

Baseline Assessment of Imposex Incidence and Severity in Coastal Gastropods within the Sarangani Bay Protected Seascape (SBPS), Philippines

Leizle B. Coronica, PhD

Notre Dame of Dadiangas University

DOI: <https://doi.org/10.51244/IJRSI.2026.1308000172>

Received: 27 August 2026; Accepted: 01 September 2026; Published: 12 September 2026

ABSTRACT

This study presents the first baseline assessment of imposex incidence and severity in coastal gastropods within the Sarangani Bay Protected Seascape (SBPS), Philippines, while evaluating sediment copper (Cu) and cadmium (Cd) concentrations as supporting environmental variables. A stratified spatial comparative research design was employed across three coastal sites with varying levels of maritime activity. Ninety gastropod specimens were examined for imposex using the Relative Penis Length Index (RPLI) and Vas Deferens Sequence Index (VDSI), while sediment samples were analyzed for Cu and Cd concentrations. Pearson's correlation analysis was performed using SPSS to determine the relationship between sediment Cu concentrations and imposex indices. Imposex was detected exclusively in Muricidae gastropods (*Drupa sp.*, *Drupina grossularia*, and *Chicoreus torrefactus*), with an overall incidence of 10.77%. The highest incidence was observed at Sites 1 and 3 (15.0%), whereas Site 2 exhibited the lowest incidence (4.0%). Overall imposex severity was moderate (RPLI = 39.48%; VDSI = 2.67). Sediment Cu concentrations ranged from 6.53 to 29.56 mg/kg, while Cd remained below the analytical detection limit (<0.03 mg/kg). Both metals were below the National Oceanic and Atmospheric Administration (NOAA) Sediment Quality Guideline thresholds. No significant relationship was found between sediment Cu concentrations and imposex incidence or severity ($p > 0.05$), whereas Cd could not be statistically evaluated because concentrations remained below the detection limit across all sites. These findings establish the first scientific baseline for imposex within SBPS and support the use of Muricidae gastropods as sentinel bioindicators for long-term coastal biomonitoring. Continued monitoring integrating imposex assessment with sediment quality evaluation is recommended to strengthen evidence-based conservation and sustainable management of the protected seascape.

Keywords: Imposex; Muricidae; Coastal biomonitoring; Heavy metals; Sarangani Bay Protected Seascape

INTRODUCTION

Marine coastal ecosystems are among the most productive and biologically diverse environments on Earth. They provide essential ecological services, support fisheries and tourism, sustain coastal livelihoods, and contribute significantly to local and national economies. However, these ecosystems are increasingly threatened by anthropogenic activities such as shipping, port operations, fisheries, industrial development, urbanization, and land-based runoff. These activities introduce a wide range of contaminants into coastal waters and sediments, altering environmental quality and affecting the health of marine organisms long before ecosystem degradation becomes visibly apparent.

One of the most sensitive biological responses to environmental contamination is imposex, a condition in which female marine gastropods develop male reproductive organs, including a penis and vas deferens (Horiguchi, 2006). Because this abnormality directly affects reproduction, imposex has become one of the most widely accepted biological indicators of pollution in marine environments. As reported by Risjani et al. (2025), increasing imposex prevalence poses a serious threat to reproductive success, as affected females may experience reproductive tract blockage, reduced fertility, sterility, and ultimately population decline. These reproductive

impairments can disrupt gastropod populations and compromise the ecological functions they perform in coastal ecosystems.

Historically, imposex has been closely linked to exposure to organotin compounds, particularly tributyltin (TBT) and triphenyltin (TPT), which were widely used as biocides in antifouling paints applied to ships and other marine structures (Horiguchi, 2006). Even at extremely low concentrations, these compounds can induce imposex, making marine gastropods highly sensitive indicators of organotin pollution. The widespread ecological impacts of TBT prompted the International Maritime Organization (IMO) to adopt the International Convention on the Control of Harmful Anti-Fouling Systems on Ships, leading to global restrictions on TBT-based antifouling paints. Despite these regulations, imposex continues to be reported in some coastal environments worldwide, consistent with persistent or renewed exposure to organotin contamination, including legacy residues in sediments.

Although the relationship between TBT and imposex has been firmly established, direct measurement of organotin compounds remains a major challenge in many developing countries, including the Philippines. Organotin analysis requires sophisticated instrumentation, such as gas chromatography–mass spectrometry (GC-MS), specialized technical expertise, and substantial financial resources. Despite extensive efforts undertaken during the conduct of this study to locate laboratories capable of analyzing TBT, access to accredited analytical facilities remained extremely limited. This limitation reflects a broader challenge faced by many environmental researchers in the country and highlights the need for practical approaches that can still provide meaningful assessments of coastal environmental quality.

In response to these constraints, environmental monitoring studies increasingly combine biological indicators such as imposex with more accessible environmental measurements. Among these, copper (Cu) and cadmium (Cd) are widely recognized as important indicators of coastal contamination because they are commonly associated with maritime activities, ship maintenance, industrial discharges, aquaculture, urban runoff, and other human activities (Beyer et al., 2022). These metals readily accumulate in marine sediments, where they may persist for long periods and become bioavailable to benthic organisms, including gastropods. Elevated concentrations of copper and cadmium have been associated with oxidative stress, endocrine disruption, physiological impairment, and reduced reproductive performance in aquatic organisms. These heavy metals are not established primary causes of imposex; rather, they were treated in this study as supporting indicators of sediment contamination and broader environmental stress that may affect organismal health. Their relatively greater accessibility for laboratory analysis also makes them valuable supporting indicators for coastal environmental assessment, particularly in regions where direct organotin determination is not feasible.

The Philippines, recognized as part of the Coral Triangle, supports some of the world's richest marine biodiversity. Its coastal ecosystems provide food security, employment, transportation, and numerous ecosystem services for millions of Filipinos. However, increasing coastal development and maritime activities have intensified environmental pressures on many coastal areas, emphasizing the need for reliable biomonitoring tools capable of detecting ecological changes before irreversible damage occurs. The use of imposex as a biological indicator offers a practical and scientifically sound approach for evaluating environmental quality and identifying areas that may be affected by pollution.

One ecologically important coastal area is the Sarangani Bay Protected Seascape (SBPS) in southern Philippines. Covering approximately 215,950 hectares, SBPS encompasses coral reefs, seagrass beds, mangrove forests, sandy beaches, and productive fishing grounds that support both marine biodiversity and the livelihoods of surrounding communities. At the same time, the bay serves as an important center for fisheries, maritime transportation, tourism, coastal settlements, and commercial activities. These multiple uses expose different parts of the bay to varying levels of anthropogenic pressure, particularly in areas adjacent to ports, shipping lanes, docking facilities, and fish landing sites.

Despite the ecological and socioeconomic importance of SBPS, information on the occurrence and severity of imposex in its coastal gastropod populations remains very limited. Likewise, no published study has investigated imposex in relation to sediment copper and cadmium concentrations within the protected seascape. This

knowledge gap restricts the use of gastropods as bioindicators for assessing environmental quality and monitoring marine pollution in the bay. Establishing baseline information on imposex incidence, severity, and sediment-associated heavy metals is therefore essential for understanding the current ecological condition of Sarangani Bay and for providing reference data for future biomonitoring and conservation initiatives.

Guided by these considerations, the study was conducted to determine the incidence and severity of imposex among selected coastal gastropod bioindicator species across sites with varying levels of maritime activity. Sediment copper and cadmium concentrations were measured as supporting environmental variables to provide additional context for the observed patterns of imposex and to establish baseline environmental information for the protected seascape. The findings also served as the basis for the development of an Information-Educational Material (IEM) intended to promote environmental awareness and strengthen coastal stewardship among stakeholders.

This study contributes to the achievement of the United Nations Sustainable Development Goal (SDG) 14: Life Below Water by supporting marine biodiversity conservation through environmental monitoring, and SDG 12: Responsible Consumption and Production by generating scientific evidence relevant to pollution assessment and the environmentally sound management of coastal ecosystems. The baseline information generated is expected to assist environmental agencies, protected area managers, local government units, researchers, and coastal communities in strengthening science-based management, pollution prevention, and long-term conservation efforts within the Sarangani Bay Protected Seascape.

LITERATURE REVIEW

This section synthesizes key scientific findings relevant to the baseline assessment of imposex incidence and severity in coastal gastropods in Sarangani Bay Protected Seascape (SBPS), Philippines. The review integrates global evidence, regulatory developments, mechanistic understanding, and biomonitoring approaches to situate the present study within established research.

Imposex in Marine Gastropods

Imposex is one of the most well-documented manifestations of endocrine disruption in marine gastropods and is widely recognized as a sensitive biomarker of coastal pollution. It is characterized by the development of male sexual organs, such as the penis and vas deferens, in female gastropods, resulting in reproductive abnormalities that may lead to reduced fertility, sterility, and population decline (Gibbs & Bryan, 1994). Because imposex directly affects reproductive success, it has become an important biological indicator for assessing the ecological impacts of contaminants in marine environments.

The occurrence of imposex has been strongly associated with exposure to organotin compounds, particularly tributyltin (TBT) and triphenyltin (TPT), which were historically used in antifouling paints applied to ships and marine structures (Horiguchi, 2006). Organotin can induce imposex even at very low concentrations and have been reported in more than 260 gastropod species worldwide (Titley-O'Neal et al., 2011). Although the International Maritime Organization prohibited the use of TBT-based antifouling systems through the International Convention on the Control of Harmful Anti-Fouling Systems on Ships, imposex remains evident in some coastal areas due to the persistence of organotin residues in marine sediments (Beyer et al., 2022).

While TBT is recognized as the primary causative agent of imposex, direct measurement of organotin compounds remains challenging in many developing countries because of the limited availability of specialized analytical facilities and the high cost of analysis. The researcher is able to reach out different laboratories in different institutions and private laboratories and even extended effort in the neighboring countries but find it limited.

Consequently, recent studies have emphasized the importance of investigating other environmental drivers and co-contaminants associated with maritime activities. Heavy metals such as copper (Cu) and cadmium (Cd) are commonly found in coastal sediments near ports, shipping lanes, and industrialized coastlines and have been

associated with oxidative stress, endocrine disruption, and reproductive impairment in aquatic organisms (Beyer et al., 2022).

Although their direct role in inducing imposex remains unclear, these contaminants may contribute to cumulative environmental stress and influence the incidence and severity of reproductive abnormalities in gastropod populations, particularly in tropical coastal ecosystems where baseline information remains limited.

Imposex, defined as the superimposition of male reproductive organs (penis and vas deferens) onto female gastropods, is one of the most well-documented examples of endocrine disruption caused by a manufactured contaminant. First described in the early 1970s and later linked mechanistically to TBT exposure (Gibbs & Bryan, 1994), imposex has since become a globally recognized biomarker of organotin contamination (Oehlmann et al., 2007). One of the most well-known biological adverse effect of TBT pollution is the female masculinization (imposex and intersex) of gastropods, a phenomenon already described for more than 260 species (Titley-O'Neal et al., 2011; Laranjeiro et al., 2018).

The ecological consequences of imposex can be severe. Progressive vas deferens development may block the female genital opening, impair egg capsule release, and ultimately cause sterility, leading to population declines in heavily contaminated areas. Importantly, gastropods can exhibit imposex at very low environmental concentrations of TBT, making them effective early-warning indicators near ports and maritime infrastructure.

Field studies consistently show that imposex prevalence and severity are highest near contamination sources and decline with increasing distance, supporting a dose–response pattern relevant to spatial management (Rial, Bellas & Ruiz 2018).

Measuring Imposex Severity: Standardized Indices

To quantify imposex, researchers apply standardized morphological indices. The Vas Deferens Sequence Index (VDSI) summarizes the mean developmental stage of vas deferens formation among female specimens which was supported by the study of Ruiz et al. (2019) while the Relative Penis Length (RPLI) compare female penis development relative to males. These indices allow consistent spatial comparisons and long-term monitoring across sites and species (Abidli et al., 2009).

The proposed Sarangani Bay assessment aligns with established biomonitoring practice by applying VDSI and RPLI to compare high-, moderate-, and reference-exposure sites. Such approaches have proven effective in mapping contamination gradients and informing coastal risk assessment.

Standardized morphological indices allow imposex severity to be compared among sites and through time. The Vas Deferens Sequence Index (VDSI) describes the progressive development of the vas deferens in females, whereas penis-based indices such as RPSI/RPLI quantify female penis development relative to males. These indices remain central to gastropod biomonitoring of organotin exposure (Gibbs & Bryan, 1994; Laranjeiro et al., 2018; Ruiz et al., 2019).

Field applications have shown that imposex indices are most informative when interpreted together with species identity, site conditions, and contaminant information. Long-term monitoring demonstrates that standardized imposex measurements can detect spatial gradients and temporal changes following regulation of organotin antifouling compounds (Laranjeiro et al., 2018; Ruiz et al., 2019).

VDSI and penis-based indices provide complementary information but are not interchangeable. Their relationship with butyltin burden can vary among species and sites, so interpretation should avoid assuming a universal one-to-one relationship between an index value and contaminant concentration (Rial et al., 2018). For the present baseline, the indices are therefore used primarily to describe biological incidence and severity across SBPS sites.

Mechanistic Basis: Endocrine Disruption and RXR Signaling

Mechanistic research has demonstrated that TBT-induced imposex involves disruption of endocrine-related signaling pathways in gastropods. Evidence points to abnormal modulation of the retinoid X receptor (RXR) pathway as a central mechanism underlying male trait development in exposed females. The study of Giulianelli et al. (2020) mentioned that the development of male sexual characteristics in female marine gastropods, known as imposex, is one of the most evident ecological signs of endocrine disruption caused by organotin compounds. Recent studies suggest that this condition is linked to specific signaling pathways in the body, particularly those involving nuclear receptors such as RXR and PPAR γ , which play a role in regulating growth and development.

Copper as a Co-contaminant Associated with Tributyltin (TBT) and Imposex

Although tributyltin (TBT) has been widely recognized as the primary environmental contaminant responsible for inducing imposex in marine gastropods, several studies have suggested that copper (Cu), another common constituent of antifouling paints, may coexist with TBT in contaminated coastal environments and contribute to overall ecological stress. Following restrictions on organotin-based antifouling compounds, copper-based paints became more prevalent, increasing the occurrence of copper in harbor sediments and prompting investigations into its possible role in imposex development. However, current evidence indicates that copper is more appropriately regarded as a co-contaminant rather than a direct causative agent of imposex.

Peralta et al. (2014) investigated imposex in the marine gastropod *Voluta musica* from the northeastern Peninsula de Araya, Venezuela, by examining imposex incidence, histological characteristics, and sediment concentrations of TBT and copper across three coastal sites. The study found that the highest imposex incidence (24.5%) occurred at Isla Caribe, the site characterized by the greatest boat traffic and the highest sediment TBT concentrations. In contrast, copper was detected primarily at Isla Lobos, where imposex incidence was considerably lower (12%), while no imposex was observed at Bajo Cuspe. Histological examination further revealed no evidence of masculinization within the gonadal tissues of affected females. These findings indicate that although copper was present in contaminated sediments, its association with imposex was considerably weaker than that of TBT. The authors concluded that the combined presence of TBT and copper in sediments represents a potential environmental threat to *V. musica* populations, but emphasized that TBT remains the contaminant most strongly associated with imposex development. They also highlighted the need for improved monitoring and regulatory measures to protect marine biodiversity in Venezuelan coastal ecosystems (Peralta et al., 2014).

Similarly, Barroso et al. (2002) experimentally evaluated the effectiveness of several antifouling paint-derived compounds, including tributyltin (TBT), triphenyltin (TPT), dibutyltin (DBT), diphenyltin (DPT), and copper sulfate (CuSO₄), in inducing imposex in the marine gastropod *Nassarius reticulatus*. Adult snails exposed to environmentally relevant concentrations of copper sulfate (500 ng Cu L⁻¹) for two to three months did not develop imposex. Likewise, dibutyltin and diphenyltin failed to induce reproductive abnormalities. In contrast, both TBT and TPT induced imposex in approximately 22–44% of exposed females and significantly increased testosterone concentrations in both male and female gastropods. These findings provide strong experimental evidence that organotin compounds, particularly TBT and TPT, directly disrupt endocrine function and induce imposex, whereas copper alone does not exhibit the same biological effect under comparable exposure conditions. Consequently, although copper frequently co-occurs with TBT in antifouling paint residues and contaminated harbor sediments, it appears to contribute primarily as an accompanying environmental contaminant rather than as a direct inducer of imposex (Barroso et al., 2002).

Taken together, these studies demonstrate that copper is commonly detected alongside TBT in coastal environments affected by maritime activities, reflecting their shared origin from antifouling coatings applied to vessels. Nevertheless, both field observations and laboratory experiments consistently indicate that copper does not directly induce imposex to the same extent as organotin compounds. Instead, copper may serve as a complementary environmental stressor that reflects anthropogenic contamination and may influence the overall physiological condition of marine organisms. Therefore, assessing sediment copper concentrations together with

imposex incidence provides valuable baseline information on coastal environmental quality and anthropogenic pressure, even though TBT remains the principal contaminant responsible for the development of imposex.

Copper and Marine Organisms

Recent ecotoxicological literature supports evaluating copper within a weight-of-evidence framework that considers both environmental concentration and biological response. Marine invertebrates differ substantially in metal uptake and sensitivity, and co-exposure to cadmium and copper can produce species-specific biomarker responses (Rainbow, 2002; Wang & Rainbow, 2008; Perić et al., 2020).

Cadmium as a Co-contaminant Associated with Tributyltin (TBT) and Marine Reproductive Effects

In addition to tributyltin (TBT), cadmium (Cd) is a persistent heavy metal frequently detected in coastal environments affected by industrial activities, urban runoff, shipping operations, and port-related discharges. Although cadmium is not recognized as a direct inducer of imposex in marine gastropods, increasing evidence suggests that it functions as a co-contaminant capable of disrupting reproductive physiology and contributing to the cumulative effects of pollution on marine organisms. Because cadmium and TBT often coexist in contaminated sediments near harbors and maritime facilities, assessing cadmium alongside biological indicators such as imposex provides a more comprehensive understanding of environmental quality and multiple contaminant exposure.

Experimental evidence by Tilton et al. demonstrated that chronic cadmium exposure significantly impairs reproductive performance in fathead minnows (*Pimephales promelas*). Adult breeding pairs exposed to cadmium for 21 days exhibited reduced spawning frequency and fecundity, alterations in male secondary sexual characteristics, and significant changes in circulating 11-ketotestosterone concentrations, indicating disruption of endocrine function. Although female estradiol concentrations and gonadosomatic indices remained unchanged, the overall reproductive success of exposed fish declined markedly at higher cadmium concentrations. These findings indicate that cadmium can interfere with hormonal regulation and reproductive processes, supporting its classification as an endocrine-disrupting contaminant. While cadmium does not directly induce imposex as TBT does, its capacity to alter reproductive physiology suggests that it may exacerbate the effects of other contaminants in polluted coastal ecosystems (Tilton et al., 2003).

Similarly, Bondgaard and Bjerregaard (2005) investigated cadmium uptake and distribution in female shore crabs (*Carcinus maenas*) during different moulting stages. Their study demonstrated that cadmium uptake increased substantially during the post-moult stage and closely paralleled calcium uptake through calcium ion channels located in the gill epithelium. Once absorbed, cadmium accumulated primarily in the midgut gland and gills, where it persisted within body tissues despite being metabolized differently from calcium. These findings highlight the strong bioaccumulative potential of cadmium in marine crustaceans and suggest that physiological conditions can influence the extent of cadmium accumulation. The study further indicates that cadmium remains biologically available in marine organisms for extended periods, potentially increasing chronic toxicity in contaminated coastal habitats.

Collectively, these studies demonstrate that cadmium is an important environmental co-contaminant commonly associated with anthropogenic pollution in marine ecosystems. Unlike TBT, which directly induces imposex through endocrine disruption in gastropods, cadmium primarily affects reproductive performance, hormone regulation, metal accumulation, and physiological homeostasis. Consequently, cadmium should not be regarded as the principal cause of imposex but rather as an environmental stressor that may contribute to the cumulative impacts of multiple contaminants on marine organisms. Measuring sediment cadmium concentrations together with imposex incidence and severity therefore provides valuable baseline information for evaluating the overall ecological condition of coastal environments and identifying areas subjected to complex contaminant mixtures resulting from maritime and coastal activities.

Cadmium Contamination and Its Effects on Marine Organisms

Cadmium (Cd) is a nonessential and persistent toxic metal that can enter coastal and marine environments through industrial discharges, mining activities, agricultural runoff, wastewater, atmospheric deposition, and the transport of contaminated sediments. Unlike essential metals, cadmium has no established biological function in most marine animals. Its persistence and capacity to accumulate in tissues make it an important contaminant in marine environmental monitoring. Coastal organisms may be exposed to cadmium through dissolved metals in seawater, contaminated food particles, suspended materials, and sediments.

Marine mollusks, particularly bivalves, are widely recognized as useful bioindicators of cadmium contamination because they are sedentary, filter large volumes of water, and efficiently accumulate metals in their tissues. Their tissue concentrations can therefore reflect the bioavailable fraction of contaminants in the surrounding environment. Recent studies have shown that cadmium accumulation varies among species, tissues, developmental stages, exposure pathways, and environmental conditions. Digestive glands, kidneys, gills, gonads, and other metabolically active tissues commonly retain higher cadmium concentrations than muscular tissues.

Cadmium Bioaccumulation in Marine Mollusks

Recent research has demonstrated that cadmium accumulation in marine bivalves is governed by both waterborne and dietary exposure. Liu et al. (2023) investigated the sources, distribution, and toxicity mechanisms of cadmium in the scallop *Chlamys farreri*. Their findings showed that scallops accumulated cadmium from seawater and dietary sources, with distinct patterns of tissue distribution and metabolic regulation. The study further demonstrated that cadmium exposure affected ion regulation, energy metabolism, cytoskeletal integrity, antioxidant defenses, immune processes, and cellular apoptosis. These results emphasize that tissue cadmium concentrations alone may not completely represent toxic effects because the biological response also depends on how the accumulated metal is stored, metabolized, or detoxified.

Huang et al. (2020) examined the internal distribution of waterborne cadmium in the clam *Meretrix meretrix*. Cadmium exposure resulted in tissue-specific accumulation and stimulated the production of metallothionein and antioxidant enzymes. Metallothioneins bind metal ions and reduce their interaction with sensitive cellular structures. However, the induction of metallothionein and antioxidant defenses also indicates that the organism is experiencing physiological stress. The study therefore supports the combined use of tissue cadmium, metallothionein expression, and antioxidant responses as indicators of metal exposure.

Field investigations likewise show that cadmium can accumulate in commercially important marine species even when concentrations in coastal water appear relatively low. Pan and Han (2023) analyzed bivalve mollusks collected from coastal areas of southeastern China and detected cadmium and other trace elements in edible tissues. Their assessment suggested that cadmium exposure through mollusk consumption may represent a potential long-term health concern, supporting the need for regular monitoring of both marine environments and seafood products.

In the Philippines, research on scallops from the Visayan Sea found that cadmium was concentrated primarily in the digestive gland. The digestive gland accounted for a substantial proportion of the total cadmium burden, while cadmium concentrations were positively associated with shell weight. This suggests that larger or older scallops may accumulate greater quantities of the metal, and that the digestive gland may serve as a useful target tissue for monitoring cadmium contamination.

Oxidative Stress and Cellular Damage

Oxidative stress is one of the most consistently reported mechanisms of cadmium toxicity in marine organisms. Cadmium does not directly generate free radicals, but it can disturb mitochondrial functions, reduce antioxidant capacity, interfere with essential metals, and promote the production of reactive oxygen species. Excessive

reactive oxygen species may cause lipid peroxidation, protein damage, DNA alteration, mitochondrial dysfunction, and cell death.

Wang et al. (2021) demonstrated that cadmium exposure caused oxidative stress in the gills of *Meretrix meretrix*. The resulting cellular disturbance activated mitochondria-mediated apoptosis, indicating that prolonged or sufficiently intense cadmium exposure can exceed the organism's antioxidant and detoxification capacity. Because gills are directly exposed to surrounding seawater and play important roles in respiration and ion regulation, damage to gill tissues may affect the overall physiological condition of marine bivalves.

Cadmium can also affect reproductive tissues. Gao et al. (2021) documented cadmium-induced apoptosis in the spermatogenic cells of *M. meretrix*, together with changes in metallothionein and apoptosis-related gene expression. Such responses indicate that cadmium contamination may impair reproductive health even before observable changes occur at the population level. Reproductive biomarkers may therefore provide important early-warning evidence of chronic metal exposure.

A more recent investigation by Zhou et al. (2024) found that short-term cadmium exposure increased cadmium accumulation, oxidative stress, and tissue damage in the ovaries of *M. meretrix*. After three days of depuration in uncontaminated seawater, ovarian cadmium concentrations declined by approximately 20%, accompanied by partial tissue recovery and reduced oxidative stress. However, the limited reduction in tissue cadmium showed that depuration may only partly reverse cadmium accumulation and toxicity.

Species-, Dose-, and Duration-Dependent Toxicity

Responses to cadmium vary significantly among marine species. Zhan et al. (2023) conducted a meta-analysis of 87 studies on cadmium toxicity in marine bivalves. The analysis confirmed that cadmium effects were strongly influenced by species, exposure concentration, and exposure duration. Clams were generally more sensitive than oysters, mussels, and scallops, although sensitivity differed according to the biological endpoint measured.

The meta-analysis identified oxidative stress and genotoxicity indicators as some of the most responsive measures of cadmium exposure. The results also demonstrated that responses were not always linearly related to exposure concentration. Some endpoints showed conventional dose-dependent increases, whereas others displayed hormetic responses in which low concentrations temporarily stimulated defensive processes before adverse effects became apparent at higher concentrations. These findings indicate that a low cadmium concentration should not automatically be interpreted as biologically harmless, particularly under prolonged exposure.

Species-specific responses were also demonstrated by Perić et al. (2020), who compared the native European flat oyster *Ostrea edulis* and the Pacific oyster *Crassostrea gigas* during combined cadmium and copper exposure. Differences in biomarker responses between the species illustrated that organisms exposed to the same contaminant concentrations may not experience equal biological effects. Consequently, monitoring programs should carefully consider the biology and sensitivity of the selected bioindicator species.

Environmental Factors Affecting Cadmium Toxicity

Cadmium toxicity is influenced not only by contaminant concentration but also by environmental variables such as salinity, temperature, pH, dissolved organic matter, sediment characteristics, and the presence of other pollutants. These factors can alter cadmium speciation, bioavailability, uptake, tissue distribution, and elimination.

Sun et al. (2024) examined the effects of salinity on cadmium toxicokinetics, oxidative stress, and metallothionein expression in *Meretrix meretrix*. Their findings showed that salinity influenced cadmium accumulation and the physiological responses of the clams. This indicates that the same measured cadmium concentration may produce different biological effects under different salinity regimes. For estuarine and coastal assessments, salinity should therefore be considered when interpreting tissue accumulation and biomarker data.

Climate-related stressors may further intensify cadmium toxicity. Experimental research involving the mud crab *Scylla serrata* found that ocean acidification increased cadmium accumulation and enhanced oxidative stress responses when the organisms were exposed to both stressors. The combined treatment produced greater biological disturbances than ocean acidification alone, suggesting that future changes in seawater chemistry may modify the toxicity of existing coastal contaminants.

Cadmium toxicity is also shaped by co-occurring environmental stressors and by exposure conditions. Consequently, sediment cadmium concentrations should be interpreted together with biological responses and site characteristics rather than as a stand-alone measure of ecological effect (Liu et al., 2023; Zhan et al., 2023).

Cadmium Biomarkers and Environmental Monitoring

Recent literature supports the use of multiple biomarkers rather than reliance on cadmium concentration alone. Useful biomarkers include metallothionein induction, lipid peroxidation, antioxidant enzyme activity, lysosomal stability, DNA damage, apoptosis-related gene expression, immune responses, and changes in energy metabolism.

Oxidative stress biomarkers such as superoxide dismutase, catalase, glutathione, and lipid peroxidation are commonly measured because they respond before mortality or population decline becomes evident. Molecular and transcriptomic methods can also identify changes in pathways associated with ion transport, mitochondrial activity, immunity, detoxification, reproduction, and apoptosis. Liu et al. (2023) showed that combining tissue distribution studies with molecular and metabolic analyses provides a clearer explanation of how marine bivalves accumulate, tolerate, and respond to cadmium.

Biomonitoring should also integrate chemical measurements from water, sediments, and biological tissues. Sediment concentrations indicate the presence and spatial distribution of contamination, while tissue measurements provide evidence of biological uptake. Biomarkers then determine whether accumulated cadmium is associated with physiological or cellular stress. This weight-of-evidence approach is more informative than assessing sediment chemistry alone because some sediment-bound cadmium may have limited bioavailability, whereas relatively low dissolved concentrations may still be efficiently accumulated by filter-feeding organisms.

Cadmium Contamination and Seafood Safety

The capacity of marine mollusks and crustaceans to accumulate cadmium creates potential risks to seafood consumers. He et al. (2023) assessed cadmium exposure from aquatic products and found that marine crustaceans and mollusks were important contributors to dietary cadmium intake. Although average exposure levels were generally below the provisional tolerable monthly intake, high consumers and young children could experience greater exposure because of consumption patterns and lower body mass.

Pan and Han (2023) similarly reported that cadmium detected in bivalve mollusks posed a potential long-term health concern. Although estimated noncarcinogenic risks were generally acceptable for average consumers, their findings supported regular surveillance because risk varies with species, collection site, contamination level, consumption frequency, and serving size.

These findings are particularly important because edible muscle may contain lower cadmium concentrations than internal organs, while some seafood products are consumed whole. Food safety assessments must therefore account for the specific tissues consumed, local food preparation practices, consumption rates, and the age or size of harvested organisms.

The most current research is Zhan et al. (2023) a meta-analysis synthesizing 87 studies on cadmium toxicity in marine bivalves. This study identified species-, dose-, and duration-dependent responses, finding that clams are more sensitive to cadmium than oysters, mussels, and scallops. It determined that oxidative stress and genotoxicity are the most reliable biomarkers for cadmium pollution, with dose-response relationships showing both linear and hormetic (inverted U-shaped) patterns depending on the endpoint measured.

Earlier updated work includes Maher and Taylor (2010) which established exposure-dose-response relationships in sediment-dwelling bivalves using biomarkers like oxidative stress and chromosomal damage.

Sarangani Bay Protected Seascape and Coastal Biodiversity

The Sarangani Bay Protected Seascape (SBPS) is in the southernmost part of Mindanao, Philippines, bordered by Sarangani Province and General Santos City. The area was declared a protected seascape in 1996 and covers approximately 215,950 hectares, including coastal waters, mangrove ecosystems, and coral reef habitats that support high marine biodiversity.

SBPS hosts diverse coastal ecosystems such as coral reefs, seagrass beds, and mangrove forests, which serve as critical habitats for numerous marine organisms. The bay is known to support more than 400 reef-associated species, along with marine mammals, sea turtles, and commercially important fish species, reflecting its ecological significance within the Celebes Sea region (Alcala et al., 2008; Agduma & Cao, 2023).

In the study of Agduma and Cao (2023) mangrove ecosystems within SBPS also contribute significantly to coastal productivity and habitat complexity. Recent ecological assessments documented more than twenty mangrove species distributed along the coastline, forming narrow fringes and patches along sandy, rocky, and riverine substrates. These mangroves serve as nursery habitats for many invertebrates and mollusks, including gastropods that inhabit intertidal and shallow coastal zones.

SBPS provides suitable environmental conditions for molluscan assemblages. Previous ecological studies conducted in seagrass beds and intertidal zones in Sarangani Province revealed a high diversity of gastropods, highlighting the importance of the area as a potential site for studying marine mollusk ecology and environmental monitoring (Herceda et al. (2016)).

The seascape supports remarkable marine diversity Manzo et al. (2014) documented 57 intertidal mollusk species from Gastropoda, Bivalvia, and Chiton classes, indicating high invertebrate richness. Alcala et al. (2008) noted that SBPS marine biodiversity is comparable to other Philippine seas, with particular significance for tuna species that contribute substantially to coastal livelihoods.

Despite its ecological importance, SBPS faces significant threats from pollution as mentioned in the study of Acot et al. (2022) and anthropogenic pressures, making its protection critically important for biodiversity conservation.

Importance of Gastropods in the Sarangani Bay Region

Gastropods are important components of tropical intertidal and shallow coastal ecosystems, where they contribute to biodiversity, ecological processes, and the functioning of benthic food webs. Depending on the species, gastropods may function as grazers, detritivores, scavengers, or predators, thereby contributing to nutrient cycling, organic matter processing, and the regulation of other benthic organisms. In coastal communities, some gastropods may also have socioeconomic value through gleaning, local consumption, ornamental shell collection, and small-scale livelihood activities.

Within the Sarangani Bay region, previous intertidal surveys have documented diverse molluscan assemblages associated with habitats such as seagrass beds, sandy substrates, rocky intertidal areas, and other shallow coastal environments (Manzo et al., 2014; Herceda et al., 2016). These records demonstrate the ecological importance of gastropods as a component of the marine biodiversity of Sarangani Bay and provide a local basis for their consideration in biodiversity assessment and environmental monitoring.

Manzo et al. (2014), for example, documented 57 molluscan species from Kawas, Alabel and Tinoto, Maasim, Sarangani Province. *Cerithium echinatum* was reported as particularly abundant in Kawas, Alabel, whereas *Cypraea annulus* was abundant in Tinoto, Maasim. Species such as *Nerita alveolus*, *Nerita maxima*, and *Nerita chamaeleon* occurred in both sampling areas, indicating similarities in the composition of their intertidal molluscan communities. Of particular relevance to environmental biomonitoring was the reported occurrence of

the family Muricidae, represented by *Thais muricoides* in Tinoto, Maasim. This provides previous evidence that muricid gastropods form part of the coastal molluscan fauna of the Sarangani Bay region.

Gastropods can also be important to coastal communities. In the Philippines, various gastropods are collected through small-scale fisheries and gleaning for food, shells, and other uses. However, despite the documented diversity of gastropods in Sarangani Bay, information specifically addressing their fisheries utilization, economic value, harvesting intensity, and contribution to local livelihoods remains limited. Thus, further investigation of their socioeconomic importance could contribute to a more comprehensive understanding of the ecosystem services provided by gastropod resources within the Sarangani Bay Protected Seascape (SBPS).

Beyond their ecological and potential socioeconomic importance, gastropods are particularly valuable in environmental biomonitoring. Their close association with benthic habitats, generally limited mobility, relatively long exposure to local environmental conditions, and sensitivity to certain contaminants allow biological responses in some gastropod groups to reflect localized environmental stress. Muricid gastropods are especially important in this context because members of the family have been widely investigated for imposex, a reproductive abnormality characterized by the development of male sexual characteristics in females and historically associated particularly with exposure to organotin compounds such as tributyltin (TBT).

The occurrence of muricid gastropods within Sarangani Bay therefore provides an important ecological basis for investigating their potential as sentinel organisms. Species encountered in the present study, including *Drupina grossularia* and *Chicoreus torrefactus*, contribute to the predatory component of the benthic gastropod community and may provide useful biological information on localized environmental conditions. However, published information specifically addressing the distribution, population characteristics, contaminant responses, and biomonitoring potential of these species within SBPS remains limited.

Despite the recognized marine biodiversity of Sarangani Bay and previous documentation of its gastropod fauna, information on imposex among muricid gastropods in the area remains particularly scarce. Establishing baseline information on the occurrence and severity of imposex in locally occurring muricids is therefore important for assessing their potential application as sentinel organisms and for supporting long-term monitoring of coastal environmental conditions. Such information can complement conventional physicochemical measurements by providing biological evidence of organism-level responses to environmental stress.

Overall, gastropods in Sarangani Bay are important not only as components of marine biodiversity and benthic food webs but also because of their potential socioeconomic and environmental-monitoring value. Documenting their diversity, ecological roles, and biological responses to environmental stress can contribute to the scientific basis for biodiversity conservation, pollution monitoring, and sustainable management of the Sarangani Bay Protected Seascape.

Relevance to Sarangani Bay

Copper and cadmium can contribute to oxidative stress and other physiological disturbances in aquatic organisms, although they should not be interpreted as direct causes of imposex. Their measurement in this study therefore provides supporting environmental context for the biological observations (Perić et al., 2020; Liu et al., 2023; Zhan et al., 2023).

Philippine coastal sediment studies have nevertheless documented potentially toxic elements in marine environments, supporting continued sediment-quality surveillance alongside biological monitoring (Bautista et al., 2024).

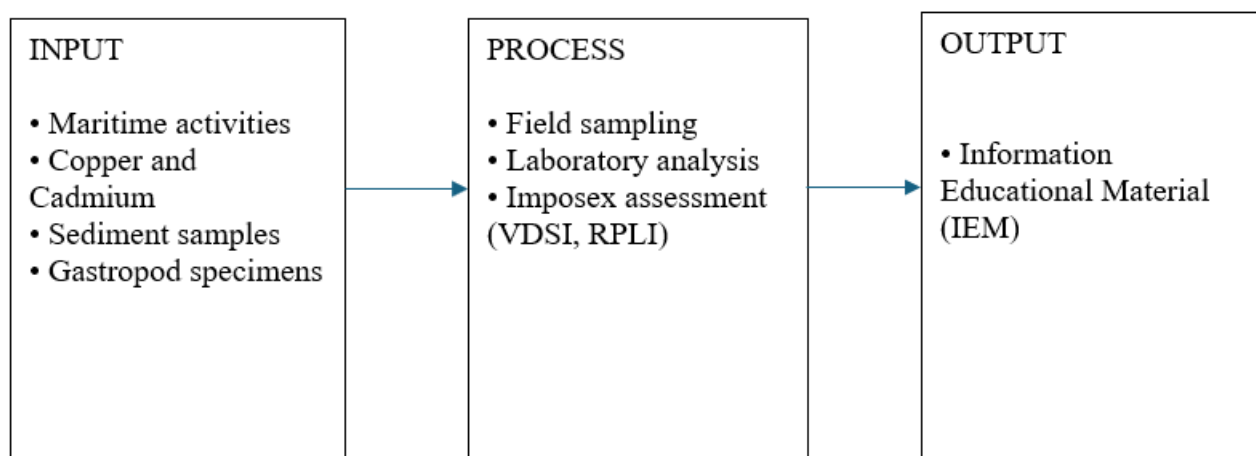
As an ecologically significant protected seascape, Sarangani Bay supports diverse marine habitats and coastal communities while simultaneously serving as an important area for maritime transportation, fisheries, coastal settlements, and other economic activities. The varying levels of maritime influence across the bay provide an ideal setting for assessing spatial patterns of imposex occurrence and severity in coastal gastropods. Establishing baseline information on imposex and examining associated environmental factors, including sediment copper

and cadmium concentrations, are therefore essential for understanding the current condition of the bay and identifying potential environmental stressors. Such information can support ecological risk assessment, biodiversity conservation, and evidence-based management strategies for the Sarangani Bay Protected Seascape while contributing to long-term biomonitoring efforts in Philippine coastal ecosystems.

CONCEPTUAL FRAMEWORK

The conceptual framework of this study follows the Input–Process–Output (IPO) model, which illustrates how environmental and biological variables are examined to generate first baseline information on imposex incidence and severity in coastal gastropods of the Sarangani Bay Protected Seascape.

Figure 1. Schematic diagram of the Conceptual Framework



The inputs comprise coastal gastropod bioindicator species collected from sampling sites representing varying levels of maritime activity within the Sarangani Bay Protected Seascape, together with sediment copper (Cu) and cadmium (Cd) concentrations as supporting environmental variables. These heavy metals were included because they are commonly associated with coastal anthropogenic activities and provide accessible indicators of environmental quality where direct organotin analysis is limited.

The process involved the collection of gastropod and sediment samples from the three study sites, identification of gastropod specimens, determination of sex, and assessment of imposex incidence and severity using the Relative Penis Length Index (RPLI) and Vas Deferens Sequence Index (VDSI). Sediment samples were analyzed to determine copper and cadmium concentrations. The resulting biological and environmental data were subjected to descriptive and inferential statistical analyses to evaluate spatial variation in imposex and examine the relationship between imposex expression and sediment heavy metal concentrations.

The outputs include baseline information on the incidence and severity of imposex in coastal gastropods, sediment copper and cadmium concentrations, and the spatial distribution of these variables within the Sarangani Bay Protected Seascape. These findings served as the basis for the development of an Information-Educational Material (IEM) to support environmental awareness, coastal biomonitoring, biodiversity conservation, and evidence-based coastal resource management.

Objective of the Study

The study aimed to assess the imposex incidence and severity of coastal gastropods within the Sarangani Bay Protected Seascape (SBPS), Philippines.

Scope and Limitation of the Study

This study focuses on the baseline assessment of the incidence and severity of imposex in coastal gastropods within the Sarangani Bay Protected Seascape (SBPS), Philippines. Copper and cadmium concentrations in sediments were included as environmental variables to determine their possible relationship with imposex incidence and severity.

The study is limited to a single baseline sampling campaign conducted during the dry season from March to April 2026. The sampling sites represent different coastal areas within Sarangani Bay and were categorized according to the intensity of maritime activities. A total of nine sampling sites were established, with three replicates per site.

Field sampling involved collection of surface sediments (0–2 cm depth) for heavy-metal analysis and gastropod specimens that were sufficiently abundant and accessible at the study sites. A total of 90 gastropods (30 individuals per site) were examined. Because imposex assessment applies specifically to confirmed females, the effective female sample size was lower than 30 at each site; therefore, site-level incidence estimates are interpreted as preliminary baseline estimates rather than precise population prevalence values. Specimens were identified to the lowest possible taxonomic level and examined through dissection and morphological assessment. Imposex severity was quantified using the Vas Deferens Sequence Index (VDSI) and Relative Penis Length Index (RPLI). Shell length was measured for each specimen to document body-size variation and to provide a morphometric variable relevant to interpretation of penis development and potential size/age-related exposure effects.

Chemical analysis in the present baseline focused on sediment copper (Cu) and cadmium (Cd). Organotin compounds, including tributyltin (TBT), dibutyltin (DBT), and monobutyltin (MBT), were not quantified because appropriate analytical facilities and reagents were not accessible during the study. Cu and Cd were therefore treated only as supporting indicators of sediment contamination and broader environmental stress and not as proxies for TBT. Consequently, the occurrence of imposex cannot be attributed to contemporary or legacy organotin exposure on the basis of the present chemical data, and no causal relationship between TBT and the observed imposex can be established from this study.

The study is further limited by its cross-sectional, single-season design. Quantitative vessel-density records, hydrodynamic and flushing-rate measurements, temperature and other water-quality parameters, sediment grain-size normalization, total organic carbon, organic-pollution indicators, tissue contaminant burdens, water-column metals, and experimental toxicity tests were not included. Maritime influence was therefore treated as a site-classification variable based on observable proximity to ports, docking/repair areas, shipping/anchorage activity, and relatively low-activity reference conditions rather than as a direct quantitative measure of vessel traffic. These unmeasured environmental and biological factors may influence contaminant retention, exposure history, and imposex expression and should be incorporated into future monitoring.

The findings of this study are intended to provide baseline ecological information on imposex incidence and severity within the Sarangani Bay Protected Seascape. The results also serve as the basis for the development of an Information-Educational Material (IEM) aimed at promoting environmental awareness, marine conservation, and future biomonitoring initiatives in SBPS.

Significance of the Study

This study is significant because it provides baseline information on the incidence and severity of imposex in selected coastal gastropods of the Sarangani Bay Protected Seascape (SBPS). Because imposex is a well-established biological response to organotin exposure and can be associated with reproductive impairment, documenting its occurrence provides useful baseline information on gastropod populations across coastal areas with different levels of maritime activity.

The study is also important in determining the levels of copper and cadmium in SBPS sediments. Although these metals are not the primary classical cause of imposex, their presence may contribute to environmental stress and may help explain possible patterns of imposex occurrence among the sampling sites. The findings can provide initial evidence on whether copper and cadmium are significantly related to imposex incidence and severity in gastropod bioindicator species.

For the Sarangani Bay Protected Seascape Administrators, the results may serve as a useful reference for coastal monitoring, pollution assessment, and conservation planning. The study can help identify areas that may require closer monitoring, especially sites with higher imposex incidence.

For local communities and coastal stakeholders, the study may increase awareness of the effects of pollution and environmental stressors on marine organisms and ecosystem health. The Information-Educational Material (IEM) developed from the findings may support environmental education, responsible coastal practices, and community participation in protecting the Sarangani Bay Protected Seascape.

For the academe and future researchers, this study can serve as a baseline reference for future biomonitoring studies involving gastropods, imposex, and sediment heavy metals. It may also guide more comprehensive studies involving organotin compounds, seasonal sampling, bioaccumulation analysis, and long-term ecological monitoring.

METHODS

Research Design

This study employed a stratified spatial comparative research design using a single baseline sampling campaign to assess the incidence and severity of imposex in selected coastal gastropods within Sarangani Bay, Philippines. Sampling sites were stratified according to varying levels of maritime influence to facilitate comparisons of imposex occurrence and severity across different coastal environments. Sediment copper (Cu) and cadmium (Cd) concentrations were likewise determined as supporting environmental variables to provide additional information on potential environmental conditions associated with imposex expression. As a baseline assessment, the study was designed to establish reference information on the status of imposex in coastal gastropod populations and to contribute to future biomonitoring and conservation efforts within the Sarangani Bay Protected Seascape.

A stratified spatial comparative design was used to capture environmental variation across sites with different levels of maritime influence. Sampling locations were grouped into predefined exposure categories so that biological responses and sediment chemistry could be compared systematically across the study area.

Sampling locations were classified according to three predefined maritime-exposure characteristics: (1) areas near ports, docking facilities, and vessel-repair activities; (2) areas influenced by active shipping lanes and anchorages; and (3) areas with comparatively minimal vessel activity and limited direct maritime influence. Three sampling replicates were established within each exposure characteristic, yielding nine georeferenced sampling units. These categories were used for spatial comparison only. Because quantitative vessel-density or automatic identification system (AIS) traffic data were not collected, the study does not claim a statistical correlation between imposex severity and vessel density.

A single sampling campaign is appropriate for this study because it is designed as a baseline assessment, establishing the current contamination status and biological response profile within SBPS. Baseline surveys are essential for identifying spatial patterns and contamination hotspots prior to long-term monitoring or intervention programs. The stratified design ensures efficient resource utilization while providing robust cross-site comparisons suitable for initial ecological risk characterization.

Furthermore, the study generates baseline ecological information that may contribute to the development of an Information Educational Material (IEM) aimed at promoting environmental awareness, coastal resource conservation, and pollution management within the Sarangani Bay Protected Seascape.

Data Sources

The data for this study were obtained from biological and environmental samples collected from selected coastal areas within the Sarangani Bay Protected Seascape (SBPS). The primary data source consisted of coastal gastropod specimens used to determine the incidence and severity of imposex. Surface sediment samples were also collected to determine copper and cadmium concentrations as supporting environmental variables since TBT test was not feasible in the country. Together, these data provided baseline information on imposex occurrence and severity in coastal gastropods and the environmental conditions of the study sites.

Sampling locations were selected based on their level of maritime exposure, including areas near ports, docking facilities, and vessel repair zones; sites located along shipping lanes and anchorages; and reference areas with minimal maritime activity. This stratified selection of sampling sites allowed comparison of contamination levels and biological responses across different environmental conditions.

Sediment samples provided information on the presence and concentration of heavy metals, while gastropod specimens served as biological indicators for evaluating imposex development. These data sources were enabled the researcher to examine the maritime-related contamination and biological responses in marine gastropods within SBPS.

The study was conducted in three sampling sites within Sarangani Bay, Philippines. Site 1 is in Tambler, General Santos City at 6°02'46" N, 125°08'52" E, Site 2 is in Alabel, Sarangani Province at 6°04'52" N, 125°16'19" E, and Site 3 is in Burias, Glan, Sarangani Province at 5°42'30.29" N, 125°15'54.80" E. The coordinates of each site were determined using a GPS-enabled mobile application during field sampling.

Research Instruments

Field sampling utilized instruments that ensure standardized and reproducible collection of gastropod and sediment samples. A Global Positioning System (GPS) device or smartphone GPS application were used to record precise geographic coordinates of sampling stations. Quadrat frames and transect tape was employed, where applicable, to standardize spatial sampling effort. Gastropods were collected using hand tools such as trowels, scoops, and forceps, while surface sediment samples were obtained using a hand corer or grab sampler depending on substrate conditions. Collected specimens and sediments were placed in properly labeled containers or zip-sealed bags and stored temporarily in coolers with ice packs to maintain sample integrity during transport. Personal protective equipment (PPE), including gloves and protective eyewear, were used throughout field activities.

Morphometric measurements were obtained using a digital caliper with 0.01 mm precision. Shell length was recorded for each specimen, and penis length was measured where applicable. Recording shell dimensions was important because body size can influence penis measurements and may also reflect differences in age or cumulative exposure history. Shell length was therefore retained as a biological descriptor for interpreting imposex measurements; however, shell length was not treated as a direct proxy for chronological age. Photographic documentation of specimens and reproductive structures was obtained using a digital camera or smartphone.

Sex determination required a complete dissection set, including scalpels, scissors, forceps, dissecting needles, and probes. Shell removal was performed carefully using appropriate tools to prevent soft tissue damage. Dissections were conducted on trays with stabilizing pins to secure specimens during examination. A stereo or dissecting microscope served as the primary instrument for identifying reproductive anatomy, including vas deferens and penis allowing accurate confirmation of sex.

Imposex detection and staging were conducted under a magnifying glass and dissecting microscope to identify penis development and vas deferens formation in female specimens. Where necessary, an ocular micrometer or calibrated image scale was used to measure small anatomical structures accurately. Standardized Vas Deferens Sequence (VDS) staging criteria will guide the classification of imposex severity to ensure consistency across observations.

Specimens were preserved in 70–95% ethanol for morphological analysis. If histological validation is required, 10% buffered formalin may be used. All samples were labeled using waterproof markers and standardized coding systems to maintain traceability. A specimen record was maintained to document collection, processing, and storage details.

Standardized data sheets were utilized to ensure systematic documentation. A Station Profile Sheet will record site characteristics, GPS coordinates, date and time, habitat description, and observable anthropogenic influences. A Specimen Data Sheet will capture specimen ID, species, shell length, confirmed sex, imposex presence or absence, penis length, VDS stage, and relevant remarks. A Sediment Sample Log Sheet documented sediment identification codes, collection depth, and handling procedures.

Spreadsheet software was used for data encoding and management. Statistical software was employed to analyze relationships between imposex indices and environmental variables.

Data Gathering Procedures

The researcher secured formal approval from the University Ethics Review Committee to ensure compliance with institutional ethical standards and environmental research guidelines.

Necessary clearance and endorsements were secured from the management of the Sarangani Bay Protected Seascape (SBPS) and other relevant government agencies.

The researcher conducted an ocular survey of the target areas where maritime activities were evident. Three sampling sites were established, each with three replicates. The sites were categorized according to the intensity of maritime activities.

Approximately 30 gastropods per site were collected whenever feasible, producing a total sample of 90 specimens. Species selection was based on local abundance, site residency, accessibility, and suitability for reproductive examination. Particular analytical attention was given to neogastropods of the family Muricidae because imposex was detected in *Drupa* sp., *Drupina grossularia*, and *Chicoreus torrefactus* and because muricid/neogastropod taxa are widely used as sensitive sentinel organisms in imposex biomonitoring. Sex was confirmed by internal reproductive anatomy before imposex assessment. The number of confirmed females, rather than the total number of gastropods collected, constituted the denominator for incidence calculations.

Sex determination was conducted using standardized dissection-based protocols for gastropods. Each specimen first underwent external screening under magnifying glass and if necessary, under dissecting microscope. Individuals exhibiting a visible penis were provisionally classified as male candidates, whereas those without a visible penis were considered female candidates. However, because females affected by imposex may also develop penis-like structures, external examination alone were not considered definitive for sex confirmation.

Definitive sex determination was performed through dissection. When necessary, specimens were briefly chilled on ice to reduce movement prior to shell removal. The shell were carefully removed either by gentle cracking using appropriate tools or by cutting around the shell lip while ensuring minimal damage to soft tissues. The visceral mass and pallial cavity were exposed to allow detailed examination of internal reproductive organs.

Male individuals were identified by the presence of a pale to cream-colored granular testis connected to a continuous vas deferens leading toward the prostate and penis. Female individuals were identified by the presence of an ovary, typically orange or greenish depending on species, connected to the oviduct and associated pallial oviduct glands such as the capsule gland and albumen gland. Sex were confirmed based on internal

reproductive anatomy. Specimens exhibiting ovary and functional oviductal structures were classified as females, whereas those with a testis and continuous vas deferens will be classified as males. Damaged or clearly juvenile specimens were recorded as indeterminate.

Imposex assessment was performed exclusively on confirmed female specimens. A female was classified as imposex-positive when male-type reproductive structures, including a penis-like structure and/or vas deferens development, were present. Penis length was measured from base to tip. Imposex incidence was calculated as the number of imposex-positive females divided by the total number of confirmed females examined at the site, multiplied by 100. RPLI was calculated as: $RPLI (\%) = (\text{mean penis length of imposex-positive females} / \text{mean penis length of males used as the site reference}) \times 100$. RPLI was used as a complementary measure of the relative degree of penis development and was interpreted together with VDS staging rather than as an interchangeable substitute for VDSI.

Vas deferens development was classified using the standardized Vas Deferens Sequence (VDS) staging approach. Stage 0 represented a normal female without penis or vas deferens development; progressively higher stages represented increasing development and continuity of male-type reproductive structures and, at advanced stages, interference with the female genital opening. For transparency in this baseline dataset, the reported site VDSI values summarize the mean VDS stage of the imposex-positive females used for severity characterization. This operational definition is stated explicitly because VDSI calculation conventions can differ among species and monitoring protocols; future repeat surveys should apply the same denominator and species-specific staging criteria to ensure temporal comparability.

For the assessment of heavy metal contamination, surface sediment samples (0–2 cm depth) were collected from each sampling station within the Sarangani Bay Protected Seascape (SBPS). Approximately 1000 g of sediment were obtained using clean, non-metallic sampling equipment to avoid contamination. Samples were placed in labeled polyethylene bags indicating the station code, sampling location, date, and exposure category. All samples were stored in an ice chest during transport and submitted to an accredited analytical laboratory for heavy metal determination.

Data were encoded and screened for completeness before analysis. Descriptive statistics included frequency, percentage, mean, shell length, imposex incidence, penis length, VDS stage, VDSI, and RPLI. Site-level Pearson correlations were used only as exploratory analyses of Cu in relation to imposex responses because only three site-level observations were available ($N = 3$). Cadmium correlations could not be estimated because all measurements were below the analytical detection limit and lacked between-site variability.

No multivariable regression model was interpreted because the number of site-level observations was insufficient for robust adjustment of potential confounders. Shell length was documented as a morphometric descriptor, and possible size/age-related effects were considered in the interpretation and identified for future studies with larger sample sizes.

Statistical tests used a nominal alpha level of 0.05 in SPSS; however, p-values from $N = 3$ site-level correlations were interpreted cautiously and were not used to claim proof of no environmental association.

Utilization of Findings

The findings of this study may serve as baseline scientific information for environmental monitoring, coastal resource management, biodiversity conservation, and pollution assessment within the Sarangani Bay Protected Seascape. These baseline data may serve as a valuable reference for environmental monitoring programs and marine resource management initiatives within the protected seascape. The results may assist the Protected Area Management Board (PAMB), Protected Area Management Office (PAMO), local government units (LGUs), and relevant environmental agencies in identifying areas that require closer ecological monitoring and management intervention. Information on imposex occurrence and heavy metal concentrations can support the formulation and enhancement of policies related to pollution control, coastal resource protection, and sustainable maritime activities within SBPS.

Furthermore, the study may contribute to the strengthening of sediment quality monitoring and biomonitoring programs through the use of gastropods as bioindicator species. The findings can also provide scientific support for the development of environmental management strategies aimed at minimizing potential sources of copper and cadmium contamination and reducing environmental stressors that may affect marine organisms and ecosystem health.

The study likewise supports the implementation of environmental education and awareness initiatives. The Information Educational Material (IEM) developed from the findings may be utilized by environmental managers, educators, community stakeholders, and local government units to promote public awareness of marine pollution, biodiversity conservation, and the ecological importance of coastal gastropods as indicators of environmental quality.

The baseline information generated by this study may serve as a foundation for future research and long-term monitoring efforts involving imposex, heavy metals, emerging contaminants, and other environmental drivers affecting the ecological integrity of the Sarangani Bay Protected Seascape.

Ethical Considerations

This study was conducted in accordance with environmental policies governing the Sarangani Bay Protected Seascape and in compliance with Republic Act No. 11038 and Republic Act No. 9275. Necessary permits and clearances will be secured from the Protected Area Management Board (PAMB), concerned local government units, and relevant environmental authorities prior to sampling.

The researcher is qualified to undertake this study by virtue of her academic training and professional experience in biological sciences, environmental research, marine ecology, and biodiversity monitoring. Her experience in conducting field-based ecological assessments and environmental studies provided the necessary competencies to carry out the research using appropriate scientific methods while ensuring compliance with ethical, environmental, and institutional standards.

Sampling followed minimal-impact principles by collecting only the number of gastropod specimens required for statistical validity and limiting sediment extraction to surface layers to reduce habitat disturbance. Disturbed substrates was restored whenever feasible. Specimens were handled humanely and processed efficiently according to accepted scientific standards for invertebrate research. Laboratory procedures was comply with biosafety protocols, including proper handling and disposal of preservatives and chemical reagents.

The coordination with local stakeholders, community representatives, or site personnel was required, informed consent was obtained prior to interviews, consultations, or information gathering. Participation was voluntary, and respondents may withdraw at any time without consequence. Personal information, if collected, was treated with confidentiality and used solely for research purposes. Data was coded and stored securely to protect privacy.

All findings were documented systematically. Results were communicated to relevant stakeholders, including local government units and protected area management authorities, through technical reports, presentations, or policy briefs. The study aimed to provide evidence-based information that supports sustainable coastal management while safeguarding ecological integrity and community interest.

RESULTS AND DISCUSSION

This chapter presents the results and discussion of the study on the baseline assessment of imposex incidence and severity in selected coastal gastropods of the Sarangani Bay Protected Seascape (SBPS). It also presents the levels of sediment copper and cadmium as supporting environmental variables and examines their possible relationship with the observed imposex patterns. The results provide baseline ecological information on reproductive abnormalities in coastal gastropods and contribute to the understanding of environmental stressors affecting marine ecosystems within SBPS.

Incidence and Severity of Imposex in Coastal Gastropods at Site 1

Table 1 presents the incidence and severity of imposex in coastal gastropods collected from Site 1 (Tambler, General Santos City).

In Replicate 1, one female *Drupa sp.* with a shell length of 36.5 mm exhibited imposex, characterized by the presence of a 6.3 mm penis and an 8.3 mm vas deferens. A male specimen collected from the same replicate measured 48.7 mm in shell length, with a penis length of 22.6 mm and a vas deferens length of 29.2 mm, serving as the reference for comparison. Although the reproductive structures observed in the female were considerably smaller than those of the male, their presence confirms masculinization.

Based on the degree of vas deferens development, the specimen was classified as Stage 2 of the Vas Deferens Sequence Index (VDSI), representing early to moderate imposex development. Stage 2 indicates the early development of the vas deferens, in which the structure is already evident but has not yet progressed to the advanced stages associated with reproductive obstruction, functional impairment, or sterility. This stage represents a moderate level of imposex severity and reflects continued exposure to endocrine-disrupting contaminants while reproductive capability is generally maintained (Ruiz et al., 2019).

In Replicate 2, three female *Drupa sp.* specimens were recorded. Two individuals exhibited imposex, with penis lengths of 11.5 mm and 8.5 mm, accompanied by vas deferens lengths of 15.3 mm and 13.9 mm, respectively. These females were classified as Stage 4 and Stage 3, indicating moderate to advanced imposex development characterized by more pronounced masculinization than that observed in Replicate 1.

Table 1. Site 1: Tambler, General Santos City Imposex Occurrence in Coastal Gastropods

Replicates	Identification	Sex	Length (mm)	Weight(g)	Vas deferens(mm)	Penis Length (mm)
Replicate 1	<i>Muricidae sp. cf drupa</i>	Male	48.7	1.0	29.2	22.6
	<i>*Muricidae sp. cf drupa</i>	Female	36.5	0.7	8.3	6.3
	<i>Nerita albicilla</i>	Female	14.1	0.1		
	<i>Clepeomonus batillariaeformis</i>	Male	25.6	0.2	6.4	5.6
	<i>Shanshiella carbonaria</i>	Female	23.9	0.2		
	<i>Clypeomorus bifasciata</i>	Male	23.4	0.2	5.1	3.6
	<i>Clepeomonus batillariaeformis</i>	Female	16.1	0.4		
	<i>Euprotomus aurisdianac</i>	Male	29.3	0.5	11.7	8.3
	<i>Clypeomorus bifasciata</i>	Female	28.5	0.1		
	<i>Shanshiella carbonaria</i>	Male	23	0.2	6.3	7.8
Replicate 2	<i>Canarium labiatum</i>	Male	34.8	0.8		5.5
	<i>Nerita albacilla</i>	Female	19.3	0.1		
	<i>Nerita albacilla</i>	Female	14.9	0.2		
	<i>Tegula eiseni</i>	Female	22.0	0.2		
	<i>Shanshiella carbonarla</i>	Female	18.4	0.2		
	<i>Canarium labiatum</i>	Male	28.7	0.3		5.5
	<i>Nerita albacilla</i>	Female	16	0.2		
	<i>* Muricidae sp. cf drupa</i>	Female	50.4	0.3	15.3	11.5
	<i>*Muricidae sp. cf drupa</i>	Female	45.71	0.27	13.9	8.5

	<i>Muricidae sp. cf drupa</i>	Female	36.5	0.1		
Replicate 3	<i>Canarium labiatum</i>	Male	29.9	1.0	3.1	5.4
	<i>Clypeomorus bifasciata</i>	Female	18.2	0.1		
	<i>Canarium labiatum</i>	Male	32.2	1.3	18.1	7.3
	<i>Tegula eiseni</i>	Female	15.3	0.2		
	<i>Clypeomorus bifasciata</i>	Female	18.8	0.1		
	<i>Canarium labiatum</i>	Female	24.5	0.3		
	<i>Clypeomorus bifasciata</i>	Female	18.9	0.3		
	<i>Nerita albicilla</i>	Female	17.2	0.2		
	<i>Tegula eiseni</i>	Female	20.7	0.3		
	<i>Clypeomorus bifasciata</i>	Male	19.9	0.2	3.6	7.1

Note : * species exhibit imposex

The remaining female specimen showed no external evidence of imposex and was considered a normal female. The occurrence of females exhibiting different imposex stages within the same locality suggests variability in individual exposure histories, physiological responses, age, or susceptibility to environmental stressors. Recent investigations have likewise reported considerable variation in imposex severity within populations, indicating that differences in contaminant exposure, environmental conditions, and species-specific sensitivity influence the expression of imposex among individual gastropods (Ruiz et al., 2019; Risjani et al., 2025).

In Replicate 3, no imposex was detected despite the presence of several gastropod species. The absence of imposex among species such as *Canarium labiatum*, *Clypeomorus bifasciata*, *Nerita albicilla*, and *Tegula eiseni* may be attributed to species-specific differences in sensitivity to endocrine-disrupting contaminants. Muricid gastropods possess biological and physiological characteristics that make them highly sensitive to endocrine-disrupting contaminants, particularly organotin compounds, resulting in a greater susceptibility to imposex development than many non-muricid gastropods. Consequently, muricid species continue to be recognized as reliable sentinel organisms for monitoring endocrine disruption and coastal environmental contamination because they exhibit consistent and measurable imposex responses even at relatively low contaminant exposure levels (Ruiz et al., 2019; Risjani et al., 2025).

Consequently, the lack of imposex in these species should not be interpreted as evidence of an uncontaminated environment but rather reflects differences in biological responsiveness among gastropod taxa.

Overall, three female *Drupa sp.* specimens exhibited imposex across the three sampling replicates, corresponding to an incidence of 15.0% among the 20 female gastropods examined at Site 1 (Table 1). The observed imposex stages ranged from Stage 2 to Stage 4, demonstrating varying degrees of reproductive masculinization. The progression from early vas deferens formation to more developed male reproductive structures indicates that affected females had experienced sufficient exposure to environmental conditions capable of inducing imposex. Although advanced stages associated with reproductive blockage and sterility were not observed, the presence of Stage 4 individuals suggests that chronic environmental exposure may already be occurring within the study area.

The occurrence of imposex exclusively in *Drupa sp.* further supports the use of muricid gastropods as reliable sentinel organisms for coastal biomonitoring. Recent studies continue to recognize imposex in muricid gastropods as one of the most sensitive biological indicators of exposure to endocrine-disrupting contaminants, particularly organotin compounds associated with antifouling paints. Consequently, the detection of imposex provides valuable baseline information for assessing the ecological status of coastal ecosystems, evaluating the effectiveness of pollution management strategies, and monitoring long-term environmental recovery (Ruiz et al., 2019; Risjani et al., 2025). In the present study, copper and cadmium were measured as supporting environmental variables to characterize overall sediment quality; however, the observed imposex cannot be directly attributed

to these metals alone because multiple environmental factors may influence reproductive abnormalities in marine gastropods.

The detection of imposex in 15% of female gastropods at Site 1 indicates that reproductive abnormalities are already present within the gastropod community, although the incidence remains relatively low compared with heavily contaminated harbors reported in the literature. Nevertheless, the presence of Stage 2 to Stage 4 individuals demonstrates that exposure has been sufficient to induce measurable masculinization in sensitive species. These findings suggest that Site 1 may be experiencing chronic but relatively moderate environmental stress associated with coastal and maritime activities. Since Tumbler serves as an important coastal area in General Santos City with fishing, navigation, and port-related activities, continuous anthropogenic inputs may contribute to the persistence of contaminants capable of affecting sensitive marine organisms.

The absence of imposex in other gastropod families should not be interpreted as the absence of contamination because susceptibility to imposex varies considerably among taxa. Muricidae have consistently been identified as one of the most responsive families to endocrine-disrupting contaminants and therefore provide a more sensitive assessment of environmental quality than less responsive gastropod groups.

The results demonstrate that *Drupa sp.* is the only species exhibiting imposex at Site 1, with an overall incidence of 15.0% and severity ranging from Stage 2 to Stage 4. These findings confirm the presence of reproductive masculinization in a sensitive gastropod bioindicator and provide baseline evidence that environmental stressors capable of inducing imposex exist within the coastal ecosystem of Tumbler, General Santos City. Although the observed severity was moderate and no evidence of reproductive blockage or sterility was detected, continued monitoring is warranted to determine whether imposex prevalence and severity increase over time.

The findings support the continued use of *Drupa sp.* as a bioindicator species for long-term environmental monitoring in the Sarangani Bay Protected Seascape. Future studies should include seasonal sampling, larger population sizes, and comprehensive analyses of additional contaminants, particularly organotin compounds where analytical facilities become available, together with other environmental variables such as sediment characteristics, organic matter, and additional heavy metals. Continuous monitoring would strengthen the baseline dataset, improve understanding of spatial and temporal trends in imposex occurrence, and provide scientific evidence to guide coastal management and pollution mitigation programs within the protected seascape.

Incidence and Severity of Imposex in Coastal Gastropods at Site 2

Table 2 presents the incidence and severity of imposex in coastal gastropods collected from Site 2 (Alabel, Sarangani Province).

A total of 30 gastropod specimens representing several species were collected across three sampling replicates. The assemblage was dominated by *Clypeomorus bifasciata*, *Canarium labiatum*, *Nerita albicilla*, *Monetaria annulus*, *Euplica sp.*, and muricidae gastropods. Among all specimens examined, 25 were females and 5 were males.

Table 2. Site 2 : Alabel Sarangani Province

Replicates	Identification	Sex	Shell Length (mm)	Body Weight (g)	Vas Deferens (mm)	Penis length (mm)
Replicate 1	Euplica species	Female	25.5	0.2		
	Nerita species	male	34.8	0.4	11.8	9.1
	Monetaria annulus	male	21.1	0.5	5.2	4.3
	Clypeomorus bifasciata	Female	29.2	0.2		

	Clypeomorus bifasciata	Female	28.2	0.3		
	Canarium labiatum	Female	36.1	0.3		
	Clypeomorus bifasciata	Female	29.2	0.2		
	Euplica species	Female	24.3	0.2		
	Muricidae sp	male	33.7	0.35	11.9	8.7
	Monetaria aluus	male	21.1	0.47	4.9	3.0
Replicate 2	Clypeomorus bifasciata	Female	46.2	0.9		
	Canarium labiatum	Female	22.3	.5		
	Clypeomorus bifasciata	Female	32.2	0.3		
	Muricid sp	Female	19.0	0.4		
	Nerita albicilla	Female	21.3	0.2		
	Monetaria annulus	Female	18.8	0.4		
	Clypeomorus bifasciata	Female	44.2	0.7		
	Canarium labiatum	Female	20.3	.47		
	Clypeomorus bifasciata	Female	29.5	0.27		
	Muricid sp	Female	17.0	0.35		
Replicate 3	Clypeomorus bifasciata	Female	31.5	0.6		
	Canarium labiatum	Female	33.9	0.4		
	Clypeomorus bifasciata	Female	28.9	0.2		
	Muricid sp	Female	18.9	0.4		
	Nerita albicilla	Female	16.8	0.1		
	Monetaria annulus	Female	29.2	0.4		
	Clypeomorus bifasciata	Female	30.4	0.56		
	Canarium labiatum	Female	33.7	0.4		
	*Muricidi sp. Cf. druppa	Female imposex	27.3	0.2	9.11	3.6
	Chicoreus torrefactus	Male	35.6	0.4	13.7	9.1

Note : * species exhibit imposex

Imposex was detected in only one female *Drupa sp.*, resulting in an overall imposex incidence of 4.0% among the female gastropods examined at Site 2. No imposex characteristics were observed in the remaining gastropod species. This finding further reinforces the recognized sensitivity of muricid gastropods as bioindicators of reproductive abnormalities associated with coastal environmental contamination. Recent studies have consistently demonstrated that muricid species exhibit a greater susceptibility to imposex than many other gastropod families because of their heightened sensitivity to endocrine-disrupting contaminants, particularly organotin compounds. Consequently, muricid gastropods remain valuable sentinel organisms for evaluating contaminant exposure, assessing ecological health, and monitoring the long-term effectiveness of pollution management in coastal ecosystems (Ruiz et al., 2019; Abidli et al., 2009; Risjani et al., 2025).

In Replicate 1, no female gastropods exhibited imposex characteristics. A male Muricidae specimen was recorded with a shell length of 33.7 mm, a penis length of 8.7 mm, and a vas deferens length of 11.9 mm, providing a reference for comparison with affected females. Likewise, male specimens of *Nerita* sp. and *Monetaria annulus* exhibited normal male reproductive structures. All female specimens examined, including *Clypeomorus bifasciata*, *Canarium labiatum*, and *Euplica* sp., displayed normal reproductive morphology without evidence of penis or vas deferens development.

Similarly, Replicate 2 yielded no imposex-positive individuals despite the presence of two female muricid specimens and several females belonging to *Clypeomorus bifasciata*, *Canarium labiatum*, *Monetaria annulus*, and *Nerita albicilla*. The absence of imposex in these specimens suggests that the females retained normal reproductive anatomy and showed no signs of masculinization. The occurrence of female muricidae without imposex further indicates that not all individuals within a sensitive species respond uniformly to environmental stressors, reflecting differences in exposure history, age, reproductive condition, or individual susceptibility.

In Replicate 3, one female *Drupa* sp. measuring 27.3 mm in shell length and weighing 0.2 g exhibited imposex characteristics. The specimen possessed a 3.6 mm penis and a 9.11 mm vas deferens, confirming masculinization of the female reproductive system. Based on the degree of vas deferens development, the specimen was classified as Stage 2 of the Vas Deferens Sequence Index (VDSI), representing an early stage of imposex development characterized by the initial formation of male reproductive structures without evidence of reproductive obstruction. A male *Chicoreus torrefactus* collected from the same replicate exhibited a penis length of 9.1 mm and a vas deferens length of 13.7 mm, providing an additional muricid reference for comparison.

The occurrence of only one imposex-positive female across all three replicates indicates that Site 2 exhibited the lowest imposex incidence (4.0%) among the three sampling sites in the present study. Moreover, the affected female exhibited only Stage 2 imposex, indicating an early stage of reproductive masculinization compared with populations from more heavily contaminated coastal environments where advanced imposex stages are more prevalent. This relatively low severity suggests that endocrine disruption has occurred but has not progressed to the higher VDSI stages commonly associated with reproductive obstruction and population-level effects. Similar observations have been reported in recent field studies, where low VDSI values reflected relatively mild contamination, whereas higher VDSI values corresponded to greater reproductive impairment and increased environmental exposure to organotin compounds (Arroyo Osorio & Braga de Castro, 2021; Ribeiro-Brasil et al., 2021). Therefore, although reproductive abnormalities were detected, the severity observed at Site 2 appears relatively limited.

The low imposex incidence recorded at Site 2 suggests that reproductive abnormalities among muricidae gastropods are present but occur infrequently within this locality. The detection of only one Stage 2 imposex individual indicates that environmental conditions may be sufficient to initiate masculinization in sensitive species but are unlikely to have caused widespread or advanced reproductive impairment within the gastropod population. This finding may reflect relatively lower exposure to endocrine-disrupting contaminants or reduced anthropogenic pressure compared with sites exhibiting higher imposex prevalence.

The absence of imposex in the majority of muricidae and non-muricidae gastropods further illustrates that imposex development is influenced not only by contaminant exposure but also by species-specific sensitivity and individual biological variability. Previous studies have demonstrated that muricidae gastropods exhibit variable imposex responses depending on contaminant concentrations, duration of exposure, reproductive condition, organism size, and local environmental factors such as hydrodynamics and maritime activities. These biological and environmental variables influence both the incidence and severity of imposex, highlighting the importance of considering site-specific ecological conditions when interpreting biomonitoring results (Ribeiro-Brasil et al., 2021; Ruiz et al., 2019). Consequently, the occurrence of a single imposex-positive female should not be interpreted as evidence that the remaining individuals were unexposed, but rather that environmental exposure may have been insufficient to induce reproductive abnormalities in most specimens.

The results also emphasize the importance of baseline biomonitoring. Even though imposex incidence was low, the detection of reproductive masculinization confirms that sensitive gastropod populations are responding to

environmental stressors within the coastal ecosystem. Continuous monitoring is therefore essential to determine whether imposex prevalence remains stable or increases over time in response to changing coastal activities.

Muricidae gastropods are widely recognized as reliable sentinel organisms for coastal biomonitoring because they exhibit highly sensitive and measurable imposex responses following exposure to endocrine-disrupting contaminants, particularly organotin compounds. Consequently, imposex incidence and severity in muricidae species continue to serve as robust biological indicators for assessing coastal environmental quality, evaluating contaminant exposure, and monitoring the effectiveness of pollution management strategies (Ruiz et al., 2019; Ribeiro-Brasil et al., 2021; Risjani et al., 2025). The occurrence of Stage 2 imposex in a single female *Drupa sp.* provides biological evidence of reproductive alteration within Site 2 despite the relatively low incidence. In this study, copper and cadmium were evaluated as supporting environmental variables to characterize sediment quality; however, the observed imposex cannot be attributed solely to these metals because reproductive abnormalities in marine gastropods are influenced by multiple environmental stressors. Thus, the present findings establish an important baseline for future ecological assessments in the Sarangani Bay Protected Seascape.

Site 2 exhibited the lowest imposex incidence (4.0%) among the three sampling sites, with only one female *Drupa sp.* exhibiting Stage 2 imposex. The predominance of normal female reproductive morphology indicates that reproductive masculinization was relatively limited within this locality. Nevertheless, the presence of an imposex-positive individual confirms that environmental conditions capable of inducing reproductive abnormalities exist within the area, although their current influence appears to be relatively low. These findings provide valuable baseline information on the reproductive health of muricidae gastropods in Alabel, Sarangani Province.

Long-term monitoring of *Drupa sp.* populations should be continued at Site 2 to determine whether imposex incidence and severity change over time. Future investigations should include seasonal sampling, larger sample sizes, and expanded analyses of sediment contaminants, including organotin compounds when analytical facilities become available, together with heavy metals and other environmental variables. Such comprehensive monitoring will strengthen the baseline dataset, improve understanding of contaminant dynamics, and support evidence-based management strategies for conserving the ecological integrity of the Sarangani Bay Protected Seascape.

Incidence and Severity of Imposex in Coastal Gastropods at Site 3

Table 3 presents the incidence and severity of imposex in coastal gastropods collected from Site 3 (Burias, Glan, Sarangani Province). A total of 30 gastropod specimens representing several species were collected across three sampling replicates. The assemblage comprised *Tectus fenestratus*, *Drupina grossularia*, *Chicoreus torrefactus*, *Canarium labiatum*, *Angaria delphinus*, *Astrarium calcar*, *Nerita albicilla*, *Clypeomorus bifasciata*, and *Euprotomus aurisdianae*. Of the specimens examined, 20 were females and 10 were males. Imposex was detected exclusively in muricid gastropods, specifically one female *Drupina grossularia* and two female *Chicoreus torrefactus*. No imposex characteristics were observed in the remaining gastropod species examined.

The occurrence of imposex in multiple muricid species further supports the well-established role of the family Muricidae as highly sensitive bioindicators of reproductive abnormalities associated with coastal environmental contamination. Recent studies have demonstrated that muricid gastropods consistently develop imposex following exposure to organotin compounds and remain among the most reliable sentinel organisms for evaluating environmental quality, even after the global restriction of tributyltin-based antifouling paints. Consequently, monitoring imposex in muricid species provides a sensitive and cost-effective approach for detecting residual contamination and assessing the long-term ecological condition of coastal ecosystems (Viana et al., 2021; França et al., 2021; Ruiz et al., 2019).

In Replicate 1, two female muricid gastropods exhibited imposex. The female *Drupina grossularia* measured 25.4 mm in shell length and 0.6 g in body weight and possessed a 1.1 mm penis and a 6.3 mm vas deferens. Based on the extent of vas deferens development, the specimen

Table 3. Site 3: Burias Glan, Sarangani Province

Replicates	Identification		Shell Length	Body Weight	Vas Deferens	Penis length
Replicate 1	Tectus Fenestratus	Female	28.7	1.2		
	*Drupina grossularia	Female	25.4	0.6 g	6.3 mm	1.1 mm
	Angaria delphinus	Female	34.6	1.4 g		
	Canarium labiatum	Male	26.2	0.9 g	6.9 mm	1.2 mm
	Angaria delphinus	Female	30.8	1.1 g		
	*Chicoreus torrefactus	Female	47.9	1.4 g	8.7mm	2.4 mm
	Actralium calcar	Male	30.7	1.4 g	19.0 mm	6.2 mm
	Chicoreus torrefactus	Male	49.7	2.1 g	15.4 mm	9.2 mm
	Chicoreus torrefactus	Male	38.6	1.2 g	15.1 mm	8.3 mm
	Nerital albicilla	Female	24.3	0.9 g		
Replicate 2	*Chicoreus torrefactus	Female	46.0	1.5 g	12.2 mm	6.8 mm
	Canarium labiatum	Male	34.3	1.2 g	8.5 mm	7.1 mm
	Tectus Fenetratus	Female	25.3	1.0 g		
	Clypeomorus bifasciata	Male	26.6	1.0 g	8.4 mm	8.9 mm
	Canarium labiatum	Female	32	1.1 g		
	Canarium labiatum	Female	26	0.6 g		
	Canarium labiatum	Male	28.2	0.5 g	8.9 mm	5.4 mm
	Canarium labiatum	Male	33.3	1.0 g		
	Angaria delphinus	Female	38.5	2.5 g		
	Canarium labiatum	Male	32.4	1.0 g	27.8 mm	5.2 mm
Replicate 3	Canarium labiatum	Male	35	1.0 g	8.5 mm	8.4 mm
	Testus Fenestratus	Female	25.1	0.6 g		
	Canarium labiatum	Female	28.4	1.0 g		
	Canarium labiatum	Male	27.9	1.2 g	20.5	9.4
	Testus Fenestratus	Female	28.2	1.3 g		
	Angaria delphinus	Female	35.3	1.8 g		
	Angaria delphinus	Female	29.9	1.6 g		
	Angaria delphinus	Female	41	3.3 g		
	Angaria delphinus	Female	36.6	3.3 g		
	Euprotomus aurisdianac	Female	57.1	3.8 g		

Note : * species exhibit imposex

was classified as Stage 2 of the Vas Deferens Sequence Index (VDSI), representing early imposex development. Another affected female, *Chicoreus torrefactus*, measured 47.9 mm in shell length and 1.4 g in body weight and exhibited a 2.4 mm penis and an 8.7 mm vas deferens. This specimen was classified as Stage 3, indicating moderate imposex characterized by a more developed vas deferens and external penis structure. Male *Chicoreus torrefactus* specimens collected from the same replicate exhibited penis lengths of 9.2 mm and 8.3 mm, providing reference measurements for comparison with affected females.

In Replicate 2, one additional female *Chicoreus torrefactus* exhibited imposex. The specimen measured 46.0 mm in shell length and 1.5 g in body weight and possessed a 6.8 mm penis and a 12.2 mm vas deferens. Based on these measurements, the specimen was classified as Stage 4 represented advanced imposex, characterized by a well-developed penis and a continuous vas deferens, indicating substantial reproductive masculinization. Although complete reproductive obstruction was not observed, this level of imposex reflects prolonged or relatively higher exposure to endocrine-disrupting contaminants and may progressively compromise female reproductive function if contaminant exposure persists. Similar observations have been reported in recent field studies, where advanced imposex stages were associated with elevated environmental contamination and greater reproductive impairment in muricid gastropods (Ruiz et al., 2019; Ribeiro-Brasil et al., 2021; França et al., 2021). The remaining female gastropods examined in this replicate, including *Canarium labiatum*, *Angaria delphinus*, and *Tectus fenestratus*, exhibited normal reproductive morphology without evidence of imposex.

In Replicate 3, no imposex-positive individuals were recorded despite the presence of several female gastropod species, including *Canarium labiatum*, *Tectus fenestratus*, *Angaria delphinus*, and *Euprotomus aurisdianae*. The absence of imposex among these taxa is consistent with previous studies indicating that susceptibility to imposex varies among gastropod families, with muricidae being considerably more sensitive than most non-muricidae species (Oehlmann et al., 2007).

Overall, three female muricidae gastropods exhibited imposex across the three sampling replicates, corresponding to an imposex incidence of 15.0% among the 20 female gastropods examined at Site 3. The affected specimens consisted of one *Drupina grossularia* and two *Chicoreus torrefactus* individuals. The observed imposex stages ranged from Stage 2 to Stage 4, yielding a mean VDSI of 3.0, which indicates a moderate degree of imposex severity within the muricid population. The distribution of one individual each in Stages 2, 3, and 4 demonstrates that reproductive masculinization occurred across multiple levels of severity rather than being confined to a single stage of development. Such variation may reflect differences in individual age, physiological condition, duration of exposure, or sensitivity to environmental stressors.

The detection of imposex in two muricid species, *Drupina grossularia* and *Chicoreus torrefactus*, indicates that reproductive abnormalities are present within the muricid population at Site 3. The occurrence of Stage 2, Stage 3, and Stage 4 individuals suggests progressive masculinization ranging from early vas deferens formation to more advanced development of male reproductive structures. The presence of Stage 4 imposex is particularly important because it reflects prolonged or repeated exposure to environmental conditions capable of inducing reproductive alteration, although reproductive blockage and sterility were not observed in the present study. Similar findings have been reported in contaminated coastal environments, where muricid gastropods exhibit varying degrees of imposex according to contaminant levels, exposure history, local environmental conditions, and species-specific biological responses. Recent field investigations have shown that differences in imposex incidence and severity among individuals and populations reflect spatial variations in organotin contamination and the ecological characteristics of each sampling site, reinforcing the value of muricid gastropods as sensitive indicators of coastal environmental quality (Ruiz et al., 2019; Ribeiro-Brasil et al., 2021; Viana et al., 2021).

The occurrence of imposex exclusively in muricid gastropods while being absent in other gastropod families further demonstrates the species-specific sensitivity of Muricidae to endocrine-disrupting contaminants. This differential response emphasizes the importance of selecting appropriate bioindicator species in coastal biomonitoring programs. The absence of imposex in non-muricid gastropods should not be interpreted as evidence of an uncontaminated environment but rather reflects differences in physiological susceptibility among gastropod taxa.

Notably, Site 3 exhibited the same imposex incidence (15.0%) as Site 1 but differed in species composition, with imposex occurring in both *Drupina grossularia* and *Chicoreus torrefactus*.

This suggests that reproductive abnormalities are affecting multiple muricid species within Burias, Glan, indicating that environmental stressors are not restricted to a single sentinel species. Such observations strengthen the ecological value of Muricidae as indicators of coastal environmental health.

Muricid gastropods are widely recognized as sentinel organisms for monitoring endocrine-disrupting contaminants because they consistently develop imposex following exposure to organotin compounds, particularly tributyltin (TBT). Their high sensitivity, together with the strong relationship between imposex severity and environmental organotin contamination, has established muricid species as reliable bioindicators for evaluating coastal ecosystem health and the long-term effectiveness of pollution control measures (Beyer et al., 2022; Viana et al., 2021; Santos & Maia, 2023). The occurrence of Stage 2 to Stage 4 imposex in both *Drupina grossularia* and *Chicoreus torrefactus* provides biological evidence that sensitive gastropod populations within Site 3 are experiencing measurable reproductive alterations. In the present study, copper and cadmium were assessed as supporting environmental variables to characterize sediment quality; however, the observed imposex cannot be attributed solely to these heavy metals because reproductive abnormalities in marine gastropods are influenced by multiple environmental stressors. Nevertheless, the detection of moderate imposex severity establishes an important ecological baseline for future environmental monitoring in the Sarangani Bay Protected Seascape.

Site 3 exhibited an overall imposex incidence of 15.0%, with affected females displaying Stage 2 to Stage 4 imposex and a mean VDSI of 3.0, indicating a moderate level of reproductive masculinization. The occurrence of imposex in two muricidae species demonstrates that reproductive abnormalities are affecting multiple sensitive gastropod taxa within Burias, Glan. Although complete reproductive blockage or sterility was not observed, the presence of Stage 4 individuals suggests that chronic environmental exposure may already be influencing the reproductive health of muricidae populations. These findings provide valuable baseline information for evaluating long-term changes in coastal ecosystem health within the Sarangani Bay Protected Seascape.

Long-term biomonitoring of *Drupina grossularia* and *Chicoreus torrefactus* should be continued to determine whether imposex incidence and severity increase over time. Future investigations should incorporate seasonal sampling, larger population sizes, and broader environmental assessments, including sediment quality, additional contaminants, and organotin analyses when analytical facilities become available. Such comprehensive monitoring will improve understanding of temporal and spatial trends in imposex development and provide scientific evidence to support conservation and pollution management strategies within the Sarangani Bay Protected Seascape.

Imposex Incidence of Coastal Gastropods Across Sampling Sites

Table 4 presents the imposex incidence of coastal gastropods across the three sampling sites within the Sarangani Bay Protected Seascape (SBPS). A total of 90 gastropod specimens representing various species were examined, consisting of 25 males and 65 females. Among the female gastropods, seven individuals exhibited imposex, resulting in an overall imposex incidence of 10.77%. The affected individuals belonged to the family Muricidae, specifically *Drupa sp.*, *Drupina grossularia*, and *Chicoreus torrefactus*. No imposex characteristics were observed in the remaining gastropod species examined. This finding is consistent with recent studies demonstrating that muricid gastropods remain among the most sensitive marine bioindicators of endocrine disruption and continue to be widely used in coastal environmental monitoring. The predictable development of imposex in response to organotin exposure, together with its strong relationship with contaminant levels, makes muricid species valuable sentinel organisms for assessing coastal environmental quality and evaluating the long-term effectiveness of pollution control measures (Beyer et al., 2022; Ruiz et al., 2019; Meza-Morelos et al., 2024).

Table 4. Imposex Incidence Across Sampling Sites

Site	Total Organisms Examined	Male	Female	Females with Imposex	Imposex Incidence (%)	
Site 1	30	10	20	3	15	
Site 2	30	5	25	1	4	
Site 3	30	10	20	3	15	
Total	90	25	65	7	10.77	

Among the three sampling sites, Site 1 (Tambler, General Santos City) and Site 3 (Burias, Glan, Sarangani Province) recorded the highest imposex incidence, with 3 of the 20 female gastropods (15.0%) exhibiting reproductive masculinization. In contrast, Site 2 (Alabel, Sarangani Province) recorded only one imposex-positive female among 25 females examined, corresponding to an incidence of 4.0%. The spatial variation in imposex incidence indicates that reproductive abnormalities are not uniformly distributed throughout the Sarangani Bay Protected Seascape. Although imposex was detected at all three sites, its prevalence differed considerably, suggesting differences in local environmental conditions, anthropogenic activities, or the extent of exposure to contaminants capable of inducing endocrine disruption.

The comparable incidence observed at Sites 1 and 3 suggests that muricidae populations inhabiting these coastal areas may be experiencing relatively greater environmental pressure than those at Site 2. Site 1 is situated adjacent to General Santos City's active coastal zone, where fishing activities, vessel operations, and port-related developments are more pronounced.

Likewise, Site 3 is influenced by coastal fisheries and maritime activities that may contribute to cumulative environmental stress. Although the present study did not directly identify the causative contaminant, the occurrence of imposex in these locations indicates that environmental conditions are sufficient to induce reproductive abnormalities in sensitive gastropod species. Conversely, the lower incidence observed at Site 2 may indicate comparatively lower contaminant exposure or environmental conditions that are less favorable for imposex development.

The exclusive occurrence of imposex in muricidae gastropods across all sampling sites further confirms the suitability of this family as a sentinel group for marine biomonitoring. Muricidae are known to exhibit high sensitivity to endocrine-disrupting contaminants, making them reliable indicators of sublethal environmental stress before broader ecological impacts become evident (Oehlmann et al., 2007). The absence of imposex in non-muricidae species should therefore be interpreted cautiously, as susceptibility to imposex varies considerably among gastropod taxa and does not necessarily indicate the absence of environmental contamination.

The overall imposex incidence of 10.77% demonstrates that reproductive abnormalities are already present within the muricidae gastropod populations of the Sarangani Bay Protected Seascape. Although this incidence is lower than those reported from highly contaminated ports and harbors where imposex may approach 100% of female muricidae, its occurrence remains ecologically significant because imposex represents a highly specific biological response to environmental endocrine disruption (Gibbs et al., 1990; Oehlmann et al., 2007). The detection of affected females across all sampling sites suggests that reproductive alterations are not isolated events but occur throughout the bay, albeit at varying frequencies.

The higher incidence observed at Sites 1 and 3 indicates spatial heterogeneity in environmental conditions within the protected seascape. Such variation may reflect differences in maritime activities, proximity to pollution sources, hydrodynamic conditions, sediment characteristics, or the cumulative influence of multiple anthropogenic stressors. Because the present study evaluated copper and cadmium as supporting environmental variables rather than primary causative agents, the observed imposex should be interpreted as evidence of overall environmental stress rather than direct proof of contamination by any single pollutant. This interpretation is consistent with the concept of imposex as an integrated biological indicator reflecting cumulative exposure to endocrine-disrupting contaminants within coastal ecosystems.

The relatively low incidence observed at Site 2 likewise provides important ecological information. While reproductive abnormalities were less frequent, the occurrence of even a single imposex-positive female demonstrates that sensitive gastropod populations are responding to environmental conditions within the locality. Continued surveillance is therefore necessary because imposex prevalence may increase if contaminant inputs or anthropogenic pressures intensify over time.

The detection of imposex in multiple muricidae species across all three sampling sites justifies the use of muricidae gastropods as biological indicators for environmental monitoring in the Sarangani Bay Protected

Seascape. Internationally, imposex has been recognized as one of the most reliable biomarkers of endocrine disruption in marine environments and has been extensively used to evaluate the ecological impacts of coastal pollution (International Maritime Organization, 2001). The present findings establish the first baseline assessment of imposex incidence across the study area, providing an important scientific reference for future environmental monitoring, ecological risk assessment, and coastal resource management. Furthermore, documenting imposex together with sediment copper and cadmium concentrations offers a broader evaluation of coastal environmental quality, even though these heavy metals are regarded as supporting environmental variables rather than direct causes of imposex.

The study revealed an overall imposex incidence of 10.77%, with Site 1 and Site 3 exhibiting the highest incidence (15.0%) and Site 2 the lowest (4.0%). Imposex occurred exclusively in muricidae gastropods, reaffirming their sensitivity to environmental stress and their value as bioindicator species. These findings confirm that reproductive abnormalities are present throughout the Sarangani Bay Protected Seascape and provide baseline evidence of environmental conditions capable of inducing imposex in susceptible gastropod populations. Although the observed incidence was relatively low compared with heavily contaminated coastal regions, its occurrence warrants continued ecological monitoring to detect future changes in environmental quality.

Regular long-term biomonitoring of muricidae gastropods should be implemented throughout the Sarangani Bay Protected Seascape to evaluate temporal and spatial changes in imposex incidence and severity. Future investigations should incorporate seasonal sampling, larger sample sizes, and expanded analyses of environmental variables, including additional heavy metals, organic contaminants, sediment characteristics, and organotin compounds when analytical facilities become available. Integrating biological responses with physicochemical assessments will provide a more comprehensive understanding of ecosystem health and support evidence-based coastal management.

In addition, the development of an Information-Educational Material (IEM) highlighting imposex as a biological indicator of marine environmental quality is recommended to increase awareness among local communities, fisheries stakeholders, local government units, and protected area managers regarding the importance of pollution prevention and biodiversity conservation within the Sarangani Bay Protected Seascape.

Imposex Severity of Coastal Gastropods Across the Sampling Sites

Table 5 presents the imposex severity of coastal gastropods across the three sampling sites in the Sarangani Bay Protected Seascape (SBPS) using two internationally recognized biological indices: the Relative Penis Length Index (RPLI) and the Vas Deferens Sequence Index (VDSI). These indices provide complementary measures of imposex severity, with RPLI quantifying the degree of penis development in affected females relative to males, while VDSI evaluates the progression of vas deferens development and overall reproductive masculinization (Gibbs & Bryan, 1994).

Table 5. Imposex Severity of Coastal Gastropods Across the Sampling Sites

Site	Female Penis Length (mm)	Male Mean Penis Length (mm)	RPLI (%)	Mean VDSI	Severity Classification*	
Site 1	8.77	22.6	38.8	3	Moderate	
Site 2	3.6	8.9	40.45	2	Moderate	
Site 3	3.43	8.75	39.2	3	Moderate	
Overall Mean	5.27	13.42	39.48	2.67	Moderate	

The RPLI values ranged from 38.80% to 40.45%, indicating relatively comparable levels of reproductive masculinization among the three sampling sites. Site 2 (Alabel, Sarangani Province) recorded the highest RPLI (40.45%), followed by Site 3 (Burias, Glan) with 39.20%, and Site 1 (Tambler, General Santos City) with 38.80%. Despite these slight numerical differences, the RPLI values were remarkably similar, suggesting that the degree of penis development among imposex-positive females was relatively uniform throughout the study area. The overall mean RPLI of 39.48% indicates that the penis length of affected females averaged nearly 40% of the corresponding male penis length, reflecting a moderate degree of masculinization within the muricidae gastropod populations.

Similarly, the Vas Deferens Sequence Index (VDSI) ranged from 2.00 to 3.00. Sites 1 and 3 recorded the highest mean VDSI (3.00), indicating more advanced imposex characterized by distinct penis formation accompanied by a well-developed vas deferens. In contrast, Site 2 exhibited a lower mean VDSI (2.00), reflecting earlier stages of reproductive masculinization in which vas deferens development had begun but had not progressed to more advanced stages. The overall mean VDSI of 2.67 likewise indicates a moderate level of reproductive alteration among the affected female muricidae gastropods. Importantly, none of the sampling sites exhibited advanced VDSI stages associated with reproductive blockage or sterility, suggesting that although imposex is present, the reproductive impairment observed has not yet reached the most severe manifestations described in highly contaminated coastal environments (Ruiz et al., 2019; Ribeiro-Brasil et al., 2021; França et al., 2021).

The combined interpretation of RPLI and VDSI demonstrates that imposex occurred consistently across all sampling sites, with moderate severity in each locality. While Site 2 exhibited the highest RPLI, its lower VDSI indicates that females possessed relatively larger penis structures compared with male references but exhibited less extensive vas deferens development than those from Sites 1 and 3. Conversely, Sites 1 and 3 displayed slightly lower RPLI values but higher VDSI scores, indicating that reproductive masculinization had progressed further anatomically. These findings highlight the complementary nature of the two indices, as RPLI reflects the extent of penis growth whereas VDSI more accurately describes the progression of reproductive tract modification.

The relatively similar RPLI values among the three sampling sites suggest that the intensity of reproductive masculinization was generally consistent throughout the Sarangani Bay Protected Seascape. The narrow range of RPLI values (38.80–40.45%) indicates that affected females developed penis structures of comparable size regardless of sampling location. This uniformity may reflect similar levels of chronic environmental exposure among susceptible muricidae populations or comparable biological responses to environmental stressors.

In contrast, the variation observed in VDSI provides additional insight into the progression of imposex development. The higher VDSI values recorded in Sites 1 and 3 indicate that reproductive modification extended beyond penis formation to more advanced vas deferens development, suggesting relatively greater progression of imposex in these localities. Meanwhile, the lower VDSI observed at Site 2 is consistent with the lower imposex incidence previously reported for this site, indicating that reproductive masculinization remained in its earlier stages. Together, these findings demonstrate that severity cannot be evaluated using penis length alone and that assessment of vas deferens development is essential for accurately describing the biological progression of imposex.

The overall classification of moderate imposex severity across all sites indicates that reproductive abnormalities are established within muricidae populations but have not yet progressed to the severe stages associated with widespread reproductive failure or population decline. Nevertheless, even moderate imposex represents an ecologically important biological response because it reflects chronic exposure to environmental conditions capable of disrupting normal reproductive development. Previous studies have shown that persistent exposure may eventually lead to higher VDSI stages, reproductive obstruction, reduced fecundity, and long-term population impacts if contaminant inputs continue (Gibbs et al., 1990; Oehlmann et al., 2007).

Although copper and cadmium were included in the present study as supporting environmental variables, the observed imposex severity should not be attributed solely to these heavy metals. Instead, the results indicate that muricidae gastropods are responding to cumulative environmental stressors present within the coastal ecosystem.

Consequently, the severity indices provide valuable biological evidence of ecosystem health that complements physicochemical assessments of sediment quality.

The Relative Penis Length Index (RPLI) and Vas Deferens Sequence Index (VDSI) remain the most widely applied quantitative indicators for assessing imposex severity in marine gastropods because they evaluate complementary aspects of reproductive masculinization and enable standardized comparisons among populations, species, and geographic regions. Recent biomonitoring studies continue to employ these indices to assess spatial and temporal patterns of imposex and to evaluate the effectiveness of environmental management measures aimed at reducing organotin contamination (Ruiz et al., 2019; Ribeiro-Brasil et al., 2021; França et al., 2021). The moderate severity recorded throughout the Sarangani Bay Protected Seascape demonstrates that muricidae gastropods are effective sentinel organisms capable of detecting sublethal reproductive alterations before severe ecological consequences become apparent. The integration of these biological indices with measurements of sediment copper and cadmium strengthens the baseline environmental assessment by combining organism-level responses with supporting environmental data. This integrated approach provides valuable scientific information for evaluating coastal ecosystem health and guiding future monitoring efforts.

The results demonstrate that moderate imposex severity occurred consistently across all sampling sites, with an overall mean RPLI of 39.48% and an overall mean VDSI of 2.67. While RPLI values were highly comparable among the three sites, Sites 1 and 3 exhibited slightly more advanced reproductive development based on VDSI. Collectively, these findings indicate measurable reproductive masculinization within muricidae gastropods throughout the Sarangani Bay Protected Seascape. Although severe reproductive impairment was not observed, the presence of moderate imposex confirms that sensitive gastropod populations are responding to environmental stressors and provides important baseline information for future ecological assessments.

Long-term monitoring of imposex severity should be continued using both RPLI and VDSI to evaluate changes in reproductive health over time. Future studies should expand spatial coverage throughout the Sarangani Bay Protected Seascape, incorporate seasonal and multi-year monitoring, and include broader environmental assessments such as sediment characteristics, organic pollutants, additional heavy metals, and organotin compounds when analytical facilities become available. Integrating biological indices with comprehensive environmental data will improve understanding of the factors influencing imposex severity and strengthen evidence-based coastal management. Furthermore, the developed Information-Educational Material (IEM) should be disseminated among coastal communities, educational institutions, local government units, and protected area managers to promote awareness of imposex as an indicator of marine ecosystem health and reinforce conservation initiatives within the Sarangani Bay Protected Seascape.

Copper and Cadmium Concentration in Sarangani Bay

Table 6 presents the concentrations of copper (Cu) and cadmium (Cd) in sediment samples collected from the three sampling sites within the Sarangani Bay Protected Seascape (SBPS). The measured concentrations were compared with the National Oceanic and Atmospheric Administration (NOAA) Sediment Quality Guidelines, specifically the Effects Range-Low (ERL) and Effects Range-Median (ERM) values, which are widely used to evaluate the potential ecological risks of sediment-associated contaminants to benthic organisms

Table 6. Copper and Cadmium Concentration in Sarangani Bay

Heavy Metals	Site 1 (mg/kg)	Site 2 (mg/kg)	Site 3 (mg/kg)	NOAA ERL (mg/kg)	NOAA ERM (mg/kg)
Copper	6.53	19.09	29.56	34	270
Cadmium	<0.03	<0.03	<0.03	1.2	9.6

Copper concentrations varied among the three sampling sites, ranging from 6.53 mg/kg at Site 1, 19.09 mg/kg at Site 2, and 29.56 mg/kg at Site 3. Although an increasing spatial trend was observed from Site 1 to Site 3, all

measured concentrations remained below the NOAA ERL guideline value of 34 mg/kg and were substantially lower than the ERM value of 270 mg/kg.

According to NOAA sediment quality guidelines, concentrations below the ERL are expected to pose a low probability of adverse biological effects on sediment-dwelling organisms, whereas concentrations exceeding the ERL indicate increasing ecological concern (National Oceanic and Atmospheric Administration). Therefore, the measured copper concentrations suggest that sediment contamination by copper within the study area remains at levels that are unlikely to produce significant toxic effects on benthic organisms.

Among the three sites, Site 3 (Burias, Glan) recorded the highest copper concentration, followed by Site 2 (Alabel) and Site 1 (Tambler). Although the measured values remain below internationally recognized ecological thresholds, the relatively higher copper concentration observed at Site 3 may reflect localized anthropogenic influences such as fishing activities, boat traffic, coastal settlements, or land-based runoff. Copper is widely used in antifouling paints, aquaculture equipment, and various industrial applications; consequently, gradual accumulation in coastal sediments may occur even when concentrations remain below ecological guideline values (Rainbow, 2002). The observed spatial variation therefore suggests differences in local environmental inputs rather than immediate ecological risk.

Cadmium concentrations were consistently reported as below the analytical detection limit (<0.03 mg/kg) across all three sampling sites. These concentrations are substantially lower than both the NOAA ERL value of 1.2 mg/kg and the ERM value of 9.6 mg/kg, indicating that cadmium contamination in the sediments of Sarangani Bay is negligible at the time of sampling. The uniformly low cadmium concentrations suggest that this heavy metal is not currently a major sediment contaminant within the study area. This finding is encouraging because cadmium is a persistent, non-essential heavy metal capable of bioaccumulation and chronic toxicity in marine organisms, particularly under prolonged exposure (Burger, 2008; Zhan et al., 2023).

The sediment quality assessment indicates that both copper and cadmium occur at low concentrations throughout the study area. Although copper exhibited measurable spatial variation, its concentrations remained below levels expected to produce adverse ecological effects according to NOAA sediment quality criteria. Likewise, the consistently undetectable cadmium concentrations demonstrate that cadmium contamination is minimal within the sediments of Sarangani Bay. Collectively, these findings suggest that the study area is presently characterized by relatively good sediment quality with respect to these two heavy metals.

Interestingly, Site 3, which recorded the highest copper concentration, also exhibited one of the highest imposex incidences (15.0%). Similarly, Site 1 exhibited an equivalent imposex incidence despite having the lowest sediment copper concentration. Conversely, Site 2, which had intermediate copper concentrations, exhibited the lowest imposex incidence (4.0%). This spatial pattern indicates that the distribution of imposex does not correspond directly with copper concentrations alone. Likewise, because cadmium concentrations remained below detection limits at all sampling sites, cadmium is unlikely to explain the observed variation in imposex incidence and severity within the present study.

These findings support the interpretation that copper and cadmium should be regarded as supporting environmental variables rather than primary drivers of imposex. Although copper is frequently associated with maritime activities and antifouling coatings, experimental studies have demonstrated that copper alone does not directly induce imposex in marine gastropods, unlike organotin compounds such as tributyltin (TBT) (Barroso et al., 2002). Similarly, cadmium has been shown to affect endocrine function and reproduction in aquatic organisms but has not been established as a direct cause of imposex (Tilton et al., 2003). Consequently, the occurrence of imposex in the present study likely reflects the influence of multiple environmental stressors rather than copper or cadmium concentrations alone.

The relatively higher copper concentration observed at Site 3 nevertheless deserves attention because copper is a persistent metal capable of accumulating in sediments through continuous anthropogenic inputs. Chronic accumulation may eventually affect benthic organisms through oxidative stress, physiological disruption, or reduced reproductive performance even when concentrations remain below acute toxicity thresholds. Therefore,

maintaining low sediment copper concentrations is important for preserving the ecological integrity of the Sarangani Bay Protected Seascape.

The comparison of sediment metal concentrations with NOAA ERL and ERM guidelines provides an internationally accepted basis for evaluating ecological risk. The results indicate that neither copper nor cadmium exceeded threshold values associated with probable biological effects, supporting the conclusion that these heavy metals are not presently major sediment contaminants within the study area. Nevertheless, including copper and cadmium in the present investigation remains scientifically justified because these metals commonly occur in coastal environments affected by maritime activities and may serve as indicators of cumulative anthropogenic influence. Their assessment complements the biological evaluation of imposex by providing supporting information on sediment quality and overall environmental condition.

The sediment analysis revealed that copper concentrations ranged from 6.53 to 29.56 mg/kg, while cadmium concentrations remained below the analytical detection limit (<0.03 mg/kg) at all sampling sites. Both metals were below the NOAA ERL and ERM guideline values, indicating a low likelihood of adverse biological effects associated with sediment contamination. Although copper exhibited an increasing spatial trend from Site 1 to Site 3, the concentrations remained within acceptable ecological limits. Furthermore, the observed pattern of imposex incidence was not directly associated with copper or cadmium concentrations, suggesting that other environmental stressors may contribute to reproductive abnormalities in muricidae gastropods. Overall, these findings indicate that sediment quality with respect to copper and cadmium remains generally favorable within the Sarangani Bay Protected Seascape.

Regular monitoring of sediment copper and cadmium concentrations should be continued to detect potential long-term accumulation associated with increasing coastal development, maritime transportation, fisheries, and urban runoff. Future studies should incorporate additional sediment quality parameters, including grain size, organic matter, and other trace metals, together with analyses of organic contaminants such as organotin compounds when analytical facilities become available. Expanding temporal and spatial monitoring will improve understanding of contaminant dynamics and their relationship with biological indicators such as imposex. Furthermore, the findings should be incorporated into the proposed Information-Educational Material (IEM) to promote awareness among coastal communities, local government units, and protected area managers regarding sediment quality monitoring and the importance of preventing marine pollution within the Sarangani Bay Protected Seascape.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS). Descriptive statistics included frequencies, percentages, means, shell-length summaries, imposex incidence, RPLI, and VDSI. Pearson correlation coefficients were calculated to explore site-level associations between sediment Cu concentration and imposex incidence/severity. Because these correlations were based on only three site-level observations ($N = 3$), the coefficients and p-values were interpreted as exploratory and not as strong inferential evidence for the presence or absence of an environmental relationship. Cadmium correlations were not estimable because all site concentrations were reported below the analytical detection limit (<0.03 mg/kg), producing no measurable between-site variability. Shell length was retained as a morphometric descriptor; the available site-level dataset did not support a robust multivariable adjustment for size/age, so potential size-related confounding was addressed in interpretation and identified as a priority for larger future datasets.

Relationship between Imposex Incidence and Copper Concentration

Table 7 presents the relationship between imposex incidence and sediment copper concentration in Sarangani Bay. Pearson correlation analysis revealed a very weak negative correlation between imposex incidence and copper concentration ($r = -0.113$), indicating that increases in copper levels were associated with only a slight decrease in imposex incidence. However, the relationship was negligible and lacked practical significance. Moreover, the computed significance value ($p = 0.928$) was substantially higher than the 0.05 level of significance, indicating that the observed relationship was not statistically significant. Therefore, the null

hypothesis was accepted, suggesting that there is no significant relationship between imposex incidence and sediment copper concentration in the study area.

Table 7. Relationship between Imposex Incidence and Concentration of Copper

		Imposex Incidence	Concentration of Copper
Imposex Incidence	Pearson Correlation	1	-.113
	Sig. (2-tailed)		.928
	N	3	3
Concentration of Copper	Pearson Correlation	-.113	1
	Sig. (2-tailed)	.928	
	N	3	3

The absence of a significant relationship may be attributed to several factors. First, the copper concentrations recorded in the sediments ranged from 6.53 to 29.56 mg/kg, which were all below the National Oceanic and Atmospheric Administration (NOAA) Effects Range-Low (ERL) guideline of 34 mg/kg, indicating that copper levels were relatively low and unlikely to cause substantial biological effects on marine organisms (NOAA, 1999). Second, imposex has been widely recognized as a biological response primarily associated with exposure to organotin compounds, particularly tributyltin (TBT), which induces the development of male sexual characteristics in female gastropods through endocrine disruption (Gibbs et al., 1990; Oehlmann et al., 2007).

Although copper may contribute to environmental stress and toxicity in marine ecosystems, its direct role in the induction of imposex remains less established than that of organotin compounds. Furthermore, environmental factors such as habitat characteristics, sediment composition, hydrodynamic conditions, species-specific sensitivity, and proximity to maritime activities may have exerted a stronger influence on imposex occurrence than copper concentrations alone. The limited number of sampling sites ($n = 3$) may have also reduced the statistical power of the analysis, making it difficult to detect significant relationships.

Based on these findings, it can be concluded that sediment copper concentration was not significantly associated with imposex incidence in coastal gastropods from Sarangani Bay. The occurrence of imposex observed in the study area is therefore likely influenced by factors other than copper contamination. While copper remains an important environmental parameter for assessing coastal pollution, the present results suggest that it was not a major driver of imposex development within the sampled sites. Similar observations have been reported in recent studies demonstrating that imposex remains more strongly associated with exposure to organotin compounds, particularly tributyltin (TBT), than with naturally occurring or moderately elevated concentrations of other trace metals. Contemporary field investigations have shown that imposex incidence and severity closely correspond to residual organotin contamination, whereas trace metals alone are generally insufficient to explain the occurrence or progression of imposex in muricid gastropods (Ruiz et al., 2019; Ribeiro-Brasil et al., 2021; Beyer et al., 2022).

Considering these findings, future studies should include a larger number of sampling sites and replicate samples to improve the reliability of statistical analyses and enhance the detection of possible relationships. Additional environmental variables, including other heavy metals, organic contaminants, sediment characteristics, and indicators of maritime activity, should also be examined to provide a more comprehensive understanding of factors influencing imposex occurrence. Continuous monitoring of imposex incidence and associated environmental conditions is likewise recommended to establish a stronger baseline for assessing the ecological health of gastropod populations and the coastal ecosystems of Sarangani Bay.

Relationship between Imposex Incidence and cadmium concentration

Table 8 reveals imposex incidence and the concentration of copper. The Pearson correlation analysis was performed to determine the relationship between imposex incidence and sediment cadmium concentration across the sampling sites. However, the correlation coefficient could not be computed because cadmium concentrations were constant across all sites, with values recorded as less than 0.03 mg/kg. As indicated in the SPSS output, Pearson's correlation requires variability in both variables, and therefore no statistical relationship could be calculated when one variable remains constant. Consequently, no correlation coefficient or significance value was generated.

The inability to compute a correlation suggests that cadmium concentrations did not vary sufficiently among the sampling sites to allow statistical evaluation of their association with imposex incidence.

Table 8 Relationship between Imposex Incidence and Cadmium Concentration

		Imposex Incidence	Concentration of Cadmium
Imposex Incidence	Pearson Correlation	1	. ^a
	Sig. (2-tailed)		.
	N	3	3
Concentration of Cadmium	Pearson Correlation	. ^a	. ^a
	Sig. (2-tailed)	.	
	N	3	3
a. Cannot be computed because at least one of the variables is constant.			

The inability to compute a correlation suggests that cadmium concentrations did not vary sufficiently among the sampling sites to permit statistical evaluation of their association with imposex incidence. This finding indicates that cadmium contamination in the study area was minimal and uniformly distributed. The measured cadmium concentrations were substantially lower than the National Oceanic and Atmospheric Administration (NOAA) Effects Range-Low (ERL) value of 1.2 mg/kg and the Effects Range-Median (ERM) value of 9.6 mg/kg, suggesting that cadmium levels were unlikely to cause adverse ecological effects on marine organisms (NOAA, 1999). Despite the uniformly low cadmium concentrations, imposex was observed in gastropods from all sampling sites, indicating that cadmium was unlikely to be a primary factor influencing the occurrence of imposex. Previous studies have identified organotin compounds, particularly tributyltin (TBT), as the principal contaminants responsible for inducing imposex in marine gastropods through endocrine disruption (Oehlmann et al., 2007). Although cadmium is known to affect reproductive and physiological processes in aquatic organisms, evidence directly linking environmental cadmium exposure to imposex induction remains limited (Burger, 2008; Wang & Rainbow, 2008).

Based on these findings, it can be concluded that the relationship between imposex incidence and cadmium concentration could not be statistically determined because cadmium concentrations were constant across all sampling sites. The uniformly low levels of cadmium suggest minimal contamination and indicate that cadmium was unlikely to have contributed significantly to the occurrence of imposex in coastal gastropods from Sarangani Bay. The observed imposex incidence is therefore more likely associated with other environmental factors, contaminant sources, or biological characteristics of the gastropod populations.

It is recommended that future studies include a greater number of sampling sites and conduct sampling during different seasons to capture possible spatial and temporal variations in cadmium concentrations. The assessment of additional environmental contaminants, including other heavy metals, organic pollutants, and residual organotin compounds, should also be considered to provide a more comprehensive understanding of factors influencing imposex occurrence. Continued monitoring of cadmium concentrations and imposex incidence is

likewise recommended to establish a stronger environmental baseline and support the long-term conservation and management of the coastal ecosystems of Sarangani Bay.

Relationship between Imposex Severity (RPLI) and Copper Concentration

Table 9 presents the relationship between imposex severity, as measured by the Relative Penis Length Index (RPLI), and sediment copper concentration in Sarangani Bay. Pearson correlation analysis revealed a positive correlation between RPLI and copper concentration ($r = 0.405$), indicating that imposex severity tended to increase as copper concentration increased. However, the relationship was only weak to moderate in strength. Furthermore, the computed

Table 9. Relationship between Imposex Severity (RPLI) and Copper Concentration

		Imposex Severity RPLI	Concentration of Copper
Imposex Severity RPLI	Pearson Correlation	1	.405
	Sig. (2-tailed)		.734
	N	3	3
Concentration of Copper	Pearson Correlation	.405	1
	Sig. (2-tailed)	.734	
	N	3	3

significance value ($p = 0.734$) was considerably higher than the 0.05 level of significance, indicating that the observed relationship was not statistically significant. Therefore, the null hypothesis was accepted, suggesting that there is no significant relationship between imposex severity (RPLI) and sediment copper concentration in the study area.

The positive correlation observed may suggest that sites with relatively higher copper concentrations also exhibited slightly higher RPLI values. However, the differences in RPLI among the sampling sites were relatively small, ranging from 38.80% to 40.45%, indicating generally similar levels of imposex severity throughout the study area. Likewise, sediment copper concentrations ranged from 6.53 to 29.56 mg/kg, all of which were below the National Oceanic and Atmospheric Administration (NOAA) Effects Range-Low (ERL) guideline of 34 mg/kg, suggesting that copper concentrations were unlikely to exert substantial biological effects on marine organisms (NOAA, 1999). The lack of statistical significance indicates that copper concentration alone cannot adequately explain variations in imposex severity among the sampled gastropod populations.

The absence of a significant relationship may also be attributed to the limited number of sampling sites ($n = 3$) and the relatively narrow range of both copper concentrations and RPLI values. In addition, imposex development is influenced by multiple environmental and biological factors, including exposure to organotin compounds, species-specific sensitivity, habitat characteristics, and historical contamination. Numerous studies have identified tributyltin (TBT) and other organotin compounds as the principal causes of imposex due to their endocrine-disrupting effects on marine gastropods (Gibbs et al., 1990; Oehlmann et al., 2007). Although copper is an important environmental contaminant and may contribute to physiological stress in marine organisms, its direct involvement in inducing imposex remains less evident. Consequently, the observed imposex severity may reflect the combined effects of several environmental variables rather than copper concentration alone.

Based on these findings, it can be concluded that sediment copper concentration was not significantly associated with imposex severity (RPLI) in coastal gastropods from Sarangani Bay. Although a positive association was observed, the relationship was statistically insignificant, indicating that copper was not a major factor influencing the degree of masculinization observed in affected female gastropods. The relatively uniform RPLI values across the sampling sites further support the conclusion that other environmental factors may have contributed more substantially to the observed imposex severity.

It is recommended that future studies increase the number of sampling sites and incorporate seasonal monitoring to improve the reliability of statistical analyses and capture potential temporal variations in environmental conditions. Additional environmental contaminants, including organotin residues, other heavy metals, and organic pollutants, should also be assessed to better understand the factors influencing imposex severity. Continued monitoring of both heavy metal concentrations and imposex indices is recommended to establish a more comprehensive baseline for evaluating the ecological health of coastal gastropod populations and the environmental quality of Sarangani Bay.

Relationship Between Imposex Severity (VDSI) and Copper Concentration

Table 10 summarizes the relationship between Imposex Severity (VDSI). The Pearson correlation analysis was conducted to determine the relationship between imposex severity, as measured by the Vas Deferens Sequence Index (VDSI), and sediment copper concentration across the sampling sites in Sarangani Bay. The results revealed a very weak negative correlation ($r = -0.182$), indicating that VDSI values tended to decrease slightly as copper concentrations increased. However, the relationship was extremely weak and of little practical significance. Furthermore, the computed significance value ($p = 0.884$) was substantially higher than the 0.05 level of significance, indicating that the observed relationship was not statistically significant.

Table 10. Relationship Between Imposex Severity (VDSI) and Copper Concentration

		Imposex Severity VDSI	Concentration of Copper
Imposex Severity VDSI	Pearson Correlation	1	-.182
	Sig. (2-tailed)		.884
	N	3	3
Concentration of Copper	Pearson Correlation	-.182	1
	Sig. (2-tailed)	.884	
	N	3	3

Therefore, the null hypothesis was accepted, suggesting that there is no significant relationship between imposex severity (VDSI) and copper concentration in the study area.

The weak negative association indicates that variations in copper concentrations among the sampling sites were not accompanied by corresponding changes in imposex severity. This finding is consistent with the observed VDSI values, which ranged only from 2.00 to 3.00, reflecting relatively similar levels of imposex development among affected female gastropods.

Likewise, sediment copper concentrations ranged from 6.53 to 29.56 mg/kg, all of which were below the NOAA Effects Range-Low (ERL) guideline value of 34 mg/kg, suggesting that copper contamination was not sufficiently elevated to induce substantial biological effects.

Previous studies have shown that imposex is primarily associated with exposure to organotin compounds, particularly tributyltin (TBT), which disrupt endocrine regulation and induce the development of male sexual characteristics in female gastropods (Gibbs et al., 1990; Oehlmann et al., 2007). In contrast, copper is generally regarded as a supporting environmental stressor rather than a direct causative agent of imposex.

Additionally, imposex severity is influenced by multiple environmental and biological factors, including species-specific sensitivity, habitat characteristics, historical contaminant exposure, and other pollutants present in the environment. Therefore, the observed VDSI values likely reflect the combined influence of several factors rather than copper concentration alone.

Based on these findings, it can be concluded that sediment copper concentration was not significantly associated with imposex severity (VDSI) in coastal gastropods from Sarangani Bay. Although a weak negative relationship was observed, the correlation was not statistically significant, indicating that copper concentration was not a major determinant of imposex severity within the sampled populations. The findings support the view that imposex development is a complex biological response influenced by multiple environmental variables and not solely by sediment copper levels.

It is recommended that future studies increase the number of sampling sites and sampling periods to improve statistical reliability and capture spatial and temporal variations in environmental conditions. Additional contaminants, including organotin residues, other heavy metals, and organic pollutants, should also be investigated to better explain the occurrence and severity of imposex. Continued monitoring of VDSI and other imposex indices is likewise recommended to establish a robust baseline for environmental assessment and to support the long-term conservation and management of coastal ecosystems in Sarangani Bay.

Relationship between Imposex Severity (RPLI) and Concentration of Cadmium

Table 11 reveals the relationship between Imposex Severity (RPLI) and Cadmium concentration. The Pearson correlation analysis was conducted to determine the relationship between imposex severity, as measured by the Relative Penis Length Index (RPLI), and sediment cadmium concentration across the sampling sites in Sarangani Bay. However, the correlation coefficient could not be computed because cadmium concentration was constant across all sampling sites. As indicated in the SPSS output, Pearson's correlation analysis requires variability in both variables; therefore, no correlation coefficient or significance value could be generated when one variable remains constant. In this study, cadmium concentrations were consistently reported as less than 0.03 mg/kg at all sampling sites, resulting in insufficient variation for statistical analysis.

Table 11. Relationship between Imposex Severity (RPLI) and Concentration of Cadmium

		Imposex Severity RPLI	Concentration of Cadmium
Imposex Severity RPLI	Pearson Correlation	1	. ^a
	Sig. (2-tailed)		.
	N	3	3
Concentration of Cadmium	Pearson Correlation	. ^a	. ^a
	Sig. (2-tailed)	.	
	N	3	3
a. Cannot be computed because at least one of the variables is constant.			

The inability to compute a correlation indicates that the available data do not permit an assessment of whether changes in cadmium concentration are associated with changes in imposex severity. The uniformly low cadmium concentrations suggest that cadmium contamination in the sediments was minimal and evenly distributed throughout the study area.

Furthermore, these concentrations were substantially lower than the NOAA Effects Range-Low (ERL) value of 1.2 mg/kg and the Effects Range-Median (ERM) value of 9.6 mg/kg, indicating that cadmium levels were well below thresholds associated with adverse biological effects in marine organisms. Despite the presence of imposex, RPLI values remained relatively consistent among sites, ranging from 38.80% to 40.45%, suggesting that factors other than cadmium concentration were more likely responsible for the observed degree of masculinization in female gastropods.

Previous studies have demonstrated that imposex is primarily associated with exposure to organotin compounds, particularly tributyltin (TBT), which disrupt endocrine processes and induce the development of male sexual characteristics in female gastropods (Horiguchi, 2006). While cadmium is recognized as a toxic heavy metal

capable of affecting reproduction and physiological processes in aquatic organisms, its direct role in the induction of imposex remains less clearly established. The occurrence of imposex in the present study despite consistently low cadmium concentrations suggests that cadmium was unlikely to be a major factor influencing imposex severity within the sampled populations.

Based on these findings, it can be concluded that the relationship between imposex severity (RPLI) and cadmium concentration could not be statistically determined because cadmium concentrations were constant across all sampling sites. The uniformly low levels of cadmium indicate minimal contamination and suggest that cadmium was not a significant environmental factor influencing the severity of imposex in coastal gastropods from Sarangani Bay. Consequently, the observed RPLI values were likely influenced by other environmental conditions or contaminant sources not evaluated in the present analysis.

It is recommended that future studies include a greater number of sampling sites and repeated sampling across different seasons to capture potential spatial and temporal variations in cadmium concentrations. Additional environmental parameters and contaminants, including other heavy metals, organic pollutants, and residual organotin compounds, should be assessed to better understand the factors contributing to imposex severity. Continued monitoring of both heavy metal concentrations and imposex indices is likewise recommended to strengthen baseline environmental data and support the long-term management and conservation of coastal ecosystems in Sarangani Bay.

Relationship Between Imposex Severity (VDSI) and Cadmium Concentration

Table 12 depicts the relationship between imposex severity (VDSI) and cadmium concentration. The Pearson correlation analysis was conducted to determine the relationship between imposex severity, as measured by the Vas Deferens Sequence Index (VDSI), and sediment cadmium concentration in Sarangani Bay. However, the correlation coefficient could not be computed because cadmium concentration was constant across all sampling sites. As indicated in the SPSS output, Pearson's correlation analysis requires variation in both variables; therefore, no correlation coefficient or significance value could be generated when one variable remains constant. In the present study, cadmium concentrations were consistently reported as less than 0.03 mg/kg at all three sampling sites, resulting in insufficient variability for statistical analysis.

The inability to compute a correlation suggests that the available data do not support an assessment of the relationship between cadmium concentration and imposex severity. The uniformly low cadmium concentrations indicate that cadmium contamination was minimal and evenly distributed throughout the study area. Moreover, the recorded concentrations were substantially lower than the NOAA Effects Range-Low (ERL) guideline of 1.2 mg/kg and the Effects Range-Median (ERM) guideline of 9.6 mg/kg, suggesting that cadmium levels were unlikely to produce adverse biological effects in marine organisms. Despite the consistently low cadmium concentrations, imposex severity was observed in the sampled gastropods, with VDSI values ranging from 2.00 to 3.00 and an overall mean of 2.86, indicating moderate development of male reproductive characteristics among affected females. This finding suggests that factors other than cadmium concentration were likely responsible for the observed imposex severity.

Table 12 Relationship between Imposex Severity (VDSI) and Concentration of Cadmium

		Imposex Severity VDSI	Concentration of Cadmium
Imposex Severity VDSI	Pearson Correlation	1	. ^a
	Sig. (2-tailed)		.
	N	3	3
Concentration of Cadmium	Pearson Correlation	. ^a	. ^a
	Sig. (2-tailed)	.	
	N	3	3
a. Cannot be computed because at least one of the variables is constant.			

The absence of a measurable relationship is consistent with previous studies indicating that imposex is primarily associated with exposure to organotin compounds, particularly tributyltin (TBT), which interfere with endocrine regulation and stimulate the development of male sexual characteristics in female gastropods (Gibbs et al., 1990; Oehlmann et al., 2007). While cadmium is recognized as a toxic heavy metal capable of causing reproductive impairment, physiological stress, and developmental abnormalities in aquatic organisms (Burger, 2008; Wang & Rainbow, 2008), there is limited evidence directly linking environmental cadmium exposure to the induction of imposex. Therefore, the occurrence of imposex in Sarangani Bay despite consistently low cadmium concentrations suggests that cadmium was not a major factor influencing the severity of imposex in the sampled gastropod populations.

Based on these findings, it can be concluded that the relationship between imposex severity (VDSI) and cadmium concentration in Sarangani Bay could not be statistically determined because cadmium concentrations were constant across all sampling sites. The uniformly low cadmium levels indicate minimal contamination and suggest that cadmium was unlikely to have contributed significantly to the development or progression of imposex. Consequently, the observed VDSI values were more likely influenced by other environmental factors, biological characteristics of the species, or contaminants not measured in the present study.

Overall, the study establishes a first baseline of imposex occurrence and morphological severity in selected Muricidae from SBPS. The observed imposex cannot be causally attributed to Cu, Cd, TBT, or any specific maritime source using the present dataset. The absence of statistically significant Cu associations should likewise be interpreted cautiously because only three site-level observations were available. These findings support continued sentinel-gastropod monitoring while emphasizing the need for larger female sample sizes, direct organotin chemistry, quantitative maritime-traffic data, and environmental co-variables in subsequent assessments.

It is recommended that future studies expand the number of sampling sites and include seasonal monitoring to capture potential spatial and temporal variations in cadmium concentrations. The assessment of additional contaminants, particularly organotin compounds and other heavy metals should also be considered to better explain the occurrence and severity of imposex in coastal gastropods. Furthermore, long-term monitoring of VDSI and sediment quality is recommended to establish a comprehensive baseline for evaluating environmental health and supporting the conservation and management of coastal ecosystems in Sarangani Bay.

Study Limitations, Management Implications, and Future Directions

The biological findings demonstrate the occurrence of imposex in selected Muricidae within SBPS, but they should not be interpreted as direct evidence of current illegal TBT use or as proof of a specific contaminant source. Direct TBT, DBT, and MBT measurements were not available, and Cu and Cd cannot substitute for organotin determination. Likewise, the small number of site-level observations, unequal numbers of confirmed females among sites, and absence of quantitative vessel-density, hydrodynamic, flushing, water-quality, sediment-organic-carbon, and tissue-residue data limit causal and source-attribution analyses. The baseline should therefore be used primarily to document biological occurrence and severity and to guide more targeted monitoring.

Future SBPS monitoring should prioritize standardized collection of at least approximately 30 adult females per site where ecologically and ethically feasible, with consistent species selection, shell-size recording, and species-specific VDS criteria. Repeated dry- and wet-season surveys should integrate quantitative maritime-traffic information, hydrodynamic or flushing characteristics, temperature and other water-quality variables, sediment grain size and organic matter, and direct chemical analysis of TBT and its degradation products (DBT and MBT) in sediments and/or gastropod tissues. These additions would allow stronger evaluation of exposure gradients, legacy contamination, and potential source areas.

For management, the Sarangani Bay Protected Seascape Protected Area Management Office (PAMO), Protected Area Management Board (PAMB), concerned local government units (LGUs), and relevant maritime and environmental agencies may use the present results as an early baseline for a tiered surveillance program. Priority

actions include periodic imposex monitoring using the same standardized indices, targeted organotin screening near ports, docking and vessel-repair areas when analytical capacity becomes available, documentation of antifouling practices and compliance with applicable regulations, and integration of biological monitoring with sediment-quality and maritime-activity information. Such surveillance should be framed as precautionary environmental management and not as evidence of regulatory non-compliance unless supported by direct chemical and enforcement data.

ACKNOWLEDGMENT

The author sincerely expresses her deepest gratitude to God Almighty for His guidance, wisdom, strength, and countless blessings throughout the completion of this study.

Special appreciation is extended to the partner agency Sarangani Bay Protected Seascape (SBPS) for their invaluable support, technical assistance, and permission to conduct this research within the protected seascape.

The author is deeply grateful to Notre Dame of Dadiangas University Research and Publication Center for providing academic guidance and institutional support. Heartfelt thanks are also extended to the research consultant Prof Ronald Sombero and technical team, panel members, colleagues, laboratory personnel, and all individuals who generously shared their expertise, assistance, and encouragement throughout the conduct of this study.

Finally, the author lovingly acknowledges her family and friends for their unwavering prayers, understanding, patience, and constant encouragement, which served as a source of strength and inspiration in completing this research.

To all who contributed in one way or another, your kindness and support are sincerely appreciated.

REFERENCES

1. Abidli, S., Lahbib, Y., & Trigui El Menif, N. (2009). Imposex and genital tract malformations in *Hexaplex trunculus* and *Bolinus brandaris* collected in the Gulf of Tunis. *Bulletin of Marine Science*, 85(1), 11–25.
2. Acot, F. T., Jr., Sajorne, R. E., Omar, N.-A. K., Suson, P. D., Rallos, L. E. E., & Bacosa, H. P. (2022). Unraveling macroplastic pollution in rural and urban beaches in Sarangani Bay Protected Seascape, Mindanao, Philippines. *Journal of Marine Science and Engineering*, 10(10), 1532. <https://doi.org/10.3390/jmse10101532>
3. Agduma, A. R., & Cao, K. F. (2023). Species richness, extent and potential threats to mangroves of Sarangani Bay Protected Seascape, Philippines. *Biodiversity Data Journal*, 11, e100050. <https://doi.org/10.3897/BDJ.11.e100050>
4. Alcala, A. C., Ingles, J. A., & Bucol, A. A. (2008). Review of the biodiversity of Southern Philippine seas. *The Philippine Scientist*, 45, 1–61. <https://doi.org/10.3860/psci.v45i0.991>
5. Barroso, C. M., Reis-Henriques, M. A., Ferreira, M. S., & Moreira, M. H. (2002). Effectiveness of some compounds derived from antifouling paints in promoting imposex in *Nassarius reticulatus*. *Journal of the Marine Biological Association of the United Kingdom*, 82(2), 249–255. <https://doi.org/10.1017/S0025315402005428>
6. Bautista, C. P. A., Punzalan, J. M., Samaniego, J. O., Gibaga, C. R. L., Tanciongco, A. M., Quierrez, R. N. M., Gervasio, J. H. C., & Reyes, R. C. G. (2024). Pollution assessment of potentially toxic elements in marine sediment from Puerto Princesa Bay, Palawan, Philippines. *Philippine Journal of Science*, 153(2).
7. Beyer, J., Song, Y., Tollefsen, K. E., Berge, J. A., Tveiten, L., Helland, A., Øxnevad, S., & Schøyen, M. (2022). The ecotoxicology of marine tributyltin (TBT) hotspots: A review. *Marine Environmental Research*, 179, 105689. <https://doi.org/10.1016/j.marenvres.2022.105689>

8. Bondgaard, M., & Bjerregaard, P. (2005). Association between cadmium and calcium uptake and distribution during the moult cycle of female shore crabs (*Carcinus maenas*): An in vivo study. *Aquatic Toxicology*, 72(1–2), 17–31. <https://doi.org/10.1016/j.aquatox.2004.11.017>
9. Burger, J. (2008). Assessment and management of risk to wildlife from cadmium. *Science of the Total Environment*, 389(1), 37–45. <https://doi.org/10.1016/j.scitotenv.2007.08.037>
10. França, M. A., Otegui, M. B. P., Zamprogno, G. C., Menário, J. M. F. S., & Costa, M. B. (2021). Imposex and ecological quality status in *Stramonita brasiliensis*: A temporal (2007 to 2018) and spatial evaluation on the southeastern coast of Brazil. *Journal of Sea Research*, 174, 102080. <https://doi.org/10.1016/j.seares.2021.102080>
11. Gao, Y. L., Hong, J. M., Guo, Y. K., Chen, M. X., Chang, A. K., Xie, L., & Ying, X. P. (2021). Assessment of spermatogenic cell apoptosis and the transcript levels of metallothionein and p53 in *Meretrix meretrix* induced by cadmium. *Ecotoxicology and Environmental Safety*, 217, 112230. <https://doi.org/10.1016/j.ecoenv.2021.112230>
12. Gibbs, P. E., & Bryan, G. W. (1994). Biomonitoring of tributyltin (TBT) pollution using the imposex response of neogastropod molluscs. In J. P. Kramer (Ed.), *Biomonitoring of coastal waters and estuaries* (pp. 205–226). CRC Press.
13. Gibbs, P. E., Bryan, G. W., Pascoe, P. L., & Burt, G. R. (1990). Reproductive abnormalities in female *Ocenebra erinacea* (Gastropoda) resulting from tributyltin-induced imposex. *Journal of the Marine Biological Association of the United Kingdom*, 70(3), 639–656. <https://doi.org/10.1017/S0025315400036638>
14. Giulianelli, S., Primost, M. A., Lanari, C., & Bigatti, G. (2020). RXR expression in marine gastropods with different sensitivity to imposex development. *Scientific Reports*, 10, 9507. <https://doi.org/10.1038/s41598-020-66402-1>
15. He, Y., Huang, Y., Zhang, L., Zhang, M., Chen, Z., & Wang, Y. (2023). Cadmium exposure in aquatic products and health risk classification assessment in the Zhejiang population. *Foods*, 12(16), 3094. <https://doi.org/10.3390/foods12163094>
16. Herceda, F. C. B., Bien, P. J. J., Palomo, J. V. A., Mamon, J. D., Mantoya, V. P. L., Cabrera, M. L. N., & Requieron, E. A. (2016). Diversity and special assessment of soft-bottom intertidal mollusks in seagrass beds of Benoni, Glan, Sarangani Province, Philippines. *Journal of Biodiversity and Environmental Sciences*, 8(2), 225–232.
17. Horiguchi, T. (2006). Masculinization of female gastropod mollusks induced by organotin compounds, focusing on mechanism of actions of tributyltin and triphenyltin for development of imposex. *Environmental Sciences*, 13(2), 77–87.
18. Huang, Y., Tang, H. C., Jin, J. Y., Fan, M. B., Chang, A. K., & Ying, X. P. (2020). Effects of waterborne cadmium exposure on its internal distribution in *Meretrix meretrix* and detoxification by metallothionein and antioxidant enzymes. *Frontiers in Marine Science*, 7, 502. <https://doi.org/10.3389/fmars.2020.00502>
19. International Maritime Organization. (2001). *International Convention on the Control of Harmful Anti-fouling Systems on Ships*.
20. Laranjeiro, F., Beiras, R., & Barroso, C. M. (2018). The use of the biomarker imposex to assess the environmental status of aquatic ecosystems regarding tributyltin pollution. *Ecological Indicators*, 95, 1068–1076. <https://doi.org/10.1016/j.ecolind.2017.12.004>
21. Liu, H., Tian, X., Jiang, L., Han, D., Hu, S., Cui, Y., Jiang, F., Liu, Y., Xu, Y., & Li, H. (2023). Sources, bioaccumulation, and toxicity mechanisms of cadmium in *Chlamys farreri*. *Journal of Hazardous Materials*, 453, 131395. <https://doi.org/10.1016/j.jhazmat.2023.131395>
22. Manzo, K., Estandarte, M. H., Dalipe, R. E., Ulangutan, J., Lecera, J. M., Acob, A., Diamalod, J., Salmo, W., & Jumawan, J. (2014). Survey and diversity of intertidal mollusks in Alabel and Maasim, Sarangani Province, Philippines. *AACL Bioflux*, 7, 449–457.
23. Meza-Morelos, D., Johnson Restrepo, B., Castro, Í. B., Fillmann, G., & Fernández Maestre, R. (2024). Imposex incidence in gastropod species from the Colombian Caribbean Coast reveals continued and widespread tributyltin contamination after its global ban. *Environmental Pollution*, 362, 125010. <https://doi.org/10.1016/j.envpol.2024.125010>
24. National Oceanic and Atmospheric Administration. (1999). *Sediment quality guidelines developed for the National Status and Trends Program*. NOAA.

25. Oehlmann, J., Di Benedetto, P., Tillmann, M., Duft, M., Oetken, M., & Schulte-Oehlmann, U. (2007). Endocrine disruption in prosobranch molluscs: Evidence and ecological relevance. *Ecotoxicology*, 16(1), 29–43. <https://doi.org/10.1007/s10646-006-0110-6>
26. Pan, X.-D., & Han, J.-L. (2023). Heavy metals accumulation in bivalve mollusks collected from coastal areas of southeast China. *Marine Pollution Bulletin*, 189, 114808. <https://doi.org/10.1016/j.marpolbul.2023.114808>
27. Peralta, A., Miloslavich, P., & Bigatti, G. (2014). Imposex in *Voluta musica* (Caenogastropoda: Volutidae) from northeastern Peninsula de Araya, Venezuela. *Revista de Biología Tropical*, 62(Suppl. 3), 199–209.
28. Perić, L., Stinga Perusco, V. T., & Nerlović, V. (2020). Differential response of biomarkers in the native European flat oyster *Ostrea edulis* and the non-indigenous Pacific oyster *Crassostrea gigas* co-exposed to cadmium and copper. *Journal of Experimental Marine Biology and Ecology*, 523, 151271. <https://doi.org/10.1016/j.jembe.2019.151271>
29. Rainbow, P. S. (2002). Trace metal concentrations in aquatic invertebrates: Why and so what? *Environmental Pollution*, 120(3), 497–507.
30. Rial, D., Bellas, J., & Ruiz, J. M. (2018). Critical analysis of the relationship between imposex and butyltin body burden in *Nassarius reticulatus* and *Nucella lapillus*. *Environmental Pollution*, 237, 523–530. <https://doi.org/10.1016/j.envpol.2018.02.090>
31. Ribeiro-Brasil, D. R. G., Castro, I. B., Petracco, M., Batista, R. M., Brasil, L. S., Ferreira, J. A., Borba, T. A. C., Rollnic, M., Fillmann, G., & Amado, L. L. (2021). Spatial distribution of butyltins and imposex in eastern Brazilian Amazon. *Marine Pollution Bulletin*, 165, 112155. <https://doi.org/10.1016/j.marpolbul.2021.112155>
32. Risjani, Y., Fitroya, Q. I., Andayani, S., Roem, M., Kadim, M. K., & Adam, M. A. (2025). Imposex phenomenon in gastropods (*Turbo crassus*) in Lamongan Regency, East Java, and the implication on food safety. *IOP Conference Series: Earth and Environmental Science*, 1557(1), 012031. <https://doi.org/10.1088/1755-1315/1557/1/012031>
33. Ruiz, J. M., Carro, B., Albaina, N., Couceiro, L., Quintela, M., & Barreiro, R. (2019). A decadal study of biometric and imposex indices in two gastropods. *Journal of the Marine Biological Association of the United Kingdom*, 99(7), 1601–1614. <https://doi.org/10.1017/S0025315419000535>
34. Santos, M. R., & Maia, R. C. (2023). Imposex in *Stramonita brasiliensis* (Mollusca: Gastropoda) in Port Terminal of Ceará: Environmental assessment and perception. *Revista AIDIS de Ingeniería y Ciencias Ambientales*, 16(3). <https://doi.org/10.22201/iingen.0718378xe.2023.16.3.83883>
35. Sun, S., Jing, Y., Zhang, T., Hu, F., Chen, Q., & Liu, G. (2024). Effect of salinity on the toxicokinetics, oxidative stress, and metallothionein gene expression in *Meretrix meretrix* exposed to cadmium. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 279, 109863. <https://doi.org/10.1016/j.cbpc.2024.109863>
36. Tilton, F., Benson, W. H., & Schlenk, D. (2003). Evaluation of cadmium effects on the reproductive success of fathead minnows (*Pimephales promelas*). *Archives of Environmental Contamination and Toxicology*, 46(1), 95–102. <https://doi.org/10.1007/s00244-003-3029-5>
37. Titley-O'Neal, C. P., Munkittrick, K. R., & MacDonald, B. A. (2011). The effects of organotin on female gastropods. *Journal of Environmental Monitoring*, 13, 2360–2388. <https://doi.org/10.1039/C1EM10011D>
38. Viana, J. L. M., Mendes, V. J. C., Costa, M. B., Otegui, M. B. P., Diniz, M. S., Santos, S. R. V., & Franco, T. C. R. S. (2021). First evaluation of imposex in *Stramonita brasiliensis* (Caenogastropoda: Muricidae) from Brazil's Legal Amazon. *Journal of Sea Research*, 174, 102064. <https://doi.org/10.1016/j.seares.2021.102064>
39. Wang, J. H., Deng, W. F., Zou, T., Bai, B. B., Chang, A. K., & Ying, X. P. (2021). Cadmium-induced oxidative stress in *Meretrix meretrix* gills leads to mitochondria-mediated apoptosis. *Ecotoxicology*, 30, 2011–2023. <https://doi.org/10.1007/s10646-021-02465-8>
40. Wang, W.-X., & Rainbow, P. S. (2008). Comparative approaches to understand metal bioaccumulation in aquatic animals. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 148(4), 315–323. <https://doi.org/10.1016/j.cbpc.2008.04.003>

41. Zhan, J., Sun, T., Wang, X., Wu, H., & Yu, J. (2023). Meta-analysis reveals the species-, dose-, and duration-dependent effects of cadmium toxicities in marine bivalves. *Science of the Total Environment*, 859, 160164. <https://doi.org/10.1016/j.scitotenv.2022.160164>
42. Zhou, J., Cai, H., Zhong, Y., Zheng, Y., Wu, Y., Chang, A. K., & Ying, X. (2024). Reversal of cadmium-induced toxicity in *Meretrix meretrix* as determined by alleviation of oxidative damage following short-term depuration. *Frontiers in Marine Science*, 11, 1444061. <https://doi.org/10.3389/fmars.2024.1444061>