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Diatom colonisation and biofilm metal bioaccumulation: Can Indigenous Knowledge Systems aid the ecological engineering of urban coastlines?

J. Ndaba[a,b,*], P. Cotiyane-Pondo[c,d,e], L. Human[c,d], E. Puccinelli[a,f], P. Pieterse[g], P. Pattrick[c], F. Porri[a,b]

[a] Rhodes University, Department of Ichthyology & Fisheries Science, Makhanda, South Africa
[b] South African Institute for Aquatic Biodiversity (SAIAB), Makhanda, South Africa
[c] South African Environmental Observation Network (SAEON), Elwandle Coastal Node, Gqeberha, South Africa
[d] Institute for Coastal and Marine Research (CMR), Nelson Mandela University, Gqeberha, South Africa
[e] Department of Biodiversity and Conservation Biology, University of Western Cape, Cape Town, South Africa
[f] Department of Coastal System, Royal Netherlands Institute for Sea Research (NIOZ), Den Hoorn Texel, the Netherlands
[g] Spectrum Analytical Facility, University of Johannesburg, Auckland Park, South Africa

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A B S T R A C T

Marine engineered structures alter the coastal ecosystems' functionality, replacing natural substrates with flat surfaces, often negatively impacting biodiversity. While providing coastal protection, artificial structures can hinder the initial colonisation by biofilm and the recruitment of coastal species. Greening the grey infrastructure through nature-based interventions is increasingly used to enhance biodiversity in artificial structures. This study explores the potential of the sedge *Cyperus textilis* and its Indigenous Knowledge applications as a substrate for coastal eco-engineering at an urbanised site on the southeast coast of South Africa. Diatom succession and metal bioaccumulation on the deployed trial-version designs (*imizi* structures) were monitored for a month, with samples collected at six, twelve, twenty-four, seventy-two hours, one and two weeks, and one month after deployment. Water quality, including dissolved nutrients and metal concentrations, were assessed near the substrates. Findings indicated that diatom colonisation occurred within twelve hours, with initial species including *Fragilaria pulchella*, *Neofragilaria nicobarica*, *Navicula* sp. and *Grammatophora undulata*, followed by a significant increase (4.6 times) in species diversity from 5 to 23 diatoms after one week. Metal bioaccumulation of aluminium, iron, zinc, manganese and arsenic was higher in the biofilm developing on the substrate compared to its surrounding environment (*imizi* substrate and water), suggesting the potential bioremediating capabilities of the biofilm on the nature-based material. These findings indicate the potential suitability of using Indigenous Knowledge-based materials for coastal eco-engineering practices as promoters of primary productivity, with the added potential of the plant *C. textilis* for bioremediation of toxic metals such as arsenic.

1. Introduction

The expansion of engineered structures, driven by local and global coastline development, has resulted in rapid and cumulative changes in the natural seascape over the years (Firth et al., 2016; Firth et al., 2024). This is evident in the proliferation of artificial structures such as seawalls and jetties, causing the deterioration of natural habitats and water quality (Fatoki and Mathabatha, 2001; Bulleri and Chapman, 2010; Gotama et al., 2024). Artificial structures are usually less structurally complex and thus have fewer microhabitats available (e.g., crevices and low surface rugosity) than adjacent natural hard habitats (Bulleri and Chapman, 2010; Strain et al., 2017).

Decreased habitat complexity in artificial structures primarily impacts the microbial colonisation and recruitment of coastal fish and invertebrates (Anderson and Underwood, 1997; Dobretsov et al., 2013). This successional alteration often leads to colonisation by non-indigenous species that are generally favoured in urban coastal environments due to their opportunistic and quick ability to colonise diverse habitats (Jackson and McIlvenny, 2011; MacArthur et al., 2020). The impacts of ocean sprawl are not limited to changes in the benthic assemblages, but also include water quality. Several studies indicate an increase in anthropogenic effluents along urbanised coastal areas. These

* Corresponding author at: Rhodes University, Department of Ichthyology & Fisheries Science, Makhanda, South Africa.
E-mail address: j.ndaba@saiab.nrf.ac.za (J. Ndaba).

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contain several elevated levels of trace elements (e.g., aluminium- Al, and copper- Cu) and toxic heavy metals (e.g., arsenic- As, and lead- Pb) either leaching from paint/substrate from artificial structures and persistent organic compounds or from impacted upstream river systems (Hingston et al., 2001; Singh and Turner, 2009; Barletta et al., 2019).

Given the multiple effects of ocean sprawl on natural coastal environments, interventions have been developed to regain and maintain habitat quality and functionality (Perkol-Finkel and Sella, 2014; Martins et al., 2016; Morris et al., 2017; Hall et al., 2018; Perkol-Finkel et al., 2018). Several ecological engineering interventions, merging ecological principles with engineering practices (see review Firth et al., 2024), have been developed, particularly in coastal environments. Eco-engineered structures, made of concrete of natural stone that mimics the natural topography of intertidal and subtidal habitats, have been designed to increase recruitment and colonisation of benthic species and increase refuge area for pelagic fish species, whilst promoting diverse functional groups (Chapman and Blockley, 2009; Martins et al., 2010; Hall et al., 2018). The choice of concrete in ecological engineering applications is often due to the strength of such material, which withstands harsh wave action and salt erosion, while concurrently being easily manipulated to a fine scale (Strain et al., 2017). Although concrete has been proven to be a suitable material for coastal eco-engineering, some disadvantages may harm benthic coastal species (McManus et al., 2018). These include the leaching of particles into the environment, an increase in the carbon footprint, and its non-degradability nature (Zhang et al., 2014; McManus et al., 2018; Cordoba and Irassar, 2023). Some adapted eco-engineered designs have however incorporated nature-based/natural components, such as natural fibres (hemp) and oyster shells within the concrete, to reduce carbon emissions in coastal environments (Perkol-Finkel and Sella, 2014; Rupasinghe et al., 2024). The benefits of using nature-based structures in coastal ecological engineering have been highlighted. For instance, nature-based substrates can support higher abundances of marine species than artificial habitats (Strain et al., 2017). A 12-month comparison of colonisation on hemp, shell and cement also indicated that hemp-and shell-incorporated blends hosted higher algal cover compared to cement structures, and those communities included several habitat engineers such as *Fucus* canopy algae, diatoms, and blue-green algae (McManus et al., 2018; Dodds et al., 2022).

Since primary productivity dictates the diversity, succession, and abundance of subsequent functional communities (Salamone et al., 2016), the mechanisms associated with the initial biofilm colonisation are of fundamental interest in eco-engineering science. Biofilms perform several vital functions in coastal environments, including mediating recruitment and settlement of larval fish and invertebrates, primary production in trophic food webs, nutrient recycling, and bioremediation of anthropogenic pollutants (i.e., metals) (Schorer and Eisele, 1997; Ferguson et al., 2021; Tuck et al., 2022). The initial colonisation on rigid substrates in coastal environments consists of ubiquitous aggregates of eukaryotic and prokaryotic cells enclosed by an extracellular polymeric substance (EPS; Salta et al., 2013). Given that the majority of anthropogenic effects noticeable in later growth phases are already prominent during early succession (Salta et al., 2013), monitoring of biofilm development is crucial for understanding the impact of ecologically designed structures during the primary stages of species settlement and succession.

The sedge *Cyperus textilis*, also known as *uluzi* (singular); *imizi* (plural) in the isiXhosa language, is a plant harvested near river streams in rural communities in the province of the Eastern Cape and Kwa-Zulu Natal, South Africa (Kepe, 2003; Makhado and Kepe, 2006). This plant is used for several Indigenous applications, including the creation of *icantsi* (sleeping mats), *inceke* (collecting basket), and in some cases for building purposes (e.g., incorporated in mud to strengthen buildings) (Kepe, 2003; Makhado and Kepe, 2006). *Cyperus textilis* is generally used by local communities because the plant is easily found and accessible, and its stems are easy to manipulate for weaving, but also for its longevity and tensile strength (Kepe, 2003). *Imizi*'s Indigenous application is essential in rural coastal communities' livelihood and economic empowerment and has recently been proposed for coastal ecological engineering applications (Porri et al., 2023).

This study serves as an initial understanding of the early stages of diatom succession and biofilm formation on *C. textilis*, to assess the potential viability of this plant, and hence provide a crucial foundation for the broader application of this Indigenous knowledge and practice to coastal eco-engineering. An understanding of the early succession of diatom colonisation and metal biofilm accumulation is essential as the initial phases of formation can significantly influence the long-term development and stability of microphytobenthic communities on the Indigenous material. In this study, we therefore aim to investigate the role of *C. textilis* as a potential candidate material for co-creating nature-based prototypes in eco-engineering interventions at urban sites along the southeast coast of South Africa. To address this, we assessed i) the benthic diatoms colonisation and successional changes on *C. textilis*; and ii) the metal accumulation on *C. textilis* and its associated biofilm from trial-version mats deployed at an urban site over a month. From a South African perspective, co-creating nature-based eco-engineered structures using Indigenous Knowledge Systems and modern ecological principles is essential to inclusively add participation by the local rural community in resolving environmental challenges. These interventions, directed at promoting coastal species diversity and urban ecosystem functionality, increase societal agency, preserving heritage while promoting economic and social empowerment of rural coastal communities (see Porri et al., 2023).

2. Materials and methods

Cyperus textilis (hereafter *imizi*) stems were collected in July 2022 at Hamburg, South Africa (33°15′17.6"S 27°25′42.4″E) under the guidance of knowledge bearers and in accordance with customary Indigenous practices. The *imizi* stems were sun-dried, sectioned and fastened with a fishing line to keep the structures intact (three horizontal lines; hereafter *imizi* structures). In October 2022, a total of twenty-one *20* × *10* cm *imizi* structures were deployed at the Royal Alfred Marina (hereafter Port Alfred), Eastern Cape (33° 36′ 1.62" S, 26° 53′ 47.80″ E), an urban site situated near the mouth of the Kowie river estuary (Fig. 1).

The Port Alfred site consists of artificial structures (seawalls) lining the channel of the Kowie river estuary, and the interior section of the marina is dominated by seawater influxes and reduced water inflow that protect the marina from intense wave action. Due to the restricted access, the marina also provides a secure site to avoid human interference and damage to the trial structures, presenting itself as a suitable environment for assessing the role of the *imizi* structures for coastal ecological engineering applications.

In the field, *20* × *10* cm *imizi* structures were deployed underwater held by a fishing line with weights to ensure they did not float. Cognisant that microorganisms that microorganisms can become established shortly after deployment, we deployed the *imizi* structures approximately five minutes apart. To characterise biofilm formation, successional changes and metal accumulation over several temporal scales, triplicate structures were collected randomly after six hours (T1) of submergence, followed by twelve hours (T2), twenty-four hours (T3), three days (T4), seven days (T5), fourteen days (T6) and thirty days (T7).

2.1. Water quality

2.1.1. Physico-chemical and nutrient variables

Physico-chemical parameters (temperature -°C, dissolved oxygen -mg l^{-1}, and pH) were measured in triplicate near (<20 cm) the deployed structures using a HI98194 multiparameter water quality meter (Hanna Instruments, Inc., United Kingdom). Salinity was measured using a HI96822 digital refractometer (Hanna instruments) at each time interval. Thereafter, triplicate 500 ml water samples were collected at the

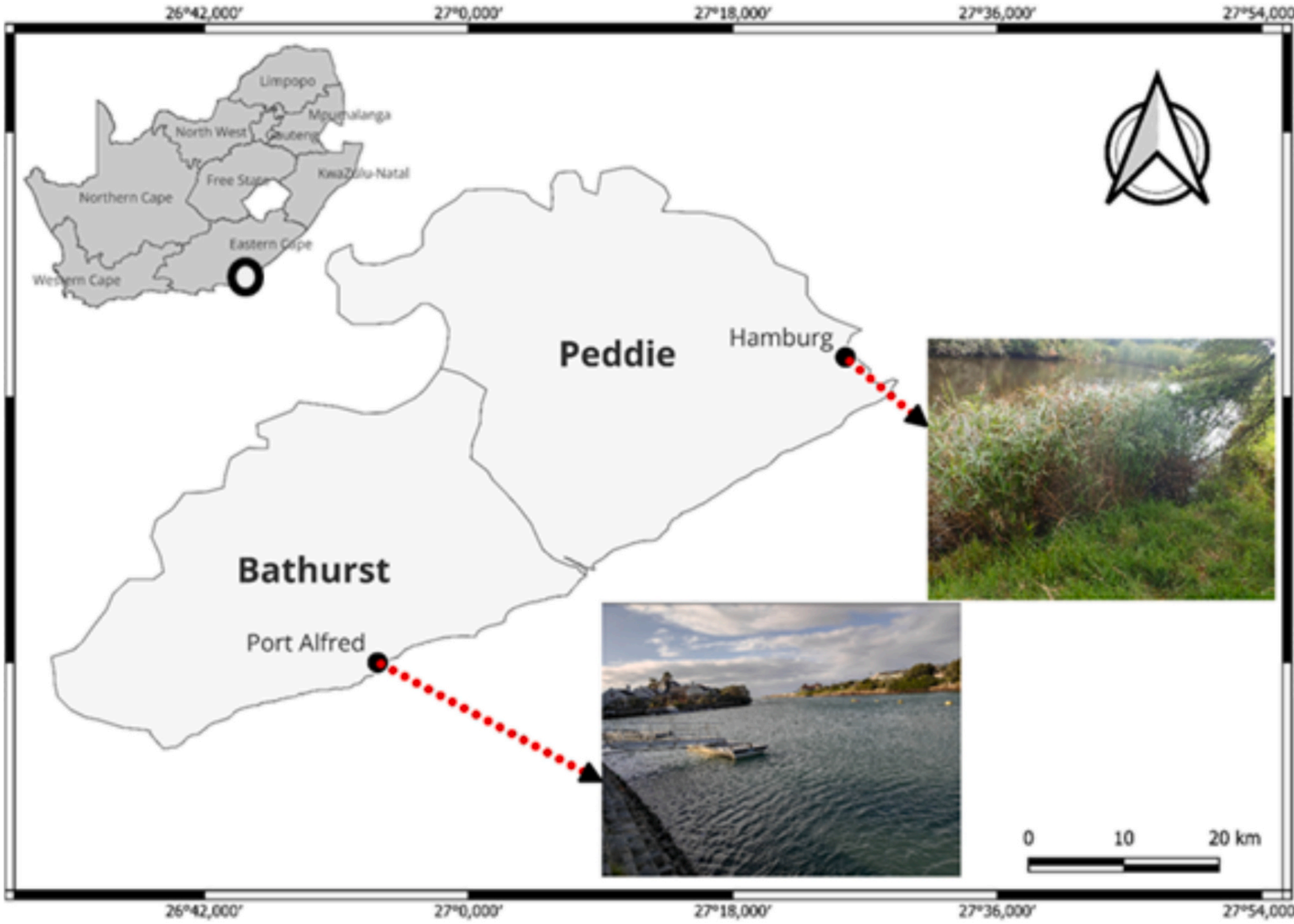

Fig. 1. Map indicating *Cyperus textilis* location in Hamburg and the deployment site at the Royal Alfred Marina (Port Alfred), both located in the Eastern Cape along the southeast coast of South Africa

same depth and gravity-filtered using Whatman GF/F filters (47-mm diameter, 0.7-μm pore size). The triplicate filtrates and filter paper containing suspended organic matter were collected into 50 ml falcon tubes and aluminium foil and used for nutrient and chlorophyll-*a* analyses, respectively. Samples were transported on ice to a − 20 °C freezer until analysis. Nutrient concentrations of dissolved nitrate, nitrite, ammonium, phosphate, and silicate were quantified using a SEAL Auto-Analyser (SEAL Analytical, Inc.), following protocols described in (Murphy and Riley, 1962; Armstrong et al., 1967; Grasshof et al., 1983). To determine the water column chlorophyll-*a* biomass, the GF/F filters were incubated in 10 ml of 95 % ethanol for 24 h at 4 °C (extraction process). The samples were then re-filtered and chlorophyll-*a* concentrations were determined before and after acidification with 1 N HCl (Nusch, 1980) using a Hitachi UH5300 Spectrophotometer.

2.1.2. *Metal analysis*

To determine the levels of dissolved metals adjacent to the deployed *imizi* structures, 500 ml aliquots of water samples were collected in the same area where structures were deployed (∼ 5 cm) and filtered on Whatman GF/F filters (pore size: 0.7 μm) at each time interval. Thereafter, 15 ml of filtered water samples were acidified with Suprapur HNO_3 to keep metals in solution and stored in a cool, dark space until analysis. At each time interval, triplicate *imizi* structures were randomly collected and the biofilm was scraped using sterile toothbrushes into containers with 300 ml distilled water. The homogenised samples were separated into two 150 ml aliquots, which were then transferred into storage containers; the first was used for metal analysis and stored at −20 °C, while the second aliquot was used for diatom identification and stored in 4 % formalin solution (see diatom method below). For metal analysis, the 150 ml biofilm samples were defrosted at room temperature, filtered on 0.65 μm nitrocellulose filter paper, freeze-dried and weighed. The very same triplicate *imizi* structures (without biofilm) were collected then placed in individual zip lock bags and transported on ice and stored at −20 °C. The *imizi* structures were oven dried at 60 °C for 24 h and homogenised using a stainless-steel blender. For each resulting *imizi* sample, ∼0.5 g aliquots were weighed. The biofilm and *imizi* samples were placed into 30 ml Teflon flasks, treated with Suprapur HNO_3, and digested for 20 min at 200 °C. The digested samples were filtered through Whatman Grade 1 filter papers into volumetric flasks and diluted to 50 ml with Milli-Q water.

Prior metal analysis, water, biofilm and *imizi* samples were diluted 10× using 1 % nitric acid. Aluminium (Al), nickel (Ni), cadmium (Cd), zinc (Zn), copper (Cu), lead (Pb), iron (Fe), arsenic (As) and manganese (Mn) were quantified using a NexION 300 Series Inductively Coupled Plasma Mass-Spectrometry (Perkin Elmar, United States of America). Internal standards (Sc, Rh and Ir) were added to correct for drift and matrix effects. The National Institute of Standards and Technology certified reference material 1640a was analysed for quality control, and all elements gave acceptable recoveries (Table S1). The sample and procedural/reagent blank were prepared using the same protocol as biofilm and *imizi* samples.

2.1.3. *Diatom analysis*

To taxonomically identify and analyse successional biofilm changes, homogenised aliquots of 25 ml were transferred into 150 ml glass beakers and repeatedly rinsed using Milli-Q®Type-1 ultrapure water to remove the formalin solution. Thereafter, samples were treated with 10 % HCl and further treated with concentrated nitric acid (HNO_3) and potassium dichromate ($K_2Cr_2O_7$) solution (Cotiyane-Pondo and Bornman, 2021). The cleaned material was mounted on glass microscope slides using Pleurax (Refractive Index: 1.7). Diatom valves were counted and identified to the lowest possible taxonomic level (genus or species) using a Zeiss AxioObserver A1 light microscope with Differential Interference Contrast (DIC) at 630× and 1000× (under oil immersion) magnification. Identification was based on a range of literature (Carmelo, 1997; Witkowski et al., 2000; Hoppenrath et al., 2009: Taylor et al., 2007; Spaulding et al., 2021; López-Fuerte et al., 2022).

2.1.4. Statistical analysis

Time differences in the physico-chemical parameters, nutrients, chlorophyll-*a* and levels of metals in water fraction and diatom community descriptors (species richness and abundance, Pielou 'J' evenness and Shannon diversity indexes) were investigated using either parametric or non-parametric tests, depending on the satisfaction of the assumptions of normality and homoscedasticity, tested using Shapiro-Wilk and Levene's tests, respectively. All analyses were performed using the '*ggstatplot*' R package (Patil, 2021). Dissolved concentrations of ammonium, nitrate, silicates, phosphate, Mn, Fe, Al, Zn and As were tested using a one-way ANOVAs with time interval as the sole factor, whereas nitrate and Pb were tested using the Kruskal-Wallis H test. When significant differences were reported, a Tukey and a Dunn-test post-hoc test for parametric and non-parametric test respectively. A p-adjusted Benjamin-Hochberg correction was applied for multiple testing (Benjamini and Hochberg, 1995). Due to the low abundance of species, resulting in numerous species having a count of less than 1 %, diatom species were pooled to genus levels and analysed for alpha diversity differences and diatom compositional differences amongst time intervals. Diatom compositional differences were assessed using the permutational multivariate ANOVA (PERMANOVA) amongst time intervals and visualised using a zero-adjusted Bray-Curtis dissimilarity coefficient non-metric multidimensional scaling (nMDS), using the '*vegan*' R package (Clarke et al., 2006). Based on significant differences observed amongst the time intervals in diatom community descriptors, a SIMPER analysis was used to assess which diatom genera most contributed to differences in compositions amongst time intervals. The ordinary least squares method was used to estimate the coefficients of the linear regression models conducted to assess the time relationship between the levels of metals in the water adjacent to the deployed *imizi* structures and their accumulation within the biofilm and *imizi* structures themselves. The goodness of fit was assessed by the adjusted R^2 and significance using the *p*-values with a confidence level set at 95 %, with p-values computed using a Wald t-distribution approximation. To determine the metal accumulation ability of the biofilm from the surrounding water and from the *imizi* structures, the following bioconcentration factors (BCF) formulae (Faburé et al., 2015) were used:

$$BCF_{water-biofilm} = C_{biofilm}/C_{water} \tag{1}$$

$$BCF_{imizi-biofilm} = C_{biofilm}/C_{imizi} \tag{2}$$

Where $C_{biofilm}$ and C_{imizi} are the mean metal concentrations in the biofilm and *imizi* (expressed as μg/kg), respectively, while C_{water} is the mean metal concentration in the water (μg/L).

3. Results

3.1. Environmental conditions

To characterise the water conditions adjacent to the *imizi* structures, differences in physico-chemical parameters, nutrients, chlorophyll-a and concentrations of metals in the water column were assessed (Table 1). No significant time differences were observed for pH ($H_6 = 9.75$, $p > 0.05$), temperature ($H_6 = 11.83$, $p > 0.05$), dissolved oxygen ($H_6 = 9.89$, $p > 0.05$), salinity ($H_6 = 12.61$, $p > 0.05$) and chlorophyll-*a* ($F_6 = 1.08$, $p > 0.05$).

Nutrient concentrations in the water adjacent to the *imizi* structures significantly decreased with time for ammonium ($F_6 = 10.57$, $p < 0.001$), nitrite ($F_6 = 9.76$, $p < 0.001$), nitrate ($H_6 = 16.93$, $p < 0.01$), silicate ($F_6 = 9.86$, $p < 0.0001$) and phosphate ($F_6 = 6.20$, $p < 0.01$). A post-hoc Tukey test revealed several significant differences between time intervals for ammonium, silicates and nitrite (Table S2). Significant differences observed for phosphate between time intervals were for T2 with T4 ($p < 0.05$) and T7 ($p < 0.05$) and T3 with T5 ($p < 0.05$). Although nitrate and nitrite significantly decreased over time, the post-hoc test only revealed significant differences for only nitrite (Table S2). The statistical comparison in metal concentrations indicated significant differences for Mn ($F_6 = 17.29$, $p < 0.0001$), Fe ($F_6 = 4.13$, $p < 0.01$), with a variably increasing trend, and Pb ($H_6 = 13.81$, $p < 0.05$) decreasing amongst time intervals (Table S3). No significant time differences were observed for Pb, Al, Zn, and As. In contrast, significant differences were observed for Mn between time intervals (Table S2).

3.2. Diatom community descriptors

The results of the one-way ANOVAs indicated a significant increase in species richness ($F_5 = 6.56$, $p < 0.001$), Shannon's diversity index ($F_5 = 6.55$, $p < 0.001$), abundance ($H_5 = 14.21$, $p < 0.05$) and Pielou J Eveness ($F_5 = 6.462$, $p < 0.001$) over the experimental period. A post-hoc Tukey test indicated significant differences ($p < 0.001$) for species richness and Shannon's diversity index and for Pielou J Evenness index (Table S4). Although the abundance of diatom species increased over time (Fig. 2), no significant differences were observed amongst time intervals based on the post-hoc Dunn's test.

A total of 100 diatom species belonging to 25 diatom genera were identified in the overall study (Fig. S1–2, Table S5). The abundance of diatom genera varied in the study, with low abundances and diversity in earlier time intervals (i.e., T1-T3; Fig. 2 and S1). Diatom abundances increased over the study period, and certain genera became dominant over time, e.g. *Cocconeis* spp. (8 species) and *Navicula* spp. (2 species) being the most abundant (>75 % of total diatom abundance) after one month (Fig. S1). The results of the PERMANOVA indicated significant differences in diatom abundance amongst time intervals (Table 2; Fig. 3). The comparison amongst time intervals using SIMPER analysis indicated that diatom genera *Grammatophora, Navicula, Nitzschia, Cocconeis* and *Achnanthes* contributed the highest with percentages ranging between 30 and 43 % (Fig. 3).

3.3. Metal concentrations and bioconcentration factors

The relationship of metal accumulation (Fig. 4) over the time intervals for biofilm a significant positive relationship was observed for Al ($p < 0.0001$), Mn ($p < 0.0001$), Fe ($p < 0.0001$), Zn ($p < 0.0001$) and As ($p < 0.0001$). Whereas for the *imizi* structure, significant positive relationship was observed for Al ($p < 0.0001$), Fe ($p < 0.05$) and As ($p < 0.0001$); significant negative relationship was observed for only Mn ($p < 0.0001$) over time intervals. The $BCF_{water-biofilm}$ indicated an increasing trend for Al, Mn, Zn and As, with the highest factors of 80.67, 33.37 and 0.61 at T7, respectively (Fig. 5). The $BCF_{water-biofilm}$ for iron was highest at T5 with a BCF of 717.92. The highest $BCF_{imizi\text{-}biofilm}$ ratio was observed for Mn at T2 for Mn and Zn with a BCF of 30.17 and 1.87 respectively, with an overall decreasing trend.

4. Discussion

In this study, we employed Indigenous Knowledge-based material (*imizi* structures – Fig. S3) to evaluate its potential as a suitable substrate

Table 1
Physico-chemical parameters pH, temperature, dissolved oxygen, salinity, chlorophyll-*a* biomass and nutrient (phosphates, silicates, nitrate, nitrite and ammonium) concentrations, measured adjacent to the *imizi* structures at each time interval (T1 = six hours, T2 = twelve hours, T3 = twenty-four hours, T4 = three days, T5 = one week, T6 = two weeks, T7 = one Month).

Time	pH	Temperature (°C)	Dissolved oxygen (mg/L)	Salinity (ppt)	Chl-a (μg/L)	PO_4^{3-} (μmol/L)	S i_4^+ (μmol/L)	NO_{3-} (μmol/L)	NO_{2-} (μmol/L)	NH_4^+ (μmol/L)
T1	8.05 ± 0.07	18.26 ± 0.62	9.03 ± 0.07	36.00 ± 0.00	0.98 ± 0.46	1.00 ± 0.19	11.99 ± 0.75	6.07 ± 0.24	0.30 ± 0.02	0.65 ± 0.14
T2	8.00 ± 0.01	20.35 ± 0.64	6.18 ± 0.76	35.50 ± 0.71	4.32 ± 2.26	1.56 ± 0.22	15.72 ± 3.32	6.07 ± 1.06	0.82 ± 0.16	7.63 ± 1.06
T3	7.99 ± 0.01	22.03 ± 0.53	7.37 ± 0.87	36.00 ± 0.00	1.12 ± 0.42	1.61 ± 0.18	15.90 ± 2.72	5.84 ± 0.45	0.81 ± 0.13	7.60 ± 1.61
T4	7.97 ± 0.01	18.69 ± 0.58	11.48 ± 0.81	38.00 ± 0.00	5.68 ± 4.94	0.98 ± 0.07	12.65 ± 4.08	5.82 ± 0.62	0.63 ± 0.27	4.47 ± 1.02
T5	8.12 ± 0.12	16.54 ± 0.80	12.20 ± 0.08	34.00 ± 0.00	2.66 ± 1.37	0.88 ± 0.14	6.53 ± 1.20	3.73 ± 0.27	0.10 ± 0.02	2.59 ± 0.97
T6	7.97 ± 0.04	20.44 ± 0.79	8.63 ± 0.08	34.00 ± 0.00	4.03 ± 0.88	1.31 ± 0.14	6.01 ± 0.22	0.93 ± 0.12	0.29 ± 0.02	5.06 ± 0.48
T7	8.05 ± 0.02	18.74 ± 0.13	7.93 ± 0.10	37.00 ± 0.00	6.39 ± 1.56	0.81 ± 0.13	2.27 ± 0.13	0.41 ± 0.01	0.15 ± 0.01	4.81 ± 0.48

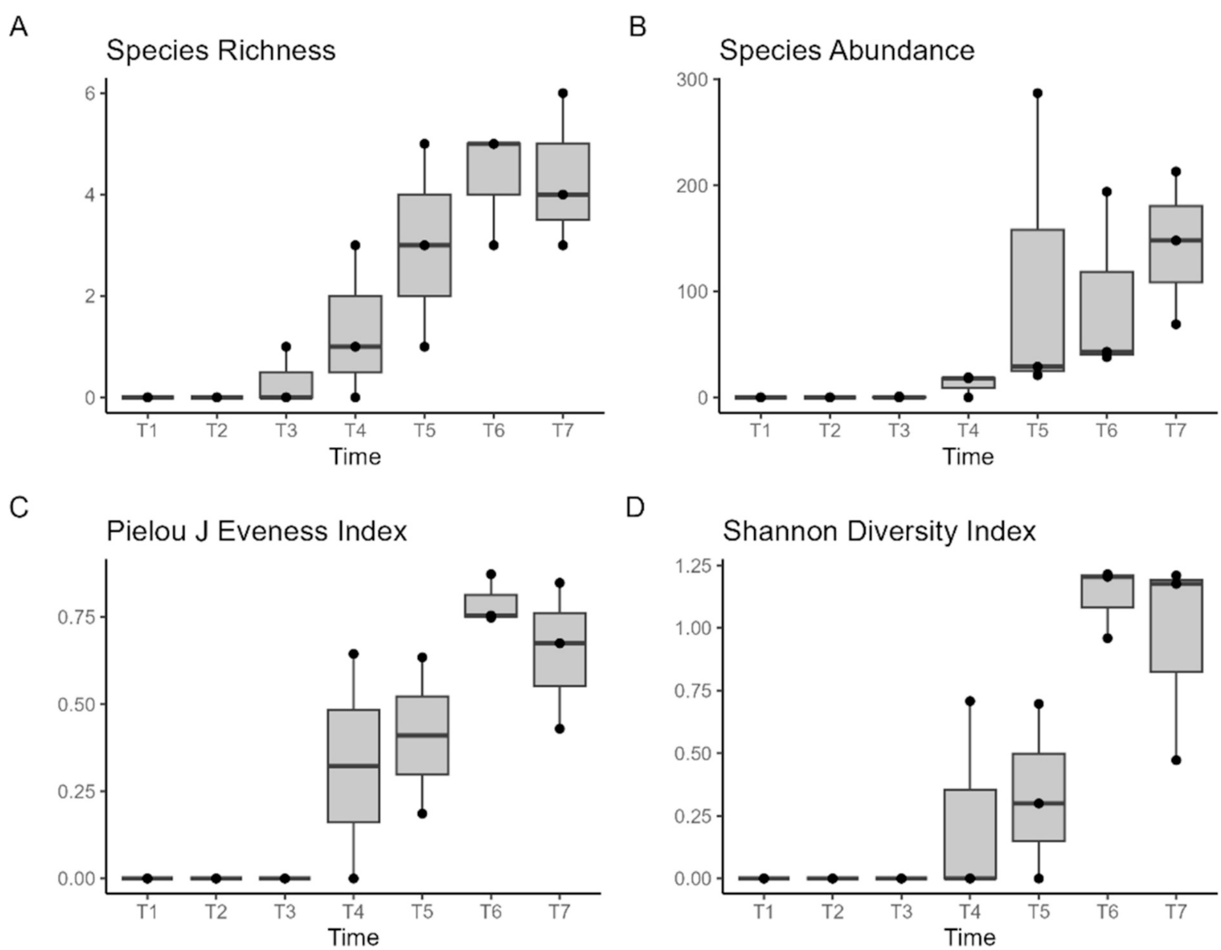

Fig. 2. Boxplots of diatom species richness (A), species abundance (B), Pielou J Evenness index (C) and Shannon diversity index (D) for the different time intervals (T1 = six hours, T2 = twelve hours, T3 = twenty-four hours, T4 = three days, T5 = one week, T6 = two weeks, T7 = one Month).

Table 2
Results of PERMANOVA for compositional temporal differences in diatom community.

	df	Sum of squares	R^2	F	Pr(>F)
Time Intervals	6	1.79	0.64	4.12	0.0001
Residuals	14	1.02	0.36		
Total	20	2.81	1		

for diatom successional growth and assess its effectiveness for metal bioaccumulation. While environmental conditions (pH, salinity, dissolved oxygen and temperature) were stable throughout the study, the concentrations of nutrients (ammonium, nitrite, nitrate, silicate and phosphate) and metal Mn showed a steady increase initially, followed by a significant decrease, with notable differences between early (<1 week) and late (>1 week) time intervals. Despite the small size of the experimental structures deployed, the consistent relatively high nutrient

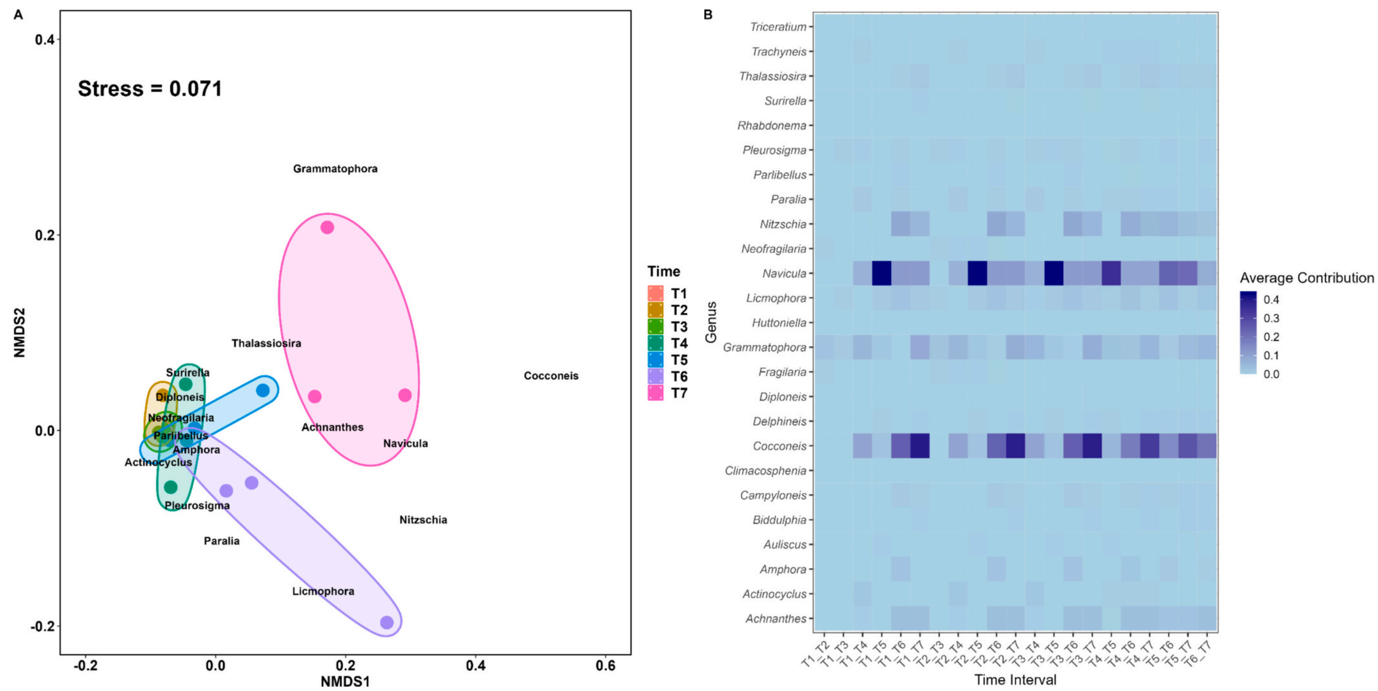

Fig. 3. A) Non-metric dimensional scaling of grouping centroids representing the 95 % confidence intervals of species diversity of diatoms at different time intervals. Scores were based on the Wisconsin square root function with the ordination of linear fit $R^2 = 0.906$ and non-metric fit $R^2 = 0.98$. B) Heatmap based on SIMPER analysis, with the average diatom genus contribution dissimilarity amongst time intervals (T1 = six hours, T2 = twelve hours, T3 = twenty-four hours, T4 = three days, T5 = one week, T6 = two weeks, T7 = one Month). Dark blue coloured blocks indicate the highest average contribution to the dissimilarities. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentrations at the study site could be due to the variable influxes of water from both the sea and the Kowie estuary. Natural sources from the permanent upwelling adjacent to the Royal Alfred marina, as well as elevated nutrient levels upstream of the Kowie river resulting from agricultural practices and a domestic wastewater treatment plant, may have contributed to the high concentrations observed (Hay, 2007). The follow-up decrease in nutrient concentrations near the *imizi* structures could be attributed to the presence of biofilm, given the requirements for trace compounds for growth, particularly silicate and phosphorus, which are essential for the development of diatoms and bacteria (Rogers and Bennett, 2004; Arrigo, 2005).

Our findings indicated that whilst diatom species appeared as early as the first 6 h (T1) from structure deployment, precise morphological identification was unsuccessful due to diatom valves being weakly silicified. Full diatom identification was however possible from T2, after 12 h. The succession process observed in the study followed a typical pattern, where the initial colonisers were gradually replaced by species that better adapt to the changing physico-chemical conditions or inter-specific competition (Dalu et al., 2016b; Cotiyane-Pondo and Bornman, 2021). The increase in diatom abundance and diversity, combined with the significant SIMPER results indicate the changes in diatom succession on the *imizi* structures, reflecting the natural progression from the colonisation (T1) to a more stable and mature community (T7). In the initial formation (T1-T2), the diatom community on the *imizi* structures was rapidly colonised by pennate diatoms such as *Fragilaria*, *Neofragilaria*, and *Grammatophora*. Similar findings along the Kowie estuary have been previously recorded (Dalu et al., 2014b, 2016b), with *Fragilaria* spp. also being identified as an initial coloniser. Such diatom genus tends to subsequently decrease over time, and it is prone to being washed out in high-wave energy environments which is explained by its relatively large size and sedentary behaviour, making it a poor competitor during the later stages of colonisation (Dalu et al., 2014b). In this study, only *Grammatophora* remained a significant contributor to the biofilm assemblages over time but showed a slight decrease in abundance after two weeks. The genera *Fragilaria* and *Neofragilaria* were observed during T1 as early colonisers on the *imizi* structures, to be later outcompeted or replaced as the community matured. After one week, the diatom community on the *imizi* structures increased substantially, and the dominance of *Navicula*, *Grammatophora*, *Actinocyclus* and *Cocconeis* became increasingly prominent. By T6- two weeks and T7- one month, the community was dominated by *Nitzschia, Cocconeis, Grammatophora* and *Achnanthes,* reflecting the development and stabilisation of the diatom community.

Given the limited studies that consider microphytobenthic species in eco-engineering approaches for coastal environments, specific comparison with previous research is limited. A parallel can, however, be drawn from ecological studies assessing coastal benthic diatom taxonomic composition. Diatom species composition found on the *imizi* structures in the present study generally reflects trends from previously reported in studies conducted in the Kowie estuary (Bate et al., 2013; Dalu et al., 2014a, 2014b, 2016a, 2016b; Nunes et al., 2021). The dominant diatom genera in this study are also consistent with those found in benthic community studies using plant substrates like wood and bamboo, indicating similar successional growth patterns of coastal diatoms (Khatoon et al., 2007; Nenadović et al., 2015). For instance, Nenadović et al. (2015) identified pennate diatoms such as *Amphora*, *Navicula*, *Halsea*, *Auricula*, and *Parlibellus* on smoothened wood after 30 days, while Khatoon et al. (2007) observed higher biofilm biomass and diatom species dominated by *Amphora*, *Cymbella*, *Gymnophonema*, *Melosira*, *Navicula*, *Nitzschia*, and *Pleurosigma* on bamboo pipes. The preference of diatoms on the plant material was attributed to the availability of essential nutrients necessary for biofilm growth, such as phosphate and silicate (Khatoon et al., 2007; Nenadović et al., 2015). These trends suggest that the natural Indigenous Knowledge-based material used in this study provided a suitable substrate for the development of diatoms, probably due to their surface texture and nutrient content of the *C. textilis* stems.

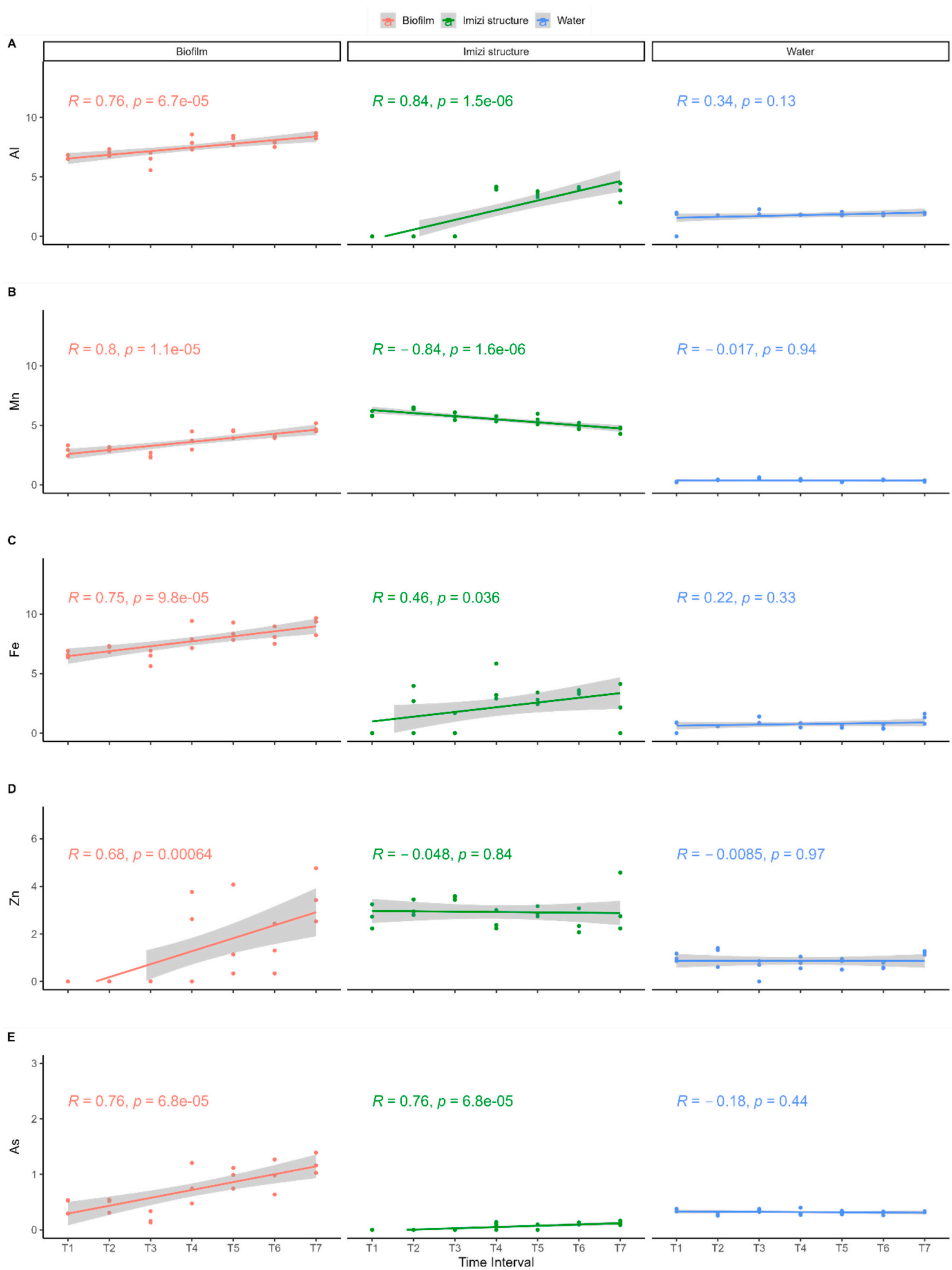

Fig. 4. Simple linear regression plots of logarithmic transformed and upscale (2 units) metal concentrations of Al (A), Mn (B), Fe (C), Zn (D) and As (E) of the biofilm (red), 20 × 10 cm *imizi* structures (green) and the surrounding water (blue) over the seven-time intervals (T1 = six hours, T2 = twelve hours, T3 = twenty-four hours, T4 = three days, T5 = one week, T6 = two weeks, T7 = one Month). Water concentrations are expressed in μg/L, while biofilm and the *imizi* structures are expressed at μg/Kg. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

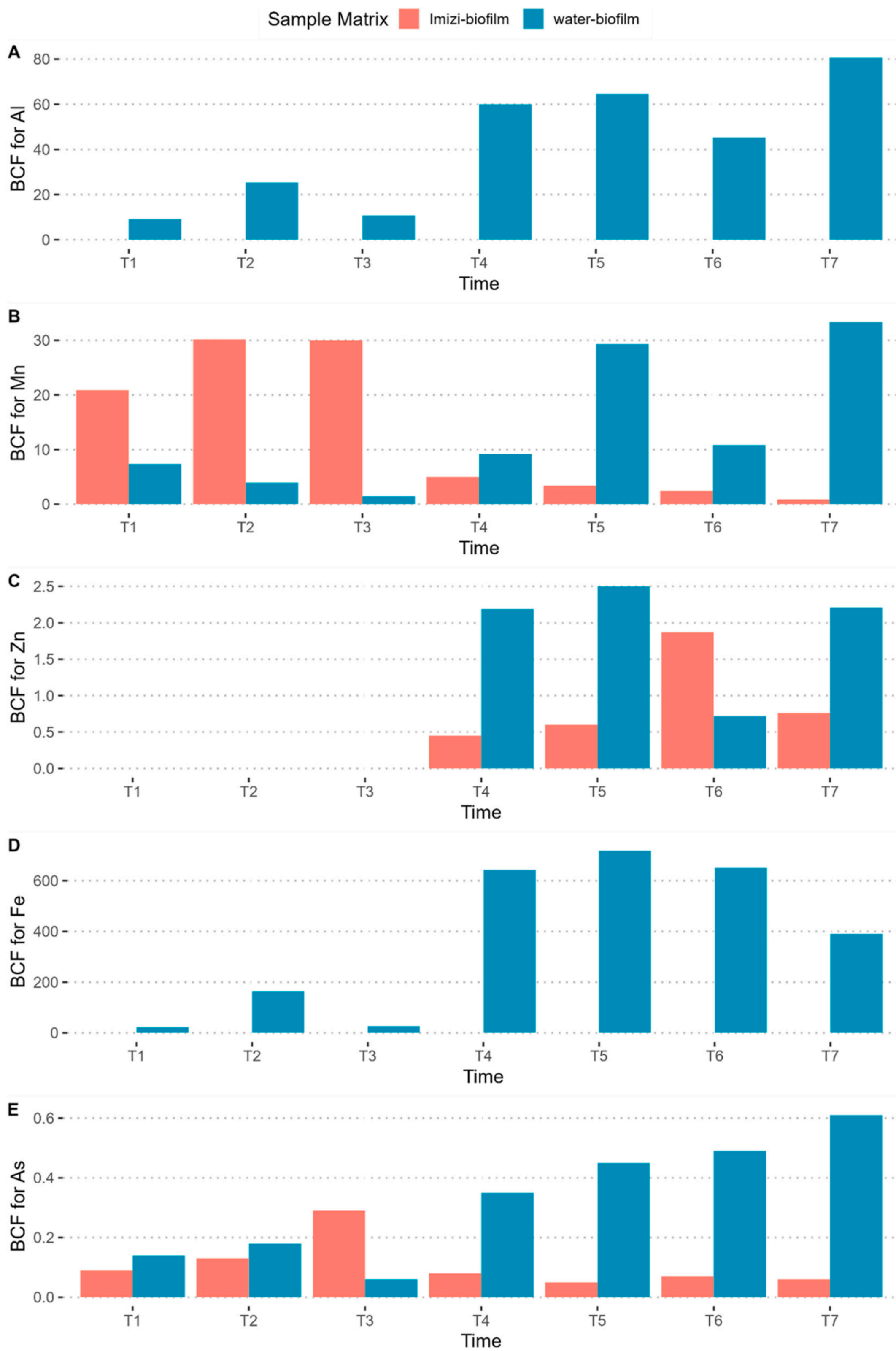

Fig. 5. Bioconcentration factors (BCF) for each time interval (T1 = six hours, T2 = twelve hours, T3 = twenty-four hours, T4 = three days, T5 = one week, T6 = two weeks, T7 = one Month) for water–biofilm (blue) and *imizi*–biofilm (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.1. Metal accumulation

Metal accumulation on biofilm collected from the *imizi* structures was variable, with significant relationships observed for metal concentrations in the structures themselves, the surrounding water, and biofilm developing on the structures, suggesting various and metal-specific accumulation modes over time. Despite the source, the biofilm developing on the *imizi* structures seem nevertheless suitable for metal accumulation. A consistent pattern of decrease in the concentration of metal in the *imizi* structures and surrounding water over time was noted, while in the biofilm, an increase in metal concentrations over time was observed. Notably, Mn and Zn levels were significantly higher (Fig. S4) in the *imizi* structures compared to the biofilm within only 6 h of deployment. These differences could be due the use of different bioaccumulation modes and the organic composition of *imizi* structures. The high abundance of cellulose in the *imizi* material (i.e., lignocellulose) could facilitate metal adsorption through the provision of metal-attachment sites, diffusion, and binding of metals (Bhunia et al., 2023; Sharma et al., 2024). Secondly, given that the *imizi* structure is non-living and porous, metal accumulation could have occurred passively, via absorption, but more rapidly as compared to biofilm absorbing metals from water.

When comparing metal concentrations in the surrounding water and biofilm, only As was significantly orders of magnitude higher in the biofilm than in water (Fig. S4) during the initial times of collection (6 and 12 h), and thereafter from three days of deployment and onwards. Such results confirm the biofilm's capability of accumulating toxic metals at low environmental concentrations (Tien and Chen, 2013). Toxic metals such as As are generally present in the water column as either arsenate or arsenite, with the former in its inorganic form being dominant in seawater environments (Neff, 1997; Kalia and Khambholja, 2015). Microorganisms, including algae and bacteria, can accumulate and detoxify As using extracellular polymeric substances (EPS) as sinks for As in periphytic biofilms (Kalia and Khambholja, 2015; Barral et al., 2022; Litter, 2022). These findings suggest that Indigenous knowledge-based material can also serve as an effective accumulator of metals and, hence, likely improve water quality by reducing toxicity levels. The steady increase in As levels over a very short time as observed in the current study could pose a risk of biomagnification, necessitating measures like frequent biofilm removal or exclusion cages to mitigate this effect. Long-term monitoring using stable isotopes would also be recommended to evaluate trophic uptake by other organisms and, hence, evaluate the dynamics of remediation of As from an eco-engineering perspective (Coclet et al., 2021; Wen et al., 2024).

Accumulation of metals by biofilm from both the *imizi* structures and water could result in a protective mechanism or a need for the development of microorganisms within the biofilm. The observed differences in metal concentrations over time could indicate a change in metal accumulation based on various factors such as metal speciation and bioavailability, physico-chemical factors (i.e., pH) and biotic factors (i.e., microbial community present) for each time interval (Singh et al., 2006; Mishra et al., 2022; Sharma et al., 2024). For example, metals, including Cu, Zn, and Fe, play an essential role in the growth of microalgal species by mediating protein structure, metabolism, and development in the community (Giri et al., 2022). Alternatively, high concentrations of As, Mn, and Fe can also induce an increase in metal-reducing bacteria, resulting in the precipitation of insoluble forms of metals in the biofilm. This mechanism provides a protective advantage for biofilm to survive in exposed environments by reducing the presence of the metal in its surrounding environment (Kielemoes et al., 2002; Bruno et al., 2012). Interestingly, Zn accumulation by the biofilm occurred after 2 weeks (T5) of deployment, possibly due to the sensitivity of younger biofilms to minimise accumulation of Zn in the earlier time intervals (Cu competitive exchange; Ivorra et al., 2000). After 2 weeks, the presence of *Ulva* sp. was observed (pers. obs.) on the *imizi* structure. Previous studies have indicated that *Ulva* species has a high affinity to bioaccumulate zinc (Filho et al., 1997; Rybak et al., 2013) which may explain the observed increase of Zn in the biofilm at T2. Essential microelements like Al, Cu, Zn, Mn, and Fe are crucial for bacterial and algal growth and metabolic pathways (Ivorra et al., 2000; Mishra et al., 2022). The biofilm's developmental stages influence metal accumulation, with shifts from loosely attached diatoms to chain-forming diatom species and green algae (Ivorra et al., 2000). This variable diatom forms could also attest to the accumulation of metals at various developmental stages of biofilm growth.

When assessing metals' $BCF_{imizi\text{-biofilm}}$, Mn was hyper-accumulated in the early time intervals (T1-T3), followed by a decrease from T3 to T7 and increase for Zn at T6. This initial high concentration factor may reflect the biofilm's affinity for Mn and the significantly higher metal concentrations in the *imizi* structure than the biofilm (Corcoll et al., 2012). As the biofilm matured, equilibrium with Mn levels may have led to metal saturation of binding sites and a reduction in Mn accumulation. One plausible explanation could be due to the fact that the metabolic activity of microorganisms within the biofilm can also alter the chemical state of metals, hence reducing their likelihood to accumulate (Ivorra et al., 2000; Corcoll et al., 2012; Tien and Chen, 2013). The contrasting trend in the $BCF_{water-biofilm}$ ratio for these metals suggests a shift in metal accumulation route from the *imizi* structure to the water fraction, observed in the increase of Mn and As concentrations in the *imizi* structure compared to the surrounding water. An increasing trend in $BCF_{water\text{-biofilm}}$ values for Al, Mn, Fe, Zn, and As as time progresses could suggest a shift in metal accumulation as the biofilm matures, compared to the *imizi* structure. Furthermore, as time goes, the entrapment of suspended particulate matter may increase metal levels of the biofilm due to organometallic complexes (Ivorra et al., 2000; Leguay et al., 2015), potentially explaining the steadily increasing and higher $BCF_{water\text{-biofilm}}$ compared to $BCF_{imizi\text{-biofilm}}$. A high correlation between suspended metals and accumulated metals in the biofilm has been reported (Morin et al., 2008; Faburé et al., 2015), confirming the significance of the accumulation route by the biofilm from the suspended fraction. This dynamic interaction between biofilm successional growth and metal accumulation explains the differences in metal levels between the biofilm, the *imizi* substrate and the surrounding water.

5. Remarks, recommendations and conclusion

The study demonstrated the potential of utilising an Indigenous knowledge-based material, the *imizi* substrate, for coastal eco-engineering practices. Over the course of one month, the *imizi* substrate effectively supported the formation and natural succession of benthic diatom species, highlighting its suitability in coastal environments. In addition, the biofilm growing on the substrate accumulated metals such as Al, Zn, Mn, Fe and As at variable levels across different time intervals. This finding therefore highlights the dual function of the *imizi* substrate as a medium for promoting primary productivity and as a material capable of promoting biofilm-metal bioremediation.

It is important to recognise that the findings are based on a short-term monitoring period of one month at a single location. While the findings and conclusion from this study may be limited, they however serve as a future step into further investigations to provide better understanding across broader spatial and temporal scales, larger and complex designs based on indigenous practices through local transdisciplinary collaborations and promotion of equitable economic and social endeavours (Porri et al., 2023). The upscaling from the current study to a fully covered seawall will indeed introduce distinct logistical and socio-environmental challenges (i.e., minimising overharvesting of *imizi* stems). For an example, in contrast to the traditional concrete-based eco-engineered structures, the Indigenous-based structures will demand unique attachment equipment adapted for its material type and flexibility. Secondly, environmental heterogeneity amongst sites (and within site variations) might bring about gradient differences with wave energy, sediment deposition and water chemistry which will likely produce differences in structural longevity and consistency in biofilm development over time. It will be necessary to refine and reinforce larger designs, monitor long-term durability, maintenance requirements and the evaluation of cost-benefit designs to assess these effects.

The study's novelty lies in the distinctive merging of Indigenous Knowledge with eco-engineering practices, particularly in contexts where secular traditions, primary producers like diatoms and the evaluation of functionality of substrates remain largely unexplored. In eco-engineering, the material's ability to support biological productivity and functionality is critical to achieve the desired ecosystem services. No studies have explored Indigenous-based materials like *imizi* in this context. This study originally proposes that the nature-based *imizi* material is suitable for eco-engineering interventions on impacted coastal sites, and manipulative studies based on this are explored.

CRediT authorship contribution statement

J. Ndaba: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **P. Cotiyane-Pondo:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Conceptualization. **L. Human:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition. **E. Puccinelli:** Writing – review & editing, Validation, Supervision, Methodology. **P. Pieterse:** Writing – review & editing, Resources, Methodology, Formal analysis, Data curation. **P. Pattrick:** Writing – review & editing, Supervision. **F. Porri:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2025.107696.

Data availability

Data will be made available on request.

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