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# Integrating multiple indices based on heavy metals and macrobenthos to evaluate the benthic ecological quality status of Laoshan Bay, Shandong Peninsula, China

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## ABSTRACT

Many different indices have been developed to evaluate habitat quality status (EcoQs) in marine ecosystems; however, few studies have concurrently considered both abiotic and biotic indices in their assessments of benthic EcoQs. Here, we propose and test a framework for integrating heavy metal pollution-related indices and macrobenthos-based indices to assess the benthic EcoQs of Laoshan Bay (Yellow Sea, China). This bay is exposed to urbanization, construction, heavy metal pollution, and land- and marine-based culturing operations for commercially valuable species like fish, scallop, and laver (seaweed). We first assessed the EcoQs of Laoshan Bay using four heavy metal pollution-related indices, i.e., the geo-accumulation index (Igeo), potential ecological risk index (R), pollution load index (PL), and Nemerow pollution index (Pn) and four macrobenthos-based indices, i, e., AZTI's Marine Biotic Index (AMBI), the multivariate AMBI (M-AMBI), BENTIX, and the feeding evenness index (*jFD*). All indices (except  $I_{geo}$ ) were then reclassified and combined to assess the overall EcoQs of Laoshan Bay. Our results show that, while some sites in Laoshan Bay have relatively high levels of heavy metal (Hg and Cd) pollution, the benthic EcoQs is acceptable across 88.90% of the bay. Kappa analysis showed that the agreement between any two indices was very low, which suggests that the composite index used here may be more robust for assessing EcoQs and more closely represent the actual status of benthic habitats than assessments based on single indices. In addition, the assessment framework proposed here is relatively flexible and can serve as a useful tool for evaluating the benthic EcoQs of similar marine ecosystems.

# 1. Introduction

Human activities in marine areas, including reclamation, sewage discharge, fishing, and aquaculture, are continuing to expand. These activities stress marine ecosystems, degrading their health and contributing to a loss of marine biodiversity (Jägerbrand et al., 2019; Losi et al., 2021; Puente and Diaz, 2015; Worm et al., 2006). Identifying optimal conservation and restoration strategies for marine ecosystems correspondingly requires studies on the impact of anthropogenic stress on the ecological quality status (EcoQs) of marine areas (D'Alessandro et al., 2020; Dreujou et al., 2021; Gorman et al., 2017). In many

countries, including China, Canada, the United States, and several European nations, EcoQs assessment has become a prerequisite and/or benchmark for ecosystem management and policy decisions (Borja et al., 2008a; Poikane et al., 2020). Numerous indices have been developed to evaluate the EcoQs of marine areas, including abiotic indices (e.g., pollutant-based indices) (Liu et al. 2015; Tian et al. 2022) and biotic indices (e.g., macrobenthos-based indices). Several such indices focus specifically on the benthic environment (Borja et al., 2019; Poikane et al., 2020). However, few studies of benthic EcoQs have considered both abiotic and biotic indices or used composite indices (Guerra-García et al., 2021; Hutton et al., 2015; Maghsoudlou et al., 2020; Pandey et al.,

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#### 2021).

Heavy metals occur naturally in marine ecosystems, however, due to human activities, large amounts of heavy metals are released into the ecosystem, resulting in heavy metal pollution. This type of pollution is one of the most common environmental problems in marine ecosystems around the world (Lu et al., 2018; Wang et al., 2020b). Because heavy metals are highly toxic, persistent, and non-degradable, heavy metal contamination can have deleterious effects on marine organisms, degrade benthic habitat and ecosystems, and eventually lead to adverse effects on humans (de Souza Machado et al., 2016; Gu et al., 2021; Hong et al., 2020). Heavy metals tend to accumulate in sediments, which is why sediment is commonly used as the matrix to evaluate heavy metal pollution (Hu et al., 2018; Pan and Wang, 2012; Wang et al., 2021). Many indices have correspondingly been developed to quantify heavy metal pollution levels in sediments and assess their risk to the environment. These indices include the geo-accumulation index (Müller, 1969), pollution load index (Tomlinson et al., 1980), Nemerow pollution index (Nemerow, 1974) and potential ecological risk index (Hakanson, 1980). Although these indices are all measures of heavy metal contamination, they all have different emphases and associated limitations. For specific study areas or sampling sites, different indices can produce different evaluation results, and some may obtain more accurate assessments of pollution levels than others. To overcome these biases, recent studies have applied multiple indices simultaneously (Franzo et al., 2022; Muniz et al., 2019; Shetaia et al., 2022; Wang et al., 2020a; Yao et al., 2021). However, few studies have considered the agreement among these indices, integrated them, or combined them with other indices (e.g., macrobenthos-based indices) to provide a comprehensive assessment result that could be used by ecosystem managers and/or governments.

Macrobenthos play a key role in energy flow and nutrient recycling in marine ecosystems and provide many advantages for EcoQs monitoring that other groups of organisms do not. Most importantly, macrobenthos have limited mobility and long lifespans (Herman et al., 1999). Macrobenthos-based indices can therefore be used to evaluate benthic EcoQs. Commonly used macrobenthos-based indices include AZTI's Marine Biotic Index (AMBI) (Borja et al., 2000), the multivariate AMBI (M-AMBI) (Muxika et al., 2007), BENTIX (Simboura and Zenetos, 2002) and the feeding evenness index (*jFD*) (Gamito and Furtado, 2009). The AMBI, M-AMBI and BENTIX indices were developed based on the sensitivity or tolerance of different macrobenthic species to disturbance (Borja et al., 2000; Pearson and Rosenberg, 1978), while the *iFD* is based on the feeding strategy of different organisms (Gamito and Furtado, 2009). These four indices have been used to assess the EcoQs of bays, lagoons, estuaries, and coastal waters around the world (Borja et al., 2019; Gamito et al., 2012; Martínez-Crego et al., 2010), and tested in a recent study in the coastal waters of Shandong Peninsula, China (Dong et al., 2021a). To ensure that they provide valuable reference information for decision-making or legislation in countries across the planet, these indices have been tested, validated, and, in some cases, modified to account for biogeographical differences in macrobenthic species pools (Poikane et al., 2020). As with the heavy metal-based indices, the final conclusions about the ecological quality of a specific area can depend on which biotic index is used. Many studies have compared the results from different indices and tested whether different indices are suitable for different marine ecosystems (Borja et al., 2008b; Brauko et al., 2016; Dong et al., 2021a; Wetzel et al., 2012), but few studies have integrated multiple indices to comprehensively evaluate benthic EcoQs (Blanchet et al., 2008; Maghsoudlou et al., 2020; Pelletier et al., 2018).

Laoshan Bay is a semi-enclosed bay of the Yellow Sea that is facing multiple external pressures, including increasing heavy metal pollution in seawater and sediments and the expansion of land-based pond aquaculture, laver (seaweed) and fish aquaculture, and port operations (Dong et al. 2021b; Liu et al., 2015; Tian et al. 2022; Wang et al. 2019). Anthropogenic activities are considered the main reason for the degradation of the Laoshan Bay ecosystem (Dong et al., 2021c; Liu et al.,

2015; Wang et al., 2019). Ever since the bay was declared a marine economic development demonstration zone in 2015, the heavy metal concentrations in its seawater and surface sediments have increased (Dong et al. 2021b; Liu et al., 2015; Tian et al. 2022; Wang et al., 2019). Heavy metals in Laoshan Bay sediments have, in turn, been shown to affect the structure and function of macrobenthos communities in both intertidal zones and subtidal zones (Dong et al. 2021b; Dong et al., 2021c). However, to our knowledge, no study has explored the benthic EcoQs of Laoshan Bay using biotic indices. In this study, we aimed to (1) investigate the concentrations of heavy metals in Laoshan Bay and quantify the potential ecological risk of heavy metal contamination; (2) explore the suitability of different indices for evaluating benthic EcoQs; and (3) assess benthic EcoQs using an integrated approach that combined heavy metal- and macrobenthos-based indices.

# 2. Materials and methods

# 2.1. Study area

Laoshan Bay is a semi-enclosed bay in the Shandong Peninsula, located on the northwest coast of the Yellow Sea (Fig. 1). Laoshan Bay is an important spawning ground for many marine organisms as well as an important culture area for many economically important species (e.g., fish, crabs, shrimps, scallops, and seaweed), which are farmed in the bay and/or in land-based aquaculture ponds within the 5 km coastal fringe of Laoshan Bay. Culture operations for these species cover an area of>20 km<sup>2</sup> (Dong et al., 2021c). In recent years, and especially since it was declared a marine economic development demonstration zone in 2015, Laoshan Bay has been facing increasing anthropogenic pressures from long-term mariculture, shipping, and population growth (Dong et al., 2021b; Wang et al., 2019).

## 2.2. Sample collection and processing

We collected samples for measuring abiotic (i.e., heavy metal pollution in sediments) and biotic (i.e., macrobenthos communities) variables from 24 sampling sites in Laoshan Bay (S1-S24) in May 2018 (Fig. 1).

## 2.2.1. Heavy metals in sediments

At each site, we collected one sediment sample using a 0.05 m<sup>2</sup> Van Veen grab. Samples were frozen at -20 °C and transported to the lab for heavy metal and total organic carbon (TOC) analysis. Seven metal(loid)s arsenic (As), cadmium (Cd), Chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), and zinc (Zn) were measured using standardized procedures according to the specifications for marine monitoring Part 5: Sediment analysis (State Bureau and of Quality and Technical Supervision of China, 2008). First, sediments (samples of 0.5 g) were digested with a mixed acid solution (HFHNO<sub>3</sub>-HClO<sub>4</sub>). Second, the content of (Cd, Cr, Pb, Cu and Zn) was determined using a graphite furnace atomic absorption spectrometer (Shimadzu AA-6300C, Shimadzu Ltd., Japan), while Hg and As were determined using an atomic fluorescence spectrometer (AFS-930, Titan Ltd., China). To ensure the accuracy of analyses, the standard reference material GBW07333 (Chinese National Standard reference material) was used. The TOC content of each sample was measured using the potassium dichromate-sulfuric acid (K2Cr2O7- $H_2SO_4$ ) oxidization method.

#### 2.2.2. Macrobenthic organisms in sediments

Using the same Van Veen grab that we used to collect samples for heavy metal analysis, we collected an additional three sediment samples from each site for macrobenthic organism analysis. Samples were sieved using a 0.5 mm mesh, fixed in 4% formalin, and then preserved in 70% ethanol. In the laboratory, we sorted and counted all macrobenthic organisms and then identified them to the lowest possible taxonomic level (generally species) using a stereomicroscope (Nikon SM2, Nikon Ltd.,



Fig. 1. Study area and locations of 24 sampling sites in Laoshan Bay.

Japan).

## 2.3. Heavy metal-based abiotic indices

# 2.3.1. Geo-accumulation index (Igeo)

To evaluate the ecological risk of heavy metal pollution and eliminate the influence of natural geological contributions, we used the geoaccumulation index  $I_{geo}$  proposed by Müller (1969):

$$I_{geo} = \log_2 \left( \frac{C_i}{1.5 \times B_i} \right) \tag{1}$$

where  $C_i$  is the measured concentration of the  $i^{\text{th}}$  heavy metal and  $B_i$  is the geochemical background value of the  $i^{\text{th}}$  heavy metal. Based on the  $I_{geo}$  value of a sample, the degree of heavy metal pollution in the sediment can be classified into one of seven categories: practically unpolluted ( $I_{geo} \leq 0$ ); unpolluted to moderately polluted ( $0 < I_{geo} \leq 1$ ); moderately polluted ( $1 < I_{geo} \leq 2$ ); moderately to heavily polluted ( $2 < I_{geo} \leq 3$ ); heavily polluted ( $3 < I_{geo} \leq 4$ ); heavily to extremely polluted ( $4 < I_{geo} \leq 5$ ); or extremely polluted ( $I_{geo} > 5$ ) (Müller, 1969).

## 2.3.2. Potential ecological risk index (RI)

This index considers not only the measured and background concentration of different metals but also their unique toxicities and combined ecological risk (Hakanson, 1980). *RI* is calculated as follows:

$$RI = \sum_{i=1}^{n} E_{r}^{i} = \sum_{i=1}^{n} T_{r}^{i} P_{i} = \sum_{i=1}^{n} T_{r}^{i} \frac{C_{i}}{B_{i}}$$
(2)

Where  $C_i$  and  $B_i$  are the same as in the formula (1),  $P_i$  is the pollution factor of the *i*<sup>th</sup> heavy metal. *T* is the toxic-response factor for the *i*<sup>th</sup> metal (defined as 1 for Zn, 2 for Cr, 5 for Cu and Pb, 10 for As, 30 for Cd, and 40 for Hg, based on the toxicity of each metal in the environment) (Hakanson, 1980).  $E_r$  indicates the potential ecological risk coefficient for the *i*<sup>th</sup> metal. For each metal,  $E_r$  can be divided into five categories: low potential ecological risk ( $E_r < 40$ ); moderate potential risk ( $40 \le E_r$ < 80); considerable potential ecological risk ( $80 \le E_r < 160$ ); high potential ecological risk ( $160 \le E_r < 320$ ); and very high potential ecological risk ( $E_r > 320$ ) (Hakanson, 1980). The risk index *RI* is calculated as the sum of the risk coefficients for each detected metal, where *n* is the total number of metals. However, because we did not measure polychlorinated biphenyls (PCBs) in this study, our values for *RI* were artificially lower than the risk evaluation criteria proposed by Hakanson (1980). We therefore adapted the classification criteria from Hakanson (1980) into four grades: low ecological risk (*RI* <120); moderate ecological risk ( $120 \le RI < 240$ ); considerable ecological risk ( $240 \le RI < 480$ ); and very high ecological risk (*RI* > 480).

## 2.3.3. Pollution load index (PLI)

The pollution load index proposed by Tomlinson et al. (1980) is used to evaluate marine sediment quality. This index considers the relative contribution of each metal to the total pollution level, as follows:

$$PLI = \sqrt[n]{\prod_{i=1}^{n} P_i}$$
(3)

In this equation,  $P_i$  is the same as in the formula (2), and n is the number of elements included in the evaluation. Tomlinson et al. (1980) propose four levels of pollution that can be classified based on the *PLI* index: unpolluted (*PLI* < 1); moderately polluted ( $1 \le PLI < 2$ ); heavily polluted ( $2 \le PLI < 3$ ); or extremely polluted (*PLI* > 3).

## 2.3.4. Nemerow pollution index (Pn)

The Nemerow index considers the extreme value and pollution coefficient of each metal, which can reflect the overall pollution degree of the sediment (Kowalska et al., 2016; Nemerow, 1974). This index can be calculated as follows:

$$P_n = \sqrt{\frac{\left(\frac{1}{n}\sum_{i}^{n} P_i\right)^2 + \left(P_{i\max}\right)^2}{n}}$$
(4)

Here,  $P_i$  is the same as in the formula (2), n is the total number of metals being considered, and  $P_{imax}$  represents the maximum  $P_i$  among

the different heavy metals at each sampling site. According to Kowalska et al. (2016), *Pn* can be divided into five categories: clean (*Pn* < 0.1); warning limit (0.7 < Pn < 1); slightly polluted ( $1 \le Pn < 2$ ); moderately polluted ( $2 \le Pn < 3$ ); or heavily polluted (*Pn* > 3).

## 2.4. Macrobenthos-based biotic indices

## 2.4.1. AMBI

The AMBI examines the response of soft-bottom benthic communities to natural and human-induced disturbances in coastal and estuarine environments. Specifically, it explores the response to organic matter loading assuming that those communities serve as indicators of the EcoQs or general health of the ecosystem (Borja et al., 2000). The AMBI is calculated using the relative abundances of macrobenthos in five ecological groups (distinguished  $EG_i$  to  $EG_v$ ) (Borja et al., 2000; Pearson and Rosenberg, 1978), as follows:

$$AMBI = 0*EG_i + 1.5*EG_{ii} + 3*EG_{iii} + 4.5*EG_{iv} + 6*EG_v$$
(5)

where  $EG_i - EG_v$  are the ecological groups defined for the AMBI index. Values for AMBI generally range from 0 to 6, but can reach 7 if the sediment contains no living macrobenthic organisms. In general, the higher the AMBI score, the lower the EcoQs. The cutoff values for different levels of EcoQs assessed by AMBI are shown in Table 1.

#### 2.4.2. M-AMBI

The multivariate AMBI (M–AMBI) is a multi-metric, macrobenthosbased index for assessing the EcoQs of marine and transitional waters (Borja et al., 2012; Muxika et al., 2007). This index considers AMBI (defined above) alongside traditional diversity and richness indices, so it can be used within the European Water Framework Directive 2000/60/ EC. Specifically, M–AMBI is calculated using a factorial analysis of the AMBI, species richness, and Shannon diversity index. Ecosystem-specific reference values for the Shannon index and species richness need to be determined before M–AMBI can be calculated (Borja et al., 2012; Muxika et al., 2007). For this study, we defined the reference value as 15% greater than the maximum value we measured, following the precedent of previous studies (Borja et al. 2012, Cai et al. 2014, Dong et al. 2021a). The values of M–AMBI range from 0 to 1, and the interpretation runs opposite to the interpretation of AMBI: the higher the M–AMBI value, the higher the EcoQs (Table 1).

# 2.4.3. BENTIX index

Similar to AMBI, the BENTIX index is a biotic index based on the relative abundances of benthic macrobenthos in three ecological indicator groups, denoted  $G_i$ - $G_{iii}$  (Simboura and Zenetos, 2002). The first indicator group ( $G_i$ ) includes both  $EG_i$  and  $EG_{ii}$  from the AMBI index,  $G_{ii}$  includes  $EG_{iii}$  and  $EG_{iii}$  corresponds to  $EG_v$ . This index can be calculated as follows:

$$BENTIX = 6^*G_i + 2^*(G_{ii} + G_{iii})$$
(6)

where  $G_i - G_{iii}$  are ecological groups defined for the BENTIX index. The values of BENTIX range from 2 to 6, but can be zero when the sediment contains no living organisms (Table 1).

## 2.4.4. Feeding evenness index

The feeding evenness index (*jFD*) is an index based on the relative abundance of different macrobenthos feeding groups (Gamito and Furtado, 2009). Based on our previous studies in Laoshan Bay (Dong et al. 2021b; Dong et al., 2021c), we classified macrobenthos into four feeding groups: carnivores, omnivores, planktivores, and detritivores. This index can be calculated as follows:

$$jFD = \left(-\sum_{i=1}^{n} P_i \log_2 P_i\right) / \log_2^n \tag{7}$$

where  $P_i$  is the relative abundance of the *i*<sup>th</sup> feeding group; *n* is the total number of feeding groups. The *jFD* values range from 0 to 1, with higher values indicating higher EcoQs (Table 1).

#### 2.5. Data analyses

## 2.5.1. Previous considerations for the use of proposed indices

To calculate some of the heavy metal-based abiotic indices of ecological quality, we required background concentrations of the different metal(loid)s we detected. However, because these background values are not available for Laoshan Bay, we established reference values using the primary grade marine sediment quality of China (MSQC) (Wang et al., 2019). Thus, the background values of As, Cd, Cr, Cu, Hg, Pb and Zn used for all calculations were 20, 0.5, 80, 35, 0.2, 60 and 150 mg/kg, respectively (State Bureau and of Quality and Technical Supervision of China, 2002). For the biotic indices of ecosystem quality, we excluded site S8 from our analysis because it contained fewer than three species.

### 2.5.2. Data analysis

The AMBI and M–AMBI indices were calculated using the freely available software provided by AZTI (AMBI 6.0; https://ambi.azti.es/) as well as their *December 2020 species list*. Calculations were performed following the guidelines of the use of AMBI. The feeding habits of the different macrobenthos species found in this study were obtained from the BIOTIC (MarLIN, 2006) and Polytraits (Polytraits Team, 2020) on-line data bases as well as from previous studies (Ni et al., 2019; Peng et al., 2013; Zhang et al., 2020). The Shannon (*H*', log<sub>2</sub>-based), Simpson (*D*), and Pielou (*J*) indices were calculated using the R package "vegan" (Oksanen et al., 2019).

Given the need to provide more direct reference information to managers and/or governments to guide decision or policies regarding benthic ecosystem management, we chose to recategorize the different pollution level classes defined by the seven indices (*RI, PLI* and *Pn*, AMBI, M—AMBI, BENTIX and *jFD*). Instead of using four or five levels, as defined by the indices, we simplified the scores to either 1 (acceptable) or 0 (unacceptable) (Table 2) (Blanchet et al., 2008; Dong et al., 2021a; Maghsoudlou et al., 2020).

The evaluation results in the studies sites (i.e., "acceptable" or "unacceptable") were not the same for all indices. We summed the recalculated (0/1) scores of all seven indices, and then used the result (a value ranging from 0 to 7) to determine the final benthic EcoQs (Table 3) (Blanchet et al., 2008; Maghsoudlou et al., 2020).

The correlation between indices was determined by Pearson coefficients. The level of agreement between indices was determined by

Table I
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Ecological quality status categories for the four benthic macrobenthos-based indices: AMBI, M-AMBI, BENTIX and jFD.

Indices	Ecological quality status (EcoQs)					Reference
	High	Good	Moderate	Poor	Bad	
AMBI	$\leq 1.2$	1.2-3.3	3.3-4.3	4.3–5.5	>5.5	(Borja et al., 2000; Muxika et al., 2005)
M-AMBI	>0.77	0.53-0.77	0.38-0.53	0.2 - 0.38	${\leq}0.2$	(Borja and Tunberg, 2011; Dong et al., 2021a)
BENTIX	$\geq$ 4.5	3.5-4.5	2.5-3.5	2.0 - 2.5	<2.0	(Simboura and Zenetos, 2002)
jFD	$\geq$ 0.8	0.6–0.8	0.4–0.6	0.2–0.4	<0.2	(Gamito et al., 2012; Gamito and Furtado, 2009)

#### Table 2

Thresholds used to convert index values into acceptable or unacceptable categories to assess EcoOs.

Indices	Thresholds	Levels of risk, pollution or EcoQs	Acceptable (1) Unacceptable (0)
RI	< 120	Low ecological risk	1
	$\geq 120$	Moderate, Considerable, or	0
		Very High ecological risk	
PLI	< 1 Unpolluted		1
	$\geq 1$	Moderately, Heavily, or	0
		Extremely polluted	
Pn	< 1	Clean, Waring limit	1
	$\geq 1$	Slightly, Moderately, or	0
		Heavily polluted	
AMBI	$\leq 3.3$	Good, High EcoQs	1
	> 3.3	Moderate, Poor, or Bad EcoQs	0
M-AMBI	> 0.53	Good, High EcoQs	1
	$\leq 0.53$	Moderate, Poor, or Bad EcoQs	0
BENTIX	> 3.5	Good, High EcoQs	1
	$\leq 3.5$	Moderate, Poor, or Bad EcoQs	0
jFD	> 0.6	Good, High EcoQs	1
	$\leq 0.6$	Moderate, Poor, or Bad EcoQs	0

Table 3

Thresholds used for identifying the final ecological quality status (EcoQs) of each site.

Sum of scores	Interpretation	Ecological quality status (EcoQs)
0	Full agreement of the seven indices on "Unacceptable" EcoQs	Unacceptable
1	Partial agreement (six of seven indices) on "unacceptable" EcoQs	Unacceptable
2	Partial agreement (five of seven indices) on "unacceptable" EcoQs	Unacceptable
3	Disagreement between the indices on the EcoOs of the station	Unacceptable
4	Disagreement between the indices on the EcoOs of the station	Unacceptable
5	Partial agreement (five of seven indices) on "acceptable" EcoOs	Acceptable
6	Partial agreement (six of seven indices) on "accentable" EcoOs	Acceptable
7	Full agreement of the seven indices on "acceptable" EcoQs	Acceptable

the weighted kappa analysis (Cohen, 1960; Fleiss and Cohen, 1973) using the "kappa2" function in the R package "irr" (Gamer et al., 2019). The level of agreement can be divided into eight categories based on the kappa value: no agreement ( $k \le 0.05$ ), very low agreement (0.05–0.20); low agreement (0.20–0.40); fair agreement (0.40–0.55); good agreement (0.55–0.70); very good agreement (0.70–0.85); excellent agreement (0.85–0.99); or perfect agreement (0.99–1.0) (Dong et al., 2021a; Landis and Koch, 1977; Monserud and Leemans, 1992).

To assess the final benthic EcoQs across all of Laoshan Bay, we first obtained the spatial distribution of the heavy metal- and macrobenthosbased EcoQs indices in the bay. For this, we applied an ordinary kriging interpolation method as in Dong et al. (2020), Qu et al. (2021) and Rufino et al. (2019) using the "autoKrige" function in the R package "automap" (Hiemstra et al., 2009). The interpolated grid size was set to  $50 \times 50$  m. The spatially continuous values for these indices as obtained by the kriging method, were then categorized as before, where 1 indicated "acceptable" and 0 indicated "unacceptable" (Table 2). The final map showing the distribution of EcoQs across the bay was obtained by (i) summing the binary scores of all indices in each grid square, and then (ii) converting the sum of the scores in each grid into a final classification of either "acceptable" or "unacceptable." As shown in Table 3, a sum of scores ranging from 0 to 4 indicates an unacceptable EcoQs.

All statistical analysis was performed using R software 4.0.2 (R Core

Team, 2020). The ordinary kriging interpolation was performed in the R package "raster" (Hijmans, 2022). The map of the study area was drawn using ArcGIS 10.4.2 (Esri, United States), and the R package "ggplot2" was used to produce other figures (Wickham, 2016).

## 3. Results

#### 3.1. Concentration of heavy metals

The average concentrations (mean  $\pm$  SD and the range of values for each metal(loid)s in Laoshan Bay are shown in Table 4. The total concentration of TOC in surface sediments was low, averaging 0.64  $\pm$ 0.15%. The distribution of heavy metal concentration in the surface sediments is presented Fig. S1. Some heavy metals appear to be highly concentrated in one or two areas; for example, As reaches a high concentration around Aoshanwei, and Hg has high concentration areas near Aoshanwei and between Ma'er and Nv Islands (Fig. S1).

## 3.2. Macrobenthos community characteristics

We identified 64 benthic macroinvertebrates in Laoshan Bay, including 51 at the species level, 8 at the genus level and 5 at the family or higher classification levels (Table S1). Polychaeta was the most species taxon in the bay with 34 species (53.13%), followed by Crustacea with 15 species (23.44%), Mollusca with 11 species (17.19%), and other groups represented by a total of four species (6.25%). The observed Shannon (*H*', log<sub>2</sub>), Simpson (*D*), and Pielou (*J*) indices across all samples were 2.66  $\pm$  0.49, 0.80  $\pm$  0.08, and 0.90  $\pm$  0.10, respectively (Table S2).

## 3.3. EcoQs of each station evaluated by single indices

## 3.3.1. Results of heavy metal-based indices

Results from the  $I_{geo}$  index suggested that the surface sediments from all sampling sites could be considered "unpolluted" ( $I_{geo} < 0$ ) with the metal(loid)s Cr, Zn, Pb, Cu and As. However, for Cd and Hg, 45.83% and 79.17% of sampling sites were classified as "unpolluted to moderately polluted" status ( $0 \le I_{geo} < 1$ ), respectively (Fig. 2a).

The results for  $E_r$  were similar to those for  $I_{geo}$ : at all sampling sites, the concentrations of Cr, Zn, Pb, Cu were considered to present low potential ecological risk, whereas the concentrations of Cd and Hg reached levels of moderate or even considerable potential ecological risk in some stations (Fig. 2b). For Cd, sediments from station S16 were at considerable potential ecological risk, and 14 other stations were at moderate potential ecological risk. Hg levels were classified as posing moderate or considerable potential ecological risk at 54.17% and 45.83% of the stations, respectively. Overall, the cumulative concentrations and potential ecological risk coefficients of the heavy metals in Laoshan Bay were low, with the exceptions of Cd and Hg at the sampling sites mentioned above.

The results from the potential ecological risk index (*RI*) showed that more than half of the stations (66.67%) were at moderate ecological risk (*RI* > 120), indicating an "unacceptable" status for these stations. Conversely, the pollution load index (*PLI*) showed that all stations could be considered unpolluted (*PLI* < 1) and therefore had an "acceptable"

### Table 4

The concentration of metal(loid)s in the surface sediment of Laoshan Bay.

Metal(loid)s	$\text{Mean} \pm \text{SD}$	Range (min – max)
As (mg/kg)	$\textbf{7.13} \pm \textbf{1.84}$	4.67-12.22
Cd (mg/kg)	$0.70\pm0.30$	0.28-1.44
Cr (mg/kg)	$62.15\pm12.62$	39.46-84.44
Cu (mg/kg)	$16.83\pm3.92$	9.67-24.72
Hg (mg/kg)	$0.40\pm0.08$	0.26-0.55
Pb (mg/kg)	$25.34\pm11.78$	13.18-39.82
Zn (mg/kg)	$54.21 \pm 11.78$	36.84-82.43



Fig. 2. Values of the geo-accumulation index (a) and potential ecological coefficient (b) for the seven metal(loid)s analyzed in surface sediments collected from Laoshan Bay. The thresholds for metal(loid)s that are not within the y-axis range are not showed.

ecological status (Fig. 3).

Results from the Nemerow pollution index (*Pn*) showed that the Laoshan Bay sediment samples ranged from clear to slightly polluted of heavy metals, with 20.83%, 62.50%, and 16.67% of stations at "clear," "warning limit," and "slightly polluted" statuses, respectively (Fig. 3). In our binary classification system, 16.67% of stations were considered "unacceptable" according to *Pn*. Detailed results for these three indices (*RI*, *PLI*, and *Pn*) are provided in Table S2.

## 3.3.2. Results of macrobenthos-based indices

The benthic EcoQs scores, as assessed using four different macrobenthos-based indices, suggested that most stations were in good

or high condition. Overall, AMBI values ranged from 1.50 to 3.99, with an average of 2.51  $\pm$  0.63. The benthic EcoQs determined based on AMBI scores was "good" or "high" at 21 of our 24 stations (91.30%); only sites S9 and S11 showed a "moderate" EcoQs as determined by AMBI, and no sites produced EcoQs results that were classified as "unacceptable" (Fig. 4).

M-AMBI values ranged from 0.47 to 0.91, with an average of 0.68  $\pm$  0.12. As with AMBI, these values corresponded to moderate to high EcoQs across all sites. Three stations were classified as "moderate" EcoQs (13.0%), resulting in a classification as "unacceptable." Thirteen stations (56.5%) produced "good" EcoQs and 7 stations (30.5%) produced "high" EcoQs, all of which were considered "acceptable."



Fig. 3. Indices of heavy metal contamination in the surface sediments of Laoshan Bay. *RI*, potential ecological risk index; *PLI*, pollution load index; *Pn*, Nemerow pollution index.

The values of BENTIX and *jFD* ranged from 2.42 to 5.0 and 0.28 to 0.95, respectively. According to their threshold levels, these results corresponded to anywhere from "poor" to "high" EcoQs. For BENTIX, the EcoQs of only one station (S9) (4.3%) was "poor;" five stations (21.7%) showed "moderate" EcoQs, 10 stations (43.5%) showed "good" EcoQs, and 7 stations (30.5%) showed "high" EcoQs. Overall, 6 stations assessed by BENTIX were in an "unacceptable" ecological state, whereas 17 stations were in "acceptable" condition. For *jFD*, the EcoQs of one station (S9) (4.3%) was "poor;" four stations (17.4%) showed "moderate" EcoQs, 15 stations (65.2%) showed "good" EcoQs, and three stations (13.0%) showed "high" EcoQs. In general, 5 stations were in "acceptable" ecological state, whereas 18 stations were in "acceptable" ecological state, whereas 18 stations were in "acceptable" ecological state, whereas 18 stations were in "acceptable" condition. More detailed results outlining the specific values of the four biotic indices at each site are provided in Table S2.

Overall, the EcoQs of most stations in Laoshan Bay, as determined using three heavy metal-based indices and four macrobenthos-based indices, was "acceptable" (Figure S2); only a few stations were considered to show "unacceptable" quality. Sites that showed "acceptable" EcoQs for *RI*, *PLI*, *Pn*, AMBI, M–AMBI, BENTIX and *jFD* accounted for 29.17%, 100%, 83.33%, 91.30%, 86.96%, 73.91%, and 78.26% of all sampling sites, respectively.

## 3.4. EcoQs across the whole Laoshan Bay

We used data from each sampling site to interpolate results for the seven indices (AMBI, M–AMBI, BENTIX, *jFD*, *PLI*, *Pn* and *RI*) across Laoshan Bay. Our results suggested that the spatial patterns of the benthic EcoQs were different for different indices (Fig. 5). For example, the spatial distributions of AMBI and BENTIX values were almost

opposite to each other because higher AMBI values, but lower BENTIX values, mean lower EcoQs. In general, AMBI and BENTIX showed that the lower EcoQs are in the coastal waters of Aoshanwei and in the area between Nv and Ma'er Islands compared to the rest of the bay. The spatial distribution of M–AMBI values was similar to that for *PLI* and *RI* (Fig. 5). However, the M–AMBI results suggested that EcoQs gradually increased from north to the south, whereas the *RI* and *PLI* results showed that EcoQs decreased from north to the south. The spatial distribution of the *jFD* index showed that the coastal waters of Aoshanwei had lower scores than other areas, indicating that the benthic EcoQs in this area were low. The spatial distribution of *Pn* values was different from the other indices; these results showed high values in the middle of Laoshan Bay, indicating that the EcoQs in these areas was low.

The combined results from our suite of seven indices showed that the areas of "acceptable" and "unacceptable" EcoQs accounted for 88.90% and 11.10% of the total study area in Laoshan Bay, respectively (Fig. 6). The areas with "unacceptable" EcoQs were mainly located in the coastal waters of Aoshanwei and in the area between Nv and Ma'er Islands.

## 3.5. Inter-index agreement

There was a high positive correlation between abiotic indices (*RI*, *PLI* and *Pn*) and a high positive correlation between biotic indices (except for the AMBI index), however, the correlation between abiotic indices and biotic indices was relatively low (Table S3). Kappa analysis showed that the benthic EcoQs assessment results showed only low, very low, or even no agreement among the seven indices we used. The only exception was "fair" agreement between AMBI and BENTIX (Table S4). Otherwise, the agreements between the assessment results of the *RI*, *PLI*, *Pn*, and *jFD* indices and the final EcoQs were null or very low, despite having a high matching rate (43.48%–82.61%). However, the agreement between the assessment results from the AMBI, M–AMBI, and BENTIX indices and the final EcoQs was fair to good, and they also had a high matching rate (82.61%–95.65%). Thus, these three indices (AMBI, M–AMBI, and BENTIX) may be more suitable for evaluating the EcoQs of Laoshan Bay than *RI*, *PLI*, *Pn*, and *jFD*.

## 4. Discussion

The assessment of benthic EcoQs is becoming increasingly necessary as more and more coastal countries focus on the appropriate management of their marine ecosystems (Borja et al., 2008a; Poikane et al., 2020). In this study, we provide the first example of how abiotic environmental factors (i.e., heavy metals) and biotic factors (i.e., macrobenthos) can be combined to comprehensively evaluate the benthic EcoQs of a coastal ecosystem like Laoshan Bay. Laoshan Bay is a model ecosystem for such studies in China because it faces disturbances from multiple human activities including land-based pond aquaculture, laver (seaweed), fish and scallop aquaculture, and port operations. Our results provide valuable outputs for future decision-making; most notably, our thematic maps of EcoQs-including three maps based on heavy metals, four maps based on macrobenthos, and one map integrating heavy metals and macrobenthos-provide an easily referenceable indication of overall ecosystem quality. This study can also serve as both a reference and a novel perspective for benthic EcoQs assessment in similar systems.

The impact of anthropogenic activities on Laoshan Bay has increased rapidly in recent years, especially since the region was declared a marine economic development demonstration zone in 2015 (Dong et al., 2021b; Wang et al., 2019). A 2011 study conducted on the southern coast of the Shandong Peninsula, including Laoshan Bay, showed that (i) metal(loid) s concentrations (As, Cd, Cr, Cu, Hg, Pb and Zn) in the surface sediments did not exceed the primary grade of MSQC and (ii) Laoshan Bay was considered non-polluted according to the *Pn* index (Liu et al., 2015). Subsequently, a 2016 study showed that Pb contamination, defined using the enrichment factor index, was present in 85.7% of sampling sites in Laoshan Bay (Tian et al., 2022). More recent studies in Laoshan



Fig. 4. Ecological quality status (EcoQs) for each station in Laoshan Bay estimated using the AMBI, M-AMBI, BENTIX and jFD indices.



Fig. 5. Spatial distribution of AMBI, M-AMBI, BENTIX, jFD, PLI, Pn and RI in Laoshan Bay.



**Fig. 6.** The overall benthic ecological quality status (EcoQs) of Laoshan Bay, as determined using a combination of heavy metal- and macrobenthos-based indices.

Bay have shown that some heavy metals (e.g., Pb, Hg, Cr, and Cd) measured in the seawater and surface sediments, in both intertidal and subtidal zones, exceeded the primary and even secondary grade of the MSQC (Dong et al. 2021b; Dong et al., 2021c; Wang et al., 2019).

Like these more recent studies, we also found that Cr, Cd, and Hg concentrations in the surface sediments were higher than the primary or even secondary grades of the MSQC. Specifically, the concentrations of Cr, Cd, and Hg exceeded the first grade at 4.17%, 70.83%, and 100% of our study sites, respectively, and Hg concentrations exceeded the second grade at three sites (12.50%). Overall, the recent results from Laoshan Bay, including those from our study here, demonstrate that heavy metal concentrations in Laoshan Bay have increased in recent years. Our study therefore addresses the urgent need for evaluating the pollution levels and potential ecological risks of heavy metals in Laoshan Bay.

Accurately identifying the source of heavy metal pollution is important for controlling these sources and implementing effective ecosystem management. Heavy metals can originate from various sources, including atmospheric deposition, surface runoff, and sewage discharge (Kim et al., 2021; Pan and Wang, 2012; Wang et al., 2021). Although there is no direct evidence for the specific sources of the different heavy metals measured in Laoshan Bay, many studies indicated that the recent increase in heavy metal concentrations is related to human activities, such as urbanization, ship repair, fossil fuel combustion, fishing, and land-based aquaculture (Dong et al., 2021c; Tian et al., 2022; Wang et al., 2019). Nevertheless, identifying the sources of heavy metal pollution is outside the scope of the work conducted here; future studies are needed to pinpoint the sources of heavy metals in Laoshan Bay and the relationship between metal accumulation and different human activities.

The heavy metal-based indices that we used (*RI*, *PLI* and *Pn*) showed varying results for whether the benthic EcoQs was acceptable in Laoshan Bay (33.3%-100.0%). Although these three indices were significantly correlated (Pearson's test, p < 0.05), the kappa analysis resulted in null

or very low levels of agreement among these indices. These results suggest that it may be inadequate to use just a single index to evaluate a system such as Laoshan Bay. One possible reason for this is lack of agreement among indices and the metal-based indices each have different emphases. For example, the geo-accumulation index  $(I_{geo})$ measures the pollution caused by a single heavy metal element, whereas the pollution load index (PLI) provides a single measure for the pollution of multiple heavy metals (Müller, 1969; Shetaia et al., 2022; Tomlinson et al., 1980). The other possible reason may be that they have different classification for evaluation results, e.g., four levels for PLI and five levels for Pn. In addition, the toxic-response factor may affect the final evaluation results of RI. These factors have been obtained through extensive experiments, however, over the past 40 years, the ecological environment condition may have changed under human activities' pressure and climate change. Future research using toxic-response factors of heavy metals measured in local species when using RI would yield a more accurate assessment.

Furthermore, the background values of metal(loid)s used in this study also affect the final evaluation results. Due to the lack of local background values, we followed information of a recent study conducted in Laoshan Bay (Wang et al. 2019) and used values of the primary grades of the MSOC standard, which is a national standard recognized by the Chinese government. The concentrations of metal(loid)s in the upper crust of East China reported by Gao et al. (1998) were also used as background values (Tian et al., 2022; Wang et al., 2020). For the latter, it should be noted that although the concentration of some heavy metals is lower than the primary grades of the MSQC, others such as Hg showed a much higher concentration (9.53 mg/kg, 46.7 times higher) than the primary grades of the MSQC. To more accurately evaluate the contamination levels of the marine sediment, we here call for strengthening the study on the background values of heavy metals in local sea areas. Given these differences, previous studies have emphasized that using multiple indices at the same time can yield more comprehensive evaluations of heavy metal pollution (Shetaia et al., 2022; Wang et al., 2021).

The results from the four macrobenthos-based biotic indices (AMBI, M-AMBI, BENTIX, and jFD) showed an "acceptable" benthic EcoQs for most stations (73.19%-91.30%). However, these indices displayed a wide range of variation in their benthic EcoQs classifications: for example, AMBI scores ranged from good to moderate, M-AMBI scores from high to moderate, and BENTIX and *jFD* scores from high to poor. Only three species could not be assigned to any AMBI ecological group (Table S1), and the number of unassigned macrobenthos individuals at each site did not exceed 5.6% of the total sampled population. This value was much lower than the threshold value of 20% proposed by Borja and Muxika (2005) in their guidelines for the use of AMBI (Borja et al., 2008c; Borja and Muxika, 2005). The range of M-AMBI scores may be related to the reference value of Shannon index that we defined, because of M-AMBI is calculated based on the factorial analysis of the AMBI score, species richness, and Shannon index. The value of 15% greater than the maximum Shannon index as the reference value has been validated in adjacent areas with similar ecological environments, e.g., Bohai Bay and Laizhou Bay (Cai et al., 2014; Dong et al., 2021a). Moreover, BENTIX and jFD indices also have been tested and validated in a nearby bay in the Yellow Sea (Dong et al., 2021a). Other studies have also reported conflicting classifications of EcoQs when these indices in a variety of marine ecosystems, including bays (Lu et al., 2021; Maghsoudlou et al., 2020), coastal waters (Dong et al., 2021a; Pelletier et al., 2018) and estuaries (Li et al., 2021; Mulik et al., 2020; Pandey et al., 2021). One possible reason for this is that these indices were developed under different principles. For example, AMBI is proposed based on the response of macrobenthos species to organic matter loading, whereas the *jFD* is proposed according to the macrobenthos feeding groups. The second possible reason is that the composition of macrobenthos in different regions may be different and may include some native species, which may lead to verying responses to specific disturbance. In addition, the dominant feeding habits of macrobenthos

may also change between different habitats, such as in seagrass sediment beds and bare sand areas.

The four indices (AMBI, M-AMBI, BENTIX and jFD) were significantly correlated with each other (Pearson's test, p < 0.05), with AMBI being significantly and negatively correlated with the other three indices, which are positively correlated between them (Table S3). It implies that using these indices may result in a relatively high consistency result. However, kappa analysis indicated that the agreement between the four macrobenthos-based indices was generally null to low; the only exception was between AMBI and BENTIX, which showed fair agreement. Although other studies have reported similar results including Dong et al. (2021a), it is the first study in these terms reported in Laoshan Bay. The relative higher agreement between these two indices may be because both indices are based in similar principles. As with the heavy metal-based indices, our results suggest that a single biotic index or the combination of only a few indices may be insufficient for accurate assessments of benthic EcoQs (Franzo et al., 2022; Wetzel et al., 2012). Furthermore, our study represents one of the few studies that consider both biotic and abiotic factors in assessments of benthic habitat quality (Guerra-García et al., 2021; Hutton et al., 2015).

Our final assessment results extrapolated across Laoshan Bay showed that the EcoQs in most areas (88.9%) of Laoshan Bay is acceptable; only a small part of the bay (11.1%) was considered to be of unacceptable quality (Fig. 6). The two "unacceptable" areas of Laoshan Bay were in the coastal waters of Aoshanwei and in the area between Nv Island and Ma'er Island. Heavy metal pollution may be the main reason for the unacceptable EcoQs between Nv and Ma'er Island; Cd and Hg concentrations in this area either exceeded or were very close to the second grade of the MSQC, potentially due to the laver (Porphyra yezoensis) and fish farming operations in the area. Previous studies have shown that red seaweed harbors higher concentrations of trace and toxic metals than other seaweed (e.g., brow seaweeds), and, more generally, Porphyra species can accumulate more metals than other seaweed genera (Chen et al., 2018; Rubio et al., 2017). In addition, the bio-deposits that are discharged from laver and fish farming operations could increase local silt content and the sedimentation fluxes of heavy metals (Jeong et al., 2020). Furthermore, we found many opportunistic species of polychaetes in this area, especially at site S19. These polychaetes included the known pollution-related indicator species Aphelochaeta multifilis, Sternaspis cf. scutata, Sigambra bassi, and Heteromastus filiformis, suggesting the general degradation of benthic habitat in this area. These results agree with two previous studies conducted in Laoshan Bay, which concluded that tolerant and/or second-order opportunistic species tend to become more abundant or common in macrobenthos communities as heavy metal pollution increases (Dong et al. 2021b; Dong et al., 2021c).

The concentrations of TOC and most heavy metals in surface sediments are positively related with the activities of land-based aquaculture ponds, and the combined effect of these three factors (TOC, heavy metals, and aquaculture) was the main driver of the composition of macrobenthos communities in Laoshan Bay (Dong et al., 2021c). Thus, land-based aquaculture may also contribute to the degradation of benthic habitats and corresponding unacceptable EcoQs in the coastal area of Aoshanwei. This effect in the Aoshanwei region is indicated by the presence of many pollution-tolerant and/or opportunistic polychaetes (e.g., Chaetozone setosa, Cirriformia tentaculata, A. multifilis, and H. filiformis). For example, at station S9, 78.95% of the total benthic macrobenthos we detected were the opportunistic species C. setosa and H. filiformis. Rapid urbanization and population growth in Aoshanwei, especially since 2015 (Tian et al. 2022; Wang et al. 2019), may also impacting the EcoQs of this area, as increased human activity comes with increased sewage discharges and shipping and recreational activities.

## 5. Conclusions

heavy metal- and macrobenthos-based indices of ecosystem health to easily but comprehensively assess the benthic EcoQs of Laoshan Bay. Our results suggest that the EcoQs determined from a single index, whether based on heavy metals or macrobenthos, should be treated with caution, because a single index may over- or underestimate benthic habitat quality status. Instead, the integration of multiple heavy metaland macrobenthos-based indices will yield more reliable and robust assessments of benthic EcoQs. The approach presented here contributes to the development of improved tools for assessing and managing marine benthic ecosystems.

# CRediT authorship contribution statement

Jian-Yu Dong: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Software, Formal analysis. Xuefeng Wang: Data curation, Supervision. Xiumei Zhang: Supervision, Funding acquisition, Writing – review & editing. Gorka Bidegain: Writing – review & editing. LinLin Zhao: Investigation, Data curation, Validation.

# **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.

## Data availability

Data will be made available on request.

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# Appendix A. Supplementary data

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In this study, we tested and integrated some of the most common

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#### J.-Y. Dong et al.

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#### J.-Y. Dong et al.

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