Efficacy of Metal Accumulation and Bioremediation Potential of Ascidians from the Thoothukudi Coast

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Abstract

This study investigated the accumulation of trace metals in ten ascidian species collected from pollution-impacted zones along the Thoothukudi coast, Southeast India. Using atomic absorption spectroscopy (AAS), we quantified seven metals K, Ca, Mg, Fe, Cu, Zn and V across three ascidian suborders: Aplousobranchia, Stolidobranchia and Phlebobranchia. The whole-body tissues of the selected ascidians were digested following EPA Method 3050B and analysed via AAS. The results revealed clear taxon-specific metal accumulation profiles: Didemnum psamathodes (Aplousobranchia) exhibited exceptionally high iron accumulation (780.68 ppm/g), Microcosmus squamiger (Stolidobranchia) concentrated calcium (>1062 µg/g) and Phallusia arabica (Phlebobranchia) sequestered vanadium (55.1 ppm/g). One-way ANOVA indicated significant interspecies differences ($F(9,54) = 42.44$, $p < 0.0001$), further supported by Principal Component analysis. Transplantation experiments confirmed site-dependent accumulation patterns with P. arabica vanadium levels increasing to 48.7 ppm/g at industrial sites over 60 days.

Biofiltration trials demonstrated about an 84% reduction in vanadium from synthetic wastewater, confirming the biofiltration potential of ascidians. Protein-binding assays revealed the presence of vanadium- and iron-affinitive proteins with high stability constants ($\log K > 6.8$), suggesting species-specific metal retention. Desorption studies further highlighted differences in metal lability: P. arabica retained 78.4% of vanadium after 28 days in clean seawater, while D. psamathodes released over 60% of its iron. These findings underscore the utility of ascidians as effective biomonitors and biofilters, offering promising avenues for marine environmental monitoring and trace metal recovery.

Keywords: Bioindicator, environmental monitoring, heavy metal pollution, marine biotechnology, metal speciation, tunicates.

Introduction

Marine ecosystems are increasingly exposed to persistent and toxic contaminants originating from both natural and anthropogenic sources. Natural inputs such as atmospheric deposition and the erosion of geological matrices, along with anthropogenic activities including industrial effluent discharge, domestic sewage, mining, coral dredging and thermal power plant runoff, introduced complex mixtures of pollutants into coastal waters[2]. Among these, trace metals are of particular concern due to their non-biodegradable nature and high potential for bioaccumulation and biomagnification[19]. Once introduced into the marine environment, these metals persist for extended periods, entering biogeochemical cycles and progressively accumulating in the tissues of marine organisms, particularly benthic fauna[36].

Numerous studies have documented that benthic invertebrates can concentrate metals in their tissues to levels far exceeding those found in the surrounding environment. This makes them reliable bioindicators for environmental monitoring. Among these, suspension feeders such as sponges[5,16,18] and bivalve molluscs including mussels, oysters and donax species have been widely studied for their metal accumulation capacity[34]. These organisms, due to their filter-feeding habits and sedentary lifestyle, remain in constant contact with suspended particles and dissolved contaminants in the water column and benthic substrate. Ascidians (Phylum Chordata: Subphylum Tunicata), commonly known as sea squirts, are another group of sessile marine invertebrates that exhibit high metal accumulation potential. They are found in a wide range of marine habitats including sea ports, harbours, coral reefs and industrial discharge zones.

Ascidians are benthic filter feeders that are particularly abundant in polluted environments[24,30]. They possess unique physiological adaptations such as a highly vascularised branchial basket and efficient water filtration systems, capable of processing incurrent volumes up to 1000 times their body size per hour. This enhances their capacity to accumulate both dissolved and particulate metals in their body tissue. A key feature contributing to their metal accumulation is the presence of specialised blood cells known as vanadocytes which selectively sequester vanadium. Moreover, the absence of a true excretory system (with the exception of members belonging to the family Molgulidae), along with the expression of intracellular metal-binding proteins, allows for long-term retention of metals within their tissues[26].

The extraordinary phenomenon of vanadium accumulation in ascidians was first reported by Henze[21]. The discovery sparked extensive global interest in their metal-binding capabilities. Over the past five decades, researchers have elucidated suborder-specific accumulation patterns: Phlebobranchia species preferentially sequester vanadium,

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Stolidobranchia tend to accumulate alkaline earth metals[12] and Aplousobranchia show a propensity for concentrating transition metals[9,11,13,26,31,52].

In India, early investigations on ascidian metal bioaccumulation in ascidians began with Krishnan[23] who reported that several species collected from the Royapuram coast of Madras (present Chennai), such as *Styela canopus*, *Microcosmus exasperatus* and *Polyclinum constellatum,* accumulated significant concentrations of cadmium, copper, nickel, lead, zinc, manganese, cobalt and iron. Subsequent studies by Meenakshi[25] revealed interspecies variation in metal accumulation, whereas a comparative study by Abdul[1] compared metal content in the mantle and test tissues of *Phallusia nigra* from Thoothukudi and Vizhinjam Bay. Tamilselvi[44] documented seasonal variations in trace metal accumulation in several ascidian species along the Thoothukudi coast.

Further, Tamilselvi et al[45] utilised the calcium-rich ascidian *Herdmania pallida* to enhance breeding performance in *Poecilia sphenops*. Radhalakshmi et al[34] demonstrated the utility of *Polyclinum nudum*-based feed in improving reproduction in ornamental fish such as swordtails. Several isolated studies have addressed species-specific accumulation, comprehensive investigations comparing multiple ascidian suborders under a common environmental stress gradient remain scarce. The present study was undertaken to address this gap by examining metal accumulation patterns in ten ascidian species representing the three suborders: Aplousobranchia, Phlebobranchia and Stolidobranchia, collected from pollution-impacted coastal zones of Thoothukudi. This region is chronically exposed to effluents from thermal power plants[4] and petrochemical industries[22], providing a suitable setting for comparative bioaccumulation analysis.

Using Atomic Absorption Spectroscopy (AAS), concentrations of essential and non-essential metals including potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn) and vanadium (V) were quantified in ascidian tissues. These metals were chosen based on their ecological relevance, prevalence in polluted waters and previously reported affinity for accumulation in tunicates[3,15,32]. Statistical analyses, including one-way ANOVA and Principal Component analysis (PCA), were performed to evaluate interspecies variation and suborder-specific accumulation trends.

To assess metal uptake dynamics, transplantation experiments were carried out using two identified accumulators: *Phallusia arabica* (for vanadium) and *Didemnum psamathodes* (for iron). These were relocated across a pollution gradient and monitored for 60 days to understand the site-dependent kinetics of metal uptake. Additionally, metal-binding proteins from ascidian tissues were extracted, fractionated using size-exclusion chromatography and evaluated through EDTA chelation and stability constant assays to elucidate the biochemical basis of metal retention.

The potential of ascidians in bioremediation was explored through a pilot-scale biofiltration experiment. *P. arabica* was exposed to vanadium-spiked synthetic wastewater in a controlled flow-through setup to evaluate their efficacy in removing metals from solution. A follow-up desorption study was conducted in metal-free seawater to examine the retention and release behaviour of vanadium and iron over a 28-day period. Furthermore, field surveys identified 23 ascidian species, of which 91% were non-native.

Pollution-tolerant taxa such as *P. arabica* and *Microcosmus squamiger* dominated in contaminated zones, while native species were more prevalent in less disturbed reference sites. These ecological patterns informed the selection of focal species for detailed experimental analysis. Thus, this study integrates ecological surveys, quantitative metal analyses, biochemical characterisation and applied filtration trials to comprehensively assess the potential of ascidians as both biomonitors and bioremediators of coastal metal pollution.

Material and Methods

Area and Sampling Protocol: The Thoothukudi Roach Park coastal region (8°47'1" N, 78°9'33" E) in Southeast India represents a tropical marine ecosystem influenced by industrial effluents and harbour activities. Sampling was conducted along a 15-km stretch encompassing industrial outfalls, shipyards and reference sites with minimal anthropogenic input. Ascidians were collected during low tide from both natural substrates (coral rubble, rocky beds) and artificial structures (harbour pilings, aquaculture floats).

Species were identified using Monniot et al[29] taxonomic keys. Voucher specimens were preserved in 10% formalin–seawater solution. The study focused on ten ascidian species across three suborders:

- **Aplousobranchia:** *Didemnum psamathodes*, *Eudistoma laysani*, *Trididemnum clinides*
- **Phlebobranchia:** *Phallusia arabica*, *Phallusia nigra*
- **Stolidobranchia:** *Microcosmus squamiger*, *M. exasperatus*, *Herdmania pallida*, *Styela canopus*

Species Selection Rationale: Species were selected based on their dominance in preliminary surveys and known associations with metal-rich sites. *P. arabica* was collected from vanadium-impacted harbour zones, *D. psamathodes* from iron-rich coastal areas and *M. squamiger* from coastal area of Thoohukudi.

Sample Preparation and Metal Analysis: Collected organisms were transported in aerated, chilled seawater and processed within four hours. After thorough rinsing with ultrapure distilled water, individuals were dissected to isolate the tunic. Samples from 15 individuals per species were pooled, oven-dried at 60 °C to a constant weight and ground to a fine powder using agate mortars to prevent metal contamination.

Acid Digestion and Metal Quantification: Acid digestion followed a modified EPA Method 3050B. One gram of body tissue was digested with 15 mL of concentrated HNO_3 (69%) and 5 mL of H_2O_2 (30%) in Teflon vessels using a MARS 6 microwave digestion system (CEM Corporation, USA). Digests were evaporated to near dryness, reconstituted in 2% HNO_3 and filtered through 0.45 µm PTFE membranes[21].

Metals (Ca, Mg, K, Fe, Zn, Cu and V) were quantified using a Shimadzu AA-6300 atomic absorption spectrometer with both flame (FAA) and graphite furnace (GFAA) modes. Calibration standards (ICP-grade, Merck) ranged from 0.1–100 mg/L. Recovery tests using NIST SRM 1566b (oyster tissue) showed 92–107% recovery; duplicate analyses exhibited <5% relative standard deviation (RSD).

Transplantation Experiment: To examine site-dependent metal uptake, *P. arabica* (vanadium accumulator) and *D.psamathodes* (iron accumulator) were transplanted from their native habitats to three pollution-gradient sites in Thoothukudi Harbour. Specimens were affixed to PVC panels and exposed for 60 days. Tissue samples (n = 5 per species per site) were collected on days 15, 30 and 60, digested and analysed by AAS. Ambient water samples from each site were also assessed to correlate environmental metal concentrations with tissue accumulation[45,51].

Protein Extraction and Metal-Binding Affinity Assays: The whole-body tissue of *P. arabica* was homogenised in ice-cold Tris-HCl buffer (pH 7.4) and centrifuged at 10,000×g for 15 min. Supernatants were fractionated using Sephadex G-75 columns[38]. Fractions containing elevated metal concentrations (as determined by AAS) were analysed by SDS-PAGE. Metal-protein complexes were confirmed through EDTA-based chelation assays. Stability constants (log K) were determined by ligand competition using 8-hydroxyquinoline[6].

Biofiltration Assay: To evaluate the bioremediation potential of *P. arabica*, a 100L flow-through system was constructed and dosed with synthetic wastewater containing 5 ppm vanadium (as $NaVO_3$). Ascidians (n = 20) were maintained in triplicate tanks; control tanks without ascidians were run in parallel[10,42]. Water samples were collected daily for 30 days and analysed using graphite furnace atomic absorption spectrometry (GFAA). Ascidian body tissues were sampled on days 0, 15 and 30 to assess uptake.

Metal Desorption Experiment: To assess retention behaviour, *P. arabica* and *D. psamathodes* collected from polluted sites were acclimated in laboratory seawater for 48h and transferred to 50L tanks containing filtered, metal-free seawater (salinity 35 ppt, pH 8.1). Tanks were replenished daily to prevent reabsorption. Five individuals per species were sacrificed on days 0, 7, 14, 21 and 28 for metal analysis[20,50]. Desorption rates were modelled using first-order kinetics.

Statistical Analysis: All data were tested for normality using the Shapiro–Wilk test prior to analysis. One-way ANOVA was used to evaluate differences in metal accumulation across species and suborders while Tukey's HSD post hoc test was applied for multiple comparisons. For multivariate analysis, Principal Component Analysis (PCA) was conducted on log-transformed metal concentration data to visualise suborder-level clustering. In the transplantation and desorption experiments, linear regression was used to model metal uptake or release over time and paired t-tests compared with initial and final metal concentrations. All statistical analyses were performed using PAST4 software with a significance threshold of $\alpha = 0.05$.

Results and Discussion

Suborder-Specific Metal Accumulation: Elemental analysis revealed distinct suborder-linked trends in metal accumulation. Magnesium was the most abundant metal across species, ranging from 458.63 ppm/g (*Trididemnum clinides*) to 1378.92 ppm/g (*Phallusia nigra)*. This was followed by calcium, potassium and iron, with vanadium, zinc and copper, present at lower tissue concentrations. The overall metal concentration hierarchy was: Mg > Ca > K > Fe > V > Zn > Cu.

Aplousobranchia species such as *Didemnum psamathodes* exhibited exceptionally high iron accumulation (780.68 ppm/g), nearly 300-fold higher than seawater background levels. Phlebobranchia members, especially *Phallusia arabica*, displayed vanadium concentrations up to 55.1 ppm/g, confirming the functional role of vanadocytes.

In contrast, Stolidobranchia, represented by *Microcosmus squamiger*, recorded the highest calcium content (1062.3 ppm/g), likely associated with structural demands of its robust tunic. These results suggest evolutionary specialisation in trace metal uptake across ascidian suborders.

The observed accumulation profiles reflect fundamental physiological and biochemical distinctions among ascidian suborders. The extreme iron concentration in *Didemnum psamathodes* (780.68 ppm/g) aligns with its tunic-localised ferritin-like storage mechanisms[52] and potentially enhanced filtration efficiency, enabling bulk water processing. The predominance of non-native ascidians (91%) in industrially stressed habitats further supports the hypothesis of adaptive metal tolerance[14,33]. *Phallusia arabica* dominance in vanadium-rich sites, with tissue loads reaching 55.1 ppm/g, underscores its capacity to sequester toxic metals without apparent physiological impairment. Native species were largely confined to reference areas, consistent with sensitivity to metal contamination[30].

Phlebobranchs such as *P. arabica* continue to demonstrate functional vanadocyte activity, a phenomenon first reported by Henze[21]. Our results suggest elevated transporter efficiency in tropical species, potentially contributing to the high vanadium levels compared to temperate populations[49]. Notably, the low copper levels observed (0.49–2.3 ppm/g) may support the theory of competitive inhibition, where vanadium displaces copper at metal-binding sites[48].

Interspecies Variability and Statistical Analysis: One-way ANOVA revealed significant interspecies differences in metal concentrations ($F = 42.44$, $p < 0.0001$), while intraspecies variation was statistically insignificant ($F = 1.221$, $p = 0.3018$). Principal Component Analysis (PCA) of log-transformed metal data accounted for 82% of the total variance, clearly separating the three suborders. PC1 (58.3%) was associated with iron and magnesium, clustering Aplousobranchia species.

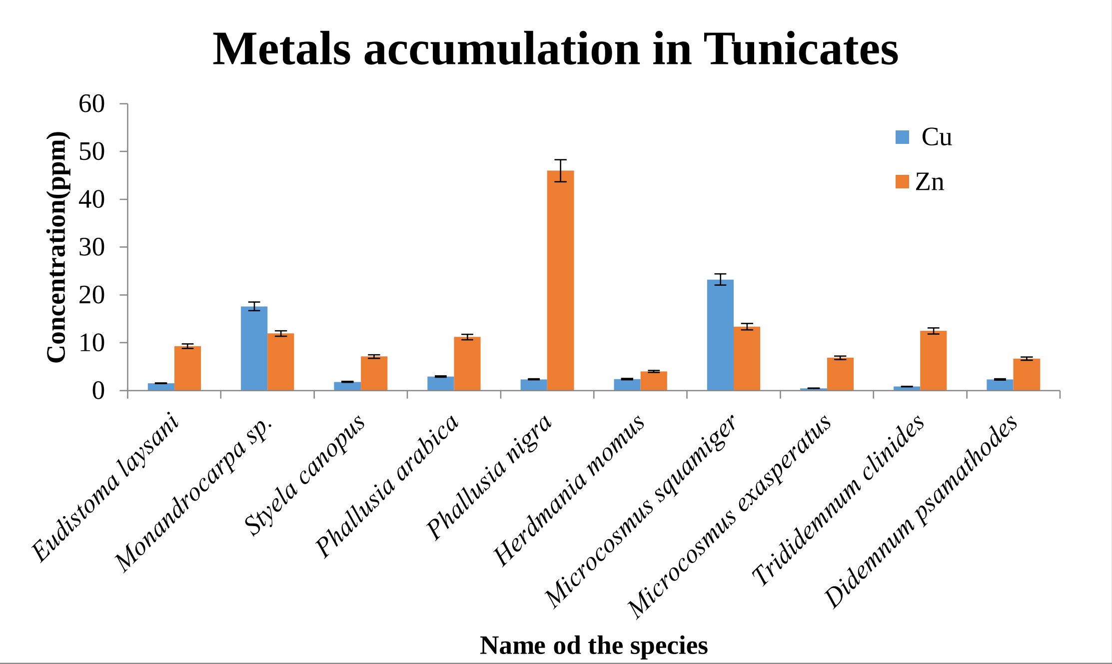

Figure 1: Cu and Zn Concentrations in ascidian tissues across species, Bar graph showing copper and zinc levels in ten ascidian species. *Phallusia nigra* exhibited the highest zinc accumulation, while *M.squamiger* showed the highest copper content, indicating species-specific uptake trends for trace metals.

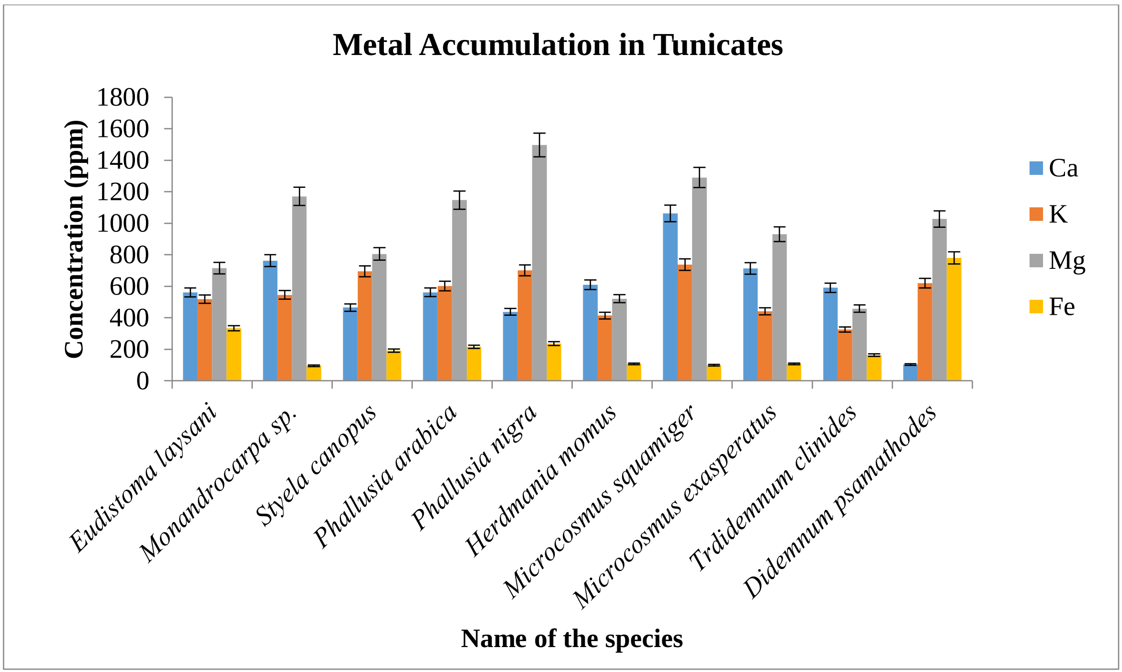

Figure 2: Comparative accumulation of Ca, Mg, K and Fe across Ascidian Species, this figure displays the concentrations of major metals (Ca, Mg, K, Fe) across species. *M. squamiger* and *P.nigra* showed peak levels of calcium and magnesium, respectively, while *D. psamathodes* had exceptionally high iron content.

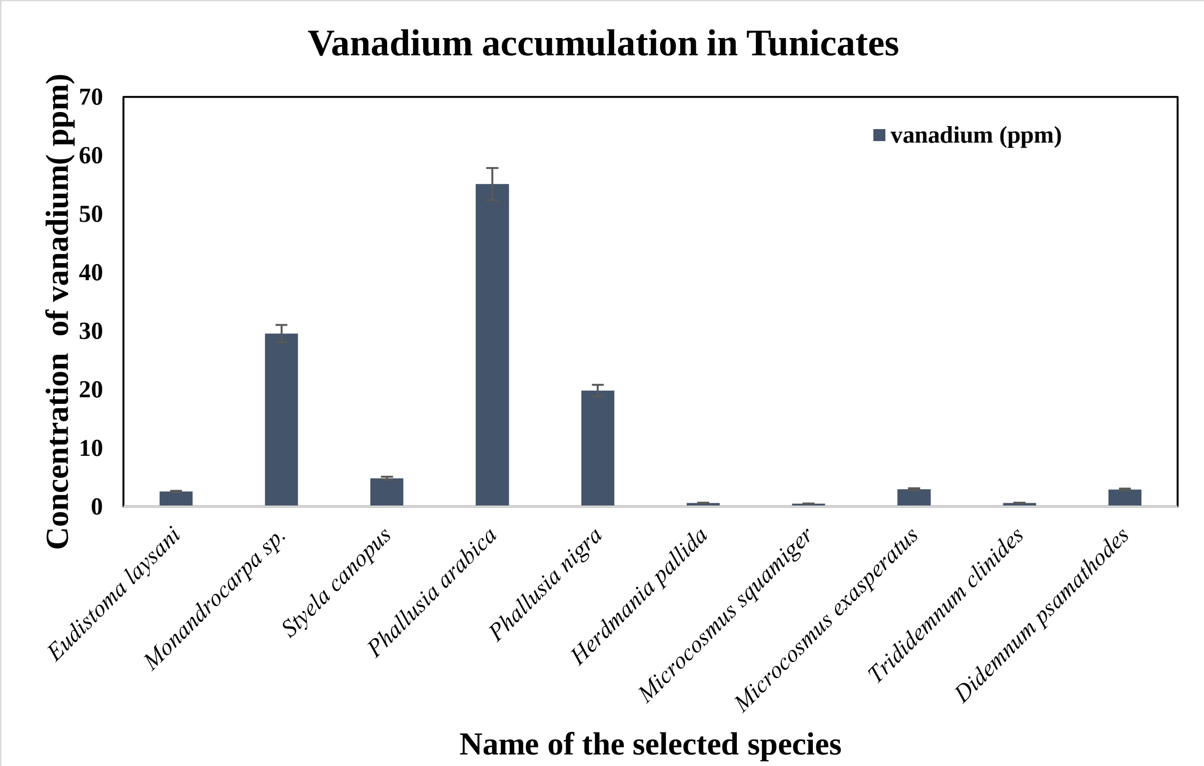

Figure 3: Vanadium concentrations in body tissues of ten ascidian species, Bar graph depicting vanadium accumulation. *P. arabica* recorded the highest levels, supporting its role as a key vanadium accumulator among the species studied.

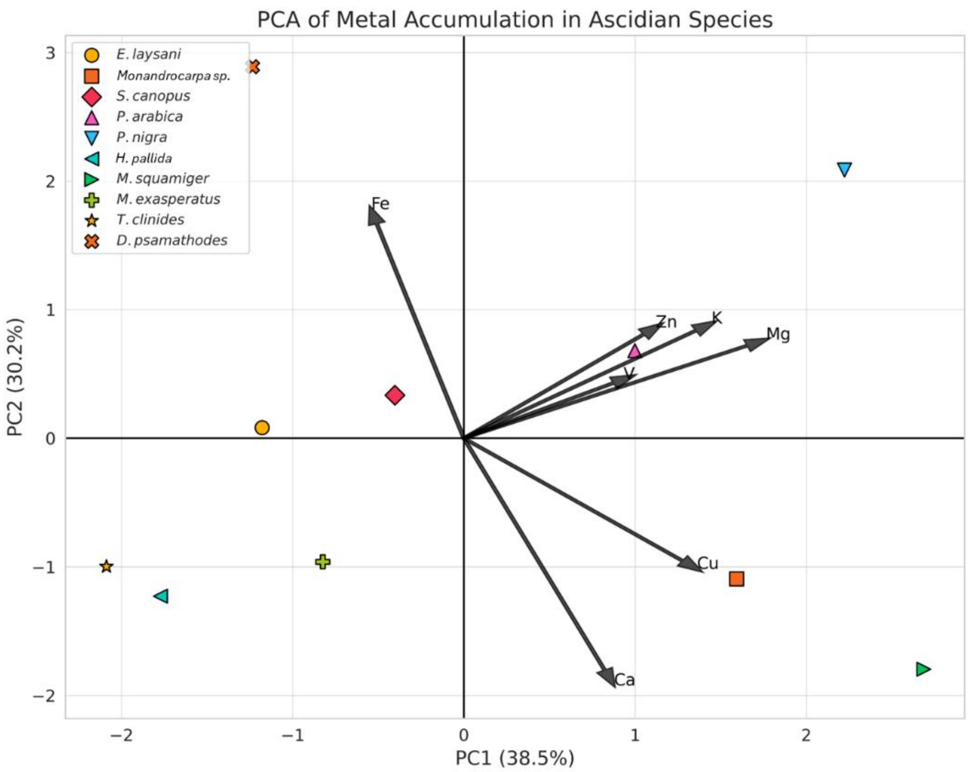

Figure 4: Principal Component Analysis of Metal Accumulation Patterns in Ten Ascidian Species Based on Seven Metal Concentrations. This PCA biplot illustrates interspecies variation and suborder-level clustering in metal accumulation (Ca, K, Mg, Fe, Cu, Zn, V). PC1 (x-axis) accounts for the majority of variance (58.3%) and primarily aligns with Fe and Mg, distinguishing Aplousobranchia. PC2 (y-axis) explains 23.7% of the variance and is associated with vanadium and zinc, clustering Phlebobranchia. Stolidobranchs group on the negative PC2 axis due to high calcium and potassium uptake. Each point represents a species, annotated for clarity.

Table 1
ANOVA results for metal accumulation among ascidian species and metal types

Source	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	356949	9	39661	F (9, 54) = 1.221	P=0.3018
Column Factor	8270943	6	1378490	F (6, 54) = 42.44	P<0.0001
Residual	1753780	54	32477		

Table 2
Trace Metal Concentrations in Ten Ascidian Species (ppm/g Dry Weight)

Name of the species	Amount of metal concentration (ppm)						
	Ca	K	Mg	Fe	Cu	Zn	V
Eudistoma laysani	561.56	518.11	715.28	333.32	1.52	9.27	2.57
Monandrocarpa sp	762.7	545.04	1170.71	95.81	17.61	11.93	29.56
Styela canopus	465.05	695.51	805.53	191.55	1.82	7.12	4.86
Phallusia arabica	562.11	601.74	1146.97	215.79	2.92	11.21	55.1
Phallusia nigra	438.12	700.89	1496.35	236.6	2.36	45.98	19.83
Herdmania pallida	609.66	414.21	521.42	108.17	2.39	4.03	0.62
Microcosmus squamiger	1062.29	737.01	1290.56	98.75	23.21	13.38	0.51
Microcosmus exasperatus	713.34	441.77	930.54	107.56	0.49	6.85	2.98
Trididemnum clinides	591.15	325.51	458.63	163.27	0.84	12.48	0.63
Didemnum psamathodes	104.06	619.8	1027.12	780.68	2.32	6.65	2.93

Table 3
Time-dependent Metal Uptake in Transplanted Ascidians (ppm/g Dry Weight)

Species	Site	Day 15	Day 30	Day 60
P. arabica	Harbor (High V)	22.1	35.6	48.7
P. arabica	Reference	5.3	8.9	12.3
D. psamathodes	Steel plant	450.2	780.0	782.4

PC2 (23.7%) correlated with vanadium and zinc, distinguishing Phlebobranchia. Stolidobranchia grouped along the negative PC2, characterised by elevated calcium and potassium concentrations. These findings support the view that metal uptake is both species-specific and phylogenetically conserved.

Site-Specific Metal Accumulation: Metal concentrations in the selected ascidians species reflected site-specific pollution profiles. *P. arabica* from Thoothukudi harbour, located near vanadium slag disposal zones, exhibited elevated vanadium levels (55.1 ppm/g). *D. psamathodes* collected near steel mill effluent zones accumulated 780.68 ppm/g iron. *M.squamiger* from cement-rich areas of Thoothukudi, recorded 1062.3 ppm/g calcium. These site-specific profiles corresponded with known effluent compositions. These species-specific accumulation patterns corresponded closely with known industrial effluent compositions. The spatial distribution of vanadium in *P. arabica* tracked proximity to slag disposal points, while iron in *D. psamathodes* aligned with steel plant discharge zones.

Notably, the 15-fold variation in vanadium levels across sites could not have been captured by spot water sampling alone, underscoring the superior ecological resolution ascidians offer as bioindicators. Different ascidian suborders demonstrated niche-specific monitoring potential: Aplousobranchs serve as early detectors of ferrous effluents[44]. Phlebobranchs are optimal for identifying refinery-associated vanadium contamination and Stolidobranchs may indicate carbonate-rich stress due to their calcium-binding matrices[41].

Bioconcentration Factors: Bioconcentration factors (BCFs), calculated as [Metal] tissue / [Metal] water, demonstrated substantial metal accumulation efficiency. *P. arabica* showed a BCF of 1.2×10^6 for vanadium, *D. psamathodes* had 4.3×10^5 for iron and *M. squamiger* registered 2.8×10^4 for calcium. These values far exceeded those observed in other sympatric invertebrates, reaffirming the exceptional metal accumulation capacity of ascidians.

Transplantation Experiment: Transplanted *P. arabica* at high-vanadium sites exhibited linear vanadium uptake ($R^2 = 0.89$), increasing from 22.1 ppm/g on day 15 to 48.7 ppm/g on day 60. In contrast, individuals at reference sites accumulated only 12.3 ppm/g. Similarly, *D. psamathodes* transplanted near steel outfalls reached saturation with iron saturation by day 30 (780.0 ppm/g), with negligible further uptake thereafter.

These results confirm that ascidian metal accumulation is dynamically responsive to environmental gradients. Transplantation experiments confirmed that ascidian metal accumulation responds dynamically to ambient exposure, as seen by the linear increase of vanadium and saturation of iron levels in *P. arabica* and *D. psamathodes* respectively, underscoring their potential as sensitive tools in active biomonitoring programmes[43].

Metal-Binding Proteins: Chromatographic analysis identified metal-affinitive proteins responsible for trace metal sequestration. A 12-kDa protein from *P. arabica* bound vanadium with a stability constant of log K = 7.2 ± 0.3. A 28-kDa tunic protein from *D. psamathodes* bound iron with log K = 6.8 ± 0.2. EDTA chelation liberated more than 85% of the bound metals, confirming protein-mediated storage. The protein-binding affinity assays reinforce the proposed mechanisms: the 12kDa vanadium-binding protein in *P. arabica* (log K = 7.2) and a 28kDa iron-binding protein in *D. psamathodes* (log K = 6.8). These biochemical specialisations further validate the retention patterns observed in the desorption study[37,39].

Biofiltration Assay: In biofiltration trials, *P. arabica* reduced vanadium concentrations from 5 ppm to 0.8 ppm within 30 days, achieving 84% removal efficiency. Control tanks without ascidians showed negligible change (4.7 ppm), confirming the role of biological uptake. Correspondingly, tissue vanadium levels in *P. arabica* increased from 2.1 to 52.4 ppm/g, validating its uptake capacity and feasibility for *in situ* remediation. This experiment demonstrated not only effective vanadium removal but also high tissue retention (78.4%), establishing *P. arabica* as a promising candidate for closed-loop bioremediation systems.

Metal Desorption: In clean seawater, *P. arabica* retained 78.4% of its accumulated vanadium after 28 days, with a first-order desorption rate constant of k = 0.008 day^{-1}. In contrast, *D. psamathodes* released 62.3% of its iron content (k = 0.032 day^{-1}), indicating a more labile storage mechanism. These results reflect species-specific physiological differences in metal retention. Retention patterns were consistent with the stability constants determined in protein-binding affinity assays, supporting a biochemical basis for interspecific variation. The strong vanadium–protein interactions in *P. arabica* account for its slower desorption rate, whereas iron appears to be more weakly bound in *D. psamathodes*. These physiological traits may be leveraged for differential metal recovery applications for instance, controlled elution of iron versus prolonged sequestration of vanadium[7,53].

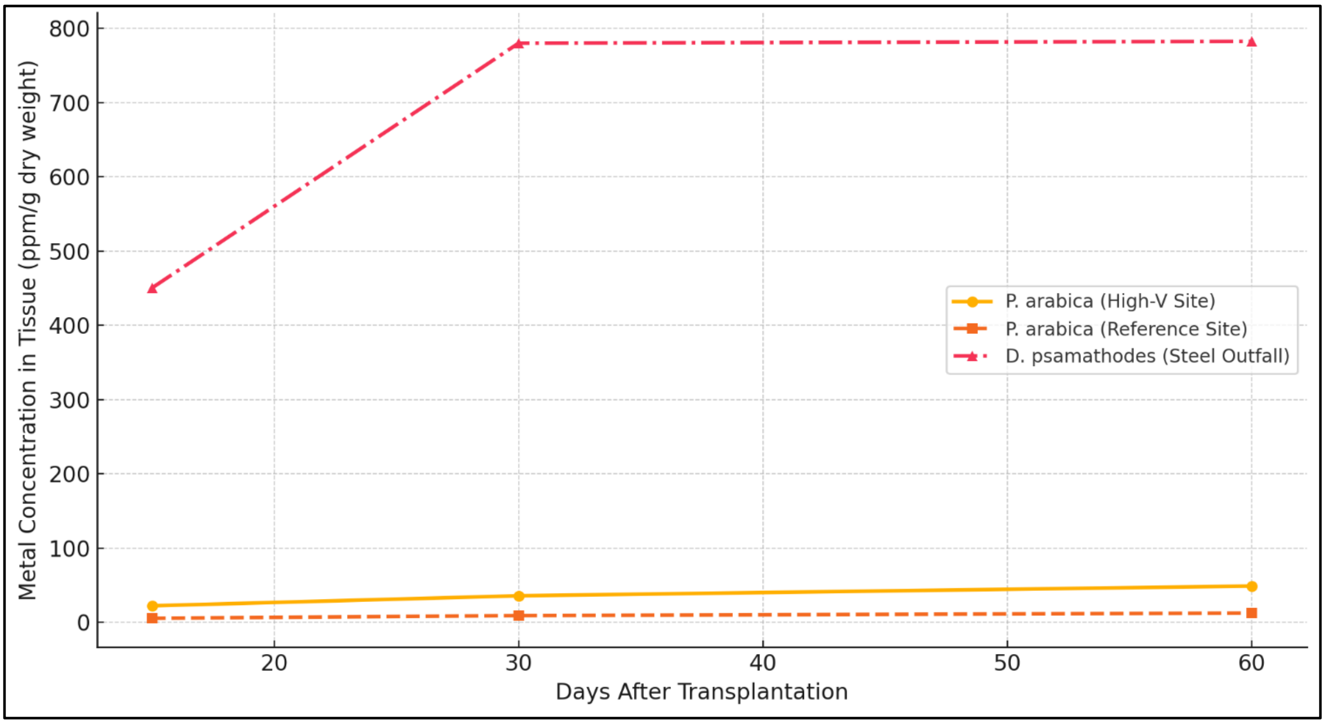

Figure 5: Time-dependent uptake of Vanadium and Iron in transplanted ascidians monitored Over a 60-Day Period. This line graph presents the kinetics of metal accumulation in *Phallusia arabica* and *Didemnum psamathodes* during transplantation across pollution gradients. Vanadium levels in P. arabica increased steadily at the high-V site (22.1 → 48.7 ppm/g), while accumulation was limited at the reference site. *D. psamathodes* showed rapid iron saturation by Day 30 near steel outfalls. These results demonstrate environmentally responsive uptake.

Table 4
Desorption Rates and Metal Retention in Ascidian Tissues After 28 Days in Clean Seawater

Species	Metal	Initial (ppm/g)	Final (ppm/g)	% Retained	Desorption Rate (k, day^{-1})
P. arabica	Vanadium	55.1 ± 2.4	43.2 ± 1.8	78.4	0.008 ± 0.001
D. psamathodes	Iron	780.7 ± 35.6	294.5 ± 22.1	37.7	0.032 ± 0.003

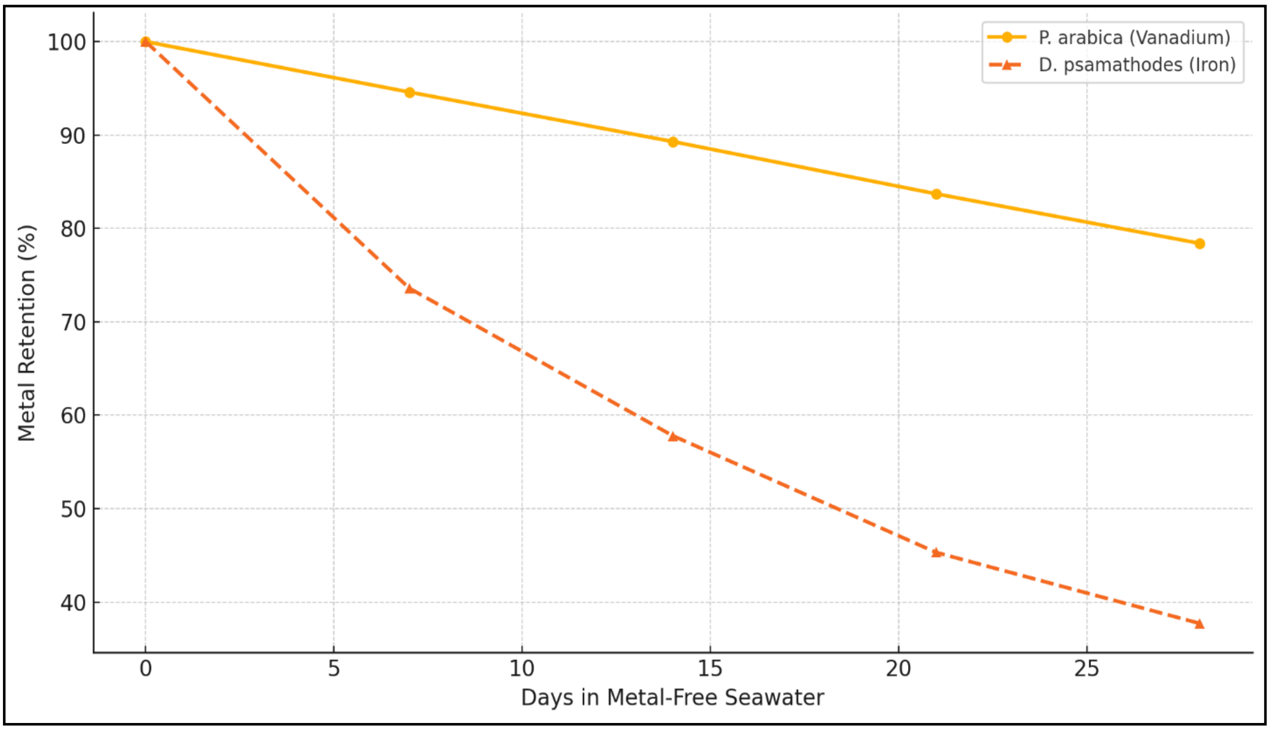

Figure 6: Desorption Kinetics of Vanadium and Iron in Ascidian Tissues during a 28-Day exposure to clean seawater. This graph depicts the percentage of retained metals in ascidian tissues over time. *Phallusia arabica* retained 78.4% of vanadium after 28 days, showing slow desorption ($k = 0.008$ day^{-1}), indicative of strong protein binding. In contrast, Didemnum psamathodes rapidly lost iron (only 37.7% retained), with a higher desorption rate ($k = 0.032$ day^{-1}), suggesting a more labile storage mechanism. These patterns reflect suborder-specific metal retention physiology.

Conclusion

This comprehensive investigation into trace metal accumulation across ten ascidian species along the Thoothukudi coast confirms their dual role as sensitive bioindicators and effective bioremediators. Suborder-specific uptake patterns were clearly observed: *Phallusia arabica* (Phlebobranchia) concentrated vanadium to levels exceeding 55 ppm/g, *Didemnum psamathodes* (Aplousobranchia) accumulated iron up to 780.68 ppm/g and *Microcosmus squamiger* (Stolidobranchia) retained over 1062 ppm/g of calcium. These taxonomic trends are driven by physiological adaptations including the presence of vanadocytes, metal-binding proteins and tissue compartmentalisation strategies.

The statistically significant interspecies differences observed across metal concentrations ($F = 42.44$, $p < 0.0001$), along with consistent intraspecies profiles ($F = 1.221$, $p = 0.3018$) indicate that ascidians respond predictably to environmental metal loads. Spatial mapping of tissue metal concentrations closely aligned with known industrial discharge sources, validating the high ecological resolution ascidians offer for coastal environmental monitoring. The transplantation trials demonstrated site-responsive uptake in real time while biofiltration assays with *P. arabica* achieved 84% vanadium removal from synthetic wastewater. Protein-binding affinity studies further supported species-specific retention mechanisms, highlighting potential applications for metal recovery or selective extraction.

These findings position ascidians as valuable resources for both passive and active biomonitoring frameworks. Future studies should prioritise:

(1) Proteomic and transcriptomic analysis of metal-binding proteins
(2) Standardisation of transplantation models for active surveillance and
(3) Field trials of ascidian-based bioremediation systems across diverse coastal ecosystems.

Additionally, desorption assays indicate differential metal lability among species. *P. arabica* retained vanadium effectively, whereas *D. psamathodes* released iron rapidly, suggesting tailored use cases in recovery and clean-up efforts. These species-specific behaviours offer a new direction in eco-engineering, where ascidians may serve both as pollution sentinels and as tools for sustainable trace metal recovery. In summary, the ascidians studied here exemplify nature’s capacity for adaptation under duress and offer an untapped biological solution to address marine metal pollution in both ecological and industrial contexts.

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