# **FINAL REPORT MEEF2021004:** Monitoring ecological impacts of heavy metal pollution on vulnerable seagrasses in Lantau Island and western Hong Kong

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# Executive Summary

This project has conducted, for the very first time, the assessment of heavy metal pollution in seagrass ecosystems in Hong Kong. As part of this assessment we have been able to identify hotspots of pollution and areas of conservation priority for seagrass meadows in our coastal waters. We have generated novel and valuable data to inform managers and policy makers in order to develop conservation and restoration efforts for seagrasses in Hong Kong. Through knowledge exchange, we have also engaged with Government, NGOs, academics and the general community around the importance of seagrasses and the need of major joint efforts to prevent their local extinction. All the work included in this project has been the foundation of a Master thesis at the University of Hong Kong, and thus, the MEEF project has also supported capacity-building and the formation new researchers in conservation biology.

# Project title and brief description of the Project

## Title

Monitoring ecological impacts of heavy metal pollution on vulnerable seagrasses in Lantau Island and western Hong Kong

## Description

Seagrasses are keystone marine plant species and are considered as important ecosystem engineers as they modify the surrounding coastal habitats for benefits of individual functioning and associated biodiversity. Seagrasses provide a wide range of fundamental ecosystem services that are critical for food security, livelihood and well-being of coastal communities worldwide. Despite their significant ecological and economic contribution, seagrass populations are rapidly declining globally and in Hong Kong due to various anthropogenic pressures. The negative effects on seagrasses in Hong Kong are magnified by the rapid increase in coastal pollution, particularly heavy metals such as zinc, copper, arsenic, and cadmium among others. These pollutants originate from industrial and municipal wastewater, sewage discharge, agricultural runoff, and antifouling coating. All these sources are highly concentrated along the Pearl River Delta, with direct impact on the Deep Bay area, the Western Hong Kong and Lantau Island, which are hotspots of local seagrasses including the IUCN Vulnerable *Halophila beccarii*.

Despite this major concern, the bioaccumulation of heavy metals in seagrasses, the functional effects on their survival, growth and physiology, as well as the ecological impacts on associated biodiversity, are largely unknown. This project aims to fill this major knowledge gap by applying cutting-edge molecular approaches to assess and monitor the ecological impacts of heavy metal pollution in populations of seagrasses in Lantau Island and western Hong Kong. Through a combination of stable isotopes, metagenomics, transcriptomics and chemical element analysis, we provide important information about the health of local populations of seagrasses, their resilience and responses to heavy metal pollution, as well as the ecological impacts on the structure and function of seagrass associated biodiversity. This project generates quantitative data and tools that will allow scientists, stakeholders and decision-makers to develop policies and management strategies for conservation and restoration of seagrass meadows and mudflat ecosystems in Lantau Island and western Hong Kong.

## Objectives

1. To determine heavy metal pollution levels in sediment and bioaccumulation in seagrasses by chemical elemental analysis and stable isotopes.
2. To quantify impacts of heavy metals on the structure and function of seagrasses-associated biodiversity.
3. To assess the resilience and responses to heavy metal pollution of local seagrasses.

# Completed activities against the proposed Work Schedule

| **Activity (including Planning, Recruitment)** | **Date** | **Time** | **Venue** | **Content** | **Anticipated no. of participants** |
|---|---|---|---|---|---|
| Recruitment of a Research Assistant | 07-2021 | 1 month | HKU | Completed | 3 |
| Logistics and preparation fieldwork | 07-2021 | 1 month | HKU | Completed | 4 |
| Field work sampling and surveys | 08-2021<br>03-2022 | 8 moths | West Hong Kong and North Lantau Island | Completed | 6 |
| Chemical elemental analysis and stable isotopes of sediments, water and tissues | 03-2022<br>07-2022 | 6 moths | HKU | Completed | 3 |
| Molecular analysis of seagrass-associated biodiversity | 04-2022<br>07-2022 | 5 moths | HKU | Completed | 2 |
| Molecular analysis of seagrass health, resilience and physiology under heavy metal pollution | 04-2021<br>08-2022 | 5 moths | HKU | Completed | 2 |
| Data analysis and interpretation | 07-2022<br>10-2022 | 4 moths | HKU | Completed | 4 |
| Reports (including Final report) | 07-2022<br>12-2022 | 6 moths | HKU | Completed | 4 |

## Study sites

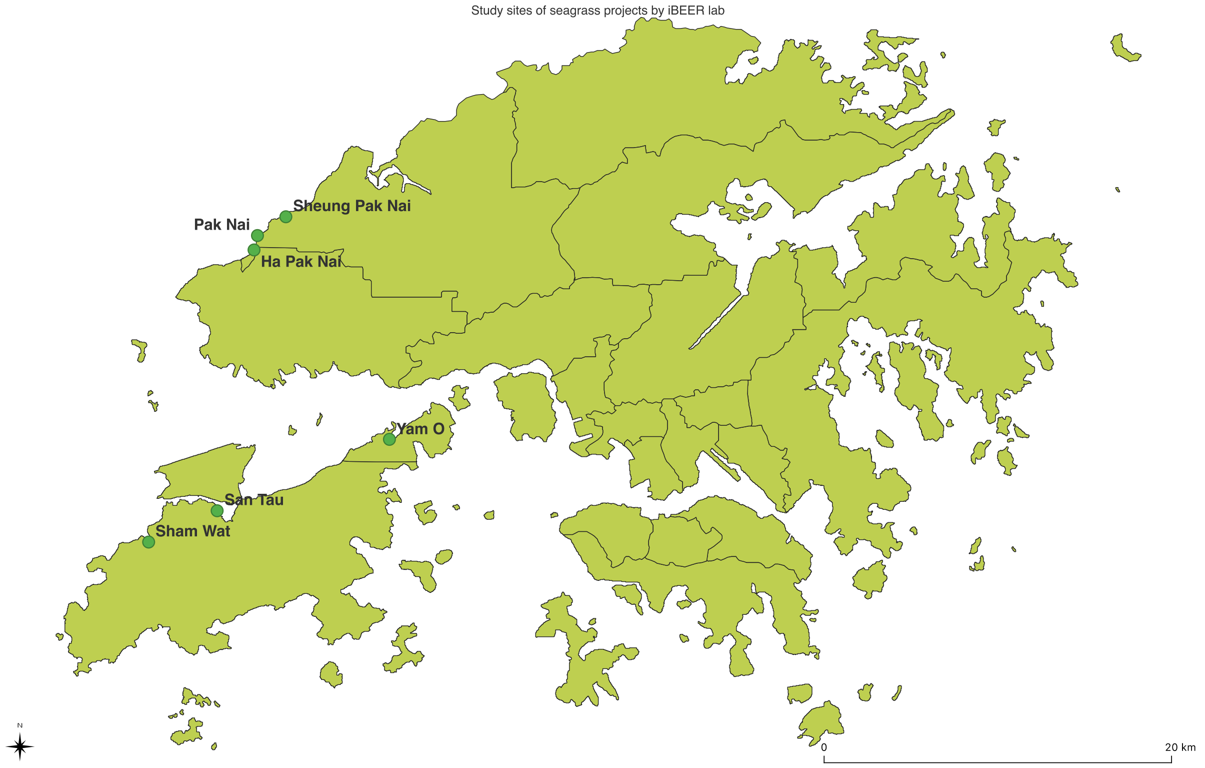

*Figure 1 A map showing the study sites of seagrasses in this project.*

### Sheung Pak Nai, Pak Nai, and Ha Pak Nai

The study sites of Sheung Pak Nai, Pak Nai, and Ha Pak Nai are intertidal mudflats 1km apart where oyster aquaculture is prominent. Pak Nai is home to a variety of mangrove and saltmarsh species, which are near to the vulnerable H. beccarii habitats. Among the different mangrove (Sonneratia apetala, Acanthus ilicifolius, Kandelia obovata) and saltmarsh (Sporobolus alterniflorous, Suaeda australis, Sesuvium portulacastrum, Sporobolus virginicus) species studied, K. obovata and S. alterniflorous were the most common on the shore. The environmental matrix of these 3 sites are dominated by mangrove and saltmarsh species on the landward side, H. beccarii in the center, and oyster beds on the seaward side. These research sites are also vital breeding grounds for the endangered Chinese horseshoe crabs (Tachypleus tridentatus). The West New Territories Landfill is located at the far end of Ha Pak Nai. Soft, muddy substratum and seagrass are spotty and rare in Sheung Pak Nai, surrounded with patchy oysters. There were no mangroves nearby, so oysters and boulders were dispersed along the mudflat. As a sewage line connecting to neighboring residences is erected within the property, an unpleasant odor was produced. A close-up of the mud flat with a small patch of *H. beccarii* seagrass surrounded by snails.

*Figure 2 Seagrass patch at Pak Nai. Taken during June 2022.*

*Figure 3 Sheung Pak Nai. Taken during August 2021.*

*Figure 4 A close up of the seagrass at Sheng Pak Nai. The patches here were scarce and small. Taken during August 2021.*

## San tau

San Tau is another prominent seagrass bed in Hong Kong. *Halophila ovalis* and *Z. japonica*, two seagrass species, co-persisted in the same ecosystem. This area follows the same pattern as Pak Nai and Ha Pak Nai, with mangroves on land, a seagrass environment in the intertidal region, and oyster beds in the seaward zone. Freshwater input is located on the site's right side. Mixed mangrove species such as *K. obovata, Avicennia marina, Bruguiera gymnorrhiza, Excoecaria agallocha, Lumnitzera racemosa, Talipariti tiliaceum,* and four saltmarshes such as *S. portulacastrum, S. australis, Limonium sinense*, and *S. virginicus* exist here as well.

*Figure 5 Seagrass meadow at San Tau. Taken during January 2022.*

## Yam O

Yam O has a sandy mud substratum, according to the data. The muddy substratum is on the right-hand side of the region where the sewage discharge is located. A vast, continuous seagrass patch (*H. ovalis*) runs the length of the intertidal zone. Human activity was shown to be very disruptive at this research location. The seagrass bed has declined rapidly in the last year, while the snail population has increased rapidly. This location follows the same pattern as Pak Nai and San Tau, with mangroves on landward and seagrass patches to the seaward, as well as oyster beds. A freshwater inflow is located in the center of the site, which is very active during the rainy season.

*Figure 6 Seagrass meadow at Yam O. Taken during August 2021.*

## Sham Wat

This site's substrate can be classified as sandy mud. Seagrass can be seen near to the roots of mangrove trees in the mangrove environment (*Avicennia marina*). The substratum was altered to sandy along the site from 2021 to 2022. Freshwater input is on the right side, which actively pumps freshwater throughout the dry and wet seasons. In recent months, we have observed a decline in the seagrass bed.

*Figure 7 The environment at Sham Wut. Taken in June 2021.*

## Tai O

We have also visited Tai O, another site with previous seagrass record. Muddy landscape is the feature of this study site. It is connected to freshwater output from the waterways of the village. However, there was no presence of seagrass nor possible landscape for seagrass to grow. We estimated that there may have been a change in landscape which caused the absence of seagrass.

# OBJ1: Heavy metal pollution levels and bioaccumulation in seagrass

## Introduction

Seagrasses in Hong Kong inhabit the intertidal area of mudflats, particularly in the western area. Like any other flora, sediment and the surrounding environment have a major effect on seagrass grow and health. As one of the primary producers and a nurturing ground in coastal ecosystem, seagrasses are considered under high risk due to metal pollution. The intertidal species of seagrasses in Hong Kong are especially under high concern as they are the frontline between inland pollution sources and the marine environment. Monitoring studies have reported bioaccumulation of metal pollutants in seagrasses growing in highly polluted regions (Conti et al., 2007, 2010; Jeong et al., 2021; Lee et al., 2019). As seagrasses and their debris are nutrient sources of many faunal species, the threat to seagrass populations can also mean a critical situation to other species (Hemminga & Duarte, 2000). Other studies have also suggested that high level of metal contaminants can exert detrimental effects on seagrasses themselves. For example, high level of copper is deleterious to the growth and photosynthetic capacity of seagrasses (Li et al., 2012; Llagostera et al., 2016).

Studies have suggested that metal pollution is one of the major threats in Hong Kong's coastal ecosystem (Blackmore, 1998; Liang & Wong, 2003; Rong et al., 2022; Wang et al., 2013), because of the significant output of industrial wastes, domestic sewages and chemically assisted agricultures. Meanwhile, extrinsic inputs such as the water coming from Pearl River are also accounted for the pollution treats. As seagrasses have shown to be susceptible to high levels of metal concentrations, there is a high chance that this metal pollution is a major factor of the seagrass decline that we have documented during the last three years in Hong Kong. Therefore, in order to develop and improve the conservation strategies on local seagrass populations, there is an urgent need to understand and characterize the conditions of local seagrasses and their habitats.

## Methods

### Sample collection

Three 15cm of sediment cores were collected using acrylic tubes within the seagrass patch (Fig. 8; core A, B and C). Another three cores representing the area without seagrasses were taken outside of the meadow from nearby area (core D, E and F). All cores were transported back to the laboratory in 2 hours. In the laboratory, each core was cut into three layers, namely 0-5cm, 5-10cm and 10-15cm. Each layer was thoroughly homogenized with sterile non-metallic tools and was subsequently stored at -80°C until further processing.

Seagrass samples were collected from station A, B and C within a 1m diameter. Collected seagrasses were washed with seawater from the site and brought back to the lab in pre-cleaned plastic containers within 2 hours. Samples were subsequently washed with MiliQ water to remove epiphytes and adhered sediments. Cleaned seagrasses were then separated into above-ground and below-ground compartments for smaller species (*H. beccarii*) or into root, rhizome and shoot for larger species (*H. ovalis*). Separated tissues were stored at –80°C until further processing.

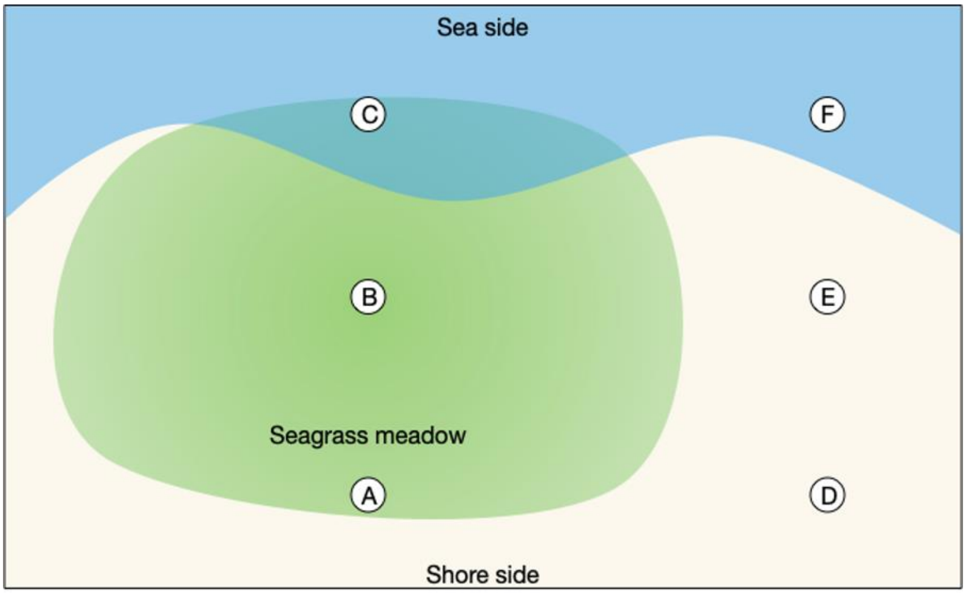

*Figure 8. A schematic diagram illustrating the locations of core collections at each seagrass site. Core A, B and C were collected within the seagrass patch while core D, E and F were collected outside of it.*

## Metal analysis

Frozen sediment and seagrass samples were freeze-dried and ground into fine powder. For sediment, we followed instructions from Method 3051A (SW-846) by U.S. EPA (US EPA, 2019) and ISO11466 (International Organization for Standardization, 1995) with adjustments for our settings. 0.2g (dry weight) of sediment was digested with 6ml aqua regia (4.5ml HCl + 1.5ml $HNO_3$) at 175°C using microwave digester (Microwave Digester, Milestone Ethos One). The digested samples were then stored in metal-free tubes (50ml MetalFree™ Centrifuge Tubes, Labcon) following filtering and dilution. Meanwhile, seagrass samples were digested with 6ml $HNO_3$ in the microwave digester (Lee et al., 2019; Maher et al., 2011; Sastre et al., 2002; Topper & Kotuby-Amacher, 1990; Wu et al., 1997; Zarcinas et al., 1987). The digested samples were subsequently filtered and diluted before detection. Certified reference materials (CRMs), namely marine sediment CRM PACS-3 (National Research Council Canada (NRC)) and ERM-CD281 (Rye Grass), were used for quality assurance of our analytical method.

Metal concentrations were measured on Inductively Coupled Plasma Mass Spectrometer (7900 ICP-MS, Agilent). Arsenic, cadmium, lead, nickel, chromium, iron, zinc, copper and manganese were measured. Indium was spiked into the samples as an internal control of measurements. The metal concentrations measured in PACS-3 (n=3) were 72-116% of the certified values while those in Rye Grass were 105-127% of the certified values.

## Stable isotope analysis

An acrylic sediment core of 15 cm height (diameter=5.5cm) was used within the transect area (n=3/transect) to collect sediment samples from each site. These sediment cores were collected adjacent to the seagrass biomass cores within each transect. After collection, sediment cores were capped at both ends, placed horizontally in an ice-box and brought back to the laboratory. In the laboratory, sediment cores were sliced at 5cm intervals (0-5, 5-10, 10-15 cm) and placed in clean aluminium foils and oven dried at 60°C for 48 hours. After drying, the weight of each sediment fraction was weighed and they dry weight was used for the dry bulk density (DBD: g DW $cm^{-3}$) calculations, following the blue carbon manual (Howard et al., 2014). The sediment $C_{org}$ density (SCD: g $cm^{-3}$) was calculated by multiplying the dry bulk density with sediment $C_{org}$ content (%).

Then each fraction of dried sediment of each core was homogenized using a mixer mill (Retsch, MM400, USA) and stored for further analysis. From this homogenized samples a fraction was used to estimate the organic matter content of the sediment by loss on ignition (LOI) method, where 5 g of sediment sample was combusted at 500°C for 4.5 hours in a muffle furnace (Howard et al., 2014). The LOI was calculated using the equation (1)

$$LOI\ (\%) = \left[\frac{A-B}{A}\right] * 100 \ldots\ldots\ldots\ldots\ldots\ldots \ (1)$$

Where A is the initial weight of the dried sediment in grams and B is the final weight of the sediment after combustion.

From another fraction (0-5, 5-10, 10-15 cm) of dried sediment, a subsample (0.30 mg) was acidified (1M HCl) to remove the carbonates. After the addition of HCl, the sediment samples were stored in a fume hood chamber, till no further bubble formation was detected. Then the sediment samples were placed in hot air oven at 60°C for 24 hours till completely dried. These dried sediment samples were analysed in duplicate for composition of carbon (C) and nitrogen (N) elemental concentrations and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) using a Flash Elemental Analyser coupled to a Delta V IRMS [isotope mass ratio spectrometer; Euro Vector (EA3028 EA-Nu)]. In-house standards, acetanilide (iACET#1, $\delta^{15}N$=1.18‰, $\delta^{13}C$=-29.53‰) were used for calibration and determination of the precision (0.2‰). Ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were expressed as the relative difference (‰) between the sample and the conventional standard, Vienna Pee Dee Belemnite (VPDB) and atmospheric air for C and N respectively.

## Statistical Analysis

### *Metal concentrations*

The statistical analysis was performed on R (version 4.1.1). With vegan package (version 2.6-4) (Dixon, 2003), non-metric multidimensional scaling (NMDS) was used to illustrate the metal concentrations across all seagrass sites in this study (Figure 9). The distance between each sample points describes the proximity of levels in metals. The vector arrows are used to show the direction and strength of correlation between the samples and each metal elements. Unpaired two-samples Wilcoxon test was used to find out if there is any significant difference in each metal element concentration with different factors in both sediment and seagrass samples. Linear regression models were performed between different metal levels in every layer of sediment cores from seagrass patch and the seagrasses collected from the respective site. The metal concentrations in aboveground and belowground tissue were taken average and added up as the total concentration to fit into the model.

### *Stable isotope analysis*

A two way-ANOVA was used to test the statistical significance between the stable isotopes ($\delta^{15}N$ and $\delta^{13}C$), total C and N content and carbon stocks of the sediment using seagrass species, seasons (dry and wet) as fixed factors. All data was pre-checked for normality and homogeneity of variance using a Shapiro-Wilk and Levene's test respectively.

In case of non-homogenous variances, data were ln (x+1) transformed. When there were significant interactions between factors, the Holm-Šídák test was performed for a posteriori comparison among factor levels. Pearson correlation was used to test the interaction between physical factors and sediment organic matter, sediment carbon density (g $cm^{-3}$), C%, N% of sediment with seagrass across the dry and wet seasons. Linear regression was used to derive the allometric relationship of sediment organic matter and $C_{org}$. Non-linear regression curve fit was used to derive the relationship between total N (%) in seagrass biomass and their C:N ratios to understand external N input into seagrass ecosystems (Duarte, 1990). All statistical tests were conducted at a significance level of $p<0.05$. GraphPad Prism (ver.9.4) software was used for all statistical analysis and graphs.

## Results

### Metal concentrations of sediments in seagrass sites

Figure 8 shows that the metal levels across all sites are not diverse while samples from the same site, regardless of collection within or outside of seagrass patch, tend to have close proximity. Some of the deeper layers of sediments are sparsely located from the center cluster and away from the arrow directions, meaning that those deeper layers contain relatively lower levels of metals comparing to the samples from the main cluster. Yet, the levels of individual metal element are different between the sites, indicated by the different directions of vector arrows. In Figure 10 and Figure 11, the concentrations of each element in sediment samples are shown. The most concerning elements are the non-essential ones, which means that trace amount of them can exhibit a high potential of toxicity to living organisms, including seagrasses (Nagajyoti et al., 2010). High levels of arsenic, lead, nickel and chromium are observed in Sheung Pak Nai and Sham Wut. With reference to the sediment quality criteria used by Hong Kong Environmental Protection Department (HKEPD) (Appendix A of the Works Bureau Technical Circular (Works) No. 34/2002 Management of Dredged / Excavated Sediment), the concentration of arsenic from all layers of sediments at Sham Wut exceeded the suggested lower chemical exceedance level (LCEL). Meanwhile, arsenic in the sediments from Sheung Pak Nai seagrass patch also highlights a relatively higher level comparing to the other sites except Sham Wut. The levels of lead at both Sheung Pak Nai and Sham Wut, at the same time, are approaching the LCEL level.

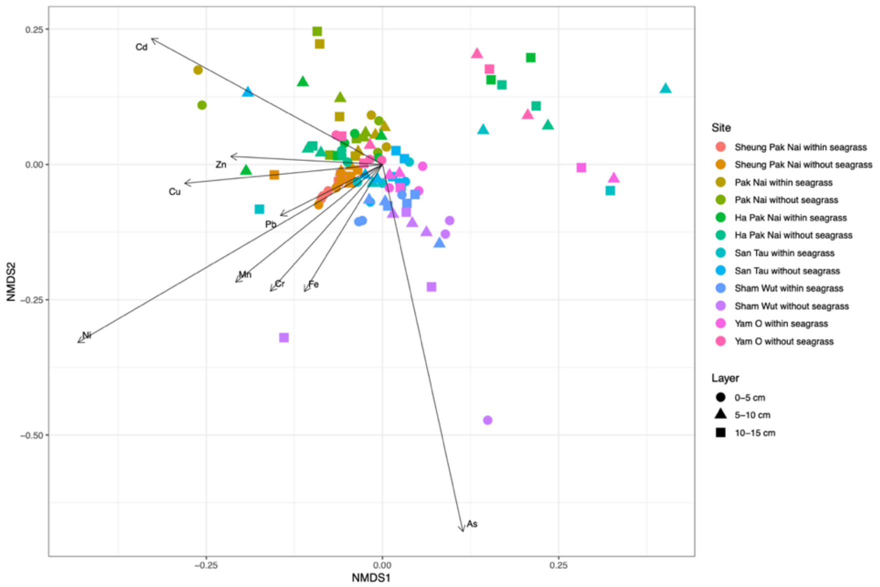

*Figure 9. A Non-metric multidimensional scaling (NMDS) figure illustrating the metal concentrations of sediments collected from different seagrass sites (colours) and in different core layers (shapes). Proximity betweåen each point describes the similarity of metal concentrations. Samples from the same site tend to be more clustered while the layers of 5-10cm and 10-15cm have higher variability to spread out from the centre cluster. The vector arrows describe the direction and strength of correlation with each element.*

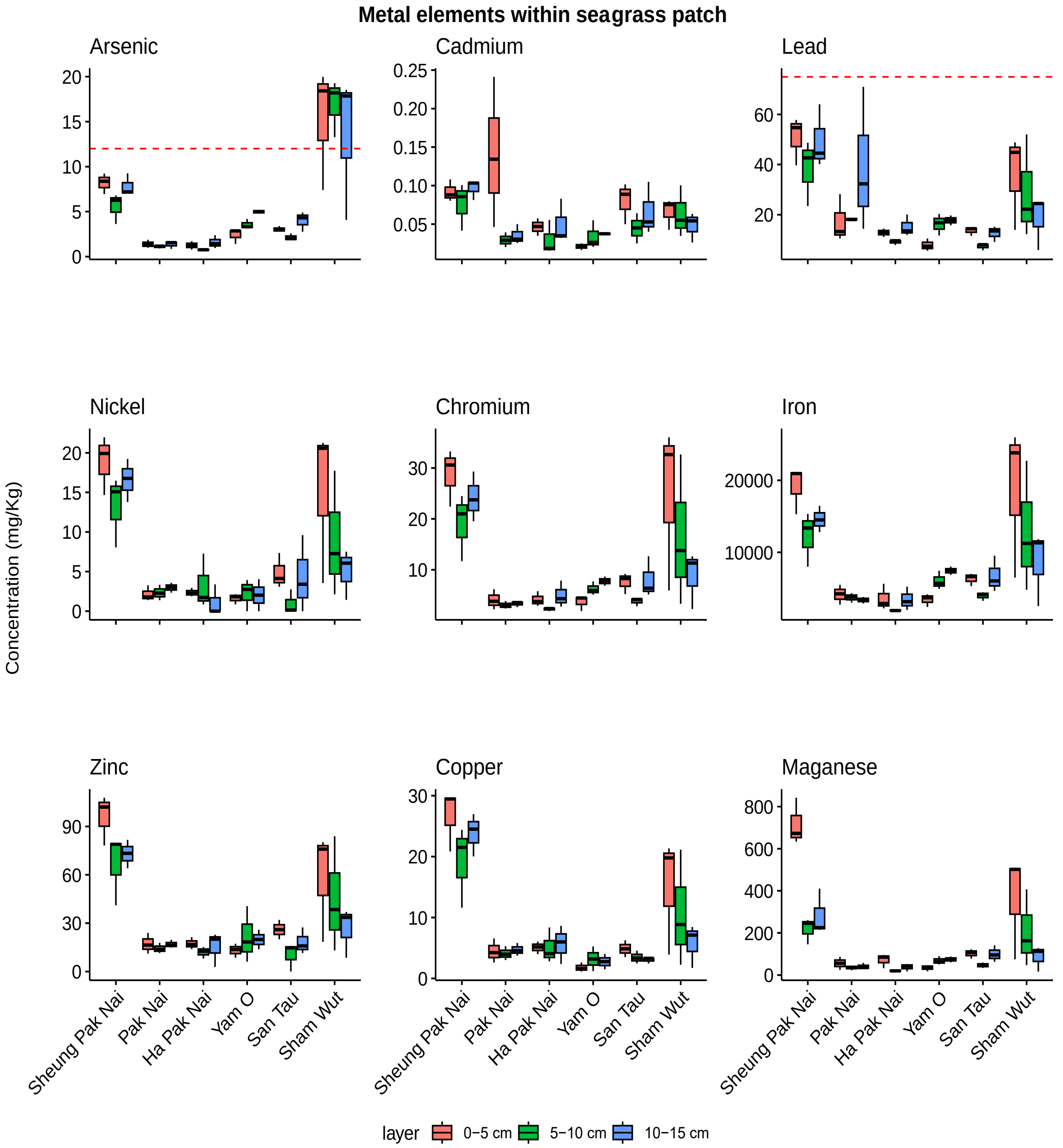

*Figure 10 The concentrations of arsenic, cadmium, lead, nickel, chromium, iron, zinc, copper and manganese in sediments collected within seagrass patch from Sheung Pak Nai, Pak Nai, Ha Pak Nai, Yam O, San Tau and Sham Wut. The red horizontal line represents the lower chemical exceedance level (LCEL) for sediment quality criteria used by the Hong Kong Environmental Protection Department (HKEPD).*

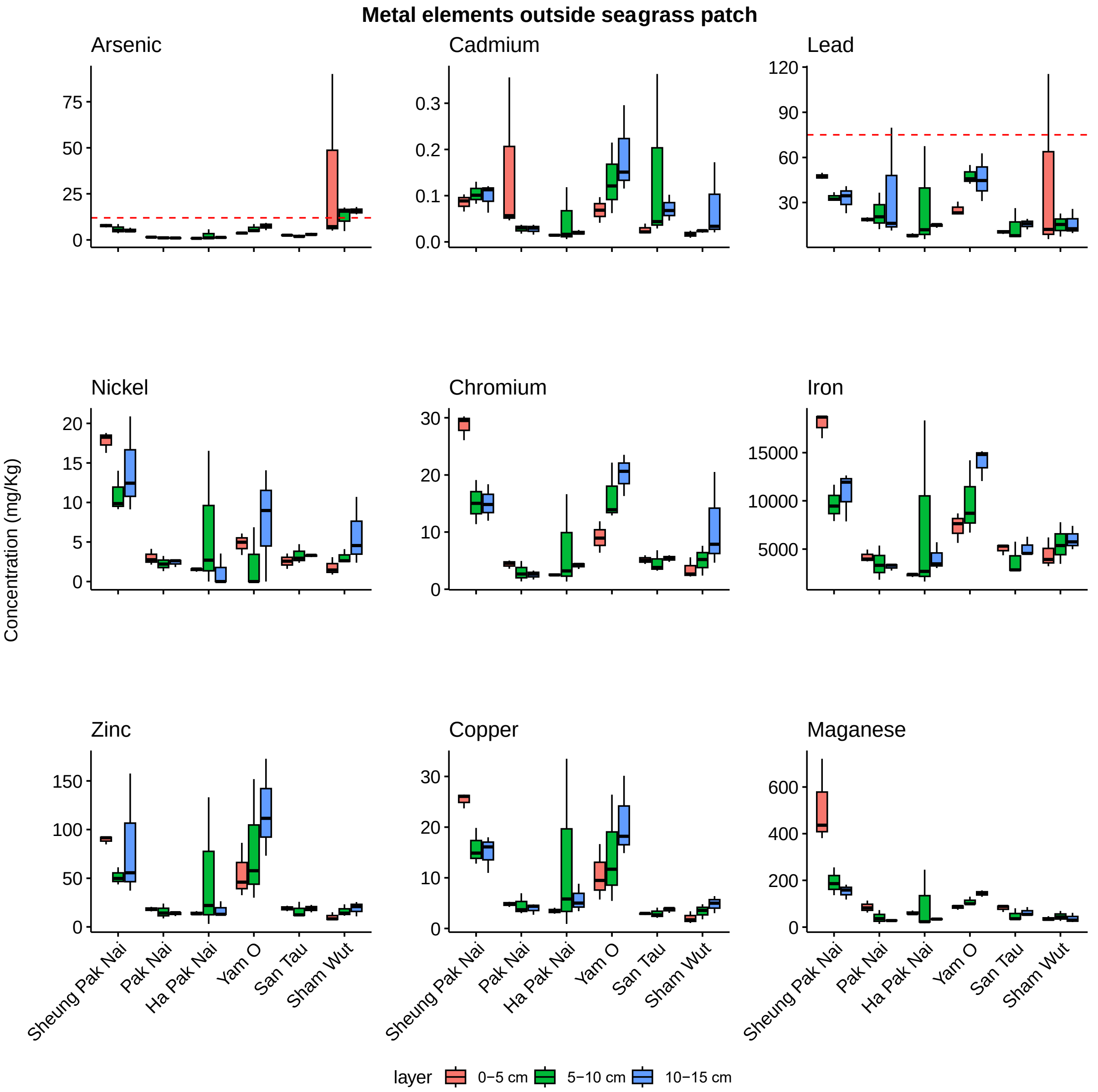

*Figure 11 The concentrations of arsenic, cadmium, lead, nickel, chromium, iron, zinc, copper and manganese in sediments collected outside of seagrass patch from Sheung Pak Nai, Pak Nai, Ha Pak Nai, Yam O, San Tau and Sham Wut. The red horizontal line represents the lower chemical exceedance level (LCEL) for sediment quality criteria used by the Hong Kong Environmental Protection Department (HKEPD).*

## Bioaccumulations in seagrasses

Only the stable seagrass patches at Pak Nai, Ha Pak Nai, San Tau and Yam O allowed us to have enough samples for metal analysis. The levels of different metal elements in seagrasses vary across different sites as well as in different parts of the plant (Figure 12 and 13). Concentrations of arsenic, iron, manganese and zinc were different in belowground and aboveground tissues (Wilcoxon, $p = 0.0077$, $p = 0.022$, $p = 0.012$, $p = 0.00012$ respectively). Meanwhile, linear regression was used to explore the correlation of each metal element concentration between sediment and seagrass. A negative correlation was found between the levels in seagrasses and in deeper sediments in terms of chromium and manganese (Figure 14). This indicates that the higher amount of the metal element found in the sediments, the less we could find in the seagrasses from the same site.

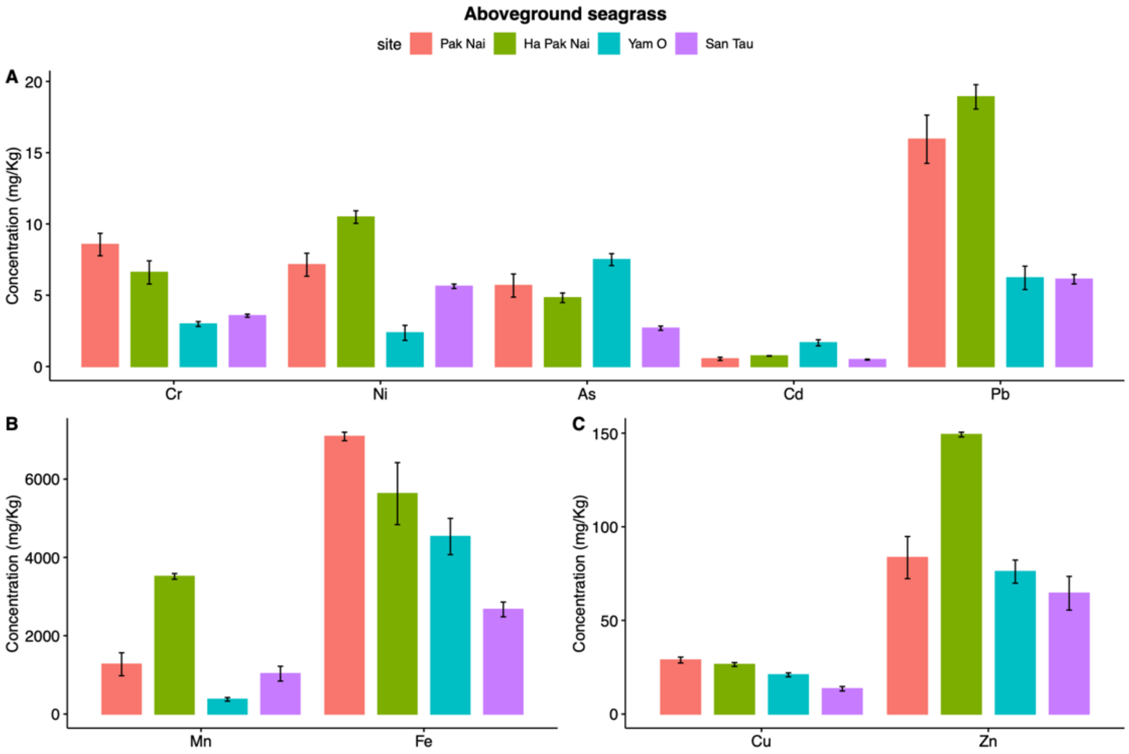

*Figure 12 Concentrations of different metal elements in seagrass **aboveground** tissues collected from Pak Nai, Ha Pak Nai, Yam O and San Tau. Panel (A) includes non-essential metal elements while panel (B) and (C) include essential metal elements.*

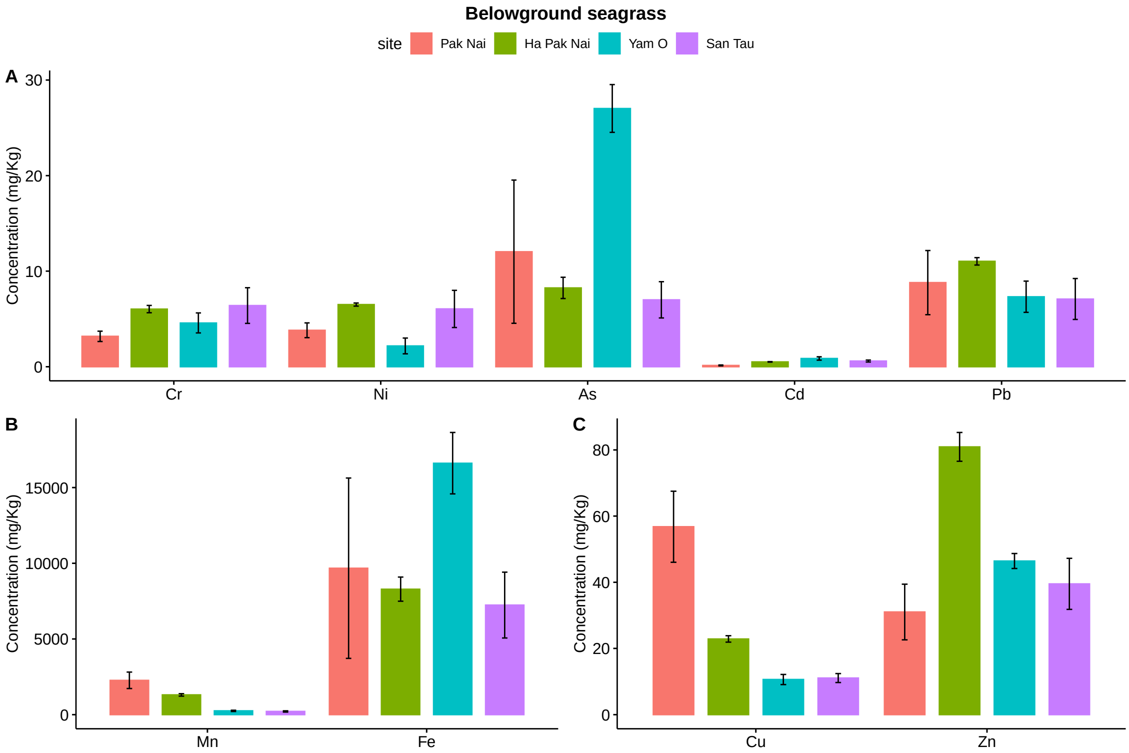

*Figure 13 Concentrations of different metal elements in seagrass **belowground** tissues collected from Pak Nai, Ha Pak Nai, Yam O and San Tau. Panel (A) includes non-essential metal elements while panel (B) and (C) include essential metal elements.*

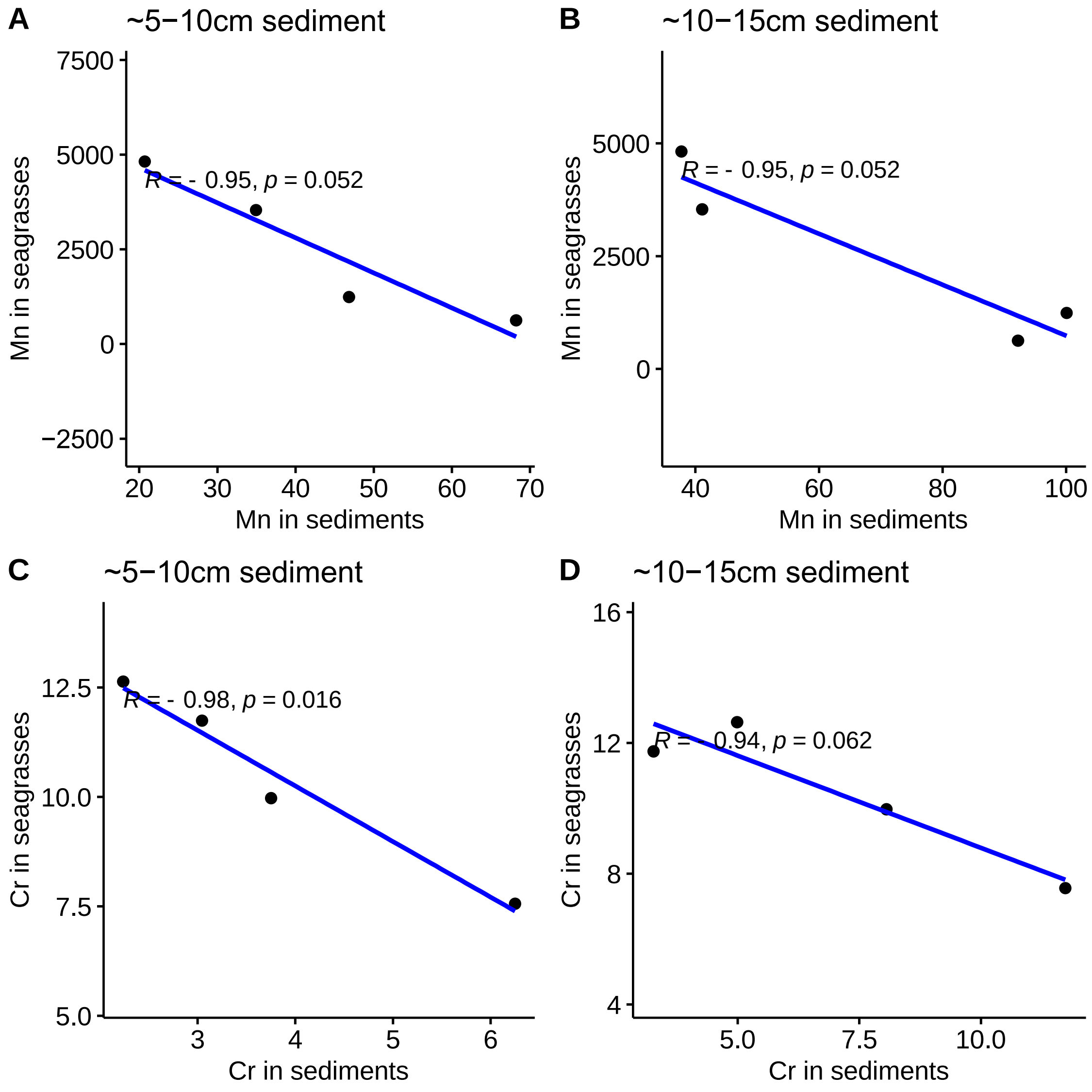

*Figure 14 Linear regression models between levels of chromium and manganese in deeper layers of sediments and in seagrasses.*

## Discussion

Overall, there is no general pattern of metal concentrations in sediments from different sites, different layer and whether it is within or outside of seagrass patch. However, there are sites with considerably high values of non-essential metal elements. For example, the high levels of arsenic, chromium, nickel and lead found in Sheung Pak Nai are concerning. Comparing to the nearby region, Pak Nai and Ha Pak Nai, the metal pollutions at Sheung Pak Nai is significantly more serious. In fact, the coverage of seagrasses from Ha Pak Nai to Pak Nai has been considerably stable over the year, despite some seasonal dynamics. Yet, the seagrass meadow always becomes patchy from Pak Nai to Sheung Pak Nai regardless to the time of the year. Although there can be more than one factor resulting in such growing pattern, the high amount of toxic metal elements found in the sediments from Sheung Pak Nai can be one of the explanations. Meanwhile, high levels of toxic metal elements were also found in Sham Wut, which is another site with little area of seagrasses. The levels of arsenic and lead here even exceeds the suggested limit by the authorities. Unfortunately, the seagrasses grown in both sites were too scarce for metal analysis. This is a general pattern we have observed in Hong Kong as part of the decline of local seagrass populations we have been documenting in the last 3 years.

The distribution of metal elements in seagrass tissues varies. We found that arsenic was more abundant in belowground seagrass tissue, suggesting that *H. beccarii* and *H. ovalis* in Hong Kong tend to accumulate this toxic metal element in their belowground tissue. This tendency aligns with the results from previous studies of other seagrass species (Jeong et al., 2021; Jeong & Ra, 2022; Lee et al., 2019; Maher et al., 2011). Root cells are often the first to encounter arsenic and takes up the element. The accumulation of arsenic in the belowground tissues of seagrass brings up a concern of hindered growth as arsenic is known to inhibit root cell metabolism. (Finnegan & Chen, 2012)

Linear models were used to investigate the potential correlation in the levels of metal elements between seagrass tissues and sediments. Manganese and chromium were two elements found to have a negative correlation in this study. Manganese is an essential element to plants while chromium is highly toxic. A negative correlation could indicate that seagrasses may have taken up the chromium in sediments and thus a higher level of this element was found in the tissue. Although some other factors such as the abundance of other living organisms may account for this trend, this remains a potential hazard as chromium is deleterious to plants (Nagajyoti et al., 2010).

# OBJ2: Heavy metal pollution and seagrass-associated biodiversity

## Introduction

One of the main priorities for improving ecosystem management is the development of early warning indicators that identify ecosystem stress. For seagrasses, the importance of microbial communities as indicators of environmental disturbance, has recently been highlighted, as well as to their efficacy in monitoring health and recovery under environmental stress (Martin et al., 2020). Microbes play a significant role in plant biochemical regulation, which is essential to maintain plant health and growth. For example, some bacteria grouped as "plant growth promoting bacteria (PGPBs)", are considered beneficial to plants by releasing phytohormones that are vital for vegetative grow (Farrar et al., 2014). It has come to researchers' attention that there are different sub-environments of microbiome in different parts of a plant because of varied levels of nutrients and oxygen available. Among them, root microbiome has been a great study interest. Root microbiome is a region of soil microbes that its composition differs from other parts because of root exudates, degraded root cells and mucilage (Turner et al., 2013). Some studies have found that microbes play an essential role in mediating plants' metal cycling, which is fundamental to stress-adaptation under metal pollution. For instance, some PGPBs can assist their host in metal mobilization and translocation in the process of bioremediation (Ma et al., 2016). For marine plants, such as seagrasses, a review in 2017 summarized that seagrass microbiomes are involved in nutrient and biochemical cycling, particularly for carbon, nitrogen and sulphur. Some of the symbionts are protective to seagrass against external toxification (Ugarelli et al., 2017). A recent study looked into seagrass root microbiome growing in polluted regions and characterized some microbial indicators that show the health condition of the seagrass population (Martin et al., 2020). Later in 2022, they applied similar technology to investigate the difference in seagrass root microbial communities under the stress of metal pollution. The results showed that there can be a shift in microbiome composition under stronger metal pollution stress with increase in some signature bacteria taxa (Martin et al., 2022).

With the advance in microbiome studies using sequencing methods, we can develop further understanding regarding the interplay between seagrasses and their associated microbiomes, which is a potential rapid tool to find out biological indicators of pollution. Thus, microbiome study offers a unique approach to assess the health conditions of seagrass, which is an essential component in conservation management. Using 16S rRNA gene sequencing, we have characterized the associated biodiversity of seagrasses. We have assessed the microbial biodiversity function and structure associated to gradients of heavy metal pollution and the health of the seagrasses in western Hong Kong and Lantau Island.

## Method

### Sample collections

The sediment samples used for assessing microbiome were the same as for metal analysis. To obtain the root microbiome, a total of nine replicates were collected from each site where there were enough seagrasses for sampling. At the site, roots were collected using sterile tools and the samples were put on ice until back to the laboratory. Samples were then kept at –80$^{o}$C until DNA extraction.

### 16S rRNA gene sequencing

Total DNA from sediment samples and root microbiome were extracted using NucleoSpin® Soil kit (MACHEREY-NAGEL) according to manufacturer's instructions. The quality of extracted DNA was checked using NanoDrop One Microvolume UV-Vis Spectrophotometers (Thermo Scientific) and gel electrophoresis following PCR amplifying V3-V4 hypervariable region. The DNA samples were sent for library preparation and 16S rRNA gene sequencing using Illumina MiSeq platform (V3-V4, 250pb, PE).

### Metagenomic analysis

Primer sequences and barcodes were removed from raw reads by the company. Quality check on the cleaned reads was done using FastQC (v0.11.9). Adaptors were removed using fastp (v.0.23.2) (Chen et al., 2018). The subsequent analyses were carried out in R (v4.1.1). The sequences were passed for quality filtering on DADA2 (Callahan et al., 2016, p. 2) and taxonomic classification using the Silva 138.1 release from DADA2 reference databases (Quast et al., 2013; Yilmaz et al., 2014). Unconstrained ordination by Principal Coordinates Analysis (PCoA) was conducted to visualize the compositional dissimilarity (beta diversity) in bacterial composition of sediment or seagrass root microbiome from different sites. Constrained ordination was done using Canonical Analysis of Principal coordinates (CAP) to show the association between the community composition and different metal concentrations. Both ordinations were conducted using R packages phyloseq (v.1.38.0) (McMurdie & Holmes, 2013) and vegan (v.2.6-4) (Dixon, 2003). Adonis in vegan was used for performing PERMANOVA to evaluate whether the variable is significant for the distances in the ordinations (with 999 permutations). iNEXT (v.3.0.0) (Chao et al., 2014; Hsieh et al., 2016) was used for rarefaction and extrapolation. Shannon and Simpson diversity were used to compute the alpha diversity indices in the layers of sediments from different sites. One-way ANOVA test was used to indicate any significant difference of alpha diversity at different sites. DESeq2 (v1.34.0) (Love et al., 2014) was used to perform differential abundance analysis to find out key taxa that can indicate a specific factor. Correlation analysis was done to find out indicative taxa with different metal elements. As Sheung Pak Nai, Pak Nai and Ha Pak Nai are adjacent to each other, we used the data from these three sites to ensure that the metal pollution levels are the major factor of a change in abundance. We investigated the taxa identifications up to order-level of the top 20 ASVs from different layers in these three sites. For root microbiome, the correlation analysis was done using top 20 ASVs with identification up to family-level with metal concentrations found in seagrass belowground tissue.

## Results

### Seagrass-associated biodiversity in sediment

The alpha diversity of sediments from seagrass patch and outside of seagrass patch varied. First, we grouped the sites by the available seagrass species. The alpha diversity of sediments from *H. beccarii* meadow was significantly different than its adjacent region without seagrasses (one-way ANOVA, $p < 0.01$) while it was similar for *H. ovalis* and its adjacent region (one-way ANOVA, $p = 0.79$)(Figures 15-16). There was also a significant difference in alpha diversity between sediments from *H. beccarii* sites and *H. ovalis* sites (one-way ANOVA, $p < 0.01$). Meanwhile, no significant difference in alpha diversity was found between different layer of sediments. PERMANOVA results revealed that the compositions of sediments were distinctive among different sites (PERMANOVA, $p = 0.001$ for all sediment layers). Community compositions between sediments from *H. beccarii* meadow and *H. ovalis* meadow also differed (PERMANOVA, $p = 0.002$ for 0-5cm, $p = 0.001$ for 5-10cm, $p = 0.002$ for 10-15cm)(Figures 17-20).

Figures 21-22 showed the relative abundance of the top 20 sequences with taxonomic assignment up to order-level in the sediment samples. The relative abundance of microbes from the order *Nitrosopumilales* showed an observable difference in the 0-5cm layer from the seagrass patch at Sheung Pak Nai (Figure 21). This observation aligns with the differential abundance analysis when comparing Sheung Pak Nai with other sites. All comparisons against Sheung Pak Nai, except the one with Yam O, resulted in a log2fold change < -1.5 with p-value < 0.01. This can indicate a significantly enriched abundance of *Nitrosopumilales* in Sheung Pak Nai.

Pearson correlation analysis between top 20 ASVs and metal concentrations found in sediments from Pak Nai region suggested that the top 0-5cm would be the most indicative among the three layers. Six out of the ten identified taxa were found to have a strong correlation (in terms of correlation coefficient) with more than one metal element in the 0-5cm layer (Table 1). *Actinomarinales, Nitrosopumilales* and *Steroidobacterales* resulted a correlation coefficient lower than -0.8 or higher than 0.8 with almost all detected metal elements (except cadmium), suggesting a strong negative or positive correlation with metal contaminants.

### Seagrass-associated biodiversity in root microbiome

Meanwhile, the root microbiome in *H. ovalis* was more diverse than that in *H. beccarii* (t-test, $p < 0.01$), with *H. beccarii* from Pak Nai having the lowest alpha diversity and *H. ovalis* from Yam O having the highest (Figures 15-16). Community compositions among different seagrass species were found to be distinct. (PERMANOVA, $p = 0.001$, Figures 17-20). Figure 23 showed the relative abundance of the top 20 sequences with taxonomic assignment in seagrass root microbiome up to family-level. The family *Xenococcaceae* was found to be more abundant in the root microbiome of *H. ovalis* than in that of *H. beccarii* (log2foldchange = -6.53, $p < 0.01$). The family *MBAE14*, on the other hand, was more prevalently found in the root microbiome of *H. beccarii* (log2fold change = 8.83, $p < 0.01$).

Pearson correlation analysis between the top 20 ASVs found in seagrass root microbiome and seagrass belowground tissue suggested some families being closely correlated with metal concentrations. *Rhodobacteraceae, Sandaracinaceae, Sedimenticolaceae, Vibrionaceae and Woeseiaceae* were found to have strong correlations with different metal elements (Table 4).

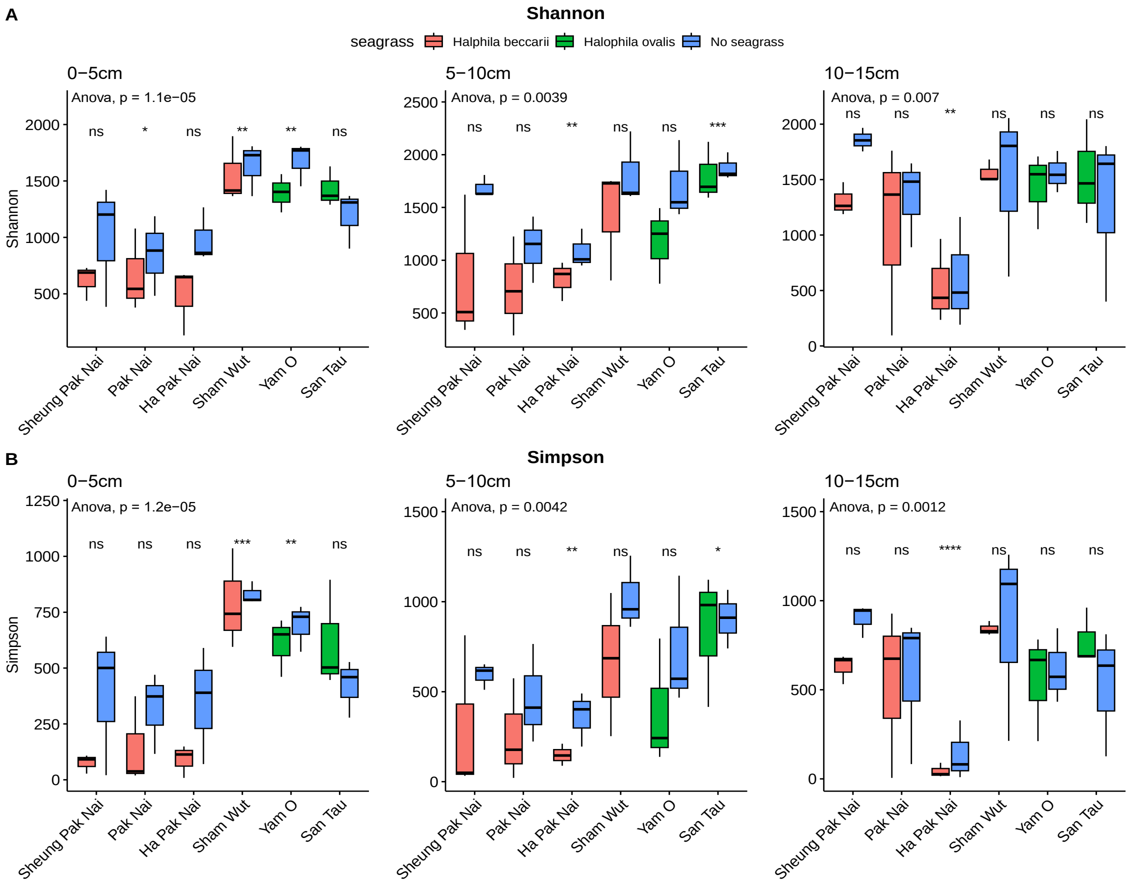

*Figure 15 Boxplots of alpha diversity indices in sediments. (A) Shannon and (B) Simpson indices show the ASV diversity in different layers of samples, collected from each site, within and outside of seagrass patch.*

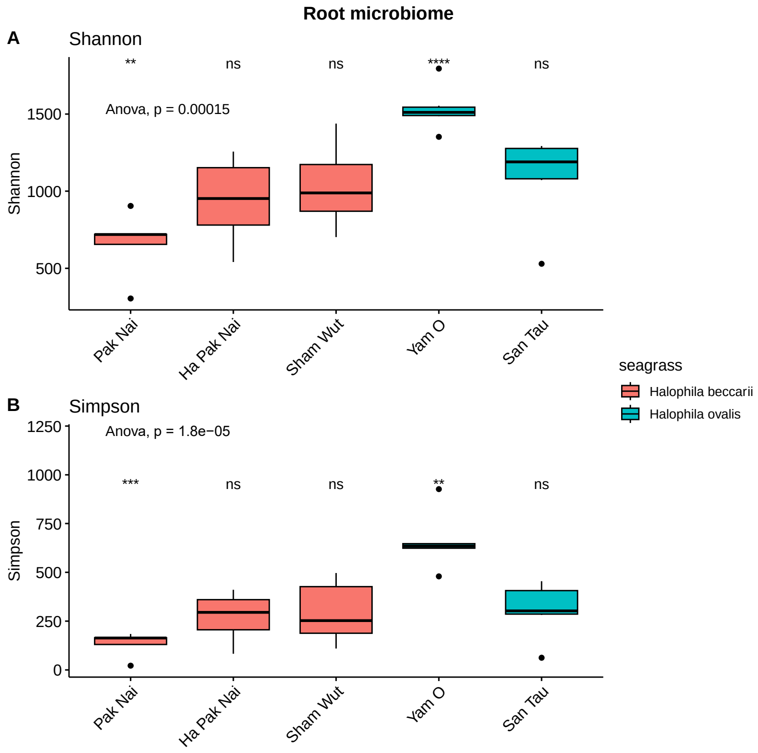

*Figure 16 Boxplots of alpha diversity indices in seagrass root microbiome.*

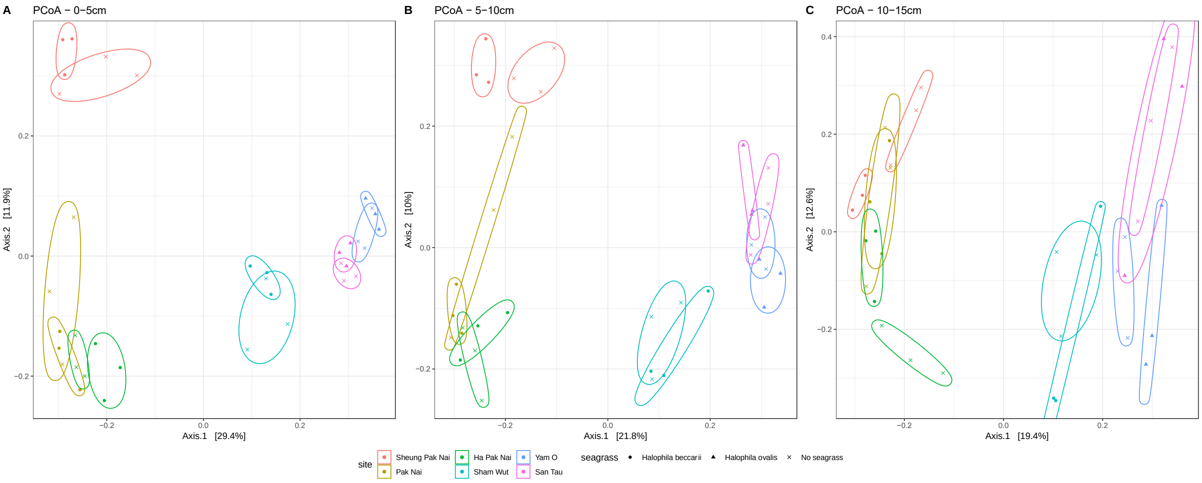

*Figure 17 Principal coordinate analysis (PCoA) plots of ASVs from sediment samples.*

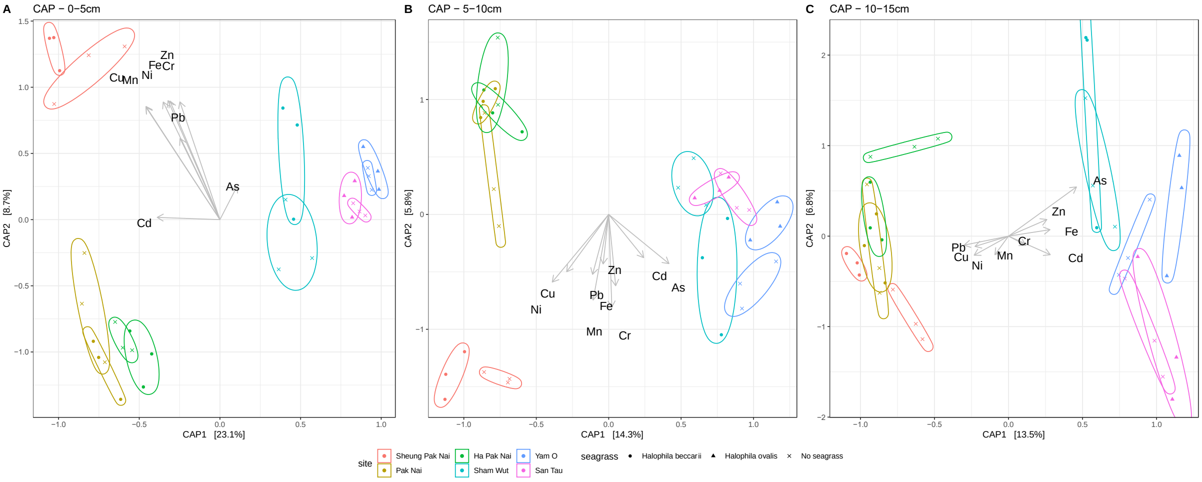

*Figure 18 Canonical analysis of principal coordinates (CAP) of Bray-Curtis dissimilatory on ASVs from sediment samples constrained by different metal concentrations.*

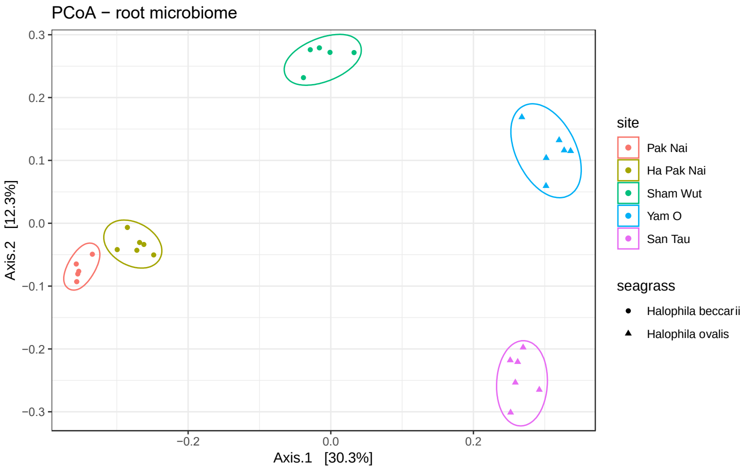

*Figure 19 Principal coordinate analysis (PCoA) plots of ASVs from seagrass root microbiome samples.*

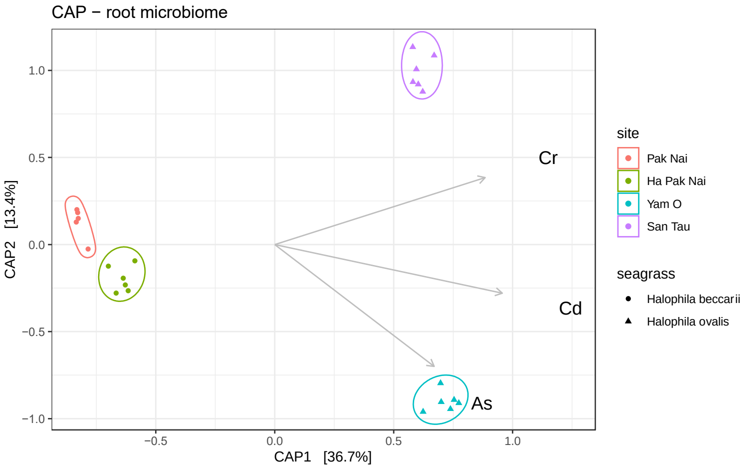

*Figure 20 Canonical analysis of principal coordinates (CAP) of Bray-Curtis dissimilatory on ASVs from seagrass root microbiome samples constrained by different metal concentrations.*

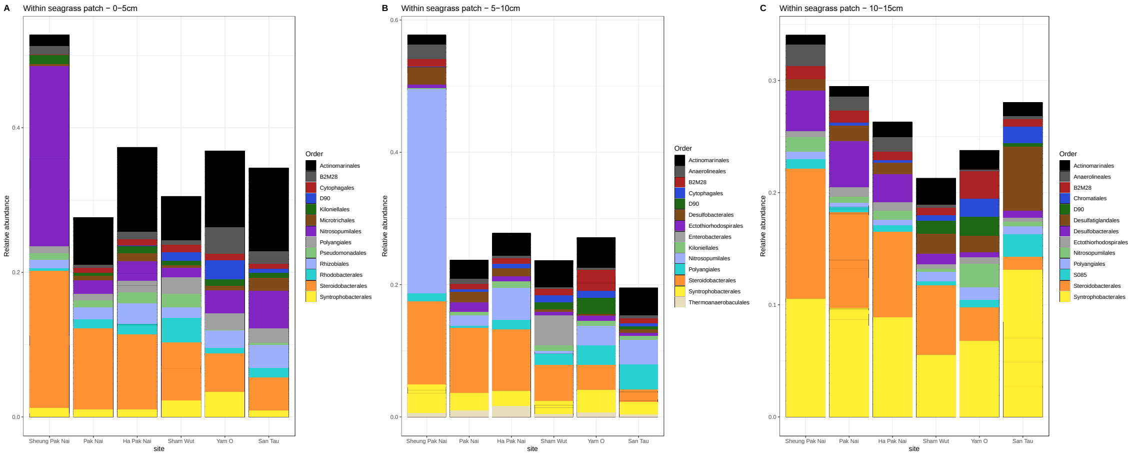

*Figure 21 Relative abundance of the top 30 sequences assigned up to order-level taxa from samples within the seagrass patch.*

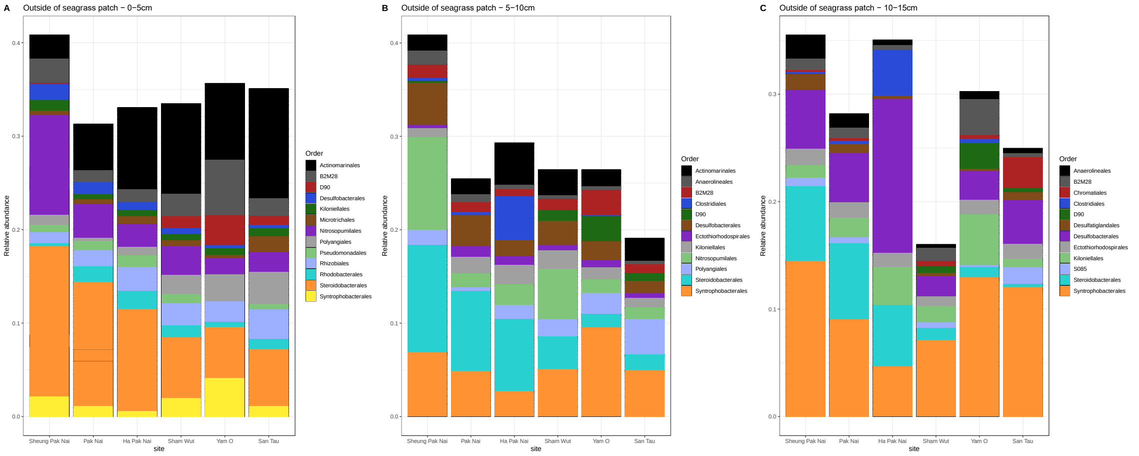

*Figure 22 Relative abundance of the top 30 sequences assigned up to order-level taxa from samples outside of the seagrass patch.*

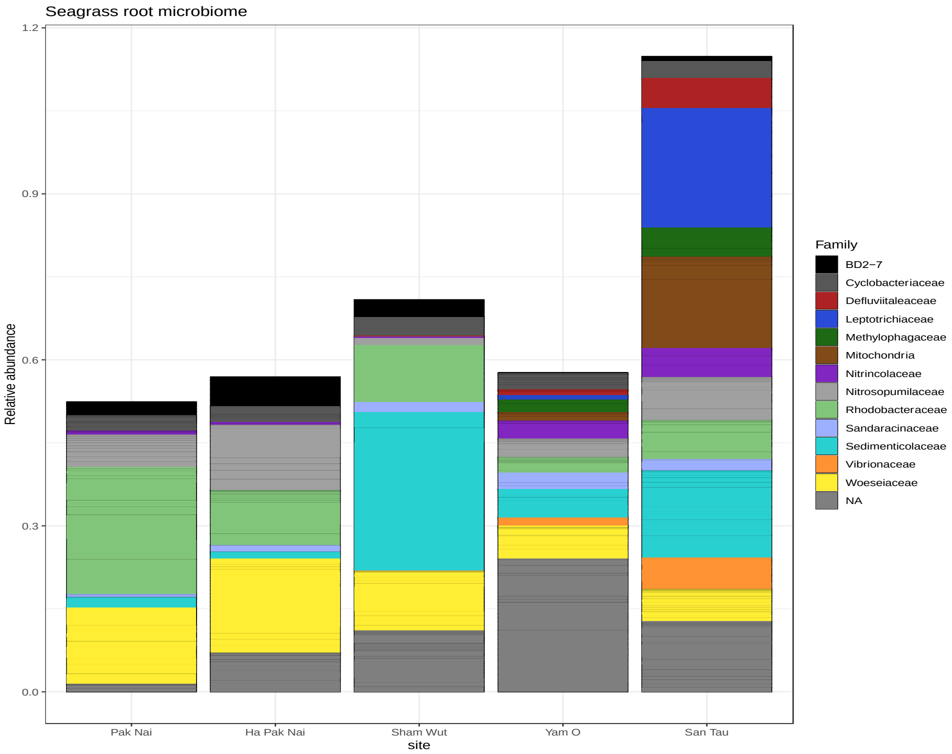

*Figure 23 Relative abundance of the top 30 sequences assigned up to order-level taxa from seagrass root microbiome samples.*

## Discussion

The analysis of community dissimilarity suggested that the structure of microbiome was more likely to be shaped by geological locations. Although we found that the overall alpha diversity from *H. beccarii* sites was lower than that from *H. ovalis* sites and at the same time, the diversity within the *H. beccarii* patch was significantly lower than outside, such differences can be specific to the Pak Nai region as three out of four *H. beccarii* sites were from this area.

Yet, differential abundance analysis of the identified taxa was able to tell us more about the conditions of the sites. The significantly enriched *Nitrosopumilales* in Sheung Pak Nai can be an indicator of pollution as it is one of the ammonia-oxidizing archaea (AOA) (Qin et al., 2020). They involve in the marine sediment nitrogen cycle by ammonia oxidation (Schleper & Nicol, 2010) and were found to be more abundant in estuarine with hypoxia history (Caffrey et al., 2007). Hypoxia often results from excessive nutrients, including nitrogen. Their enrichment in Sheung Pak Nai suggested that there may be a nitrogen flux, resulting in more AOA for the nitrogen cycling as a coping mechanism.

Such observation of high *Nitrosopumilales* abundance in Sheung Pak Nai is complementary to the correlation analysis (Table 1). From the 0-5cm sediment of the Pak Nai region, *Nitrosopumilales* was found to be positively correlated with arsenic, chromium, copper, iron, manganese, nickel, lead and zinc, meaning that site with more nitrogen pollution was likely to be more polluted with metal elements as well in this model. Other orders of microbes found to be closely correlated with metal concentrations such as *Actinomarinales* and *Steroidobacterales* are not well studied yet. *Actinomarinales* belongs to the phylum *Actinobacteria*, which is known to be more abundant with rich organic matter. Many of the members under this phylum can degrade organic matter (Barka et al., 2015). It is possible that the presence of microbes from *Actinomarinales* is related to a higher amount of organic pollution. *Steroidobacterales* come from phylum *Gammaproteobacteria*. This phylum consists of many sulfur-oxidizing bacteria (Dyksma et al., 2016; Lenk et al., 2011). Although we do not have enough information to draw a detailed conclusion, it is possible that both of them are related to pollution. They are worth to note in any future study on their correlations with metal pollution because of our findings.

The Pearson correlation analysis with seagrass root microbiome and their belowground tissue indicated that *Sandaracinaceae* was positively correlated with arsenic, cadmium and lead, but negatively correlated with manganese. Arsenic, cadmium and lead are non-essential elements that trace amount of them is already toxic to living organisms including plants (Nagajyoti et al., 2010). Manganese is an essential micronutrient to plant instead. This suggests that *Sandaracinaceae* could be an indicator when the seagrass belowground tissue has accumulated with more toxic metal elements but with depleted nutrients. Meanwhile, *Woeseiaceae* was negatively correlated with cadmium, chromium and lead but positively correlated with manganese. It was suggested that some *Woeseiaceae* members are potentially chemolithoautotrophy, meaning that they can oxidize inorganic compounds (Mußmann et al., 2017). It may have an opposite role comparing to *Sandaracinaceae* that they would be more abundant when the seagrasses accumulated less harmful metal elements.

*Table 1 Pearson correlation coefficient between the top 20 ASV abundance (assigned up to order-level) with metal concentrations found in the 0-5cm layer of sediment in Sheung Pak Nai, Pak Nai and Ha Pak Nai. Positive values > 0.8 are labelled by green. Positive values > 0.7 but < 0.8 were labelled by yellow. Negative values < 0.7 were labelled by red.*

| Order-level of top 20 ASVs in 0-5cm | Cr | Mn | Fe | Ni | Cu | Zn | As | Cd | Pb |
|---|---|---|---|---|---|---|---|---|---|
| ***Actinomarinales*** | -0.86 | -0.82 | -0.87 | -0.87 | -0.85 | -0.85 | -0.87 | -0.34 | -0.87 |
| ***Anaerolineales*** | 0.79 | 0.70 | 0.79 | 0.79 | 0.79 | 0.80 | 0.77 | -0.13 | 0.78 |
| ***B2M28*** | 0.72 | 0.62 | 0.70 | 0.72 | 0.72 | 0.72 | 0.70 | 0.00 | 0.66 |
| *Clostridiales* | -0.55 | -0.50 | -0.59 | -0.55 | -0.53 | -0.54 | -0.57 | -0.49 | -0.63 |
| *Desulfobacterales* | 0.66 | 0.51 | 0.65 | 0.68 | 0.65 | 0.65 | 0.63 | 0.35 | 0.65 |
| ***Nitrosopumilales*** | 0.81 | 0.87 | 0.84 | 0.83 | 0.84 | 0.84 | 0.84 | 0.04 | 0.83 |
| *Peptostreptococcales-Tissierellales* | -0.55 | -0.51 | -0.58 | -0.55 | -0.53 | -0.54 | -0.57 | -0.49 | -0.61 |
| *Pseudomonadales* | -0.45 | -0.37 | -0.43 | -0.47 | -0.43 | -0.44 | -0.43 | -0.55 | -0.43 |
| ***Steroidobacterales*** | 0.83 | 0.82 | 0.84 | 0.83 | 0.82 | 0.84 | 0.83 | 0.05 | 0.84 |
| ***Syntrophobacterales*** | 0.79 | 0.59 | 0.79 | 0.80 | 0.78 | 0.78 | 0.75 | 0.25 | 0.79 |

*Table 2 Pearson correlation coefficient between the top 20 ASV abundance (assigned up to order-level) with metal concentrations found in the 5-10cm layer of sediment in Sheung Pak Nai, Pak Nai and Ha Pak Nai. Positive values > 0.8 are labelled by green. Positive values > 0.7 but < 0.8 were labelled by yellow. Negative values < 0.7 were labelled by red.*

| Order-level of top 20 ASVs in 5-10cm | Cr | Mn | Fe | Ni | Cu | Zn | As | Cd | Pb |
|---|---|---|---|---|---|---|---|---|---|
| *Actinomarinales* | -0.45 | -0.41 | -0.29 | -0.34 | -0.26 | -0.18 | -0.46 | -0.48 | -0.29 |
| ***Anaerolineales*** | 0.80 | 0.68 | 0.61 | 0.66 | 0.54 | 0.45 | 0.64 | 0.54 | 0.50 |
| ***Burkholderiales*** | 0.83 | 0.76 | 0.65 | 0.71 | 0.58 | 0.53 | 0.76 | 0.64 | 0.51 |
| *B2M28* | 0.46 | 0.34 | 0.25 | 0.36 | 0.22 | 0.14 | 0.37 | 0.41 | 0.13 |
| *Clostridiales* | -0.04 | 0.03 | 0.19 | 0.17 | 0.29 | 0.37 | -0.04 | 0.01 | 0.20 |
| *Desulfobacterales* | 0.53 | 0.39 | 0.31 | 0.42 | 0.26 | 0.17 | 0.43 | 0.42 | 0.21 |
| *Nitrosopumilales* | 0.18 | 0.22 | 0.12 | 0.13 | 0.08 | 0.08 | 0.20 | 0.05 | 0.01 |
| *Peptostreptococcales-Tissierellales* | -0.14 | -0.10 | 0.05 | 0.06 | 0.14 | 0.23 | -0.16 | -0.14 | 0.04 |
| *Steroidobacterales* | 0.53 | 0.50 | 0.32 | 0.42 | 0.24 | 0.19 | 0.55 | 0.40 | 0.21 |
| *Syntrophobacterales* | 0.50 | 0.36 | 0.29 | 0.39 | 0.25 | 0.17 | 0.39 | 0.37 | 0.20 |

*Table 3 Pearson correlation coefficient between the top 20 ASV abundance (assigned up to order-level) with metal concentrations found in the 10-15cm layer of sediment in Sheung Pak Nai, Pak Nai and Ha Pak Nai. Positive values > 0.8 are labelled by green. Positive values > 0.7 but < 0.8 were labelled by yellow. Negative values < 0.7 were labelled by red.*

| Order-level of top 20 ASVs in 10-15cm | Cr | Mn | Fe | Ni | Cu | Zn | As | Cd | Pb |
|---|---|---|---|---|---|---|---|---|---|
| ***Anaerolineales*** | 0.63 | 0.57 | 0.65 | 0.74 | 0.62 | 0.65 | 0.69 | 0.78 | 0.28 |
| *Clostridiales* | -0.23 | -0.22 | -0.25 | -0.41 | -0.26 | -0.30 | -0.29 | -0.38 | -0.44 |
| *Desulfatiglandales* | 0.34 | 0.29 | 0.32 | 0.34 | 0.31 | 0.21 | 0.39 | 0.54 | 0.25 |
| *Desulfobacterales* | -0.14 | -0.13 | -0.16 | -0.20 | -0.13 | -0.14 | -0.17 | -0.17 | -0.18 |
| *Kiloniellales* | -0.43 | -0.40 | -0.45 | -0.45 | -0.39 | -0.34 | -0.49 | -0.60 | -0.44 |
| ***Steroidobacterales*** | 0.63 | 0.70 | 0.59 | 0.70 | 0.66 | 0.54 | 0.62 | 0.43 | 0.40 |
| *Syntrophobacterales* | 0.46 | 0.42 | 0.46 | 0.49 | 0.45 | 0.35 | 0.52 | 0.64 | 0.39 |

*Table 4 Pearson correlation coefficient between the top 20 ASV abundance (assigned up to family-level) in seagrass root microbiome with metal concentrations found in their belowground tissue. Positive values > 0.8 are labelled by green. Positive values > 0.7 but < 0.8 were labelled by yellow. Negative values < 0.7 were labelled by red.*

| Family of top 20 ASVs in root microbiome | Cr | Mn | Fe | Ni | Cu | Zn | As | Cd | Pb |
|---|---|---|---|---|---|---|---|---|---|
| *Defluviitaleaceae* | 0.61 | -0.45 | 0.05 | 0.62 | -0.27 | 0.20 | -0.05 | 0.31 | 0.43 |
| ***Leptotrichiaceae*** | 0.63 | -0.42 | -0.07 | 0.72 | -0.25 | 0.14 | -0.18 | 0.23 | 0.38 |
| ***Methylophagaceae*** | 0.77 | -0.64 | 0.26 | 0.64 | -0.40 | 0.34 | 0.15 | 0.53 | 0.63 |
| *Nitrincolaceae* | 0.51 | -0.44 | 0.24 | 0.38 | -0.26 | 0.24 | 0.17 | 0.40 | 0.45 |
| *Nitrosopumilaceae* | -0.10 | 0.16 | -0.50 | 0.21 | -0.03 | -0.06 | -0.50 | -0.37 | -0.26 |
| ***Rhodobacteraceae*** | -0.50 | 0.69 | -0.50 | -0.16 | 0.76 | -0.79 | -0.46 | -0.65 | -0.67 |
| ***Sandaracinaceae*** | 0.62 | -0.79 | 0.85 | 0.09 | -0.62 | 0.72 | 0.80 | 0.91 | 0.85 |
| ***Sedimenticolaceae*** | 0.84 | -0.66 | 0.16 | 0.78 | -0.40 | 0.32 | 0.04 | 0.49 | 0.63 |
| ***Vibrionaceae*** | 0.85 | -0.65 | 0.13 | 0.82 | -0.40 | 0.30 | 0.00 | 0.47 | 0.62 |
| ***Woeseiaceae*** | -0.76 | 0.75 | -0.60 | -0.41 | 0.49 | -0.51 | -0.51 | -0.77 | -0.79 |

# OBJ3: Resilience and responses of local seagrasses under heavy metal pollution

## Introduction

Plants are responsive to their external environment. Under stress condition, they adopt physiological changes to increase their chances of survival. Underlying these feedbacks, there is a complex signaling network modulated at the molecular level. A review in 2019 summarized the feedbacks of plants under metal pollution stress, including their molecular changes (Ghori et al., 2019). Changes in gene expression of plants are expected to happen when they are exposed to stressful conditions. For instance, expression of antioxidative enzymes is expected to increase because metal stress causes productions of reactive oxygen species (ROS). Under these conditions, we can also expect an increase in phytohormones expressions be- cause theses hormones are useful to regulate other signaling pathways involved in producing antioxidants and detoxification (Ghori et al., 2019). In seagrasses, Buapet et al. found that increased copper exposure caused elevated expression of antioxidant-activities-related genes (Buapet et al., 2019). Some other similar studies concluded that apart from antioxidant genes, metal chelators were also induced in their experiments(Alvarez-Legorreta et al., 2008; Greco et al., 2019; Lin et al., 2016). Apart from the mentioned pathways, metabolic and growth-related signaling network may also worth the attention as studies have shown that photosynthesis and growth rate can be hindered under experimental exposure of metal stress (Ambo-Rappe et al., 2011; Llagostera et al., 2016; Macinnis-Ng & Ralph, 2002; Prange & Dennison, 2000; Ralph & Burchett, 1998).

The signaling pathways mentioned above are the mechanisms utilized by plants to survive despite the critical situations. When plants are consistently exposed to such an abiotic stress, there is a chance that the population may achieve certain degree of resilience by epigenetic modification. This ability allows plants to quickly respond to stressful events, enhancing survival under stressful conditions (Sharma et al., 2022). To understand how our local seagrasses may react under metal pollution, the first step is to adopt metal exposure experiments. We have performed experiments using Hong Kong local seagrasses *H. beccarii* to understand specifically the mechanisms used by them to cope with and respond to the physiological challenges imposed by metal pollution in Hong Kong, one of the most highly urbanized coastal areas on Earth.

## Method

### Seagrass collection

*H. beccarii* is one of the most common seagrass species in Hong Kong, mainly growing in Pak Nai and Ha Pak Nai. The seagrasses used in the experiment were collected from Pak Nai on 12th August 2022. Three 20x20cm quadrats of *H. beccarii* were collected randomly over the site. Collected seagrasses were sent back to the laboratory and cleaned with seawater and MiliQ water to remove sediments and epiphytes. 10 replicates of seagrasses were collected for metal analysis.

### Experimental design

The cleaned seagrasses were acclimated in the laboratory for 7 days before the experiment started. They were kept at 25°C ± 2°C in 20 ‰ artificial sea water prepared using distilled water. The light intensity was kept at 80-100 µmol $m^{-2}s^{-1}$ with a 12h:12h light-dark cycle. After 7 days, another 10 replicates of seagrasses were collected for metal analysis. Then, the same wet mass of acclimated seagrasses was put into plastic containers, which were pre-washed with 10% $HNO_3$. Stocks of treatment artificial seawater with corresponding metal concentrations were prepared and aliquoted to each container. The selected metal treatments were copper and lead because of their significant impacts on plant and seagrass species (Barwick & Maher, 2003; Greco et al., 2019; Lin et al., 2016, 2018; Llagostera et al., 2016; Macinnis-Ng & Ralph, 2002) and their significant amounts from marine sediment in Hong Kong reported by HKEPD (Annual Marine Water Quality Reports, HKEPD). The seagrasses were exposed in 50 µg/L and 250 µg/L of copper, while the concentrations of lead were 50 µg/L and 500 µg/L. The concentrations were chosen based on the ambient concentrations (Blackmore, 1998; Rong et al., 2022) and a representative concentration that have shown chronic toxicity in plants and seagrass in previous studies (Buapet et al., 2019; Greco et al., 2019; Lin et al., 2016; Macinnis-Ng & Ralph, 2002; Prange & Dennison, 2000; Shah Mohammadi et al., 2019). The temperature, pH, light intensity and salinity of each tank were maintained at the same level throughout the experiment. To maintain consistent treatment levels, the treatment waters were changed every 2 days. The timeline of the experiment was as shown in Figure 24.

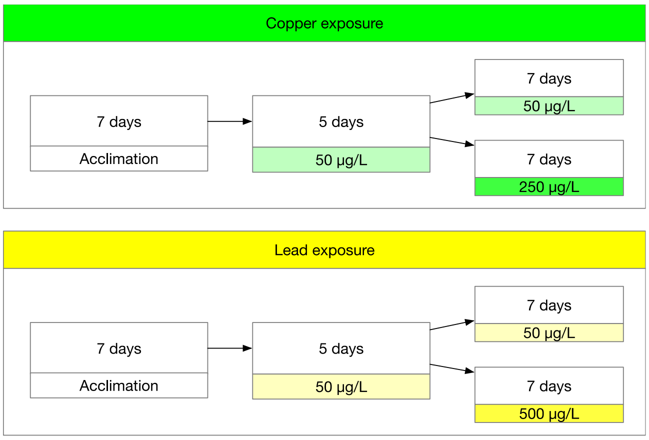

*Figure 24 A schematic figure of the experimental design. H. beccarii collected from Pak Nai was acclimated in the lab for 7 days and exposed to low concentrations of metal treatments, copper and lead, for another 5 days. Then, they were separated into two cohorts, exposed to low and high concentrations of treatments respectively for 7 more days.*

## Physiological parameters

Hansatech Pocket PEA (Hansatech Instruments Ltd) was used to measure their chlorophyll a fluorescence parameters every day, to represent photochemical efficiency. Five leaves from each treatment replicate were dark acclimated for 15 minutes using leaf clips before being measured under 3500 µmol/$m^2$/s light intensity for 1 second.

Dissolved oxygen (DO) concentrations were measured at the end of the experiment to assess the effects of metal exposures on their primary productivity. 0.2g of seagrasses were put in a sealed glass vial attached with Oxygen Sensor Spot SP-PSt3-NAU (PreSens Precision Sensing GmbH, Germany). PreSens Multi-Channel Oxygen Meter (PreSens Precision Sensing GmbH, Germany) was used to detect the levels of dissolved oxygen in the vials. The DO concentrations were measured every minute in µmol. The measurement began with a one-hour dark incubation achieved by wrapping aluminum foil on the vials. The difference between initial and final DO concentrations in dark incubation is the amount of respiration (R). After dark incubation, the bottles were then placed under 100 µmol $m^{-2}s^{-1}$ light for another hour to obtain the net primary production (NPP). Gross primary production is calculated according to Howarth & Michaels (Howarth & Michaels, 2000).

### Metal analysis

10 replicates of seagrass were collected before and after the acclimation period and at the end of the experiment. Seagrass individuals were divided into above and below-ground tissues and dried at 60°C in oven until constant weight. The samples were digested at 180°C with 6ml $HNO_3$ using microwave digester. The metal concentrations were measured on ICP-MS.

### RNA sequencing and Transcriptomic analysis

At the end of the experiment, seagrasses in each treatment replicate were collected and divided into above and below-ground tissues. The samples were subsequently snap-frozen using liquid nitrogen. Total RNA was extracted using TransZol Up Plus RNA Kit (TransGen Biotech Co., Ltd.). The quality of the samples was randomly checked using Tape Station and they achieved satisfactory RIN values. Extracted RNA was sent to Novogene for sequencing on Illumina NovaSeq 6000 Sequencing System. Sequence processing, de novo transcriptome assembly, gene prediction, functional annotation and gene expression was analyzed using the following steps: QC cleaning, Trinity assembly, RSEM, Blastx, KEGG, GO, GSEA, DeSeq2. Gene expression comparison between different physiological states and levels of stress allowed us to identify gene clusters, co-expressed genes and regulated pathways that are part of the adaptive molecular toolkit of seagrasses to cope with heavy metal stress. To validate the RNAseq expression data and to assess the transcriptional responses, we assessed the relative expression of the top 20 up- and top 20 down-regulated genes using qPCR and primers developed for those specific genes.

### Statistical analysis

All statistical analysis was performed on R (v.4.1.1). The difference of metabolic rates between seagrasses under different treatments was analyzed using one-way ANOVA and post-hoc Tukey HSD.

## Results

### Metal concentrations in the seagrasses

The concentrations of copper and lead decreased in seagrasses decreased after the 7-day acclimation (Figure 25A). There was no significant decrease of copper in the aboveground tissue (one-way ANOVA, $p > 0.05$) but a great drop in the belowground (one-way ANOVA, $p < 0.001$). The decrease was opposite for the concentration of lead, meaning that there was a significant drop of lead in the aboveground tissue (one-way ANOVA, $p < 0.001$) but no change in the belowground ones (one-way ANOVA, $p > 0.05$).

The metal concentrations in the seagrasses after experiment indicated that the seagrasses have accumulated the metal elements in their tissues with respect to the concentrations they were exposed to (Figure 25B). The results also proved that there was no contamination between different treatment replicates. We observed that there was no statistical difference in copper concentration between the belowground and aboveground tissue coming from both low and high copper treatments. Yet, a higher concentration of lead was found in the belowground tissues after both lead treatments (one-way ANOVA, $p = 0.008$ for Low Lead, $p = 0.002$ for High Lead).

### Effects of metal exposures on seagrass metabolism

Respiration rates of seagrasses after metal exposure were shown in Figure 26.The respiration rate of seagrasses exposed to copper treatments were lower than that of the control replicates (post-hoc TukeyHSD, $p = 0.01$ for Low Copper, $p < 0.001$ for High Copper). For the seagrasses under exposure of lead, only the ones received high concentration of lead resulted in lower respiration rate than the control ones (post-hoc TukeyHSD, $p = 0.003$).

Figure 27 showed the results of GPP after the experiment. The seagrasses received high concentration of copper were having lower GPP than that of the controls (post-hoc TukeyHSD, $p = 0.001$). There was also a difference between seagrasses having low and high copper exposure (post-hoc TukeyHSD, $p = 0.02$). Yet, for those exposed to lead, there was instead an increase of productivity when they received low level of lead (post-hoc TukeyHSD, $p = 0.01$). Those exposed to high level of lead did not declare a change in GPP when they were comparing to the controls. However, we do see a drop of GPP when the seagrasses were exposed to a high concentration of lead (post-hoc TukeyHSD, $p = 0.01$).

After optimized protocols for RNA extraction and assessment of the quality and quantity were developed, we obtained 64.8M readings from each library. After quality trimming and de novo assembly, we identified a total of 110,096 unigenes. Our results show high transcriptomic flexibility (changes in transcriptional regulation), with strong differences in expression between treatments and high consistency between biological replicates. We found marked differences in expression of genes associated with metabolic pathways, immune response and oxidative stress. We also identified over-expressed transcripts related with oxidoreductase activity, mainly including Cu/Zn superoxide dismutases (Cu/Zn SOD, c67144_g1_i1) reaching an expression of 172-fold, glucose dehydrogenase (c77507_g1_i1) with 786-fold, aplysianin-A (c58387_g1_i1) with 235-fold, peroxidasin (c58853_g1_i1) with 141-fold, and chorion peroxidase (c3791_g1_i1) with 137-fold. This is consistent with signals of oxidative stress as a result of heavy metal stress. Moreover, we found elevated fold change values for genes involved in lectin and catecholamine pathways, which are critical components of the innate immune response in plants. The transcriptional machinery controlling these responses involved in heavy metal tolerance for seagrasses is currently under development.

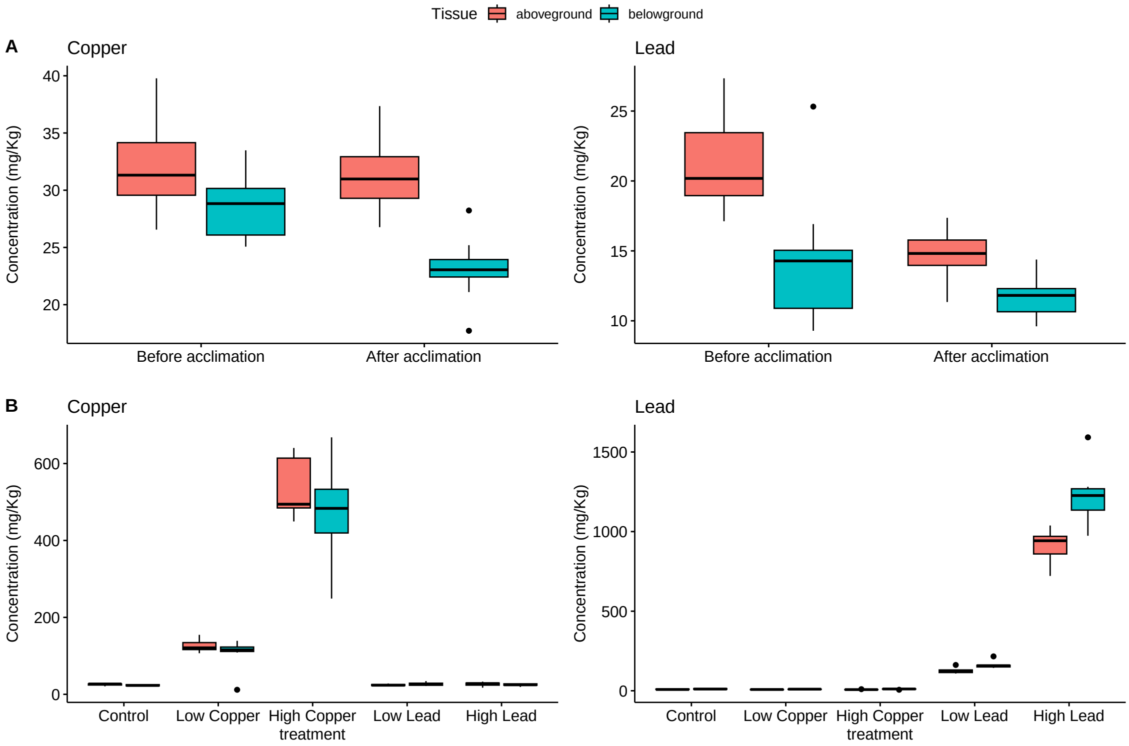

*Figure 25 Metal concentrations in seagrass tissues collected (A) before and after acclimation in lab (B) after metal exposures.*

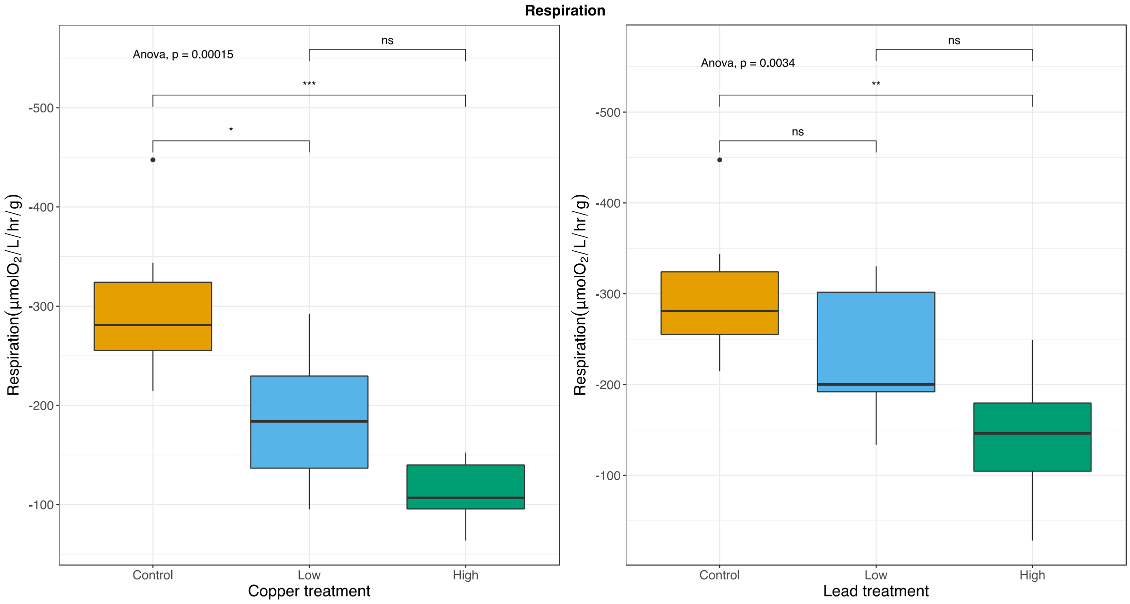

*Figure 26 Boxplots showing the respiration rates of H. beccarii under different levels of metal exposure. (Control, low copper, high copper, low lead, high lead)*

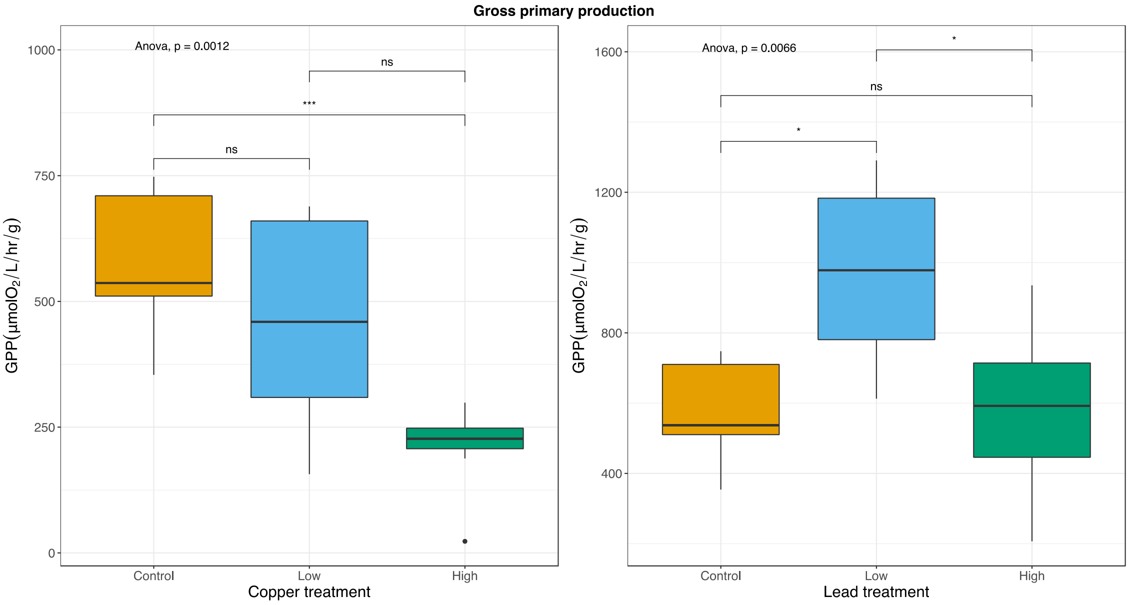

*Figure 27 Boxplots showing the gross primary production rates of H. beccarii under different levels of metal exposure. (Control, low copper, high copper, low lead, high lead)*

## Discussion

Our results suggest that Copper and Lead have different physiological effects on local seagrasses. High concentrations of these elements were more detrimental for respiration and photosynthesis. However, no major mortalities were observed, which is contrasting to other studies with temperate seagrasses. This may be associated to the historical exposure of local populations to high levels of metal pollution in Hong Kong. Our transcriptional analysis suggests that local seagrasses are highly resilient to metal pollution and use fine tune regulation of gene expression in order to buffer the stress of chemical contamination. This regulation involves pro-survival pathways and the control of oxidative stress. It is important to highlight that despite the lack of differences in gene expression between control and low heavy metal pollution, the elevation of chemical concentration may trigger cellular damage in the long-term. In fact, we maintained seagrasses for longer experimental exposure and were able to observe bleaching and loss of tissue in the high-level treatment. This ultimately led to mortality due to long-term stress exposure in which seagrasses are unable to maintain the transcriptional regulation of oxidative stress. These experimental findings may explain the rapid decline of seagrasses in western sites of Hong Kong were heavy metal pollution is elevated.

# Social media and outreach activities

## The Nature Conservancy (TNC)

*January 2022 & June 2022*

With The Nature Conservancy (TNC) on-going oyster reef restoration project occurring in Pak Nai, one of Hong Kong most consistence seagrass site, we saw the importance of sharing the knowledge and nature of seagrasses to people who have also contributed their time in conservation. In order to let the public know more about the seagrasses in Hong Kong and their roles in the habitat, two presentations and workshops about Hong Kong seagrasses were held for TNC volunteers and students' helpers.

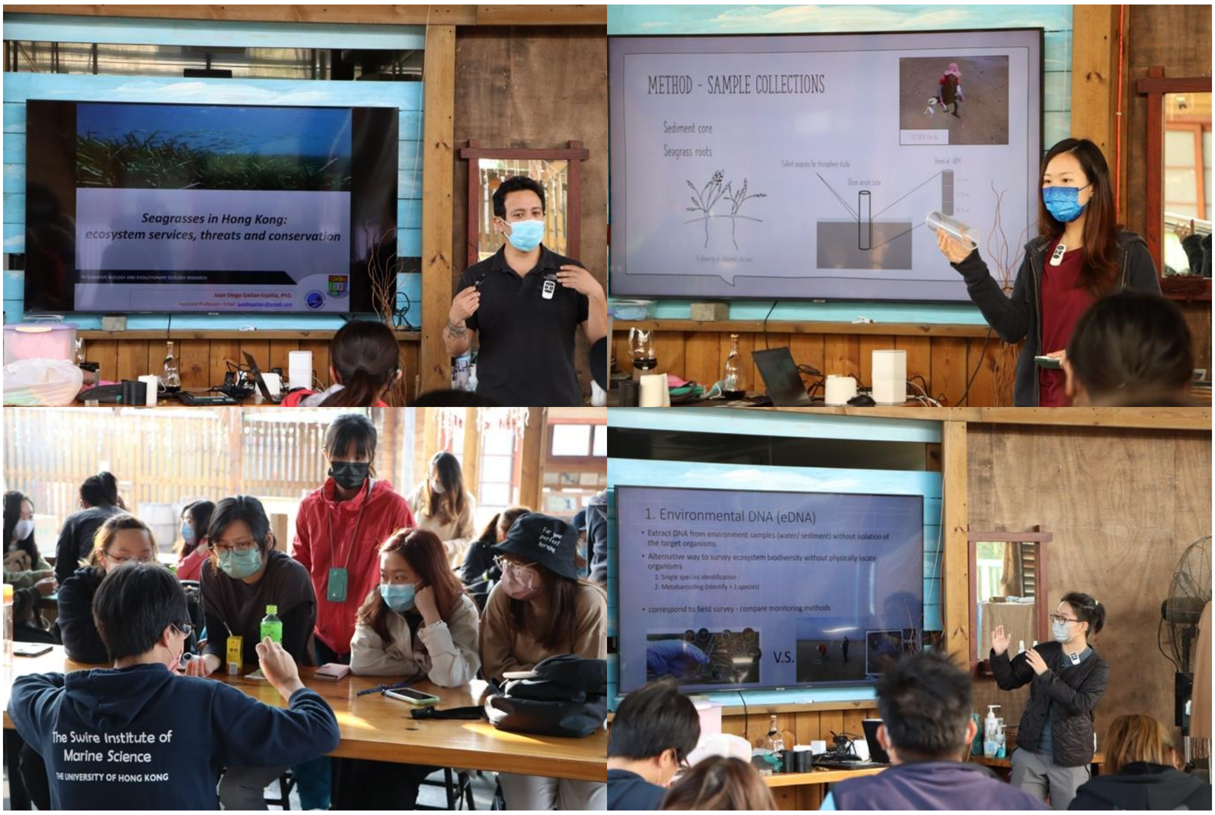

*Figure 28 Public engagement and outreach activities in Pak Nai*

## Agriculture, Fisheries and Conservation Department (AFCD)

*May 2022*

Having limited research and study done on Hong Kong seagrasses, exchange and update of information is crucial. We developed knowledge exchange with different stakeholders in Hong Kong interested in seagrass management and conservation. Here, we conducted a workshop with the Agriculture, Fisheries and Conservation Department (AFCD), aiming to attract the attention of the Government to the serious concerns regarding seagrass declines and the potential effects of heavy metals on these trends.

*Figure 29 Workshop with 30 officers from AFCD where we develped capacity-building on seagrass research*

In the second half of the year, we were invited by the Radio Television Hong Kong (RTHK) for the shooting on one episode of the documentary《大自然生態人 3》in podcasting in Cantonese. This is a documentary on ecology that introduce the work related to ecological study. In this episode, the RTHK focus on our study in seagrass conservation throughout our daily work in the field and laboratory, describing our projects on assessing pollution impacts on ecological responses in the function of seagrasses and the conservation interventions on the populations. As the documentary would be podcasted to the public, this would promote the understanding on seagrass study and protection to the community (Fig 30-31). https://youtu.be/GzDY7M9XW0M

**Figure 30**. RTHK documentary filming day in San Tau on 30th November 2022. One of the team members was working on surveying while the RTHK crew were filming.

**Figure 31.** One of the RTHK documentaries is on seagrass conservation and restoration in Hong Kong. https://youtu.be/GzDY7M9XW0M

## Case of change for historic court

The former North Kowloon Magistracy in Sham Shui Po will be revitalised in the next four years, with the site's operator planning to reopen it as a centre offering universal legal education and conducting outreach efforts. The Society of Rehabilitation and Crime Prevention said areas in the historic court building that would be changed included the second courtroom, several holding cells and the layout for the third and fourth floors. Photos: Yik Yeung-man

ENVIRONMENT

# CALLS GROW TO PROTECT CRITICAL SEAGRASS BEDS

**Alliance of conservationists demands urgent action to save rare habitat for endangered horseshoe crabs and winter home of the black-faced spoonbill**

**Ezra Cheung**
ezra.cheung@scmp.com

The city should designate 800 hectares in the northwestern New Territories as a marine reserve before 2030 to protect the last stronghold in Asia of a vulnerable species of seagrass, an alliance of more than 30 conservation groups has proposed.

The Hong Kong Marine Protection Alliance said the seagrass in Pak Nai supported vast marine biodiversity, with the area comprising the city's largest wetland habitat of mudflats, mangrove forests, seagrass fields and oyster reefs.

The seagrass beds are said to be home to more than 60 per cent of the endangered horseshoe crab population, and are a key wintering ground for the endangered black-faced spoonbill.

The alliance said urgent protection was necessary as Pak Nai was under threat from local tourism and invasive species, but authorities had not taken any action to protect the area.

"When residents search Pak Nai on the internet, most results identify it as a hotspot for photography, watching sunsets and clam digging," alliance spokesman Stan Shea Kwok-ho said. "However, its ecological value is often overshadowed."

Tourists had stepped on the mudflats and unknowingly caused damage to the ecology, he added.

Founded in August, the alliance consists of more than 30 environmental groups, academics and corporations, including the Nature Conservancy, the Maritime Museum and the Swire Group Charitable Trust. It aims to preserve local waters and raise public awareness of marine conservation issues.

The alliance's appeal echoed former chief executive Carrie Lam Cheng Yuet-ngor's suggestion to set aside about 2,000 hectares of land for conservation in her Northern Metropolis plan, an economic engine relying on proximity with bordering Shenzhen to yield more than 650,000 jobs.

The Environment and Ecology Bureau yesterday said it was considering developing a coastal protection park between Tsim Bei Tsui and Pak Nai, "with a view to proactively conserving the existing natural coastline and the ecosystem of … mangroves".

*NGO-proposed area for marine protection in Pak Nai*

Source: The Hong Kong Marine Protection Alliance and the Hong Kong government SCMP

The Development Bureau conducted a feasibility study on the development potential of the area in lockstep with proposals from late August by the Environment and Ecology Bureau to reserve sections of Tsim Bei Tsui, Lau Fau Shan and Pak Nai for the park.

Lam said in her final policy address that she would set aside 145 hectares for conservation purposes, falling short of the 800 hectares suggested by the alliance.

Assistant professor Juan Diego Gaitan Espitia, a biostatistician at the University of Hong Kong's Swire Institute of Marine Science, said Pak Nai had the most stable and largest population of Beccari's seagrass across Asia. The institute is a member of the alliance.

"If you go to Indonesia or the Philippines, it's totally extinct; you can't find these plants any more," he said. "If you go to India, Malaysia or Thailand, their populations are declining."

In Pak Nai, the size of the area where the vulnerable seagrass species was located varied between 2.4 and four hectares, depending on the season, Gaitan Espitia said.

Native to the South China Sea and the Bay of Bengal, Beccari's is the smallest seagrass in its family known to science, with leaves growing up to just 5mm.

Research suggests it is a critical food source for horseshoe crabs, among other animals, and helps stabilise coastlines and sediments. The alliance also said the habitat it helped create could capture four times more carbon dioxide than other terrestrial vegetation given the same surface area.

But Gaitan Espitia expressed worry that habitat changes, such as land reclamation and water pollution, would threaten seagrass health. "We look at how the seagrass is changing through time, and changes in the habitat use are affecting it," he said.

Pak Nai is home to the endangered tri-spine horseshoe crab and the round-tailed horseshoe crab. The latter is so rare that scientific data has been too sporadic to determine its conservation status.

The alliance pointed out that only part of Pak Nai was listed as a site of special scientific value in the 1980s, and the rest of the area was under no legal protection. It also warned that without statutory protection from the government, efforts made by conservation groups and the public were limited.

"A synergistic effect can only be achieved when authorities, society, environmental groups and others work together," alliance spokesman Shea said. "We hope Pak Nai can have better legal protection and management."

COURTS

**Fig 32)** Press media outreach and community engagement by lobbying conservation of seagrasses in Hong Kong. South China Morning Post Dec 10, 2022 *https://www.scmp.com/news/hong-kong/health-environment/article/3202781/call-mark-800-hectares-northwest-hong-kong-marine-reserve-protect-last-stronghold-vulnerable*

## More media coverage regarding restoration and conservation of seagrass *H. beccarii* in Hong Kong

| Date | Publication | Type | Headline |
|---|---|---|---|
| Dec 10, 2022 | I-Cable News | Broadcasting media - Online and aired news | Tourists stepping on mudflats would jeopardise endangered species. The green group advocates the planning of conservation areas and entry restrictions during the breeding season<br>稱白泥任踏足危害瀕危物種<br>環團促劃保育區、繁殖季節限制進出 |
| Dec 10, 2022 | Oriental Daily News | Newspaper-Printed news | Tourists stepping on mudflats would jeopardise endangered species. Efforts made by conservation groups and the public were limited. Green group advocates the planning of protection area.<br>白泥任遊人踏足 瀕危物種陷煉獄 民間維護事倍功半 促設保護區規管 |
| Dec 10, 2022 | Oriental Daily News | Newspaper-Online news | Tourists stepping on mudflats would jeopardise endangered species.<br>白泥任遊人踏足 瀕危物種陷煉獄 |
| Dec 10, 2022 | Oriental Daily News | Newspaper-Online news | Tourists stepping on mudflats would jeopardise endangered species. The green group advocates the planning of protection areas.<br>白泥任遊人踏足危害瀕危物種　環團促設保護區規管 |
| Dec 10, 2022 | Sing Tao Daily | Newspaper-Printed news | Green groups advocate zoning Pak Nai as a "Marine Protection Area"<br>環團倡白泥列作「海洋保護區」 |
| Dec 10, 2022 | Sing Tao Daily | Newspaper-Online news | Green groups advocate zoning Pak Nai as a "Marine Protection Area"<br>環團倡白泥列作「海洋保護區」 |
| Dec 10, 2022 | Sing Tao Daily USA | Newspaper-Online news | Green groups advocate zoning Pak Nai as a "Marine Protection Area"<br>環團倡白泥列作「海洋保護區」 |
| Dec 10, 2022 | Sing Tao Daily Canada | Newspaper-Online news | Green groups advocate zoning Pak Nai as a "Marine Protection Area"<br>環團倡白泥列作「海洋保護區」 |
| Dec 10, 2022 | Headline Daily | Newspaper-Online news | Green groups advocate zoning Pak Nai as a "Marine Protection Area"<br>環團倡白泥列作「海洋保護區」 |
| Dec 10, 2022 | Bastille Post | Newspaper-Online news | Green groups advocate zoning Pak Nai as a "Marine Protection Area"<br>環團倡白泥列作「海洋保護區」 |
| Dec 10, 2022 | South China Morning Post | Newspaper-Printed news | Calls grow to protect critical seagrass beds |
| Dec 10, 2022 | South China Morning Post | Newspaper-Online news | Call to mark 800 hectares in northwest Hong Kong as marine reserve to protect last stronghold of vulnerable seagrass species |

**Publication: I-Cable News**

**Title:** 稱白泥任踏足危害瀕危物種　環團促劃保育區、繁殖季節限制進出

**Date: Dec 10, 2022**

**Link:** https://www.i-cable.com/新聞資訊/77395/稱白泥任踏足危害瀕危物種-環團促劃保育區-繁/?utm_source=icable-web&utm_medium=referral

稱白泥任踏足危害瀕危物種　環團促劃保育區、繁殖季節限制進出

i-Cable · 2022年12月10日 · 港聞, 新聞資訊

【有線新聞】元朗白泥有全港最大的海草床，不少瀕危物種在此棲息。有環保團體促請政府，將白泥列為海洋保護區，保育這些珍貴物種，同時亦可推動生態旅遊。

這些黑色一點點並非泥灘的污染物，是「貝克喜鹽草」。白泥這裏的海草床是全港最大，有4萬平方米，更是馬蹄蟹「中國鱟」、黑臉琵鷺等瀕危物種的聚居地。

環保團體說白泥是看日落的打卡熱點，遊人可以自由出入泥灘，有機會騷擾這裏的生物。海洋公園保育基金社區教育經理溫翰芝：「我們見過一些踩單車的人，他們可能是一群朋友，把單車帶到泥灘。其次，也有市民來這裏挖蜆，當他翻起泥土的時候，可能一些底棲動物受到傷害，甚至很難恢復一個情況，適合馬蹄蟹這類動物在上面移動、覓食。」

阻礙馬蹄蟹覓食，還有散落一地的蠔殼。大自然保育協會保育教育經理陳梓健：「因為我們看到一個荒廢泥灘，蠔民會把很多水泥柱放在泥灘，霸佔了一個很大的面積。原本這個地方有馬蹄蟹、瀕危馬蹄蟹和海草在生活，因為他們用以養蠔，使這個地方主要是一個蠔的結構，其他生物居住的範圍少了。」

白泥有一公頃的荒廢蠔田，相當於大約一個半標準足球場的面積。有機構建議將蠔田修復成為蠔礁，可以過濾海水、改善水質，堆成蠔礁後可以防止海浪侵蝕、穩定海岸線。陳梓健續指：「蠔礁復修的工作，就是我們需要在廢棄的蠔田，撿起水泥樁柱，然後把它們疊在固定位置，讓它們能自然些生長。」他建議政府將白泥劃為海洋保護區，加強監管，在馬蹄蟹繁殖的季節，限制遊人進出，減少對環境的傷害。

元朗白泥有全港最大的海草床，不少瀕危物種在此棲息。有環保團體促請政府，將白泥列為海洋保護區，保育這些珍貴物種，同時亦可推動生態旅遊。

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**Publication: Oriental Daily News**

**Title:** 白泥任遊人踏足 瀕危物種陷煉獄 民間維護事倍功半 促設保護區規管

**Date: Dec 10, 2022**

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■黑臉琵鷺於冬季會飛抵白泥過冬。（李志湧攝）

生態災難

元朗下白泥為欣賞日落勝地，亦是全港最大潮間帶泥灘，擁有紅樹林和海草床、多種瀕危動物棲息。不過白泥未如米埔，至今仍沒有法規或政策管理，公眾可自由出入，每逢假日便擠滿遊人，有保育組織擔心，泥灘生物會被踏死，加上亂拋垃圾，恐造成生態災難，惟政府未有保育措施或執法，僅依賴環保團體及村民維護，坦言事倍功半，促當局將白泥列為「海洋保護區」保育。

■被譽為「活化石」的馬蹄蟹年幼時會於潮間帶泥灘生活。（大自然保護協會）

# 白泥任遊人踏足 瀕危物種陷煉獄

## 民間維護事倍功半 促設保護區規管

### 物種豐 亞洲最後海草寶庫

泥灘物種豐富，生長着「秋茄」紅樹，以及大片海草，包括「貝克喜鹽草」，香港大學生物學系副教授Dr. JD Gaitan-Espitia表示，白泥海草面積一般約2.4公頃，高峰期可達4公頃，是本港海草最後的穩定生長地，亦是亞洲最後的海草寶庫。

而紅樹及海草均提供居所、食物予海洋動物，包括馬蹄蟹。大自然保護協會保育教育經理陳梓健指，統稱馬蹄蟹的瀕危中華鱟及數量稀少的圓尾鱟均棲息於白泥，而年幼馬蹄蟹更佔了全港的60%。冬季亦有候鳥棲息，例如瀕危的黑臉琵鷺及白胸翡翠。香港觀鳥會高級區域項目主任林釗說，白泥是黑臉琵鷺於香港僅餘的落腳點之一。

### 打卡掘蜆捉蟹「你踩我又踩」

白泥「鳥語花香」，卻面對人為災難，陳梓健稱，大眾對白泥的認知停留於看日落、打卡、掘蜆及捉蟹等，忽略生態價值，尤其周末假日充斥遊客，未能控制人數，「你踩我又踩」，傷害了泥灘上的生物，衝擊地區生態系統。

香港海洋公園保育基金社區教育經理溫翰芝亦說，泥灘生長了入侵物種「大米草」會使泥灘乾旱，危害泥灘上的物種。陳梓健補充，大米草現時僅靠保育團體與義工清理，10名義工每兩小時只能拔除9平方米的草，欠缺政府支援及協調，難以全面清除。陳又提到，協會與港大太古海洋科學研究所合作安排義工、學生重整逾2,000平方米的荒廢而零散的蠔礁，方便生物移動覓食，而當中的「香港蠔」每小時亦可過濾30公升海水，期望能改善后海灣水質。

世界自然基金會香港分會代理海洋保育主管彭莉恩指，下白泥生態獨特，生物多樣化，對受威脅物種及其生長十分重要，生態容易受外來因素而受損，需重點保護。香港海洋保育聯盟促港府於2030年前將白泥列為「海洋保護區」，彭補充，當局可按生態敏感度劃分成區域管理，設計生態友善步道，減少遊客直接踏足泥灘。

環境及生態局回覆指，白泥部分地區已被納入具特殊科學價值地點，漁護署會定期巡視。發展局已於今年8月底探討尖鼻咀、流浮山及白泥一帶發展潛力，環境局正探討可預留作海岸保護公園的範圍，並考慮將白泥地區的生態保育納入該範圍，保育生態系統。

■大米草僅依賴義工用手清除。

■香港海洋保育聯盟促政府將白泥列為「海洋保護區」。

元朗下白泥為欣賞日落勝地，亦是全港最大潮間帶泥灘，擁有紅樹林和海草床、多種瀕危動物棲息。不過白泥未如米埔，至今仍沒有法規或政策管理，公眾可自由出入，每逢假日便擠滿遊人，有保育組織擔心，泥灘生物會被踏死，加上亂拋垃圾，恐造成生態災難，惟政府未有保育措施或執法，僅依賴環保團體及村民維護，坦言事倍功半，促當局將白泥列為「海洋保護區」保育。

### 物種豐 亞洲最後海草寶庫

泥灘物種豐富，生長着「秋茄」紅樹，以及大片海草，包括「貝克喜鹽草」，香港大學生物學系副教授 Dr. JD Gaitan-Espitia 表示，白泥海草面積一般約 2.4 公頃，高峰期可達 4 公頃，是本港海草最後的穩定生長地，亦是亞洲最後的海草寶庫。

而紅樹及海草均提供居所、食物予海洋動物，包括馬蹄蟹。大自然保護協會保育教育經理陳梓健指，統稱馬蹄蟹的瀕危中華鱟及數量稀少的圓尾鱟均棲息於白泥，而年幼馬蹄蟹更佔了全港的60%。冬季亦有候鳥棲息，例如瀕危的黑臉琵鷺及白胸翡翠。香港觀鳥會高級區域項目主任林釗說，白泥是黑臉琵鷺於香港僅餘的落腳點之一。

### 打卡掘蜆捉蟹 「你踩我又踩」

白泥「鳥語花香」，卻面對人為災難，陳梓健稱，大眾對白泥的認知停留於看日落、打卡、掘蜆及捉蟹等，忽略生態價值，尤其周末假日充斥遊客，未能控制人數，「你踩我又踩」，傷害了泥灘上的生物，衝擊地區生態系統。

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## Summary and Way Forward

Seagrasses are rapidly declining in Hong Kong. Populations in western waters are clearly impacted by different Anthropogenic pressures, including the effects of heavy metals from human-related activities. Heavy metal pollution is similar across seagrass sites in the New Territories and Lantau Island. However, the levels of pollution show temporal trends associated to seasonality. Seagrass bioaccumulate heavy metals in tissues, particularly below-ground biomass. This seems to have an effect of the associated biodiversity. For example, microbial communities, which are important for the health and functioning of seagrasses, are affected in sites where seagrasses are present and bioaccumulate heavy metals compared to mudflats were no seagrasses are detected. This suggests potential functional effects through biogeochemistry of soils and the role that microbes have on these processes. Considering the long-term exposure of seagrasses to heavy metals in Hong Kong, we hypothesized potential local adaptation of seagrass populations. Our transcriptomic analysis potentially supports this hypothesis as local seagrasses show very unique gene expression regulation under different levels of heavy metal pollution. No major effects on survival and physiology can be observed at the short-term but detrimental effects can be seen when seagrasses are maintained under high levels of chemical pollution for longer periods. These findings may explain the rapid decline of local seagrass populations we have observed during the last three years in Hong Kong. It is important to continue monitoring the levels of heavy metal pollution and the seagrass dynamics in order to better understand demographic trends. Through this project we have highlighted the urgent need of conserving and monitoring seagrasses in Hong Kong, engaging different stakeholders, from NGOs to local communities and the Government.

## Interim evaluation of project effectiveness and impact

**OBJECTIVE 1:** To assess levels of heavy metal pollution in sediments, water and bioaccumulation in seagrasses:

**Evaluation**: We have generated a spatial-temporal assessment of heavy metal pollution in seagrass areas in Western waters of Hong Kong. This assessment has been successfully completed, documenting temporal and spatial trends of heavy metals and their bioaccumulation in local seagrass populations. With this information, we have developed different knowledge exchange activities, engaging with local communities and the Government, and calling for more efforts conserving seagrasses and the associated areas. We successfully conducted 3 workshops, 2 seminars and participated in several media coverage, including one episode of the documentary《大自然生態人 3》transmitted by Radio Television Hong Kong (RTHK). The objective impact and effectiveness is 100%

**OBJECTIVE 2:** To quantify impacts of heavy metals on the structure and function of seagrasses-associated biodiversity:

**Evaluation**: This is the first study in Hong Kong documenting the link between heavy metal pollution and the dynamics of seagrasses and their associated biodiversity. We have generated massive ecological and genetic data as part of the project, which is included as part of the 1st chapter of a Master thesis at the University of Hong Kong. All the data will be available in public repositories at HKU (ecological surveys) and the NCBI (microbiome and transcriptome data). In addition, we are currently working on the scientific manuscripts associated to this project which will expand the impact of the conducted research. Such publications will also provide direct access to the data and information compiled in this study. This information includes overall status of seagrass populations, associated biodiversity, spatial and temporal changes in this biodiversity as a function of heavy metal pollution, and genetic tools/resources for management of seagrass microbiomes in Hong Kong. Examples of the impact and coverage of this project and objective can be seen in the news of the South China Morning Post Dec 10, 2022 https://www.scmp.com/news/hong-kong/health-environment/article/3202781/call-mark-800-hectares-northwest-hong-kong-marine-reserve-protect-last-stronghold-vulnerable

The objective impact and effectiveness is 100%

**OBJECTIVE 3:** To assess the health, resilience, tolerance and responses to heavy metal pollution of local seagrasses:

**Evaluation**: We have successfully provided the first study of physiological and molecular responses of seagrasses to heavy metal pollution in Hong Kong. This information is part of the second chapter of a Master thesis at HKU. We are currently developing the scientific manuscript associated to this objective, which combines the physiological and transcriptional dynamics under heavy metal stress, characterising the regulatory mechanisms of seagrasses and providing molecular markers that can be used in rapid assessment of stress and health of local populations in the field. These aspects have been discussed in workshops and covered by the media as previously highlighted. The objective impact and effectiveness is 100%

**Attendance record of the project staff**

Attendance record are not disclosed due to confidentiality reasons.

**Recruitment record of the project staff**

Recruitment record are not disclosed due to confidentiality reasons.

**Declaration**:
*I hereby irrevocably declare to the MEEF Management Committee and the Steering Committee of the relevant Funds including the Top-up Fund, that all the dataset and information included in the completion report has been properly referenced, and necessary authorisation has been obtained in respect of information owned by third parties.*

*Any opinions, findings, conclusions or recommendations expressed in this report do not necessarily reflect the views of the Marine Ecology Enhancement Fund or the Trustee.*

*Signed by:*

Gaitán E. Juan Diego

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