Standard classifies algal toxins chemically into 3 groups *viz*. saxitoxin (STX), okadaic acid (OA) and domoic acid

(DA) (Garthwaite, 2000), and to be discussed for each in the next subsection.

4.3.1 | Paralytic shellfish toxins (PST)

Food containing saxitoxin (STX) is responsible for PST poisoning produced by genus dinoflagellates *Alexandrium*, namely *Gymondinium catenatum* and *Pyrodinium behamense* (Cusick & Sayler, 2013). Exposure to high doses of PST can lead to death mostly due to respiratory paralysis (Toyofuku, 2006). According to the severity of these biotoxins, the Tasmanian regulatory authorities have confirmed using the bivalve shellfish maximum level to be the permitted level for crustaceans' consumption set at 0.8 mg STX.2HCl eq/kg (Melorose et al., 2015). Southern Rock lobster (*J. edwardsii*) was reported to accumulate the paralytic shellfish toxins at a level of 4.05 mg of STX.2HCL eq/kg in their hepatopancreas (Whittle & Gallacher, 2000).

4.3.2 | Diarrheic shellfish poisoning (DSP)

DSP is a non-lethal gastrointestinal disease caused by okadaic acid (OA) containing food. OA is produced by the toxic microalgae *Dinophysis* and *Phalacroma* (Reguera et al., 2014). When ingesting food contaminated with DSP, gastrointestinal problems such as diarrhea, due to the effects of OA and its analogues on inducing the intestine secretion cells to release sodium as a result of inhibiting protein phosphatase enzyme. Usually, symptoms appear within 30 min after ingestion of contaminated food and can be cured within 3–4 days (Whittle & Gallacher, 2000). The maximum permissible level of OA is 160 μg of okadaic acid equivalent/kg, No 854/2004 (Commission Regulation, 2004). In Norway, brown crabs (*Cancer pagurus*) were reported to be intoxicated by *ca*. 290 μg/kg okadaic acid which is above the permissible limit (Torgersen et al., 2005). This contamination was justified by the previous feeding of crabs on blue mussels that were contaminated with OA (Castberg et al., 2004).

4.3.3 | Amnesic shellfish poisoning (ASP)

ASP toxicity occur due to DA that is produced from diatoms (genus *Pseudo-nitzschia*) (Visciano et al., 2016). It is responsible for the induction of glutamate receptors and disruption of neurochemical signaling (Jeffery et al., 2004). Symptoms after ingestion range from mild to severe based on the period after consumption; for example, within the first 24 h the human body would suffer from headache, coma, respiration difficulty, followed by permanent memory loss at 48 h (Lehel et al., 2021). The maximum permissible level of DA in food is 20 mg/kg, No 853/2004 (Commission Regulation, 2004). Further, studies were conducted to estimate DA level in crustaceans and their accumulation rate within crustaceans' tissues. DA was measured in the swimming crab (*Polybius henslowii*) collected from Portugal, found to exceed the permissible level as it contained 323 μg/g in the whole body and 571 μg/g in the visceral tissues (Costa et al., 2003).

In contrast, and being of non-protein nature, algal toxins cannot be destroyed by some post-harvest processing techniques such as smoking, drying, salting and cooking. Accordingly, regular monitoring of harmful algae in coastal water is an important control measure to mitigate against crustaceans' algal contamination (Fernandes, 2009), and further to limit such toxic algal growth.

4.4 | Pesticides

Pesticides are widely used to protect organisms against different infections. However, their residues can accumulate inside the tissues of marine creatures without being degraded due to their lipophilic nature. The toxicity level of 14C-DDT pesticides was monitored in white shrimp samples that consumed various doses of 14C-DDT after 24 h which showed hyperactive state, paralysis, and finally, death at a level of 2.2, 9.4 and 37 ng/ml, respectively (Wandiga et al., 2003). Fishes have a higher ability to accumulate organochlorine pesticides than crustaceans in their tissues, which makes the later less likely to cause problems from these contaminants. DDTs were one of the most abundant pesticides that were detected in shrimp prawn at a level of 6.51 μg/kg, though much less than that in seabass fish detected 247 μg/kg, and hence confirming that organic pesticides accumulate more in fishes (Shi et al., 2011)

4.5 | Veterinary drugs

Veterinary drugs that are used in aquaculture have a crucial role in preserving marine animals by preventing pathogenic infestations. However, the problem occurs when these drugs cannot be cleared from them and hence can reach the consumers. The most common drugs used in aquaculture are chloramphenicol, nitrofurans, quinolones, and malachite green that definitely would expose human health to risk as forming bacterial resistance against antibiotics (Cabello, 2006). These drugs can also affect negatively the body functions for instances; chloramphenicol can distort blood cells that may lead to leukemia and plastic anemia (Hanekamp & Bast, 2015), malachite green can induce liver tumor (Bilandžić et al., 2012), quinolones such as fluoroquinolone can expose human health to risk because it can cause kidney and cardiac dysfunction, blood hemolysis, and decrease in the platelets count (Stahlmann, 2002).

Veterinary drugs are widely applied in aquaculture farms to preserve marine animals' health. Quinolones residues in shrimp tissues were reported to exceed the permissible limits reported at 21.8, 55.9, 56.3 and 81 ng/g for ciprofloxacin, enrofloxacin, oxolinic acid and flumequinine, respectively (S. Zhao et al., 2007). Shrimps among crustaceans that have the potential to accumulate veterinary drugs that is why the implementation of these drugs should be monitored by establishing the maximum residual limit based on the acceptable daily intake. The EU Commission has set the maximum residual permissible limits (MRPL) for the banned veterinary drugs in seafood such as chloramphenicol and malachite green, to be of MPRL at 0.3 and 2 ng/g, respectively (Quesada et al., 2013).

5 | MONITORING OF CRUSTACEAN BIOSAFETY: DETECTION METHODS

Several methods of biological and chemical contaminants detection in crustaceans, *viz*. microbiological, molecular, and analytical methods to safeguard consumer safety are presented in the following subsections highlighting their merits and limitations (Figure 4).

5.1 | Detection of microbial pathogens

Although there are multiple methods for detecting the presence of bacteria in food, the microbiological method, *that is*, microbial culturing is still the most popular due to its high sensitivity, precision, and effectiveness (Gugliandolo et al., 2011). Microbial culturing are employed under many protection and hygiene protocols, such as the protocol for *Salmonella* and *Listeria* detection (de Assis Castro et al., 2017). One of the limitations of microbial culturing is that being less specific and time consuming as incubation, detection, and identification takes from 5 to 7 days. Additionally, culturing requires a fully aseptic environment to avoid sample contamination as well as non-destructive isolation and purification steps to store the strains for future analysis (Zhao et al., 2014).

Since microbial pathogenicity cannot be revealed by microbiological techniques, immunological methods for nucleic acid amplification methods, such as polymerase chain reaction (PCR) are still required as a confirmation step. PCR is a more sensitive and faster technique that provides more accurate identification of the species and their pathogenicity than microbiological methods. Quantification is also applicable by qPCR method with the determination of optimal detection limits. The virulence genes encoding microbial toxins are targeted by both PCR and qPCR methods. Three main Vibrio species, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*, are potentially pathogenic to humans and responsible for a dramatic increase of seafood-borne infections worldwide. Hence, early detection of total and pathogenic Vibrio is needed and should rely on quick and effective methods (Bonnin-Jusserand et al., 2019).

A multiplex PCR method was used for the detection and amplification of a wide range of microorganisms and their virulence factors in crustaceans with merits of detecting several microbial species simultaneously. For detection of *Vibrio* spp., this method uses primers that target the *tdh* and *trh* genes encoding hemolysins and the phylogenetic marker, *that is*, groEL gene, which is experimented in artificially contaminated shellfish with *V. parahaemolyticus* strains. The virulent and non-virulent *V. parahaemolyticus* strains can also be differentiated by multiplex PCR using *tdh* and *trh* and *hns* genes (Bonnin-Jusserand et al., 2019).

A more comprehensive method was reported by Espiñeira et al. (2010) who employed a sequential method based on several multiplex PCR assays to distinguish between five *Vibrio* species in shrimp and crabs by targeting the *ctxA*, *tlh* gene for detection of *V. cholera* and *V. parahaemolyticus*, respectively, and *dnaJ* gene for *V. vulnificus*, *V. alginolyticus*, and *V. mimicus* (Espiñeira et al., 2010).

One of the drawbacks of real-time PCR is that it is only applied to a limited number of viruses; however, it can be used to quantify virus copy numbers. For this purpose, real-time quantitative PCR assay was used for the detection of white spot syndrome virus (WSSV) viral load in the body of moribund juvenile shrimps (*P. vannamei*, *P. stylirostris* and *P. monodon*), and post-larval *P. stylirostris*. Further, viral detection by PCR does not provide any information about the susceptibility to infection as it only detects the viral genome elements not its location

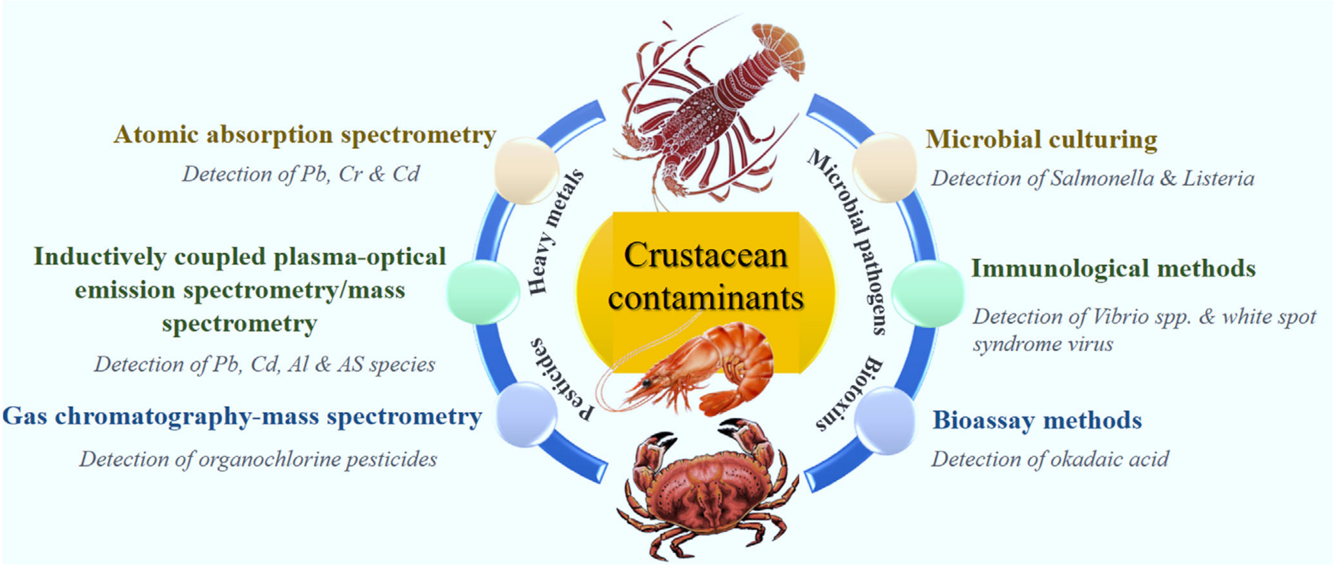

FIGURE 4 Methods of detection of crustacean contaminants *viz*. microbial pathogens, biotoxins, heavy metals, and pesticides

TABLE 4 Chromatographic and spectroscopic methods for detection of chemical contaminants in crustaceans

Detection methods	Chemical contaminants	Food type	Advantages	Disadvantages	LOD	LOQ	Reference
Graphite furnace atomic absorption spectrometry	Cd Pb	White shrimp (*Litopenaeus schmitti*) Blue crab (*Callinectes exasperatus*)			0.001 0.042 μg/g	0.001 0.102 μ/g	da Araújo et al. (2016)
Atomic absorption spectrometry	Pb, Cr Cd	*Crustaceans*	Sensitive and simple		1.0 0.05 0.2 mg/kg	N/A	Gu et al. (2016)
Atomic fluorescence spectrometer	As Hg	*Crustaceans*	Sensitive and simple		0.05 0.001 mg/kg	N/A	Gu et al. (2016)
Capillary electrophoresis (CE)-ICP-MS	Arsenic species	Biological samples	Short time (single run), selective, low detection limit (ng/g)	Expensive	N/A	N/A	Azizur Rahman et al. (2012)
Hg-analyzer instrument-atomic spectrometry	Hg	Shrimp	Selective, low mercury interference		0.0021 μg/g	0.0063 μg/g	Habte et al. (2015)
ICP- OES	As Cd, Pb Hg	Warty crab (*Eriphia verrucosa*)	Low detection limit (ppm)		1.4 0.2 1.8 0.01 ppm	N/A	Bayrakli (2021)
ICP-MS	As Pb Cd Cr Hg	Muscles, exoskeleton, and skin of shrimp	Low detection limit, sensitive, multi-element detection	Expensive	0.035 0.07 0.004 0.33 0.006 μg/kg	0.10 0.20 0.01 0.01 0.95 μg/g	Kaya and Turkoglu (2017)
HPLC-UV	Domoic acid	Swimming crab (*Polybius henslowii*)	Sensitive		0.06 mg/ml	N/A	Costa et al. (2003)
HPLC-MS/MS	Chloramphenicol (enrofloxacin and flumequine)	Shrimp soft tissues	Sensitive, high peak resolution	Expensive	N/A	0.25 1 ng/g	Tsai et al. (2019)
Gas chromatography–mass spectrometry (GC/MS)	Organochlorine pesticides	Chinese prawn Shrimp	High separation rate, sensitive		0.040–0.182 μg/kg	N/A	Shi et al. (2011)

whether intracellularly (infection) or on the surface or in the gut content of the animal (contamination). Hence, histological examination, *in situ* hybridization, immunohistochemistry or transmission electron microscopy is needed (Durand & Lightner, 2002).

5.2 | Heavy metals detection

The commonly used chromatographic and spectroscopic methods for detection of heavy metals in crustaceans are enlisted in Table 4. Graphite furnace atomic absorption spectrometry was applied in a study conducted in Ataru bay Brazil to detect Cd and Pb in white shrimp (*Litopenaeus schmitti*) and blue crab (*Callinectes exasperatus*) samples. A significant better LOD and LOQ of both shrimps (0.001 and 0.001 μg/g and 0.042 and 0.102 μg/g, respectively) were recorded (da Araújo et al., 2016).

Atomic absorption spectrometry is typically applied to detect heavy metals such as Pb, Cr, and Cd after sample digestion using microwave with detection limits of 0.1, 0.05 and 0.2 respectively. Atomic fluorescence spectrometer was used for the detection of As and Hg with detection limits of 0.05 and 0.001 mg/kg in digested samples of crustaceans *viz. Penaeus penicillatus*, *Oratosquilla oratoria*, *Oratosquilla nepa*, *Trachypenaeus curvirostris*, *Metapenaeus ensis*, *Portunus sanguinolentus*, *Portunus pelagicus* (Gu et al., 2016).

Arsenic is mainly found in its organic form with Arsenobetaine (AsB) is the main arsenic species in many marine organisms such as fish, mollusks, and crustaceans, and was also identified in the Western rock lobster using vapor generation atomic absorption spectrometry following sample digestion. Other analytical approaches involve the use of separation techniques coupled with a sensitive atomic detector such as high performance liquid chromatography (HPLC) coupled to inductively coupled plasma-optical emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) are widely used to estimate trace metals in biological specimens (Azizur Rahman & Hasegawa, 2012).

ICP-MS detection was reported for the determination of arsenic species *viz.* inorganic arsenic (iAs), methylarsonic acid (MA), dimethylarsinic acid (DMA), arsenobetaine (AB), trimethylarsine oxide (TMAO), and arsenocholine (AC) in Spanish pawns (*Aristeus antennatus*, *Aristaeopsis edwardsiana*) and shrimp (*Crangon crangon*). Arsenobetaine was the main arsenic species found in all the samples. The total extracted arsenic from prawns and shrimps ranged from 0.01–2.9 mg AS kg^{-1} dry mass exceeding the maximum level of inorganic arsenic of 1.0 mg kg^{-1} (dry weight) for shrimps and crabs set by the Republic of China (Zmozinski et al., 2015). ICP-MS offers high detection rate, produces high-resolution peaks with multi-elements detection at low detection limits in ng/g, however, the only limitation lies in the expensive cost of the equipment (Sneddon & Vincent, 2008).

Toxic metal levels were determined in edible tissues of 3 commonly available shrimp species consumed in South Korea using ICP-MS for Pb, Cd and As, however, Al and Hg were determined by ICP-OES and direct mercury analyzer, respectively. These methods were proved to be accurate and reproducible for use in determination of toxic heavy metal levels in seafood (Habte et al., 2015). A study was conducted to determine levels of heavy metals in warty crab (*Eriphia verrucosa*) samples collected from southern coasts, Black sea, Turkey using ICP-MS-OES (Bayrakli, 2021). Optical emission spectrometry showed LOD of As, Cd, Pb and Hg with 1, 4, 0.2, 1.8 and 0.01 ppm, respectively. Although OES technique has a prominent role in identifying trace metals, mass spectroscopy appears more sensitive than OES. Inductive coupled plasma- mass spectrometry (ICP-MS) was applied to determine heavy metals level in muscles, exoskeleton, and skin of selected shrimp species collected from İskenderun Bay in Turkey (Kaya & Turkoglu, 2017).

Assessment of tolerable daily/weekly/monthly intake values is another perspective which is specific for the consumers, depending on their locations and available species in the market. In order to estimate the potential public health risk, values of target hazard quotient (THQ) should be measured and assured to be less than 1. THQ calculation is applied through the equation $\text{THQ} = \text{EDI}/\text{RfD} \times 10^{-3}$. RfD is the oral reference dose that has been calculated by the international organization, whereas EDI stands for the estimated daily intake that depends on the body weight (μg/kg bw/day) (Capar et al., 2007). THQ is used to detect the health risk consequences resulting from ingesting food containing heavy metals. THQ does not provide quantitative data, but it indicates the health level of the trace metals. For example, insignificant level ($\text{THQ} \leq 1$), low level ($1 < \text{THQ} < 9.9$), moderate level ($20 < \text{THQ} < 99$), high level ($20 < \text{THQ} < 99$), and above 100 is considered a severe level (Petroczi & Naughton, 2009).

5.3 | Biotoxins detection

Bioassay methods have been widely used for the detection of biotoxins in animals, though with several disadvantages being insensitive and not highly specific as it does not provide the required data about the toxins injected into mice, however it also legislates using mice which is against animal rights and ethical codes. These reasons rationalize using other analytical methods for biotoxins detection including biosensors or chromatographic methods.

Biosensor is a highly advanced detector that is used intensively with biological samples as being cost effective, small, and transportable devices. Its principle is based on a reaction between the target cell and the bio-receptor (*e.g.*, DNA, Enzyme and cell) (Farag, Tanios, et al., 2021). The product makes a specific signal transformation that can be detected using a transductor, such as optical, electrochemical or mass-based. Another feature of the biosensors is that they can be easily modified depending on the target activity (Neethirajan et al., 2018). Biosensors are reported to serve as a tentative screening tool of presence of biotoxins before applying other sophisticated techniques (Farag, Mesak, et al., 2021). OA activity results from the inhibition of the phosphatase enzyme. From this prospective an enzyme based biosensor is used to measure the inhibition activity of OA (Hamada-Sato et al., 2004). Development of biosensors for detection of biotoxins in crustaceans as well as other marine organisms should now follow considering its health risks.

PSP biotoxins are separated firstly using high-performance liquid chromatography (HPLC) coupled to a highly sensitive detection unit such as a fluorescence detection unit or mass spectrometer. Coupling of hydrophilic interaction liquid chromatography (HILIC) with electrospray ionization tandem mass spectrometry (MS/MS) showed better results in detection of PSP toxins (Dell'Aversano et al., 2005), in addition to the MS/MS structural elucidation power to confirm toxin chemical structure (Hegazi et al., 2022). In 2003, along the Portaguous coast, a study was conducted to determine domioc acid accumulation within swimming crab (*Polybius henslowii*) by HPLC-UV. The study confirmed the high rate of DA accumulation within the crab visceral tissues by 571.6 mg DA/g exceeding the regulatory limit (20 mg DA/g) in public health protection, however the detection limit of HPLC was 0.06 mg/ml (Costa et al., 2003).

5.4 | Pesticides and drug residues detection

In Taiwan, a study was conducted on 3 species of farmed shrimps *that is*, whiteleg, grass shrimp, and giant river shrimps to show the power of LC–MS/MS in detecting the residues of chloramephenicol and two other quinolone drugs (enrofloxacin, and flumequine) with LOQ of 0.25 and 1 ng/g (lower than the that set by TFDA, which are 0.3 and 10 ng/g respectively) inside the shrimp soft tissues (Tsai et al., 2019). For detection of organochlorine pesticides, gas chromatography–mass spectrometry (GCMS) was used with a detection limit of 0.040–0.182 μg/Kg to detect DDTs and their metabolites in prawn shrimp tissues, with high recovery values ranging from 66.8% to 149% (Shi et al., 2011).

6 | CONCLUSIONS

Crustaceans might present a potentially hazardous seafood being more prone to various chemical and biological contaminants, thus biosafety assurance of crustaceans is of prime importance. Implementation of rapid analytical and detection methods for pathogenic microbes, biotoxins and heavy metals can significantly contribute to ensuring crustaceans safety.

As it has been reviewed, bacterial pathogens *that is*, Vibrio and Salmonella spp. are found to be the most prevalent class of biological hazards in crustaceans due to consumption of raw or undercooked crustaceans. Improperly cooked crustaceans can also increase the risk of parasite and virus hazards. Cooking at high temperature (55°C), irradiation and deep freezing at −20°C are the best preventive measures against these biological hazards.

Microbiological methods have obviously some limitations compared to molecular methods due to the close genetic and phenotypic relations between certain microbial species. However, the alteration of the biochemical composition and or sensory characteristics of crustaceans that could be resulting from their possible contamination with different biological hazards has yet to be experimented using metabolites profiling approaches and/or sensory analysis (Farag, Abib, et al., 2021). However, multiplex PCR method is of advantage in the simultaneous detection and amplification of a wide range of microbial species in crustaceans as well as their virulence factors.

Crustaceans are considered accumulators of heavy metals as they live in a close contact with contaminated sediment, with arsenic being the most toxic element to human that has the highest accumulation rate in shrimp muscles. Cadmium and lead accumulate more in shrimps, whereas, crabs pose higher risk of accumulating more chromium and mercury than other crustaceans. Levels of toxic heavy metals in seafood has to be assessed to ensure being within permissible levels so as not to pose any hazard to consumers. For the sake of rapid on-site detection of microbial pathogens, miniaturized diagnostic tools might provide more suitable rapid methods to release crustacean batches.

Mishandling of fresh crustaceans since the collection till the consumption contributes to its contamination, hence proper processing, culinary, and storage processes are deemed necessary to be implemented. Thus, adopting Hazard Analysis Critical Control Point (HACCP) system, a 7-step management protocol, is highly needed to improve the quality of farmed crustaceans and their processed products as it addresses food safety through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement, handling to manufacturing, distribution, and consumption of the processed product (Venugopal & Gopakumar, 2017).

Different processing and cooking procedures eliminate heavy metals and microbial pathogens from crustaceans, however, only a limited number of studies were concerned about examining the impact of different culinary procedures on the chemical safety of cooked crustaceans. Comparison of different cooking methods in different crustaceans with regards to removal of biological or chemical contaminants has yet to be determined on a large scale.

Allover, different cooking methods appear to have different detoxifying effect in different crustaceans which though needs to be tested on a large scale on different metals to be conclusive.

Future endeavors need also to be directed toward optimizing the storage conditions and the use of intelligent packaging devices that can further aid in assuring crustaceans safety and provide a real time monitoring of their freshness to consumers (Khattab et al., 2019).

AUTHORS CONTRIBUTIONS

MAF organized the review theme and supervised the writing, screening and extraction were done by all authors. Data collection, interpretation, tables, and figures were done by all authors equally. All authors contributed to the manuscript writing and approved the final version.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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