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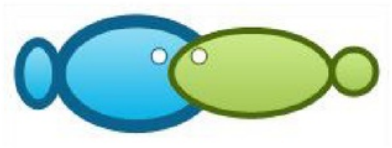

Imposex phenomenon in gastropods from Bitung waters, North Sulawesi, Indonesia

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Abstract. Tributyltin (TBT) bioaccumulation study used imposex character of 3 gastropod species, *Thalessa aculeata*, *Monodonta labio*, and *Nerita exuvia* collected from Bitung waters, particularly in shipping activities concentration. Sediment sampling was done from the bottom surface, and TBT measurements used Gas Chromatography-Mass Spectrometry (GC-MS), while imposex character assessment was done by comparing percent imposex among species in the sampling point. The highest imposex (I) and relative penis size index (RPSI) values were recorded in *Thalessa aculeata,* 60% and 71%, respectively, from Bitung harbour and fisheries ports. This condition is indicated with the TBT concentration presence in the sediment, 0.17 µg g^{-1} and 0.3 µg g^{-1}, respectively. Thus, Bitung waters in high shipping and sea transportation activities have been contaminated with TBT as imposex cause.
Key Words: tributyltin, imposex, sediment, port, shipping activity.

Introduction. Marine environment with its various living components is not free of surrounding environmental influences or pressures. Organic matters, heavy metals, domestic wastes, or industrial disposals yield marine pollution that can disturb the animal condition. Marine pollution is one of the problems of nearly all maritime countries, including Indonesia, and it can appear from marine conditions in many areas, particularly marine ports (Rumampuk 2018).

Marine pollution and toxicity of organotin (OT) compounds have become a specific concern worldwide since 1960s, because the use of tributyltin (TBT) as biocide of antifouling paint used on the bilge is found to be highly effective to prevent and slow down the attachment of the fouling organisms (De Mora 1996). It, however, has dangerous effects on the non-target organisms in long-term application. One of these is disturbance of the endocrinal system and the reproductive function in gastropods, in which the imposex syndrome occurs, the appearance of male reproductive organ (penius/vas deferens) on females (Gibbs & Bryan 1986; Bryan et al 1986; Bryan et al 1987; Oehlmann et al 1998). The imposex phenomenon from TBT generally occurs in the areas of high marine transportation or industrial activities, such as marine ports and ship docks (Bigatti & Penchaszadeh 2005; Kirli 2005; De Castro et al 2008).

International Maritime Organization (IMO) has recommended global prohibition worldwide for TBT utilization as antifouling paint of the ship body since 2008. Even, to prevent bigger disturbance of marine environment, several countries, such as United States, England, Holland, Canada, and Japan, have long banned the use of TBT as antifouling. Even though TBT pollution level declines in several parts of the countries that have implemented regulations on TBT usage prohibition, TBT is still detected in sediment and organisms (Rumengan & Ohji 2012)

Several studies reveal that the TBT is still applied in Indonesia (Razak 2004; Sudaryanto et al 2005; Kusumastuti et al 2013), especially Minahasa peninsular waters, North Sulawesi (Harino et al 2012; Noor et al 2013; Rumampuk et al 2018). Imposex phenomenon has also been studied in Indonesian gastropods (Ellis & Patissina 1990; Pandey & Evans 1996; Kusumastuti 2013; Rumampuk et al 2018), so that TBT

contamination has certainly occurred. This study aims to analyze the TBT concentration in the sediment and evaluate the imposex occurrence in several gastropod species in Bitung waters, particularly the area of high shipping activities.

Material and Method

Sampling location. Sampling activities and laboratory analyses were carried out for one month, from July to August 2018. Sediment and gastropod samples were taken from 5 different localities in Bitung waters, Polytechnique port (B1), MAFS controlling port (B2), Fisheries port (B3), public crossing port (B4), Harbour Bitung (B5). Sampling was based on the gastropod occurrence and geographic position indicated with Global Positioning System (GPS) (Figure 1).

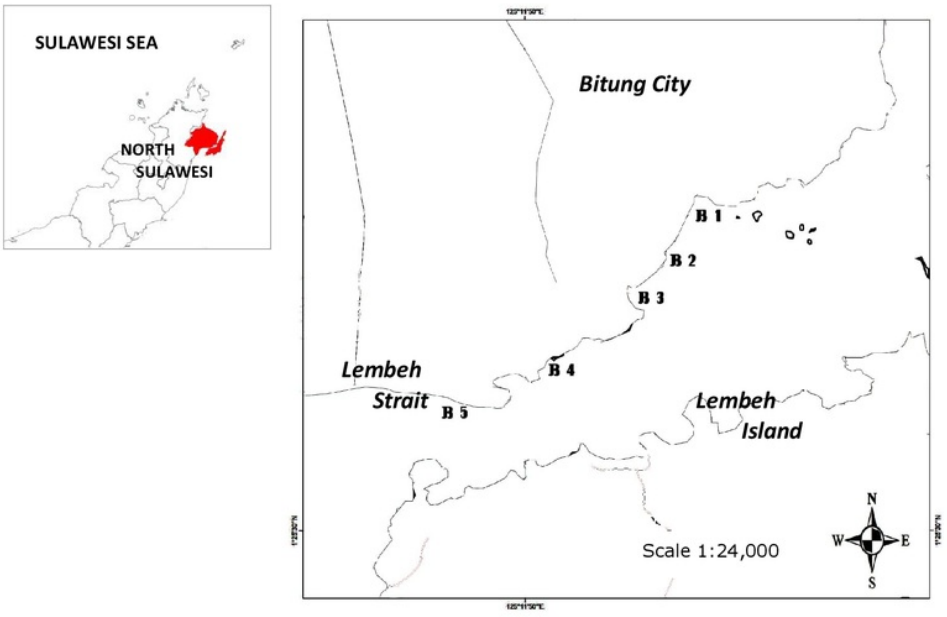

Figure 1. Sampling locations.

Sample collection. Sediment samples were taken from the bottom substrate (about 0-5 cm thick) at the depth of 50 m using polyethelene (PE) spoon and put into sample bottles. For sediment organotin measurement, each sampling point was represented by a "composite sample". The sample was put in the styrofome with the ice pack and brought to the laboratory. These were put into labelled sample bottles, then in the iced cool-box, and brought to the laboratory for further analyses. Gastropod samples (*Thalessa aculeata*, *Monodonta labio*, *Nerita exuvia*) were randomly hand-collected at 5 sampling points each of which were 30 individuals. The specimens were taken to the laboratory and identified following Dharma (1988) and Dharma (2005).

Organotin concentration measurement. TBT concentration of the sediment was conducted in the Water Laboratory Nusantara (WLN) using a Gas Chromatography-Mass Spectrometry (GC-MS). The sample was added with 1 mg of surrogate standard and homogenized with 1 N HCl-methanol/ethyl acetate (1:1), then shaken, centrifuged, and added with NaCl. It was then extracted using ethyl acetate + haxane (3:2), devitatized sodium tetraethylborate, added with 1 N KOH, and stirred for 1 hour, and centrifuged. The extract was evaporated up to reaching 2 mL and pipetted into a vial, then analyzed in the GC-MS.

Imposex analysis. The imposex character testing o*halessa aculeata, Monodonta labio,* and *Nerita exuvia* was done following Vasconcelos et al (2010) and nandez et al (2002). The gastropod samples were collected from 5 sampling sites, identified, and measured the shell length using a 0.1 mm caliper. Before observing the imposex, the samples were anesthetized by immersing them in 7% $MgCl_2$ solution for about 15 min, then the shells were broken, the body removed, and laid on the petridisc for the imposex observation.

It was done by looki at the female sex based on the presence of light yellow-colored ovary, while male sex was based on the presence of dark yellow or orange-colored testis. Samples with imposex character were observed whether there was penis beh d the tentacle or not. The index used to measure the imposex was percent imposex (I) and RPSI (relative peni ize index). The former is an index to calculate the percent female imposex [(number of imposex females/total number of normal fem es) x 100], and the latter is an index to measure the relative size of male penis (MP) [(mean female penis size)^3/(mean male penis size)^3 x 100] (Bryan et al 1987).

Results

Organotin concentration in the sediment. TBT concentration in the sediment varied from 0.17 to 0.31 µg g^{-1} (Figure 1). The highest concentration was found in the Polytechnique port and the lowest in the Fisheries port. These concentrations are 1.8-3.1 folds higher than 3 other sampling points. It could result from that the TBT is often employed as antifouling paint, so that port activities, such as ship moori , docking, and sea transportation, could increase the TBT concentration in the waters. The use of TBT-based antifouli paint and waste treatment in the ship dock are causes of high TBT concentration (to et al 2007; Anastasiou et al 2016; Ytreberg et al 2016). Besides, waste disposal into the marine environment could become source of increased TBT (Sudaryanto et al 2005; Lasut et al 2008).

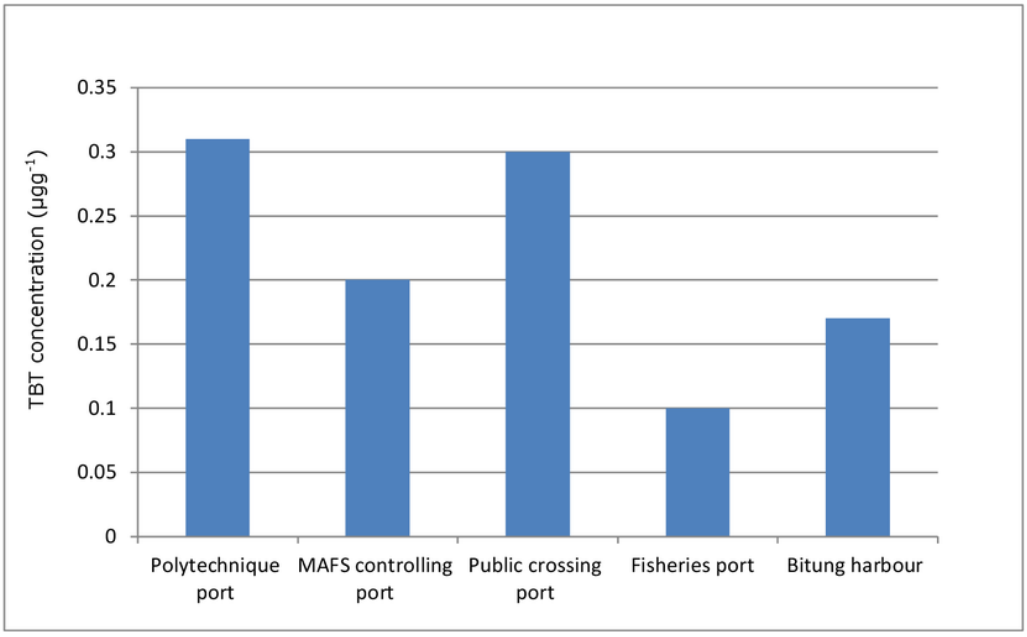

Figure 1. TBT concentration (µg g^{-1} ww) in the sediment in Bitung waters: 1) Polytechnique port (B1); 2) MAFS controlling port (B2); Public crossing port (B3); Fisheries port (B4); Bitung harbour (B5).

The sediment TBT concentration in Bitung waters was detected relatively similar to that reported by Harino et al (2012), 160 µg kg^{-1} and Rumampuk et al (2018), 0.167-0.313 µg g^{-1}, while the waters with no shipping activities like Gangga island, the TBT concentration was found to be lower, 0.7 µg g^{-1}. It reflects that TBT is still released into the sediment in the port area despite not continuously done.

Compared with studies in several regions of Indonesia, it is apparent that the TBT concentration in the sediment sample of Bitung waters be higher than that detected in several large ports in Jakarta, Surabaya, and Medan, 2.3-190 ng g^{-1} (Sudaryanto et al 2005), Jakarta Bay, 0.4-52 µg kg^{-1} (Harino et al 2012), and Banten Bay, 12 ng g^{-1} (Razak 2004).

Imposex incidence in gastropods. TBT enters the gastropod through sediment, water column, or food, and then will be accumulated in the tissue. The exposure duration highly affects the TBT accumulation in the body to make the imposex occur. The present study found that the imposex (I) in *N. exuvia* ranged from 47.83 to 60% and from 47.83 to 54.17% in *M. labio* ranged from, in all sampling locations, with RPSI range of 9.73 to 43.77% for *N. exuvia* and 38.81 to 46.72% for *M. labio*, while *T. aculeata* had the highest imposex in almost all localities (Tables 1-3). Nevertheless, high values in *T. aculeata* was found lower than that in *Thais tuberosa* in Singapore and *Thais* sp. in Malaysia penninsula that reach 100% (Tan 1999; Wagiman 2004).

Table 1

Biometric, imposex quantification, and penis size index data of *Thalessa aculeata*

Sampling point	*Mean SL (mm)*	*T*	*IF*	*NF*	*I (%)*	*MP (mm^3)*	*IFP (mm^3)*	*RPSI (%)*
Polytechnique port	19.69	30	13	11	54.17	32.76	18.82	57.43
MAFS controlling port	23.18	30	15	10	60	42.87	21.71	50.65
Fisheries port	23.20	30	14	10	58.33	56.18	40.00	71.20
Public crossing port	21.74	30	13	9	59.09	61.62	25.67	41.65
Samudra port	23.18	30	14	10	58.33	43.61	23.63	54.20

Note: SL - shell length; T - total snails collected; NF - normal females; IF - imposexed females; I% - percentage of imposex; MP - male penis; IFP - imposexed female penis; RPSI%: percentage of relative penis size index.

Table 2

Biometric, imposex quantification, and penis size index data of *Monodonta labio*

Sampling point	*Mean SL (mm)*	*T*	*IF*	*NF*	*I (%)*	*MP (mm^3)*	*IFP (mm^3)*	*RPSI (%)*
Polytechnique port	21.74	30	11	12	47.83	53.58	20.79	38.81
MAFS controlling port	21.12	30	11	12	47.83	52.31	23.39	44.71
Fisheries port	20.43	30	13	11	54.17	52.73	24.64	46.72
Public crossing port	20.46	30	12	13	48	35.28	15.43	43.74
Samudra port	21.51	30	12	12	50	35.28	16.19	45.89

Note: SL - shell length; T - total snails collected; NF - normal females; IF - imposexed females; I% - percentage of imposex; MP - male penis; IFP - imposexed female penis; RPSI%: percentage of relative penis size index.

Table 3

Biometric, imposex quantification, and penis size index data of *Nerita exuvia*

Sampling point	*Mean SL (mm)*	*T*	*IF*	*NF*	*I (%)*	*MP (mm^3)*	*IFP (mm^3)*	*RPSI (%)*
Polytechnique port	19.69	30	13	11	54.17	39.30	9.88	25.15
MAFS controlling port	23.18	30	11	12	47.83	39.80	9.750	24.49
Fisheries port	23.19	30	14	10	58.33	35.39	15.49	43.77
Public crossing port	21.74	30	12	12	50	109.44	10.64	9.73
Samudra port	23.15	30	15	10	60	45.11	10.94	24.24

Note: SL - shell length; T - total snails collected; NF - normal females; IF - imposexed females; I% - percentage of imposex; MP - male penis; IFP - imposexed female penis; RPSI%: percentage of relative penis size index.

Imposex observations have been done, especially in European countries, more on gastropod than bivalve. According to Kirly (2005), gastropods are more sensitive to TBT pollution than bivalves. Observations in Japan revealed that the impact of TBT occurred in gastropod *Thais clavigera* (Horiguchi 2006). In Latin America, the imposex was firstly observed in Chile by Goding in 1990 (Bigatti & Penchaszadesh 2005), and in Brazil, it was done on Muricid, *Stromonita haemastoma* (De Castro et al 2008).

Bryan et al (1987) stated that the sediment TBT concentration of 1 ng g^{-1} could cause the imposex phenomenon in *Nucella lapillus.* Ellis & Pattisina (1990) reported that *Thais luteostoma* from Ambon has experienced 30-100% imposex, while Pandey & Evans (1996) found I value of 75% in adult *Thais* sp. from Bitung waters and 85% in *Morula granulata* from Manado port.

High imposex found in this study indicates high shipping activities in the port area that enable to result in accumulation and bioaccumulation of OT compounds in Bitung waters. The imposex phenomenon from TBT generally occurs in the protected coastal areas with low transportation level. Moreover, according to De Castro et al (2008), the imposex phenomenon from TBT generally occurs in the waters where marine transportation or industrial activities are high as in the sea port and ship docks.

High RPSI value reflects that the imposex phenomenon in Bitung waters, particularly in the port area is a very poor condition because the imposex value is above 50%. Compared with previous studies in the waters of high shipping or sea transportation activities as Bitung waters, there is no decline in the imposex value identified due to high use of TBT material-based antifouling paints beside no regulations for TBT utilization. Based on the description above, it is obvious that TBT is still a serious problem challenged by several countries, including Indonesia.

Conclusions. Tributyltin (TBT) compounds were still detected in the sediment in Bitung waters, and the gastropods, *Thalessa aculeata*, *Monodonta labio*, and *Nerita exuvia* had high imposex index, even though it did not reach 100%. This condition enables to be improved through periodic monitoring activities.

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