

Research Article

Public health risks posed by the invasive Indo-Pacific green mussel, *Perna viridis* (Linnaeus, 1758) in Kingston Harbour, Jamaica

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Abstract

The control strategies for any marine invasive species that is edible may include their use as food for human consumption. The potential and realized use of the invasive Indo-Pacific green mussel, *Perna viridis* (Linnaeus, 1758) for food in Jamaica, from wild populations in Kingston Harbour, underscored the importance of investigating the potential public health risks to consumers from mussels fished from different areas of Kingston Harbour. Levels of bacterial coliforms and heavy metals were examined during this investigation due to the nature of pollutants already documented in Kingston Harbour. Bacterial coliforms showed high levels (15 – >16,000 MPN/100mL) at five stations during the investigation, with the highest values at the station located adjacent to the Hunts Bay Power station (HBPS). Heavy metals, especially chromium and cadmium were also high (43.3–70.3 mg/kg (chromium) and 17–60 mg/kg (cadmium)), with highest value at the station GC (Goodbody's Channel) for chromium and Station DT (Kingston Waterfront) for cadmium. The risks to public health through consumption of these green mussels, *Perna viridis* from Kingston Harbour are significant. If consumption of wild populations from Kingston Harbour is to be promoted to reduce the population number of this invasive species, then depuration of the mussels prior to consumption should be carried out.

Key words: heavy metals; coliform bacteria; marine invasive species; public health risk

Introduction

The first documented sighting of the green mussel in Jamaica, presumed to be *Perna viridis* (Linnaeus, 1758), was in February 1998. This sighting was made during routine collection activities by the Department of Life Sciences - University of the West Indies in the Port Royal Mangroves. The identification of the mussel as *Perna viridis* has been confirmed (Buddo et al. 2003). It is very likely that the mussel arrived in Jamaica via the transport and subsequent discharge of ballast water containing larvae of the mussel from ships arriving in Kingston Harbour from an undetermined location.

Prior to its discovery in Jamaica in 1998, *Perna viridis* had been documented in Trinidad in 1990 (Agard et al. 1992) and Venezuela in 1993 (Rylander et al. 1996). One year after its

discovery in Jamaica, *P. viridis* was discovered in the Tampa Bay area, Florida in 1999 (Ingrao et al. 2001).

The fact that mussels are filter-feeding animals poses significant risks to public health as they can accumulate water contaminants including human pathogens and heavy metals from the area in which they are found (Yap et al. 2005). The highly polluted nature of Kingston Harbour (Webber and Wilson-Kelly 2003) increases public health risks associated with consumption of these green mussels.

Perna species, like other bivalves are filter feeders, and are mostly cultured in coastal and estuarine areas. These areas are prone to industrial and sewage discharges. *P. viridis* has been suggested as a potential biomonitoring agent of heavy metals (Ismail and Yap 2000). This is due to its widespread distribution in

Malaysia, and because it remains relatively stationary and is a suspension-feeder.

Bacterial Contamination

Sewage pollution usually leads to the presence of bacterial and viral pathogens (Lewis et al. 1986). Faecal coliform counts in tissues of green mussels, seawater and sediments are higher during the monsoon rain season (Sasikumar and Krishnamoorthy 2010).

Mary et al. (1994) used *P. viridis* to study bacteriostatic compounds in extracts of marine animals from the Indian Ocean. Eighteen bacterial isolates from five genera were isolated from the biofilm associated with *P. viridis*. The five genera included *Aeromonas*, *Alcaligenes*, *Flavobacterium*, *Pseudomonas* and *Vibrio*. The bacterial isolates were tested for sensitivity to methanol and methylene-chloride extracts of *Spirastrella inconstans* (Dendy, 1887) and *S. officinalis* and showed 56–94% sensitivity. *Vibrio* is of special importance to public health.

Martinez and Oliveira (2010) studied the bacterial loading in *Perna perna* (Linnaeus, 1758) in the coastal waters of Brazil, near an urbanized area similar to the situation in Kingston Harbour. It was found that the bacterial loads in the mussels' flesh were between 50 and 4,300 times greater than in a sample of water taken in the vicinity of the mussels. The study also showed that clearance rates are important for making the best decision in situations where, for example, it is desired to introduce mussels for aquaculture. These clearance rates represent the rate at which the mussels remove bacterial cells from the seawater in which they are living. In some instances during this study, it was demonstrated that mussel samples showed high bacterial contamination even in areas where no restriction exist for mussel aquaculture. *Perna perna* therefore demonstrated its usefulness as a species for bacterial biomonitoring.

Heavy Metals

Kamaruzzaman et al. (2011) conducted a study on the bioaccumulation of some essential and non-essential heavy metals in *Perna viridis* collected from Pekan, Pahang in Malaysia. The bioaccumulation of metals in the soft tissue followed an Fe>Zn>Cu>Co>Pb>Cd order indicating that the essential heavy metals accumulate at a faster rate than in non-essential metals.

Previously in Malaysia, Yap et al. (2005) examined the presence and concentrations of Cd, Cu, Pb and Zn. From a consumption point of view, it was found that the concentrations of these metals in the tissues of the green mussels were acceptable according to the Malaysian Food Regulations (1985). The study also showed that the consumption of mussels was not risky and any risk is dependent on the amount of mussels consumed and the sites they were collected from.

A similar study was conducted by Dimalagan et al. (2010) on *Perna viridis* samples taken from selected seafood markets as well as an analysis of the water that supplies the mussels in the Manila metropolitan area. It was found that only copper and lead exceeded local standards and therefore represent a risk to public health from consumption.

Yap et al. (2007) performed laboratory investigations of the tolerance of high inorganic mercury of *Perna viridis* based on its accumulation, depuration and distribution. Green mussels showed high tolerance to very high concentrations of mercury (100µg/L), as none of the mussels dies after a 4-day exposure. The green mussels showed a high bioaccumulative capability and a high tolerance to inorganic mercury since the soft tissues can accumulate inorganic mercury. With respect to depuration, the byssus was found to have the highest depuration rate coefficient, indicating that it could act as one of the main excretion routes for mercury. The faecal materials released by the mussels had elevated levels of mercury indicating that the mussels acted as a mercury retention mechanism on the coastal ecosystem.

P. viridis also poses a public health risk to persons who consume the mussel. Due to its high filtration rate, it will often accumulate toxic dinoflagellates and other pathogens and contaminants from the water in which it has grown (Wong and Cheung 1999). As Kingston Harbour is recognized as one of the most eutrophic harbours in the Caribbean (Webber et al. 2003), the risk to the public is significant (Buddo et al. 2003).

Buddo et al. 2003 showed the presence of four potentially toxic microalgal species in the gut contents of *Perna viridis* specimens collected from eleven stations within Kingston Harbour, Jamaica. These were *Dinophysis caudata* (Saville-Kent, 1881), *Prorocentrum minimum* (Schiller, 1933), *Prorocentrum lima* (Dodge, 1975) and *Prorocentrum rathymum* (formerly *Prorocentrum mexicanum* – Tafall, 1942). If

farming or collection of wild mussels for sale and consumption is to be considered from Kingston Harbour, the public health risks will have to be carefully evaluated.

The objective of this investigation was to determine the levels of coliform bacteria and heavy metals in the mussels, and therefore the public health risks associated with the animals from different areas of the Kingston Harbour. These two categories of pollutants were chosen subjectively based on historical data (Buddo et al. 2003) as well as the type of industries and urban influences on Kingston Harbour.

Depuration

Depuration is recommended to remove public health risks associated with mussels. Highly polluted mussels in Singapore have been successfully depurated to acceptable limits of *Escherichia coli* within 48 hours (Cheong and Monzil 1982) using a recirculating bacteria-free water system. De Guzman and Mabesa (1985) reported that *P. viridis* depurated by using stagnant water and sucrose solution technique did not affect the organoleptic properties, however, they noted a change in the fat and protein content within 24 hours of depuration. Li et al. (2005) examined the depuration of toxic dinoflagellates from *P. viridis*. The transfer coefficients during the depuration phase from viscera to gill, hepatopancreas, adductor muscle, and foot were 0.01, 0, 0.01, and 0.003 per day, respectively.

Heavy metal elimination during depuration of *P. viridis* showed that metals in the soluble fraction mediated depuration, whereas metals in the insoluble fraction acted as a final storage pool. Redistribution of heavy metals among the tissues may also occur between the metal-sensitive and inactive tissue pools without significant depuration as a secondary protective mechanism (Ng and Wang 2005).

Methods

Coliform Bacteria

Mussels were collected by hand on two different occasions from the 11 selected stations (Figure 1); however, there was enough replication of stations and close proximity to each other to allow for temporal comparisons as outlined in Table 1.

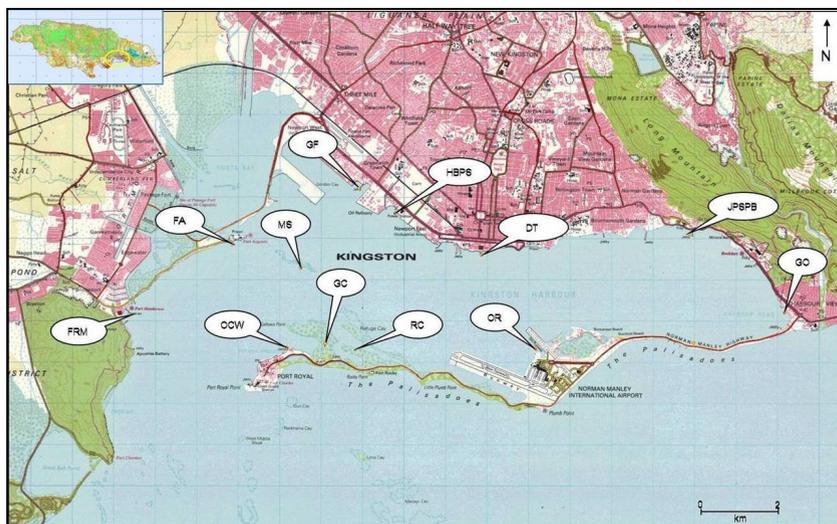
The specimen containers (wide-mouth glass bottles) were prepared by the Ministry of Health – Environmental Health Laboratory (EHL) in Jamaica. The bottles were cleaned using a suitable detergent and rinsed thoroughly with hot water to remove all traces of the detergent. They were then finally rinsed with distilled water and air-dried on a drying rack. 0.1mL of a 10% solution of Na₂S₂O₃ was placed in each bottle. The tops of the bottles were then covered with aluminum foil and sterilized in an autoclave at 121°C for 15 minutes. The prepared bottles were received just prior to field trip.

The sampling events were chosen close to the end of the first dry season for the calendar year in Jamaica i.e. March. However, rainfall events in the general area of the Kingston Harbour occurred prior to sampling, which increased the amount of run-off to the sampling stations. Based on the findings of the first sampling event (see results), focus was given to fewer stations and only faecal coliforms were targeted for the second sampling event. In addition, reports on shellfish removal for consumption were observed at these stations. At each station visited, ten mussels were collected in the size range 70-80mm. The mussels were placed individually in clean Ziploc® bags and taken back in a cooler on ice to the laboratory immediately to maintain viability. At the laboratory, each animal was treated separately. Each mussel was carefully dissected using clean latex gloves and the cavity water drained into a 500ml beaker, prepared in the same manner as the collecting bottles. The stomach was also dissected, the contents washed out using a syringe and the cavity water collected. The resultant liquor was also allowed to drain into the beaker. Each mussel from each sample was collected in a different beaker. The contents were then transferred to the collecting bottles. Care was taken not to cross-contaminate each sample by using a different pair of latex gloves for each sample, and also soaking the dissecting instruments in 70% alcohol before use. An air space of approximately 2.5cm was left at the top of the bottle to facilitate mixing. The cover of the bottles was replaced immediately.

The samples were immediately placed in an iced cooler and then transported immediately to and analyzed by the Environmental Health Laboratory (EHL) of the Ministry of Health's Public Health Laboratory, Government of Jamaica. The technique used was The Multiple-Tube Fermentation Technique. This technique was based on lactose fermentation to indicate all

Figure 1. Map of Kingston

Harbour showing stations used to assess public health risks (FRM=Forum Hotel; FA=Fort Augusta; GF=Greenwich Farm; HBPS=Hunt's Bay Power Station; DT=Downtown Kingston; JPSPB=Jamaica Public Service Power Barge; GO=Gypsum Office; OR=Old Runway; RC=Refuge Cay; GC=Goodbody's Channel; OCW=Old Coal Wharf; MS=Mammee Shoal) [Source: National Land Agency – Jamaica.

**Table 1.** Stations used for sampling and physicochemical characteristics.

Station	Depth (m)	Temperature (°C)	Salinity	Substrate Type	GPS Coordinates	
					Latitude, N	Longitude, W
FRM	0.6	26.77	34.24	Submerged Rocks	17.950648	76.873190
FA	1.1	26.88	34.96	Metal Wharf Pilings (Pylons)	17.965356	76.850037
MS	0.6	27.11	34.33	Seagrass Bed (Muddy/Sandy)	17.961464	76.838848
GF	1.3	26.94	34.12	Metal Wharf Pilings (Pylons)	17.977403	76.820199
HBPS	1.1	27.02	34.30	Concrete Wharf Pilings (Pylons)	17.971761	76.811826
JPSPB	1.4	27.10	34.06	Metal Wharf Pilings (Pylons)	17.967050	76.755122
GO	0.6	26.92	33.75	Concrete Pier Wall	17.950066	76.723084
OR	0.4	26.95	34.23	Mangrove Prop Roots	17.943235	76.780602
RC	0.3	26.86	34.33	Mangrove Prop Roots	17.942288	76.821395
OCW	0.9	26.66	33.51	Concrete Wharf Pilings (Pylons)	17.942060	76.836829
DT	0.4	26.88	33.61	Concrete Pier Wall	17.963409	76.792896

aerobic and facultative anaerobic, gram negative, non-spore forming rod-shaped bacteria that ferment lactose with gas and acid formation within 48h at 35°C. The results of the examination of the replicate tubes and dilutions were reported in terms of the Most Probable Number (MPN) of organisms present. The samples were analyzed using the Standard Method for the examination of water and wastewater 21st edition, 9221B and 9221E (APHA 2005).

Heavy Metals

Four stations were used for this investigation; these stations were Station GF, Station DT, Station GC and Station OR (Figure 1). These stations were subjectively chosen based on

proximity to industrial operations as well as evidence of the mussel being removed for human consumption or fish bait. Three random replicate samples were taken at each station. To ensure consistency, ten mussels in the size range of 70-80mm were taken for each replicate sample at each station. The mussels were removed by hand only, i.e. no knives were used, and transported back to the laboratory in plastic containers.

Each sample was kept separate during the tissue removal process to prevent contamination of the samples. A wooden mallet was used to crack the shell initially and subsequently, the entire flesh was removed using a plastic knife. The flesh was placed into glass beakers. The flesh from the ten mussels in each station sample was stored together, but separate from the other samples. All instruments (including the glass

beakers) were rinsed with 10% nitric acid and then with distilled water before the removal process for each sample and also between samples. Clean latex gloves were used for handling each sample.

Each sample was weighed using a digital metric scale (Carolina Analytical Balance 702029) to nearest 0.01g to establish the fresh weight of each sample. Each sample was then placed into large petri dishes to maximize surface area and then dried in a Gravity Convection Oven at 80°C for a period of 72 hours.

After the 72hr drying period, the mussels were reweighed to determine the dry weight and then packaged into separate Ziploc bags and sent off to the International Centre for Environmental and Nuclear Sciences at the University of the West Indies for heavy metal analyses.

A dried substrate sample (0.5g) was weighed into a 60ml polyethylene vial and 20 ml of a 1:3 mixture of trace metal grade concentrated HCl and HNO₃ (reverse aqua regia) was added. The mixture was allowed to stand overnight and was then digested at 110°C for 2 hours in a metal free, electrically heated graphite digestion block (MODBLOCK, CPI International, California). The vials were covered with polyethylene watch glasses to aid reflux.

The digestate was allowed to cool and the volume made up to 30ml with water. The elements reported were analyzed by Flame (Cu, Mn, & Zn) and Graphite Furnace Atomic Absorption Spectrometry (Cd, Cr, & Pb) (Perkin Elmer 5100ZL). Blanks, replicates and certified reference materials (BCR 278R, Trace Elements in Mussel Tissue) were used for quality control (Community Bureau of Reference (Belgium)).

Results

Coliform Bacteria

The results shown in Table 2 and Table 3 represent coliform analyses on two occasions (March 17, 2001 and March 21, 2005 respectively). These values are compared to the Trade Effluent Standards of the Natural Resources Conservation Authority (NRCA) in Jamaica. These are <500 MPN/100mL for Total Coliform and <100 MPN/100mL for Faecal Coliform (NRCA, 1995).

On the first occasion (Table 2), five of the ten stations exceeded the NRCA Standards (highlighted in red) for both the Total and Faecal

Table 2. Total and Faecal Coliform results for ten stations on March 17, 2001 (values exceeding standards = bold type).

Station	Total Coliform (MPN/100ml)	Faecal Coliform (MPN/100ml)
FRM	80	<20
FA	<20	<20
MS	3000	260
GF	130	40
HBPS	>16000	5000
JPSPB	1300	170
GO	2400	220
OR	1300	110
RC	500	80
OCW	170	40

Table 3. Faecal Coliform results for five (5) stations on March 21, 2005 (values exceeding standards = bold type).

Station	Faecal Coliform (MPN/100ml)
GF (Greenwich Farm)	23
OCW (Old Coal Wharf)	15
DT (Kingston Waterfront)	240
RC (near Refuge Cay)	40
OR (behind Old Runway)	280

Coliform counts. The highest value was seen at Station HBPS of >16000 MPN/100ml and 5000 MPN/100ml for total and faecal coliforms respectively. Of the five stations that showed the values within the NRCA limits, Station FA showed the lowest values of <20 MPN/100ml for both total and faecal coliforms.

On the second occasion on March 21, 2005 (Table 3), two of the five stations exceeded the NRCA Standards (shown in bold font) for the faecal coliforms. These were Stations DT and OR. Station OR, as on the first occasion, exceeded the standards. Stations GF, OCW and RC all showed values within the standards as in the first occasion, showing a value of 15 MPN/100ml with Station OCW.

Heavy Metals

The presence and concentrations of six heavy metals, cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), lead (Pb) and zinc (Zn) in the tissues of the green mussels are shown in Table 4.

Traces of all six heavy metals were found at all stations used in this investigation. Cd and Cr showed the highest levels of concentrations in the mussel tissues for all stations. Cd ranged

Table 4. Results of heavy metal analyses of tissues of *Perna viridis* (n/r = no result).

	Station OR		Station GF		Station GC		Station DT	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cd (mg/kg)	50.0	7.5	38.0	1.7	17.0	6.6	60.0	1.7
Cr (mg/kg)	57.7	4.0	58.7	7.0	70.3	9.0	43.3	6.4
Cu (mg/kg)	2.2	0.2	3.6	0.0	2.0	0.6	3.3	1.3
Mn (mg/kg)	7.4	0.4	3.7	0.5	6.8	0.8	4.1	0.9
Pb (mg/kg)	0.2	0.0	0.3	0.0	0.3	0.1	0.4	0.0
Zn (mg/kg)	18.3	0.6	21.3	4.2	19.3	1.5	21.7	3.1

from 10-62mg/kg and Cr ranged from 36-76mg/kg. Pb showed the lowest concentrations of all the metals ranging from 0.2-0.44mg/kg of mussel tissues (Table 4).

According to the European Standards and Guidelines for Trace Metals in fish and shellfish (Brown and Balls 1997), the acceptable levels for shellfish are as follows:

- Lead: 7.5mg/kg
- Cadmium: 5.0mg/kg
- Zinc: 4000mg/kg
- Copper: 400mg/kg
- Chromium: 6.0 mg/kg

According to the standards above, cadmium and chromium exceeded the standards at all stations. The values obtained for all other metals satisfied this standard. There are currently no European Standards for Manganese.

Discussion

The two categories of contaminants examined in this investigation, coliform bacteria and heavy metals showed varying but equally important findings.

Coliform bacterial levels found in the combined cavity water and the gut contents are of great concern regarding potential consumption of the mussel from Kingston Harbour. On both occasions of testing for coliforms, approximately 50% of the stations showed values that exceeded the national standards, most by a significant margin. The presence of land-borne influences via sewage, storm and industrial outfalls in Kingston Harbour is the likely contributor to the high bacteria levels in the mussels at some of these stations (Webber and Wilson-Kelly 2003).

At Station HBPS (Hunts Bay Power Station), the values of coliform bacteria were exceptionally high (>16000 MPN/100mL (TC) and 5000 MPN/100mL (FC)). This station receives input from nearby storm drains. It may

also be influenced by the Greenwich Farm Wastewater Treatment Facility outfall which is approximately 400m from this station. Station MS also showed high values (3000 MPN/100mL (TC) and 260 MPN/100mL (FC)). This station is located in the middle of the harbour, and would receive sewage influences from a variety of possible sources. Martinez and Oliveira (2010) studied the bacterial loading in *Perna perna* (Linnaeus, 1758) in the coastal waters of Brazil, near an urbanized area similar to the situation in Kingston Harbour. It was found that the bacterial loads in the mussels' flesh were 50 to 4,300 times greater than in the sample of water in the vicinity of the mussels. Though the surrounding water was not tested in this study, the mussels in Kingston Harbour do show similar high levels of bacterial contamination compared to *P. perna* in Brazil. A comparable study in Malaysia showed that the mussels showed higher loads of bacterial coliform than the surrounding waters (Sasikumar and Krishnamoorthy 2010).

Station GO is close to an outflow from the Harbour View Sewage Facility/Pumping Station, as well as storm drains from the neighbouring communities. This would account for the coliform values in the mussels of (2400 MPN/100mL (TC) and 220 MPN/100mL (FC)). Station DT showed excessive values (2400MPN/100mL FC) in the mussels in the 2005 sampling event. This station is close to waterways leading from the urbanized and informal (unplanned and unregulated) residential areas, and would receive some level of under-treated wastewater from numerous soak-away pits in the area. These values are comparable to those found in tissues *P. perna* in Brazil's coastal waters (Martinez and Oliveira (2010) due to under-treated wastewater.

The heavy metals analyses showed that for cadmium and chromium, the mussel poses significant risk to public health if consumed from all stations studied. Though all other trace

metals showed levels well within the acceptable European limits (Brown and Balls 1997), Kingston Harbour possesses industries around its perimeter that are possible sources of heavy metal contamination. Station OR is close to the airport, which contains large fuel storage areas and well as other storage areas for discarded machinery. Station GC is close to boating activity, and receives direct boating activity from especially fishermen. This would be a possible source of cadmium due to fuel leaks, and may explain the high levels obtained. This would also be applicable to the other two stations, GF and DT. Stations GF and DT however, are close to various industries, such as those involved in metal electroplating, tanning, wood preservation and corrosion inhibitors, which are potential sources of these heavy metals. All these industries are found around Kingston Harbour, and likely to have leaching into the Harbour. *Perna viridis* has shown the capability to accumulate, concentrate and retain heavy metals in its tissues (Kamaruzzaman et al. 2011; Yap et al. 2005; Dimalaggan et al. 2010).

The green mussel in Kingston Harbour has been used as a food source. These mussels have been collected from the wild and do not undergo any form of depuration prior to consumption (Andre Kong, per comm.). As shown in this study, the consumption of green mussels from Kingston Harbour poses a significant threat to human health. If future consumption is to be encouraged, especially in an effort to minimize the population number of this invasive species, then depuration must be a feature of any effort. Clearance rates shown in *Perna viridis* of heavy metals are reasonable, and the mussels would be able to clear the heavy metal contaminants (Kamaruzzaman et al. 2011).

Collection and consumption of mussels after periods of heavy rainfall should be discouraged and depuration at an established facility prior to consumption should be carried out to reduce the risks to public health, from both heavy metals and bacterial coliforms. A monitoring programme should be put in place to test mussels on an ongoing basis to track changes in the levels of the contaminants particularly coliform levels and heavy metal levels. A public education and outreach programme is important so that persons will know the possible risks as well how to avoid these risks. Based on this study, the consumption of green mussels *Perna viridis* from Kingston Harbour should not be encouraged.

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