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Modified, optimized method of determination of Tributyltin (TBT) contamination in coastal water, sediment and biota in Sri Lanka

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ABSTRACT

Tributyltin (TBT) is a toxic organotin compound that belongs to the group of Persistent Organic Pollutants (POPs) and it is documented to cause severe sexual disorders development in aquatic fauna. According to the present study, The TBT concentration in coastal water ranged from 303 ± 7.4 ngL^{-1} to 25 ± 4.2 ngL^{-1} wherein sediment was from 107 ± 4.1 ngKg^{-1} to 17 ± 1.4 ngKg^{-1}. TBT in *Perna viridis* was found to range from 4 ± 1.2 ngKg^{-1} to 42 ± 2.2 ngKg^{-1} wet weight and in ascending order of the body weight. The highest TBT level in water and sediment was found in the Colombo port where the highest level of TBT in *P. viridis* (42 ± 2.2 ngKg^{-1}) was recorded from the Dikkowita fishery harbor. A positive correlation between the number of male *P. viridis* and TBT level ($p < 0.05$) suggests possible reproductive impairment in aquatic animals exposed continuously to a high concentration of TBT.

1. Introduction

Organotin compounds (OTs) including Tributyltin (TBT) are a set of toxic Persistent Organic Pollutants (POPs) in the environment and they persist 10–40 years due to their chemical and structural nature (Guruge et al., 2005; Ohura et al., 2015; Guruge et al., 2015). Tributyltin has biocidal properties and could be used in different industries such as agricultural pesticides, wood preservatives, and antifouling paints on ships and boats (Barbosa et al., 2018; Cho et al., 2012). Though the International Maritime Organization has prohibited the use of TBT-based paints (CD, 2002; IMO, 2001), different industrial sectors in developing countries are still using TBT with poor environmental monitoring (Santillo et al., 2002). Due to its wide spread use as an antifouling agent in boat and ship paints, TBT is recorded as a common contaminant of marine and freshwater ecosystems (Antizar-Ladislao, 2008). TBT contamination of the local breeding waters or maricultural water was found to be responsible for the failure to reproduce and for anomalies occurring in the shell calcification of adult oysters leading to stunted growth (Neuparth et al., 2017). Numerous investigations have proven that TBT is toxic to aquatic organisms in general and to bivalve mollusks and gastropods in particular, for which the No Observed Effect Levels (NOELs) are below 1.0 ngL^{-1} (Alzieu, 1998). Mollusks are known to be the most sensitive species to TBT exposure, thus the effects on larval development of bivalves as well as disturbances in gastropod sexuality were recorded at ngL^{-1} level in seawater (Haydee and Dalma, 2017). OTs are environmental hormones (Guruge et al., 2008), which disrupt reproductive cycles of invertebrates (Gibbs and Bryan, 1996), humans, wildlife (Guruge and Tanabe, 2001; Hiromori et al., 2016) and the toxicity depends on the concentration of the main groups of organotin compounds and the number of organic (tetra-, tri-, di- and mono-organotin) molecules in the chemical structure (Tomza-Marciniak et al., 2019; Fang et al., 2017; Sunday et al., 2012). TBT has a long half-life in marine water (>2 years), sediment (>5 years) (Hassan et al., 2019; Stolarek et al., 2019), and having a high specific gravity of 1.2 KgL^{-1} at 20 °C. Further, it has been shown that rapid removal of the chemical from water column to sediment bed (Yozukmaz et al., 2011) and its adsorption on sediment was reversible (Parmentier et al., 2019). Thus, the TBT contaminated sediment could act as a long term source of dissolved-phase contamination to the upper water column (Sahoo and Oikari, 2016) as a renewable pollutant source and not immediately be

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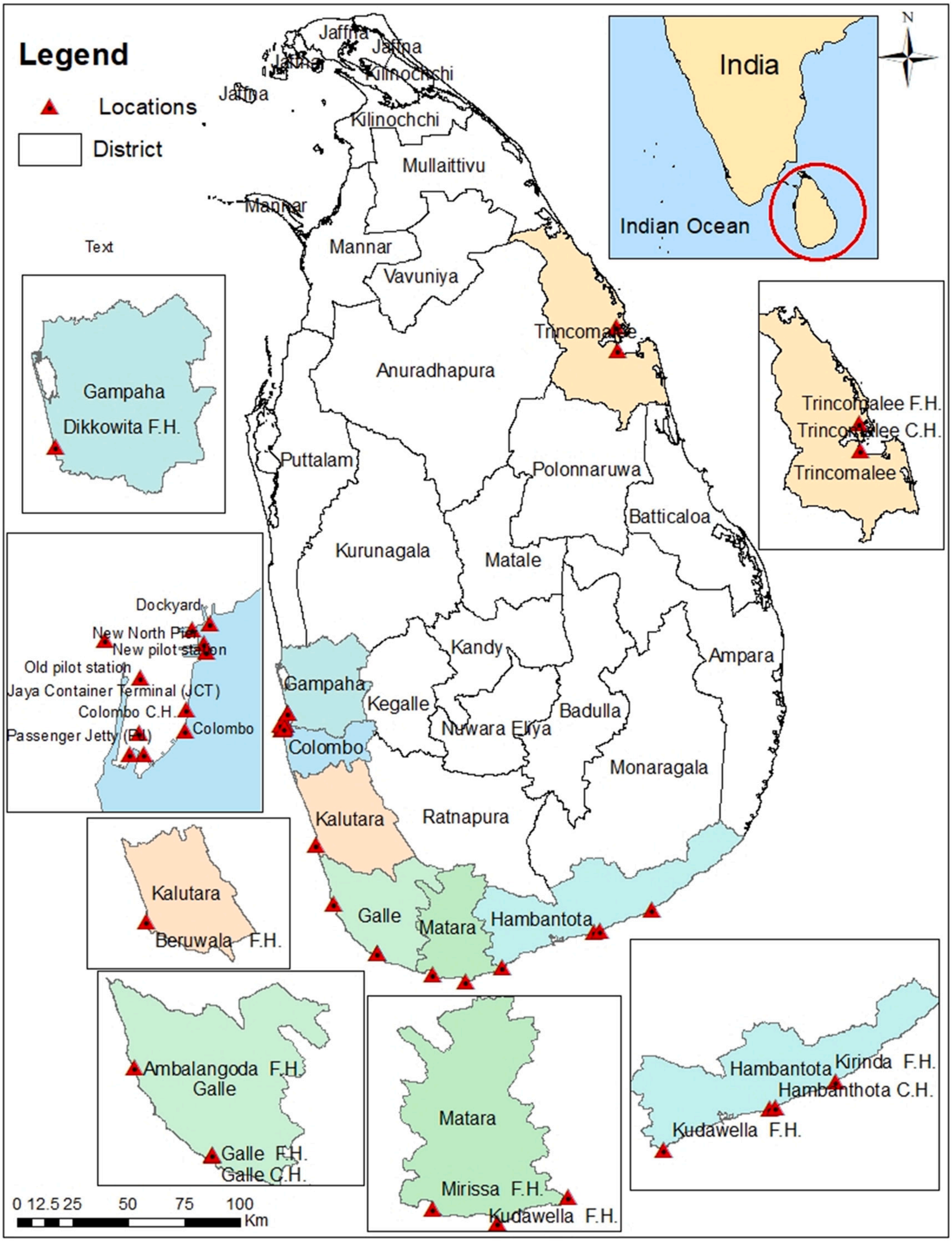

Fig. 1. Water, sediment and *P. viridis* sampling locations of the present study and different water sampling locations of the Colombo port (C.H: Commercial harbor, F.H: Fishery harbor).

removed from the marine environment (Yozukmaz et al., 2011). Wide distribution, high hydrophobicity, and persistence of OT compounds have raised concerns about their bio-accumulation, potential bio-magnification in the food webs, and their adverse effects to the environment and human health (Galloway, 2006; Nakanishi, 2007; Takahashi et al., 1999; Veltman et al., 2006).

The appearance of male characters in females in some group of aquatic invertebrates has been recorded (Grilo and Rosa, 2017; Alzieu, 2000; Matthiessen and Gibbs, 1998) and irreversible development of imposition of male sex characteristics in female genitalia via masculinization in some aquatic fauna is referred to as imposex (Smith, 1980). Imposex can be caused by the accumulation of TBT and recently such characters in aquatic animals have been recorded (Schoyen et al., 2019; Hassan et al., 2019). Imposex has been described in over 200 species belonging to 50 genera and serves as a bio-indicator of TBT exposure in many monitoring programs (Axiak et al., 1995; Ni et al., 2019). Recent studies showed that the several species of gastropods exposed to TBT, contain a high concentration of testosterone in females (Rossato et al., 2016) and some of the in vitro studies have shown that the injection of testosterone to females was able to produce imposex

(Giraud-Billoud and Castro-Vazquez, 2019; Ni et al., 2019). Further, calcification anomalies of mollusks have been recorded even when the concentrations of TBT as low as 2 ngL^{-1} (Alzieu, 2000; Bray and Langston, 2006). Prolonged exposure to OT chemicals causes immune (Iqbal et al., 2016), neuro (Barbosa et al., 2018), genotoxicity (Hunakova et al., 2019), and metabolic dysfunction including obesity and cancer on wildlife have also been recorded (Li et al., 2019; Barbosa et al., 2018).

Understanding TBT pollution in coastal areas of certain Asian and Oceanian countries is crucial due to the challenge of lack of research and advanced detection methods of xenobiotics (Ho and Leung, 2017). Sri Lanka is situated strategically at the cross roads of major shipping routes to South Asia, a maritime hub in the Indian Ocean, which is recognized as a heavy shipping traffic line in Southeast Asia. On the other hand, Sri Lanka's position in the Indian Ocean means that fishing activities take place in almost every direction off the country's coast. The busy maritime and heavy boating and shipping activities in coastal areas all around Sri Lanka may lead to heavy usage and contamination of OT in the coastal environment. However, to date there is very limited information regarding OT contamination in marine water, sediment and biota. Thus, in the present study, *P. viridis* was selected as a biological indicator based on their wide geographical distribution, sessile lifestyle, easy sampling, tolerance of a considerable range of salinity, resistance to stress and high accumulation of a wide range of chemicals, and worldwide demand as a commercial seafood (Antizar-Ladislao, 2008). Therefore, this paper discusses a sensitive, cost-effective, and precise optimized method to identify and quantify TBT at ngL^{-1} level in water, at $ngKg^{-1}$ level in sediment, and biota in selected commercial and fishery harbors in Sri Lanka.

2. Materials and methods

2.1. Reagents and materials

Standard TBT chloride and other chemicals (HPLC grade) were purchased from Sigma-Aldrich in Germany. Manual fiber assembly of Solid-Phase Micro Extraction (SPME) with an extraction fiber coated with Polydimethylsiloxane/Divinylbenzene (PDMS/DVB), PDMS and Polyacrylate fibers with various thicknesses were purchased from Supelco (Tokyo, Japan).

2.2. Preparation of samples for extraction of TBT

2.2.1. Standards and calibration curves

Tributyltin chloride (1.2 g/mL) was dissolved in deionized water to make a stock solution at a concentration of 1 mgL^{-1} and stored at 4 °C. The diluted standard samples were prepared using deionized water to get different standard concentrations; 25, 50, 100, 500, 1000 ngL^{-1} of TBT following the preparation of the calibration curve. Ten milliliter aliquot of each standard was derivatized to more volatile tributyltin hydride in headspace SPME vial by using 0.5 g of KBH_4.

2.3. Study area and sampling

Water and sediment samples were collected from commercial and fishing harbors along the Southern coastal belt from the Colombo port to Trincomalee harbor (Fig. 1). Four major commercial and ten fishing harbors were selected to collect water and sediment samples from June 2018 to April 2019. In the Colombo port, water samples were collected from ten different locations. *P. viridis* samples were collected from Colombo port, Dikkowita, Galle, Mirissa, Dewundara, Kirinda, and Trincomalee fishery harbors using a professional diver at each site (Fig. 1).

2.3.1. Collection of water and sediment samples

Water samples were collected into autoclaved amber glass bottles (2.5 L) and preserved adding 1 M Hydrochloric acid. Sediment and biological samples were collected into sealed polyethylene bags and samples were placed in ice boxes (4 °C), and transported to the laboratory within 8 h, stored in the dark at 4 °C, and analysis was performed within 5 days.

Fig. 2. *P. viridis* (green mussel).

Fifty grams of well mixed wet sediment sample was freeze dried (ShinBioBase, Korea) until become a constant weight and 10 g of freeze dried sediment sample was used to extract TBT.

2.3.2. Collection of biological samples

In each location, approximately 40 *P. viridis* (Fig. 2) of three weight size classes; 0–15 g, 15–30 g and 30–45 g were collected by quadrat sampling method and separated to sizes and sex. In the laboratory, shells were washed three times using distilled water, shells removed, and the soft tissue in each individual was weighed and divided into each size class and freeze-dried until extraction of TBT.

2.4. Extraction of TBT

Water samples (10 mL) were placed in headspace vials and pH was adjusted to 5, and 0.5 g of KBH_4 was added as the derivatizing agent. The mixture was properly agitated following vortex for 3 min and then the HS-SPME/GC–MS analysis was done.

Ten grams of each freeze-dried sediment and mussel samples were treated with 10 mL of deionized water as the solvent for TBT extraction. The mussel tissues were homogenized and sediment samples were shaken for 24 h at 4000 rpm (Multishaker MMS, Japan), centrifuged for 15 min at 4000 rpm, and 10 mL of supernatant was removed into a headspace vial. Then 0.5 g of KBH_4 was added and sealed with a twist cap, vortexed, to absorb volatile Tributyltin hydride to HS-SPME (Mattos et al., 2017). The extraction procedure was repeated three times to ensure complete extraction of TBT from the environmental sample.

2.5. Headspace Solid-Phase Micro Extraction (SPME)

SPME which is a relatively new technique for extracting chemical compounds in the ultra-trace level (Wilson et al., 2018) was used. The sample and derivatization agent was transferred to a headspace vial and sealed with a screw cap. The septum of headspace vial cap was pierced and the SPME fiber was exposed to the headspace without touching the sample until analytes get adsorbed to the fiber. After that, the fiber was retracted into the metal needle and it was removed from the septum following thermal desorption of the analyte to the hot GC injector.

2.5.1. Optimization of headspace Solid-Phase Micro Extraction (SPME)

The different extraction times (5 min, 10 min, 20 min, and 30 min), temperature (25 °C, 30 °C, 40 °C, and 60 °C) and pH (2, 5, 8, 10, and) were studied modifying the method described by Wilson et al. (2018).

2.6. Recovery tests for TBT extraction

Recovery tests were carried out to verify the extraction procedures of water, sediment, and biological samples by spiking the known

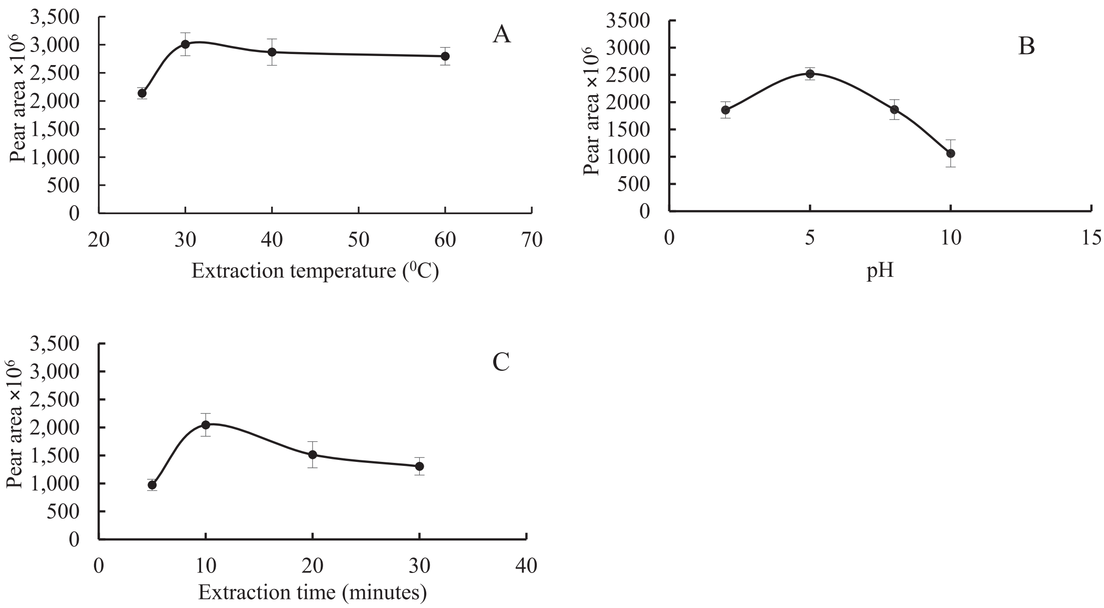

Fig. 3. The effect of temperature (A). pH (B) and extraction time (C) on extraction of TBT (standard concentration 2 μgL^{-1}).

concentration of TBT chloride (2 μgL^{-1}) to artificial seawater, reference sediment samples and cultured reference mussel samples following the HS/SPME extraction procedure described (Mattos et al., 2017; Liyanage and Manage, 2016).

2.7. *Quantification of TBT using Gas Chromatography–Mass Spectrometry (GC–MS)*

Samples were analyzed by the Gas Chromatography (Agilent Model 7890A, USA) system coupled to a Mass Spectrometer detector (Agilent Model 7890A, USA) (GC–MS). Chromatographic separation was achieved on a fused-silica capillary column with cross-linked 5% Phenyl Methyl Siloxane of HP-5 MS (30 m × 250 μm × 0.25 μm film thickness). The carrier gas used in the study was helium (99.9999% purity) and the column flow was maintained at 1 mLmin^{-1}. The GC operating conditions used to detect TBT were as follows: injector temperature 270 °C; helium carrier gas flow rate was 1.0 ml/min. The oven was initially held at 100 °C for 1 min, then increased by 15 °C/min to 160 °C and held for 4 min. The full scan mass spectra were obtained at an m/z range of 33–550 D. Scan mode detection and quantification for TBT was selected at $m/z = 179$ and identification was done with NIST reference library. These were monitored alternatively at dwell times. The correlation area was measured to construct the calibration curve to determine the concentration of TBT in samples.

TBT levels in water, sediment, and biological samples were measured in triplicate with the instrument and procedural blanks. Every set of 10 environmental samples, standard samples were run to confirm the accuracy of the procedure (Chen et al., 2017) and the calibration curve was used for quantitative analysis of TBT.

2.8. *Statistical analysis*

Statistical comparison of the data was carried out using a Pearson correlation of Minitab 14 for windows. The correlation value of p less than 0.05 was considered to be a statistically significant linear relationship.

3. Results and discussion

A number of studies on TBT distribution in water column, sediments, and biota from Japan, Korea, Canada, USA, UK, Spain, and France, etc. have been recorded (Evans et al., 1995; Murai et al., 2005; Rodríguez-González et al., 2006; Kim et al., 2008; Zeidan and Boehs, 2017; Rumampuk et al., 2018; Laranjeiro et al., 2018). TBT in bottom sediments has been reported as hot spots associated with ship channels, ports, harbors, and marinas in Galveston Bay, USA (Wade et al., 1991), Hong Kong (Ko et al., 1995), Netherlands (Ritsema et al., 1998), Iceland (Svavarsson and Skarphédinsdóttir, 1995), Israel (Rilov et al., 2000) and Japan (Harino et al., 1998). No information about TBT contamination in the coastal environment in Sri Lanka is documented to date. The most obvious routes of organotin exposure to biota and consequently to the food chain is through the diet and accumulation from surroundings (Kucuksezgin et al., 2011; Lee et al., 2006; Strand and Jacobsen, 2005; Bryan et al., 1989).

However, with the rapid economic progress in Sri Lanka as a developing country, a set of restrictive environment quality standards for chemical pollutants is a timely requirement. Therefore, it is necessary to develop green analytical methods to monitor environmental pollutants even in the ultra-trace levels to protect the aquatic environment and human beings. Thus, the present study was aimed to record the contamination status of TBT in commercial and fishing harbors of Sri Lanka by the newly optimized HS/SPME-GCMS method which is a sensitive, simple analytical method; requiring fewer reagents, reduce waste and is less time consuming than traditional methods employed for detection of OTs, such as Soxhlet and solid-liquid extraction, liquid-liquid extraction and solid-phase extraction, etc. As such, the procedure connects with the principles of green analytical chemistry.

3.1. *Optimization of HS/SPME extraction*

Several effective factors of the SPME procedure were studied to identify the best-operating conditions yielding the highest SPME recovery. The major significant variables affect the extraction efficiency of TBT; SPME fiber type, extraction pH, temperature, and time were studied.

3.1.1. *SPME fiber type*

The principle of HS/SPME extraction is based on the equilibrium partition process of analytes among the liquid phase (sample matrix), headspace, and the solid phase of the fiber coating. To optimize the best extraction conditions for HS/SPME, the selection of fiber coating is of foremost important one (Cardellicchio et al., 2001; Raza et al., 2019;

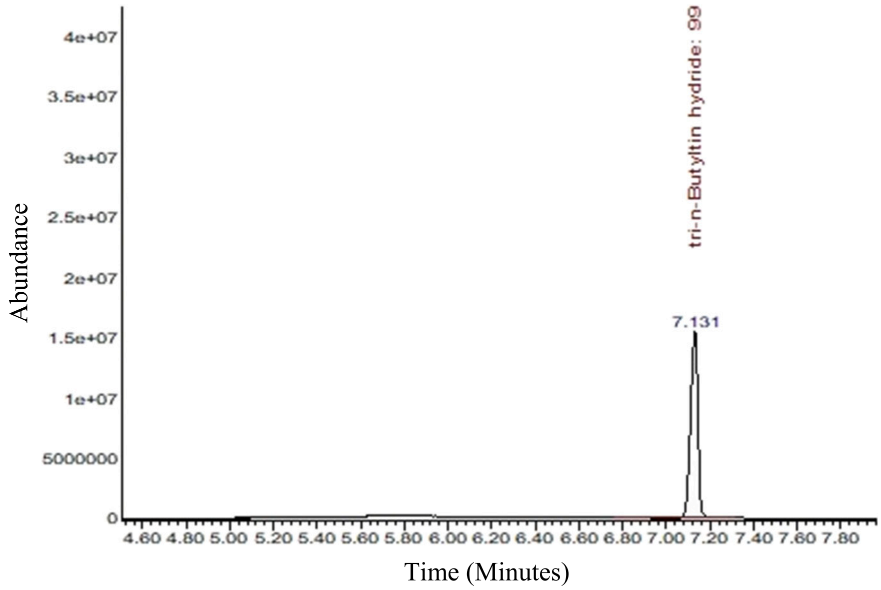

Fig. 4. Total ionic current spectrum of TBT hydride. Retention time is at 7.13 min and 99% matching with NIST library spectra.

Spietelun et al., 2013), thus, three types of SPME fibers; Polar (polyacrylate), Nonpolar (Polydimethylsiloxane) and Medium polar (Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)) were tested for TBT extraction from water, sediment and biological samples after the derivatization to TBT hydride. Tributyltin hydride peak was obtained for both medium polar and non-polar SPME fibers. The best conditions obtained for the efficiency of each of the SPME fiber was based on the maximum peak area of the target compound (TBT hydride) isolated according to each of the conditions analyzed (Garcia et al., 2019). The greater peak area of the medium polar SPME fiber (Polydimethylsiloxane/Divinylbenzene (PDMS/DVB, Fused Silica)), microfiber with the film thickness of 65 μm showed the most efficient SPME fiber to absorb TBT hydride. Nevertheless, a standard peak was not obtained for the polar fiber at GC–MS. Before using the fiber, it was conditioned for 30 min at 250 °C in the GC injector to clean (bake-out) and prepare it for the analysis. It will help to protect the GC injection port from contamination and free up the port after thermal desorption (Cardellicchio et al., 2001).

3.1.2. Extraction conditions (temperature, pH and time)

Four different temperatures (25 °C, 30 °C, 40 °C, and 60 °C) were tested to find the most effective temperature for the TBT extraction (Beceiro et al., 2009) and no significant difference was found ($p < 0.05$). Thus in the study, room temperature (28–30 °C) was selected for TBT quantification (Fig. 3A). As in many reported studies, the acidity of the solution plays a key role in the extraction of TBT from the different matrix (Richter et al., 2016; Jiang and Liu, 2000). Thus, to explore the best pH for the extraction efficiency, 2–10 pH ranges were studied and pH 5 gave the highest extraction yield for Acetic acid-Sodium acetate buffer solution (Fig. 3B). The fiber should be exposed to the vapor phase above the sample for a particular time to extract the analytes and the extraction time is considered optimum when the equilibrium between the fiber coating, the headspace gas phase, and the sample solution is added. Therefore, different extraction times; 5, 10, 20, and 30 min were tested according to Beceiro et al. (2009) and a maximum peak was recorded at 10 min extraction time following gradual reduction after 10 min onwards (Fig. 3C).

3.2. Detection and quantification of TBT

Standard TBT chloride solution (2 μgL^{-1}) was used to identify and quantify TBT hydride (Tributyltin hydride) through scan mode at GC–MS as per Fig. 4 at the retention time of 7.13 min for TBT hydride peak. Mass spectra of TBT hydride peak was found to be 99% match with NIST library spectra. The selected ions for the quantification of TBT hydride is m/z 179. Excellent linear correlation of the peak area and level of TBT hydride was obtained ($R^2 = 0.9955$) over the concentration range from 1 to 1000 ngL^{-1}. Minimum Quantification Level (MQL) was obtained as 1 ngL^{-1} and Minimum Detectable Level (MDL) was 0.3 ngL^{-1}. Optimized TBT extraction methods by HS/SPME gave an

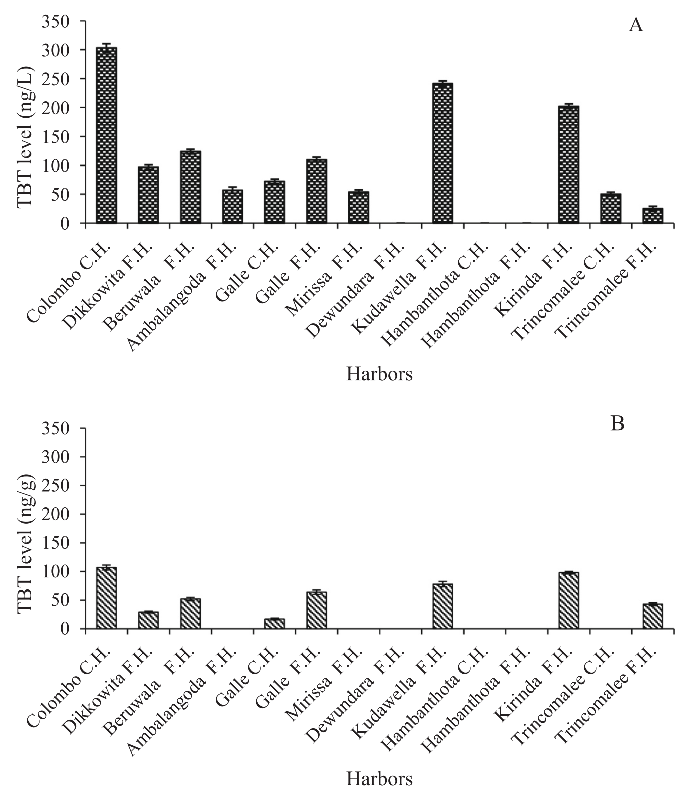

Fig. 5. TBT concentration in coastal water (A) and sediment (B) in different harbors. C.H; Commercial harbor and F.H; Fishery harbor.

Table 1
TBT concentration in water at different working stations in Colombo port.

Ref no.	Working stations	TBT level in water (ngL^{-1})
C1	Dockyard	295 ± 7.4
C2	Unity Container Terminal (UCT)	100 ± 4.3
C3	Prince Vijaya Quay (PVQ)	12 ± 4.1
C4	Jaya Container Terminal (JCT)	<MQL
C6	New North Pier	Not detected
C7	Colombo Port Expansion Project (CPEP)	<MQL
C8	Bandaranaike Quay (BQ)	Not detected
C9	Passenger Jetty (PJ)	202 ± 5.0
C10	Old pilot station	7 ± 4.3
C11	New pilot station	Not detected

excellent recovery percentage for water; 87 ± 2.1%, sediment; 78 ± 1.7% and biological samples; 81 ± 2.6% respectively. The recorded recovery values were greater than the international standard level (75%) given by International Organizations (Atnafe et al., 2019; Liyanage and Manage, 2016).

3.3. TBT levels in water, sediment and biota

Fig. 5 shows TBT levels in marine water and sediment samples collected from commercial and fishery harbors in coastal areas of Sri Lanka (Fig. 1). The highest TBT concentration in water was recorded in Colombo commercial harbor (303 ± 7.4 ngL^{-1}). TBT concentrations in Dewundara and Hambanthota fishery harbors were less than the level of MQL (<1 ngL^{-1}) (Fig. 5a).

The highest TBT concentration in sediment samples was recorded in Colombo port (107 ± 4.1 ng Kg^{-1}) where the lowest was found in the Galle commercial harbor (17 ± 1.4 $ngKg^{-1}$) (Fig. 5b). TBT concentrations in sediment collected from Ambalangoda, Dewundara, and Hambanthota fishery harbors were less than the MQL level and no TBT was recorded in sediment samples collected from Hambanthota, and Trincomalee commercial harbors and Mirissa fishery harbor (Fig. 5b).

TBT concentrations in different sampling locations of the Colombo commercial port varied drastically from 295 ± 7.4 ngL^{-1} to 7 ± 4.3 ngL^{-1} where the highest was detected in the Dockyard (C1) (295 ± 7.4 ngL^{-1}). The Unity Container Terminal (UCT) (100 ± 4.3ngL-1) and Passenger Jetty (PJ) (202 ± 5.0 ngL^{-1}) also recorded high concentrations of TBT compared to the other sampling locations of the port (Table 1).

Boat painting, building and repairing activities in the dockyard of the Colombo port was identified as the major point source of TBT. Hoch (2001) recorded the maximum leachate rate of biocide occurring from the newly painted hulls could cause the accumulation of TBT in biota at high concentrations, as the example; the range of TBT concentrations reported in biota was 0.01–3 mg/kg while 5–300 ng/g levels of total butyltin in muscle of fish. Dockyard (C1) is the panting and repairing site of the Colombo harbor that recorded the highest level of TBT. It was found that the level of TBT in coastal water at Colombo port varied according to the different marinas. UCT and PVQ which are located in the close vicinity of the dockyard area had relatively higher levels of TBT was detected (Table 1). Being a busy commercial port, Colombo is home to a large fleet of fishing boats, a shipbuilding unit, and a dry dock facility involved in the construction, repair, and painting of various commercial and naval vessels. Du et al. (2014) reviewed that a three-day docking of a commercial ship at a harbor can release more than 200 g TBT to water and if freshly painted, this amount can reach more than 600 g and results of the present study showed the TBT level in the surrounding water ranged from 100 ± 4.3 to 300 ± 2.3 ngL^{-1}. Relatively high levels of TBT in coastal water collected from the Colombo port probably reflect the combination of these sources and particularly the effect of dockyard activities. Thus, the levels of TBT in water and sediment exceeded the levels TBT recorded in the other harbors (Fig. 7A and B). Colombo port is a rapidly growing maritime hub of the South Asia Region and it supplies shipbuilding, ship stores, fuel, and fuel changing facilities for national and international ships and it caters annually to more than 4000 ships (Statistical report of SLPA, 2018). Hence, this might be the factor that would be responsible to record the highest level of TBT in water and sediment in the Colombo port. Guruge and Tanabe (2001) recorded that butyltin contamination in rabbit fish collected from the Colombo dockyard complex is the highest ever reported for butyltin contaminated fish in the world and these findings supports the results of the present study. The reason for low levels of TBT in other harbors may be due to the small boatyard and fewer marinas compared to the Colombo port. Further, it was found that at boat activity sites, the TBT levels ranged from 0 to 300 ± 2.3 ng/L, reflecting the presence of different marina activities and boat densities. In the Hambanthota commercial harbor that was recently constructed in 2010, TBT was not recorded. In Asian countries where no specific legislation controlling the use of TBT has been adopted, particularly high levels of contamination are still recorded in harbor waters (Frouin et al., 2010). Undap et al. (2013) showed that TBT usage has been regulated in Japan since 1990 around northern Kyushu, the concentrations of TBT have been found to range from 5 to 94 ng/L and 7 to 1100 ng/g dry weights in seawater and sediments respectively and from 8 to 135 ng/g in bivalves. TBT in bivalves from fishery harbors in Malaysia, Thailand and India were recorded as 1100 ng/g, 1250 ng/g, and 1190 ng/g respectively. In the present study, the highest TBT levels were found to be in the areas where Colombo, Kudawella, Kirinda, Bruwala, and Galle harbor with shipyard and boat activities occur.

In the present study, the level of TBT in water was found to be greater

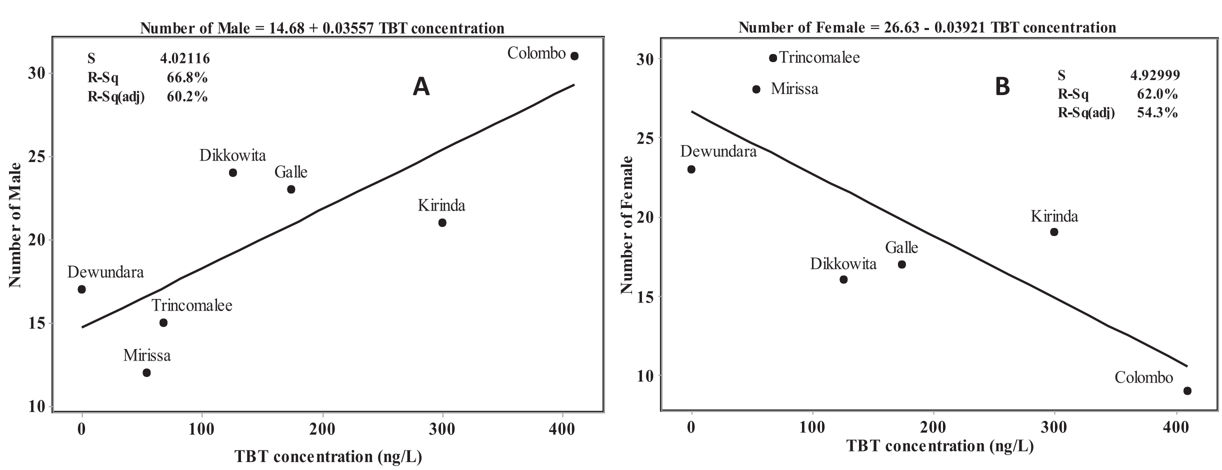

Fig. 6. Regression fitted line; number of male versus TBT concentration (p = 0.025) (A); number of female (p = 0.036) versus TBT concentration (B).

Table 2
Description of the sites where *P. viridis* was collected with sex differences.

Location	Year of commission of the harbor	Features of the harbors	TBT in water and sediment W - ng/L S - ng/g	Number of collected individuals	
				Number of males	Number of females
Colombo	1517	Heavy boat densities, dockyard, ship repairs, shipyards, fuel exchanging	W - 303 ± 7.4 S - 107 ± 4.1	31	9
Dikkowita	2013	Moderate boat densities, boat and hull repairing, gel coat application to boats, mooring	W - 97 ± 4.3 S - 29 ± 1.6	24	16
Galle	1965	Heavy boat densities, next to Galle commercial harbor, mooring, service and fuel filling facilities	W - 110 ± 4.1 S - 64 ± 3.7	23	17
Mirissa	1966	Heavy boat densities, Fuel filling facility, developing marina for yacht	W - 54 ± 3.6 S - ND	12	28
Dewundara	1980	Heavy boat densities, Fuel filling facility, boat repairing and workshop	W - <MQL S - <MQL	17	23
Kirinda	1985	Low boat densities, Transit harbor for craft fishing in Easter coast, boat repairing and workshop	W - 202 ± 4.3 S - 98 ± 2.3	21	19
Trincomalee	1965	Moderate boat densities, natural harbor, not used from 1990 to 1998. Fuel filling facility, boat repairing and workshop	W - 25 ± 4.2 S - 43 ± 2.5	15	30

Boat densities - low: less than 50 boats in the area; moderate: 50 to 300 boats in the area; heavy: over 300 boats in the area. W - water S - sediment.

than that of the TBT levels recorded in harbor sediment. Similar results were recorded by Landmeyer et al. (2004), reasoning out that this may due to TBT has high affinity to bind with organic matters and harbor water contain more organic matters than sediment layer (Hoch and Schwesig, 2004). Most researchers have been found that TBT concentration in sediment is greater than water (Neuparth et al., 2017) However, some studies accept the results of the present study (Harino et al., 1998). This may due to the variation dissolving factors of TBT; temperature, salinity, and pH in marine water. In sediments, clams had disappeared in areas where TBT levels were approximately 800 ng/g dry wt was recorded (Ayanda et al., 2012; Pent and Hunn, 1995). The polychaete *Armandia brevis* presented moderate to a severe reduction in growth for sediment TBT concentration in the range of 100–1000 ng/g dry wt. (Neuparth et al., 2017), and the bivalves *Macoma balthica* and *Scrobicularia plana* disappeared in sediment at the TBT concentrations over 700 ng/g dry wt. (Neuparth et al., 2017). The results of the present study also show that the lesser number of mussels with a high concentration of TBT in the Colombo port and adjacent area of Dikkowita fishery harbor and the high number of mussels in Trincomalee fishery harbor with a low concentration of TBT (68 ± 4.2 ng/L) (Fig. 6).

As indicated in Table 2, these sites varied widely concerning to the degree of marinas, boat densities as well as the presence of organotin contaminations. Ports with dry-docking and large-scale repair facilities are considered as TBT hot-spots, indicating the finding of a high male population.

There was a significant correlation between the number of males and females against TBT concentrations found at different locations (Fig. 6). The correlation coefficient of the number of male and female were significant ($p < 0.05$) and relatively high r^2 (0.66 and 0.62 respectively) value was strong enough to show the linear relationship. Accordingly, the male population of *P. viridis* increases with increasing the TBT concentration in water and sediment in the marine environment, and consequently decrease of the female population was recorded. Research was undertaken since the early 1970s has shown that TBT is very toxic to a large number of aquatic organisms (Frouin et al., 2010) and causes impairments in growth, development, reproduction, and survival of many marine invertebrate species (Hagger et al., 2005). In particular, concern has been the decline of marine mollusks in coastal areas due to imposex (Giraud-Billoud and Castro-Vazquez, 2019; Ni et al., 2019). In Fig. 7, the results of the study showed the highest concentration of TBT in the highest bodyweight of the mussels. The present study showed a significant relationship between the number of male *P. viridis* and TBT concentration ($p < 0.05$) indicating that the high TBT contamination enhances the production of a more male population of the *P. viridis*. This would be due to reproductive impairment within the *P. viridis* population particularly in the TBT contaminated environments. The bivalves, *P. viridis* are the focus of this research due to its limited ability to detoxify or excrete TBT with their benthic habitat in which contaminated sediments are ingested and accumulated by them.

TBT contamination in different size classes of the *P. viridis* varied from 4 ± 1.2 ngKg^{-1} to 42 ± 2.2 ngKg^{-1} (wet weight) and the highest level was recorded as 42 ± 2.2 ngKg^{-1} for 30-35 g size class in Dikkowita harbor which is situated adjacent to the Colombo port. The TBT concentrations in *P. viridis* were recorded by ascending order of 12 ngKg^{-1}, 22 ngKg^{-1} and 42 ngKg^{-1} following the average body weight ranges of; 0–15 g < 15–30 g < 30–45 g respectively.

Fent (2006) also recorded the positive correlation of TBT with the high body weight, fatty tissues that accumulate a high level of TBT in male bivalves. Similarly, high levels of the TBT were found in the large size class of male mussels in the present study. Imposex is the most severe effect of TBT exposure. Gibbs and Bryan (1996); Gooding et al. (1999); Huaquín et al. (2004); Oehlmann et al. (1998); Terlizzi et al. (2004), have performed numerous studies on such monitoring and biological effects (Alzieu, 2000). The mechanism of the reproductive impairment by TBT in bivalves is still not fully understood. However, previous studies have proposed mainly two mechanisms of reproductive

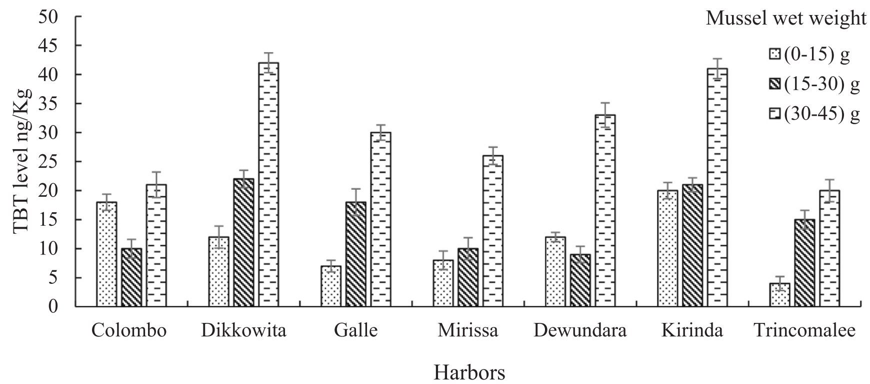

Fig. 7. TBT concentration in different size classes of the *P. viridis* samples collected from different harbors.

impairment due to TBT in gastropods. Bryan et al. (1989) and Park et al. (2015) described that penis occurs in female gastropods as a results of the continuous exposure of TBT, accumulation of TBT in ganglia and TBT functioning as a neurotoxin by abnormally releasing Penis Morphogenic Factor termed 'PMF' and a neuropeptide hormone causing the development of male secondary sex characteristics. The proposed second mechanism was that TBT acts to inhibit the conversion of androgens to estrogens mediated by the aromatase cytochrome P450 enzyme (CYP19A1) activity (Fent, 2006). Horiguchi et al. (1997); Morcillo and Porte (2000); Abidli et al. (2012) have reported steroid hormone levels in TBT-accumulated gastropods and bivalves with increased testosterone levels and decreased estradiol levels.

Often, the occurrence of imposex correlated significantly with TBT levels in marine biota, and exposure to trace levels of TBT (1–2 ngL^{-1}) induced imposex (Li and Collin, 2009). The reported TBT level in the study was remarkably greater than that of the threshold levels given for the imposex development of gastropods (1 ngL^{-1}). Despite regulations banning the use of TBT in Sri Lanka, current TBT contamination in coastal areas frequently reach concentrations likely to cause imposex. Accordingly, imposex has resulted in the decline or extinction of local populations worldwide, including coastal areas all over Sri Lanka. Alzieu (2000) has recorded that the TBT concentration as low as 2 ngL^{-1} is responsible to cause shell anomalies, abnormally thickened shell, stunt growth and calcifications in gastropods and the most adverse case is the oyster and bivalve production that suffered severe disturbances due to progressive decline of reproduction and of juvenile recruitment leading to the decline of biodiversity in the marine environment (Kanda, 2019; Daigneault and Latham, 2020; Matthiessen and Gibbs, 1998). Thus, the accumulation of TBT via consumption of marine seafood in humans is also possible. Research on TBT accumulation by aquatic invertebrates has been mostly confined to mollusks (bivalves) and crustaceans (decapods) because these groups are important seafood resources and are ecologically dominant in many habitats (Frouin et al., 2010). Thus, the contamination levels recorded in the present study would adversely affect the country's economy both by the collapse of seafood production of the country and the tourism industry. The issue is complex; and its impact has not been determined in Sri Lanka due to the lack of analytical technology. As a result of extensive environmental distribution and non-discriminatory biotoxicity, organotin biocide use in antifouling coatings has been restricted for ships <25 m in length by the Organization for Economic Cooperation and Development member countries; United Kingdom, Sweden and Denmark since 1988, with a total ban of TBT use taking place internationally on 17th September 2008. Following the ban of TBT in the United Kingdom in 2001 on vessels <25 m in length, water and sediment sampling in southern England during 2004–2005 demonstrated significant reduction of the concentration of TBT compare to earlier studies (2000–2004) (Evans et al., 1995), indicating that control measures by restricting the use of TBT has effective in reducing its concentrations in coastal waters and sediment within the region. As this data are not sufficient and further studies need to be conducted and course of action needs to be worked out.

4. Conclusion

Dietary consumption of contaminated seafood is the main pathway of TBT exposure and accumulation in humans. Thus, it is worth highlighting that TBT contamination poses a risk to human populations who are regularly exposed to TBT contaminated seafood. The present study attempted to detect a low-cost green chemistry modified method to detect TBT and evaluated the environmental contamination levels of TBT in major potential susceptible areas of Sri Lanka. This is the first record on TBT contamination in water, sediment, and invertebrates in ship/boat traffic zones in Sri Lanka as a baseline reference record.

The study concludes that TBT is one of the toxic pollutants in coastal water, sediment, and biota. The optimized HS/SPME-GCMS method is sensitive, simple, and solvent-free owing to the green analytical chemistry approach. SPME optimized conditions, the maximum yield of TBT was obtained at a 10 min extraction time, and the best extraction temperature and pH were found to be 28 °C and 5 respectively quantifying TBT up to the 1 ppt level which is far below level given for TBT contamination in water, sediments, and biota by the WHO. The results of the study revealed that TBT contamination in coastal water ranged from 303 ± 7.4 ngL^{-1} to 25 ± 4.2 ngL^{-1} where in the sediment and biota ranged from 107 ± 4.1 $ngKg^{-1}$ to 17 ± 1.4 $ngKg^{-1}$ and 4 ± 1.2 $ngKg^{-1}$ to 42 ± 2.2 $ngKg^{-1}$ respectively. There was a positive correlation between the number of male *P. viridis* with TBT concentrations ($p < 0.05$) was found. Further, the study revealed that the ship/boat traffic zones are likely to be hotspots of TBT contamination in the coastal environment in Sri Lanka.

Moreover, according to the relative infancy of knowledge on the dangers of TBT on biological pathways of many marine organisms, it is timely important to explore other species such as marine mammals, sea birds and coastal invertebrates outside of merely mollusks. The scientific community should take into consideration that larger more complex mammals, both oviparous and viviparous in coastal region, to study not merely the exterior sexual biology but also the role of TBT in heritable genetics and epigenetics, as well as reproductive biology.

CRediT authorship contribution statement

K.R.V. Bandara: Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **S.D.M. Chinthaka:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – review & editing, Supervision. **S.G. Yasawardene:** Resources, Writing – review & editing, Supervision. **Pathmalal M. Manage:** Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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