



Research Article

Investigation of Imposex in a Marine Gastropod *Tibia curta* (Sowerby II, 1842) at Gadani Ship Breaking Yards

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Abstract | Imposex is a well-known phenomena that has been documented over more than fifty years (50) in several marine gastropod species world around. The Phenomenon of imposex is directly connected with toxicity of tributyl (TBT) contamination and the source of this contamination is shipping traffic due to the use of TBT-based paints on ship hulls and the leaching of content into the water column. So, during the present study specimens of *Tibia curta* (Sowerby II, 1842), collected and investigated for the planned study from Gadani ship breaking yards (25.0361° N, 66.7136° E) which is the third largest ship dismantling site across the globe. Specimens were procured for a period of February 2019 to October 2021 in different intervals from the four selected sites along the Pakistan coast. Imposex was recorded only in the specimens of Gadani. The intensity of Imposex determination was based on the presence of and psudopenis and VDS (*vas deferens sequence*) in imposex female individuals. Imposex remained promising only at Gadani where imposex intensity was found 100% in females as when compared to other three sites. Whereas 1 to 4+ Vas deferens sequence (VDS) stages were observed in imposex females. Therefore, studies revealed the presence of imposex phenomena in targeted species at Gadani due to high concentration of leached out organometallic compound Tributyltin Tin (TBT) from the ship breaking scrap which is responsible to change hormonal levels and subsequent physiological change related to sexuality in animals as well as also responsible to bring morphological changes in other body parts like antenna and eye spot of affected individual.

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Introduction

Pakistan largest ship breaking industry is located at Gadani, which is 50 km northwest of Karachi. It has a capacity of more than 6000 workers. Ship breaking gives boost to the economy of the country but it leaves very much negative impacts on the local population, wildlife and to the environment specially

marine life, like as ship breaking is related with famous gender bender phenomenon in marine gastropods, which is known to occur due to accumulation of toxic paints used on ship hull, other than that oil and metal pollution, and pathogenic contamination is also associated with ship dismantling practice and municipal waste in breaking yards and adjoining areas (Afsar and Siddiqui, 2015; Afsar *et al.*, 2016; Hassan

et al., 2019).

Environmental bio-monitoring is a technique which provides the low cost and easy bioassay of anthropogenic stressors by analyzing the marine organisms for impact assessment in terms of taxonomic assessment or by examining morphological, anatomical and histological changes in organs and tissues that are connected to specific harmful waste either organic, inorganic, Organometallic, parasitic in nature (Degiam and Abas, 2010; Afsar and Siddiqui, 2015). Maciel *et al.* (2018) investigated imposex phenomena caused due to contaminants of Organotin from two estuaries of north eastern coast of Brazil. Biodiversity at such sites includes endangered, invasive, endemic, threatened and commercially important life forms therefore: Monitoring programs are used to evaluate the physical health status of coastal zones and to formulate legislation in order to protect valuable marine. There are so many marine species that have been used successfully to resources. monitor environmental health issues and among them molluscan species are very much popular i.e., *Ruditapes decussata*, *Cerastoderma glaucum*, *Thais clavigera*, *T. carinifera*, *T. bufo*, *Morula granulata*, *Leucozonia nassa* and *Leucozonia ocellata* (Titley-Oneal, 2011; Castro *et al.*, 2012; Abidli *et al.*, 2012, 2013; Afsar and Siddiqui, 2015; Maciel *et al.*, 2018).

Several earlier workers have defined biomonitors as biological objects (animals and plants) which take up contaminants from their surroundings and accumulate in their tissues and organs which leads to serious health damage and leaves harmful impact collectively on marine environment. Process of quantification of such contaminants in fish, crustaceans, bivalves, gastropods etc is biomonitoring which reflects the presence and impact of hazards on the ecosystem by means of ecological distraction which results in ultimate harm to human populations. The ship breaking industry generates a huge quantity of solid waste in the form of broken wood, rubber, insulation materials, paper, metals, glass and ceramics, plastics, leather, textiles, food waste, chemicals, paints, thermocol, sponge, ash, oil mixed sponges, organometallic contaminants, miscellaneous combustible and non-combustible hazardous toxicants (Oehlmann *et al.*, 1992; Ragi *et al.*, 2016; Nuraini *et al.*, 2020; Rodríguez-Grimon *et al.*, 2020). Ship dismantling industry pollution has been recognized as a serious environmental threat. Toxic pollutants are present in such areas where it

can accumulate all along the food chain. Moreover, small amounts of absorbed contaminants are either stored in a metabolically available form for essential biochemical processes or detoxified into metabolically inert forms or held in the body either temporarily or permanently (Mann *et al.*, 2006; Nuraini *et al.*, 2020; Rodríguez-Grimon *et al.*, 2020). Since anthropogenic stressors e.g. radionuclides, tributyltin (TBT) triphenyltin (TPT) and bioinvasives are well known within biomonitoring perspective and tend to cause physiological changes in marine objects besides ecological web distraction. Thus, to monitor these changes there are many techniques are available to quantify the impact with respect to biomonitoring surveillance.

Biomonitoring endeavors by means of morphological and histological examination related to the well-known phenomenon of imposex (imposition of male sex characteristics onto females) in gastropod populations have been carried out successfully to address this global issue. Imposex is caused by leaching organotin content found in antifouling paints that have been used on ship hulls since decades (Titley Oneal, 2011; Afsar and Siddiqui, 2015). Some of the basic research on the subject of environmental biomonitoring related to toxic organotin contamination and the associated phenomenon of imposex from Pakistani waters had been carried out by the following workers (Afsar *et al.*, 2012a, b, c, 2013a, b, 2015; Hassan *et al.*, 2019). Due to international ban imposed by IMO (international maritime organization) to restrict the use of organotin based antifouling paints on ship hulls from 2003 to 2008 now this global issue has turn into control overall in port areas. Other hand unfortunately this type of contamination is still present in areas subjected to ship breaking activities and among these Gadani is the one which holds the third position in global ship breaking industry which has never been investigated thoroughly (Afsar and Siddiqui, 2015; Hassan *et al.*, 2019). Only two imposex gastropod species have been reported from Gadani (Hassan *et al.*, 2019). Up till now any published report of imposex in *Tibia curta* is not available. However, Ragi *et al.* (2016) have done some work on biochemical composition of the species and studies have revealed the presence of alkanes, aminoacids, fatty acids and sterols in *Tibia curta* from Neendakara south west coast of India. Similarly, Degiam and Abas (2010) studied antimicrobial activity through extraction of three marine molluscan species of *Tibia curta*, *Sepia* and *Loligo* at bacterial species belongs to

family Enterobacteriae. In all three species of marine molluscans *Tibia* and *sepia* have more antimicrobial activities as compare to species of *Loligo*.

In general, study is subjected to biomonitoring and use of biological objects involve mainly in impact assessment of hazardous organometallic contamination which is still present in large quantities at. The objective of the planned study is imperative in terms of establishing baseline and to acquire basic knowledge in an environmental health perspective.

Materials and Methods

Collection of gastropod species were made from intertidal area of Gadani (25.0861° N, 66.7308°E), Jiwani (25.0538° N, 61.7707° E), Damb, Somiani (25.4484° N, 66.5685° E) and Keti Bandar (24.1429° N, 67.4509° E) (Figure 1) during February 2019 to October 2021, through random sampling collection method at low tide mark. Shells were collected by hand- picking methods at low tide mark from the selected sites. The collected samples were brought to the laboratory live and kept in freezer for further detailed analysis. Snails were initially identified taxonomically by the help of literature (Nazneen and Begum, 1988; Liverani, 2014; Mollusca Base, 2023) biometric and morphometric study was carried out using vernier calliper. Shell length (mm), shell width (mm), other than that penis/pseudopenis length (mm) and vas deference sequences (VDS) stages were noted in all effected individuals. Identification of animal sex and imposex, stages made by followed by method described by earlier workers (Smith, 1971; Oehlman et al., 1992) shown in Figures 2 and 3.

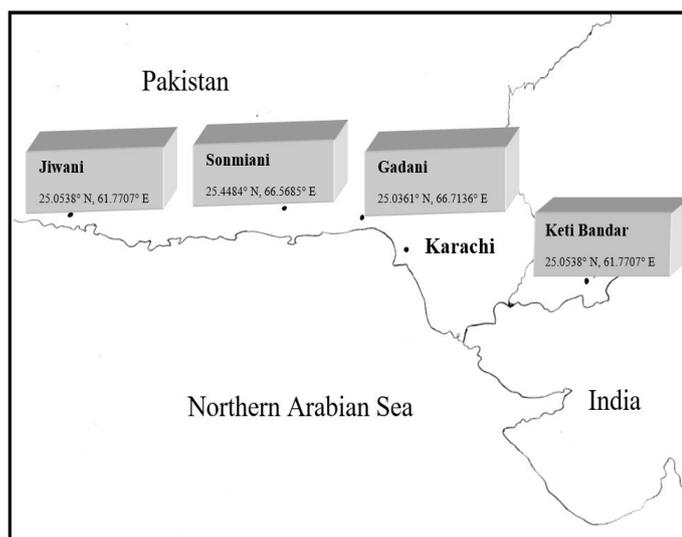


Figure 1: Map is showing collection sites (1-4).

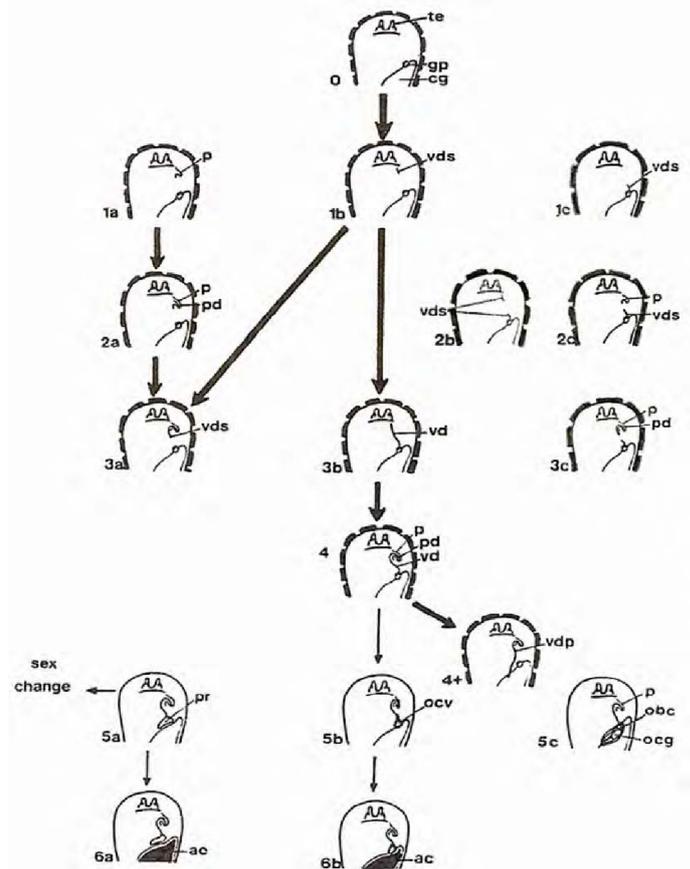


Figure 2: Showing scheme of imposex and Vas Deferens stages (VDS) (Oehlman et al., 1992).



Figure 3: A- *Tibia curta* (dorsal and ventral view), B- Male with penis length (>70 mm), C- Imposex female with dissimilar tentacles, D and F- imposex with VDS formation, E- imposex female with pseudopenis, VDS formation and unequal eye spot.

Results and Discussion

In the presence investigation sample of *Tibia curta* were collected during February 2019 to October 2021 at low tide and identified by the help of literature as mentioned above and classified below:

- Kingdom: Animalia
- Phylum: Mollusca
- Class: Gastropoda (Cuvier, 1795)

- Subclass: Caenogastropoda (L. R. Cox, 1960)
- Order: Littorinimorpha (Golikov and Starobogatov, 1975)
- Superfamily: Stromboidea (Rafinesque, 1815)
- Family: Rostellariidae (Gabb, 1868)
- Genus: *Tibia* (Röding, 1798)
- Species: *Tibia curta* (G. B. Sowerby II, 1842)

June 2020 and October 2021 (Tables 1 and Figure 2).

Overall, total collected number of individuals at all four site was amounted three hundred and two (302) contributing 9.93% from Sonmiani, 78.48% from Gadani, 4.30% from Jiwani and 7.28% from Keti Bandar. Phenomenon of imposex was found only in the individuals of *Tibia curta* collected from the Gadani site during February 2019 to October 2021, where intensity of imposex found 100% in females. Male individuals found only 13.92% of the overall population at Gadani. Imposex females contributed 86.08% as shown in Table 1 and Figure 4. At Sonmiani samples were procured during April 2019 and March 2021 where male, female ratio was 23.33% and 76.67%. Similarly, at Jiwani male, female contribution was found 30.77% and 69.23% and samples were obtained only in March 2021. Whereas, from Keti Bandar calculated male, female ratio was 22.73% and 77.27%, respectively during

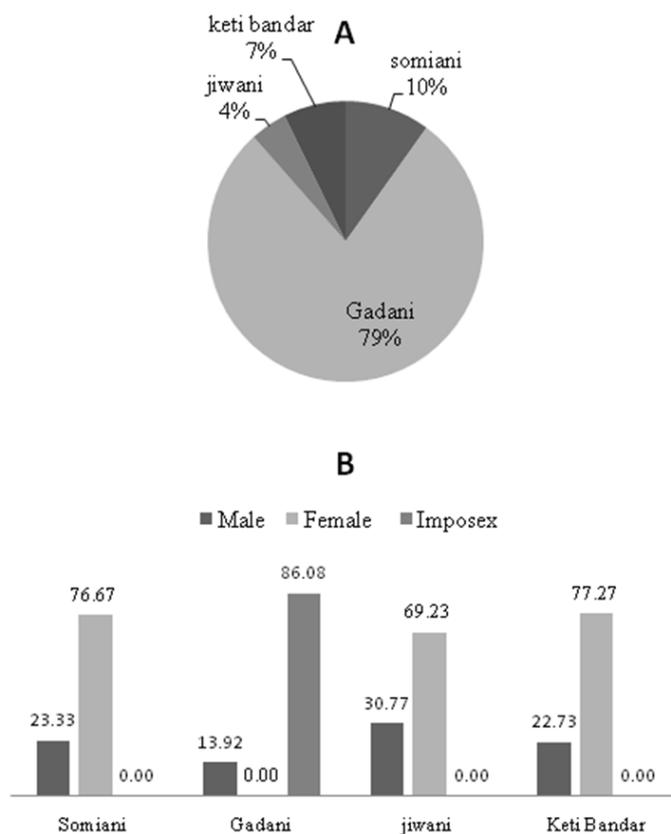


Figure 4: A showing % total number of individual at all four sites and B showing % male, female and imposex at collection sites.

Table 1: Showing *Vas deferens* sequence stages (VDS) Average±Standard deviation of shells length (mm), width (mm) and penis size (mm) at four (4) collection sites.

S. No.	VDS stages	Sites	Total No.	Shell length ± SD mm	Shell width ± SD mm	Penis length ± SD mm
1	1a	Gadani	28	125.91± 13.35	31.59 ± 5.16	11.71 ± 21.58
2	1b		22	129.73± 11.64	33.01 ± 4.92	0.61 ± 1.96
3	1c		18	124.53 ± 12.00	31.48 ± 5.71	0.38 ± 1.64
4	2a		20	121.05 ± 14.18	29.86 ± 4.83	6.10 ± 2.99
5	2b		15	128.76± 12.11	32.54 ± 4.53	2.6 ± 4.11
6	2c		7	124.06 ± 14.85	31.20 ± 6.29	3.28 ± 3.25
7	3a		16	126.16 ± 13.18	30.42 ± 4.75	5.12 ± 3.34
8	3b		35	124.74 ± 13.02	31.40 ± 5.90	1.67 ± 3.59
9	3c		16	127.81 ± 14.96	32.50 ± 3.79	5.25 ± 3.25
10	4		19	126.33 ± 13.12	31.26 ± 3.96	10.26± 4.43
11	4+		8	117.78 ± 16.74	29.89 ± 6.06	9.25 ± 0.70
12	Male		33	126.14 ± 14.00	32.03 ± 6.08	57.82±22.55
13	Male	Jiwani	4	128.54±15.36	34.027±3.74	70.0 ±1.63
14	Female		9	124.06±14.40	32.36±3.91	0.00±0.00
15	Male	Keti-Bandar	5	130.30±11.83	34.85±3.68	66.20±6.68
16	Female		17	128.79±10.20	34.78±3.34	0.00±0.00
17	Male	Somiani	7	129.28±10.100	30.06±7.12	69.28±9.99
18	Female		23	128.07±9.63	35.60±23.16	0.00±0.00

From Pakistan phenomenon of imposex has been reported in marine gastropods by (Afsar, 2009; Afsar et al., 2012a, b, c, 2013a, b, 2015). Imposex found in species belongs to the families Babyloniidae, Muricidae, Bursidae and Turridae that have been collected from the Manora channel (shipping activities site) during 2003 to 2008 from Manora Channel, the largest shipping hub in Pakistan. Hassan et al. (2019) have also reported the phenomenon of imposex from Gadani ship breaking yards due to exposure of gastropods to tributyltin (TBT that used as biocidal agent in antifouling paints, applied on the ship hulls and marine submerged structures such as fishing gears and buoys. Hassan et al. (2019) carried out the bioassay experiment to determine imposex inductive and endocrine disruptive effect of TBT in two species of gastropods of genus *Thais* which are namely *Thais bufo* and *T. rudolphi* that were exposed to three different concentrations (100, 500 and 1000ngl⁻¹) of TBTCI for four weeks in laboratory and at the end of experiment level of free testosterone and TBT body burden was estimated by radioimmunoassay and gas chromatograph coupled with a flame photometric detector respectively. In both tested species exposed to 500 and 1000ngl⁻¹ of TBT imposex stages developed, while in 100ngl⁻¹ and control groups showed no imposex condition. Elevation of free testosterone level in imposex females has also been observed. These observations indicate that the TBT act as potential imposex inducer and endocrine disruptor in the targeted gastropod species and these species can be used as sensitive biomonitoring tool for TBT contamination. Giulianelli et al. (2020) describe the RXR expression in marine gastropods with different sensitivity to imposex development.

During present studies other than presence of pseudopenis and VDS other anatomical changes or deformities have also been noted in the individuals of *Tibia curta* for the first time from Pakistan coast. Deformities related to unequal tentacles and eye spot were evident only in individuals collected from the Gadani ship breaking yards which is the world's third largest ship dismantling hub provides the clear evidence of presence of TBT contamination due to antifouling paints coatings on scrap ships. Among these effected individuals seventy-seven (77) have shown difference in eyespot size whereas thirty-nine (39) found with absence of eyespot. Similarly, Antenna or tentacle differences were also recorded, and one hundred and ten (110) individuals have been

recorded with difference in their antenna size, as well as forty-one (41) exhibited deformities or absence of antenna. Previously, alongside the pseudopenis and VDS development Afsar (2009), Afsar and Siddiqui (2015) also observed abnormality in a number in imposex females that have been found with differences in eye spot and at tentacles right side in both male and imposex females as well as spermatogenesis was also observed in histological sections of ovaries containing imposex stages 3a and 3b in *Babylonia spirata*. Ovarian spermatogenesis and imposex development was only observed at Manora channel due to elevation in concentration of Tributyltin tin which used extensively in past decades in shipping industries and this content was responsible for contamination at Manora channel which is the largest shipping hub in Pakistan. Primost et al. (2015) have also carried out some work on the shell shape as indicator of pollution in edible marine snail *Buccinanops globulosus* using geometric morphometric analysis that was found affected by imposex, cause of TBT contamination which was associated with maritime traffic and human activities. Similarly, Giulianelli et al. (2020) provided the evidence of the retinoid X receptor (RXR) expression in marine gastropods with different sensitivity to imposex development.

During recent studies biometric data was also procured and on average shell length (mm), shell width (mm) and penis length (mm) of male individuals of *Tibia curta* found 126.148±14.002, 32.03±6.081 and 57.82±22.55, respectively from Gadani. Similarly 128.54±15.362, 34.027±3.749, 70.0±1.639 from Jiwani, 130.30±11.836, 34.852±3.681, 66.2±6.685 from Keti Bandar and 129.28±10.1005, 30.065±7.125 and 69.285±9.995 from Sonmiani as given in Table 1. Overall, slightly larger shells were found at Keti Bandar as aforementioned. Moreover, largest penis size found in males collected from Jiwani (70.0±1.639) and Damb, Sonmiani (69.285±9.995). Whereas smallest size was found 57.82±22.55 in males procured from Gadani. Generally, disparity in penis size is directly connected with their breeding season. Similarly average shell length (mm) width (mm) and pseudopenis length (mm) of imposex females was also calculated for individuals found with 1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 3c, 4 and 4+ VDS stages as prescribed by earlier workers (Afsar and Siddiqui, 2015) shown in Figure 3 and Table 1. VDS sequence 3b found notably in 35 individuals among 204 females. 28 individuals shown 1a, 22

found with 1b, 19 with stage 4 and only 8 with 4+. Whereas, largest penis length 11.71 ± 21.588 found in individuals with 1a stage and 10.263 ± 4.43 with stage 4 and 9.25 ± 0.70 with stage 4+. Least count 0.388 ± 1.649 and 0.619 ± 1.961 found in individuals with 1b and 1c, respectively due to endocrine disruption as there are several hundred reports are available on the topic from across the globe. For instance Vasconcelos *et al.* (2011) investigated monthly variation in penis length of Murex (*Bolinus brandaris*) also find out reproductive cycle during this research they investigated reproductive cycle, gonado somatic index (GSI) and penis length variation in gastropods species of family Muricidae from Ria Formosa lagoon (Algarve Coast) southern Portugal. Other than that they have found positive allometric correlation in males and imposex females. According to their findings in males penis length grows faster than shell length and this variation randomly found throughout the year connected with their specific breeding season in order to increase or decrease in penis length while in imposex female penis length variation does not show significant relationship they shows random variation which is results of endocrine disturbance. Mann *et al.* (2006) reported the occurrence of imposex and seasonal patterns of gametogenesis in the invading veined rapa whelk *Rapana venosa* from Chesapeake Bay, USA. So, penis length is related to seasonal patterns or their respective breeding season and it's not directly correlated to shell height or age factor. So, the research carried out is based on the hypothesis keeping in view the biomonitoring efforts should be made at Gadani coast which has been least explored and is very much vulnerable to a number of pollutants like oil, metal, organometallic and related Imposéx, dioxins, natural radiation hazards, pathogenicity and bioinvasive intrusion to find out the actual reasons of health hazards and risk assessment to figure out environmental management measures.

Conclusions and Recommendations

Results of the present investigation clearly indicates that the imposex individual are present only at Gadani ship breaking yards, where ship scrap arriving from all over the world to dismantle and these older ships still contain antifouling paint coatings on ship hull composed of TBT content. This content leached out directly in water column and results in endocrine disruption and consequent phenomenon of imposex in marine gastropods which are normally dioecious.

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Novelty Statement

Imposéx in *Tibia curta* is recorded first time from Gadani beach. In view of recent finding *Tibia curta* is found as one another suitable biomonitoring tool to assess such environmental hazard and environmental health in a cheap cost and easy way forward.

Author's Contribution

Sheharish Munawar: Conducted experiment, collected data and prepare initial draft.

Nuzhat Afsar: Supervise the experiment and did scrutiny of final manuscript. Help in collection of data and preparation of manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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