Spatial and temporal distribution of toxic compounds in sediments and potential ecological effects on macrobenthic faunal species in Hangzhou Bay from 2003 to 2015

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ABSTRACT

The development of toxic compounds in sediment and macrobenthos species in Hangzhou bay (2003–2015) was evaluated. Concentrations were compared to Chinese sediment quality guidelines (CN-SQG) and risk assessed by the ecological risk index (ERI) and t-Distributed Stochastic Neighbour Embedding (t-SNE). To study seafood contamination, sediment and swimming crabs were collected.

Chromium, copper, and arsenic exceeded CN-SQG. Organic contaminants did not exceed CN-SQG; however, t-SNE revealed a negative relationship with benthic species numbers. Since 2003, half of the benthic species have disappeared. Species sensitive to contamination were not observed after 2003–2007, while crustacea species are more tolerant: cadmium levels in crabs were 5–17 times those in the sediment, demonstrating strong bio-accumulation. These results suggest that metals and organic pollutants pose ecological and seafood risks.

For good environmental management in HZB, it is important to analyze sediment, benthic biota, and seafood species for compounds known to pose toxic risks.

In China, marine contaminants and the related ecological impacts on coastal regions, are a significant issue (Liu et al., 2012a, 2012b, Pan and Wang, 2012). A densely populated and highly industrialised area, the Yangtze River Delta (YRD) contributes approximately 24% of the national gross domestic product (GDP) (Tang, 2008). Hangzhou Bay (HZB) lies downstream of the YRD region, and includes cities with several millions of inhabitants and international harbours, such as Shanghai (SH), Ningbo (NB), and Jiaxing (JX). Therefore, HZB can used to study the sediment quality and marine conditions of the YRD, and as a representative of Chinese coastal areas with large urbanizations and industrial harbours. The national seawater quality has been reported over the past decade (Li et al., 2013, Zhang et al., 2016, Xie et al., 2018), and demonstrates how contaminants, such as metals and pesticides, continue to impair the seawater quality of HZB. Recent studies have also shown high levels of several toxic compounds in the marine sediments of HZB. For example, dichloro-diphenyl-trichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) have been found at 5.1, 172, and 63 ng/g dry weight, respectively (Adeluyi et al., 2016). Hong-Jiao Pang et al. (2015) reported high levels of heavy metals, such as cadmium, chromium, copper, nickel, lead, zinc, and arsenic in sediments, and traced these back to the anthropogenic sources (Pang et al., 2015). However, most of these studies took samples over one year; therefore, it is not possible to assess the development of contaminant concentrations over time, nor the response of benthic communities. Longer sampling periods are needed to provide a complete picture of the development of sediment contamination levels and the presence of benthic species over time in HZB, and to enable environmental safety assessments.

Good-quality sediments are essential for healthy aquatic ecosystems and, especially in marine environments, they are an important basis for the food web. Sediment quality guidelines have been largely employed to determine sediment quality, by comparing chemical concentrations with estimated safe levels (Birch, 1996, Kolt et al., 2006, Cheung et al., 2008). These guidelines are practical but broad, and usually do not take into account complex factors, such as ecological context and combined effects in the case of multiple compounds. Therefore, ecotoxicological assessment tools and statistical approaches have been developed to provide a more comprehensive benthic ecotoxicological effect.
evaluation. For example, the ecological risk index (ERI) is an assessment tool that integrates the chemical levels and ecotoxicological no effect concentrations for many compounds in sediments (Hakanson, 1980). In addition to the predicted risk, the actual impact can be assessed. For this, multivariate statistical tools, such as principal components analysis (PCA) and t-Distributed Stochastic Neighbour Embedding (t-SNE) approaches can be applied to relate the biological and chemical data (Reid and Spencer, 2009).

In HZB, the Zhoushan Marine Ecological Environmental Monitoring Station has sampled marine sediments and macrobenthos every two years from 2003 to 2015, at four locations close to the largest coastal cities. In addition, sediment and swimming crab (*Portunus trituberculatus*) samples were collected in 2016 in same sampling region. The concentration of several toxic compounds was analyzed, e.g., metals and organic pollutants, and the presence of benthic species in the sediment samples was recorded. This datasets offers a unique opportunity to study the development of these parameters over time. This research aims to evaluate the spatial and temporal distribution of toxic compounds and benthic species in the sediments, and assess the potential ecotoxicological effects in HZB.

Most of the sampling was conducted as part of regular environmental surveys performed in April every two years, from 2003 to 2015 by the Zhoushan Marine Ecological Environmental Monitoring Station. Four sampling locations were chosen for this research, namely three locations close to the largest coastal cities in the region, JX (121.04, 30.425), NB (121.7170, 30.1670), SH (121.7120, 30.7608), and Zhoushan (ZS) (122.2620,30.3968), representing a relatively remote location (Fig. 1). The depths of those sampling locations were 10, 14, 6, and 8 m, respectively. Surface sediments were collected with a Van Veen grab sampler, around 20 cm deep in 0.1 m². Benthic macrofauna was sampled with an epibenthic sledge (1 m wide) dragged over 1 m with a slowly moving boat. Sediment samples were kept in a refrigerator at 4 °C, and the benthic macrofauna samples were kept in a freezer at -20 °C until analysis. Additional sampling was conducted in April 2016 to collect swimming crabs (*P. trituberculatus*) and sediment at four additional sampling locations, close to the ZS site, using the same methods. The exact sampling locations were 0908 (122.26, 30.39), 0912 (121.71, 30.16), 0904 (122.07, 30.6100), and 0402 (121.04, 30.4250). (See Fig. 1. Map of four sampling locations in Hangzhou Bay, China. JX (121.04, 30.425), ZS (122.2620,30.3968), NB (121.7170, 30.1670), SH (121.7120, 30.7608).)
Firstly, 10 g (wet weight) sediment samples were dried at 50 °C. Concentrations of toxic compounds and nutrients (mg/kg dry weight) and core properties of sediments sampled at four locations (JX, ZS, NB, SH) in Hangzhou Bay of the Yangtze River Delta, China from 2003 to 2015 (n.d. = no data; n.a. = not applicable). The Chinese Sediment Quality Guideline (CN-SQG) given per compound are the official Chinese marine sediment safe levels and the lowest effect level (LEL) for ecotoxicological. As published in the Chinese governmental Marine SQG (SEPA, 2002). In this study, the Chinese SQG (CN-SQG) used in this study was encoded with a spectrometer (Agilent 6320 series) at 545 and 880 nm for TOC measurements, using a national standard reference (GBW07343) and the lowest effect level (LEL) reported for ecotoxicological effects was used, obtained from a classical study that focused on sediment contamination in urban estuaries (Williamson and Morrissey, 2000).

Four approaches were applied to evaluate the ecological safety of the collected sediments: 1) comparison with Sediment Quality Guidelines (SQGs); 2) calculation of the ERI; and 3) determination of the relationship between animal species and chemical compounds, using the t-SNE method. SQGs provide maximum levels for chemicals in sediments that still can be considered safe for aquatic life (Peddicord and Booth, 1977, Kwok et al., 2014). The Chinese SQG (CN-SQG) used in this study was published in the Chinese governmental Marine SQG (SEPA, 2002). In addition, the lowest effect level (LEL) reported for ecotoxicological effects was used, obtained from a classical study that focused on sediment contamination in urban estuaries (Williamson and Morrissey, 2000).

The ERI is a diagnostic tool to assess the potential ecological risk of individual toxic compounds, or mixtures thereof, based on the LELs for ecotoxicological. ERI is calculated by Eq. (1):

\[ ERI = \sum_{i=1}^{n} E_{i} = \sum_{i=1}^{n} Tr_{i} \times C_{i} \]  

(1)

where Ci is the concentration (mg/kg dry weight) of each contaminant in sediment, and is multiplied with a compound-specific toxic-response factor (Tr). The Tr combines the bioavailability and toxicity of each compound (Hakanson, 1980). The ERI is calculated by Eq. (1):

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compound in sediment (Hakanson, 1980). The Tr numbers of the eight metals included in this study are copper = 5, chromium = 2, lead = 5, zinc = 1, cadmium = 30, arsenic = 10, and mercury = 40 (Hakanson, 1980). The multiple compound risk for the local benthic ecosystem (ERI) is calculated by summing up all individual ER values (Hakanson, 1980).

Following the t-SNE approach, the relationship between benthic animal species and chemical compounds is investigated. The principle of the t-SNE is visualization of high-dimensional data by giving each data point a location in a two dimensional graph, to enable better understand relationship between multiple factors (Maaten and Hinton, 2008).

Trend analyses of compound levels were made and presented in GraphPad (Prism 7). The relationships between contaminants and benthic species were analyzed by t-SNE using the t-SNE package in R (version 3.5.3). Details of the codes applied in R for the t-SNE approach can be found in the supplementary information, S7.

The concentrations of seven heavy metals, TPH, lindane, and DDT in sediments from four locations in HZB in the YRD, China, sampled between 2003 and 2015 are presented in S8. The nutrient levels (N and P) and characteristics of the sediments are also listed. Every sample measurement was performed in triplicate, and is reported as the mean value because of the governmental monitoring station data policy. In 2003 and 2005, chromium, DDT, lindane, N, P, and sediment types were not yet

Fig. 2. Copper, Chromium, Arsenic, Cadmium, Lead and Mercury concentrations in sediments at JX, NB, SH, ZS locations in Hangzhou Bay, from 2003 to 2015 suggested in the more milligram per kilogram per dry weight(in milligram per kilogram dry weight). The dotted lines indicate the Chinese sediment quality guideline (CN-SQG) and the lowest effect level for ecotoxicological effects (LEL).
measured. The results (S8) also present the Chinese SQG (CN-SQG) (SEPA, 2002) plus the LEL for ecotoxicological effects (based on (Williamson and Morrisey, 2000).

In general, there was an increasing trend in sediment levels of most heavy metals from 2003 to 2007–2009, followed by a slight decrease. This may be the result of the Water Pollution Prevention and Control Law (CLI.1.2007) that came into force in 2007. This environmental law forced industries to treat their wastewater and should have reduced the levels of metal compounds in the effluents of factories in the upper regions of the YRD. The decrease, however, does not seem to continue much since 2013. It is advisable to continue monitoring the success of the Water Pollution Prevention and Control Law in the sediments.

The organic contaminants analyzed did not exceed, or even come close to the Chinese safe levels, as provided in SEPA, 2002. The highest concentrations found in this research were for lindane (0.003 mg/kg), DDT (0.005 mg/kg), and TPH (17.1 mg/kg), which are 5000, 4, and 100,000 times lower than the CN-SQG, respectively (S8). Therefore, further risk assessments and the discussion is more focussed on heavy metals than organic pollutants.

Fig. 2 shows the levels of seven heavy metals at the four locations over time, and how they relate to the CN-SQG and the LEL. Chromium and copper levels exceed the CN-SQG in several samples and chromium, arsenic, and copper exceed the LEL for ecotoxicological effects. Arsenic, copper, and chromium cause several adverse effects to the marine benthic ecosystem, including teratogenicity, oxidative stress, reduced fertility, and genotoxicity. These metals have also been shown to reduce benthic animal species diversity (Baldwin et al., 2003, Ventura-Lima et al., 2011). The levels found are higher than previous studies on healthy marine sediments. For example, mean concentrations of arsenic, chromium, and copper in sediments of European estuaries were approximately 5 mg/kg/dry weight (Wilson and Jeffrey, 1987), while a 2005 study found that in sediment of the Sado Estuary, Portugal, these metals were found at concentrations of 7.41, 1.85, and 26.8 mg/kg/dry weight, respectively (Caeiro et al., 2005). The average levels of arsenic, chromium, and copper in the sediments in this study were 9.5, 48.2, and 26.8 mg/kg/dry weight, respectively, and are regarded as risk compounds in HZB. The concentrations of lead and zinc were just below the LEL, while cadmium and mercury were under the LEL. Previous research has indicated that lead, cadmium, and mercury have reached very high levels in some Chinese marine environments (up to 42.5, 7.15, and 453 mg/kg, respectively), and pose serious ecotoxicological threats (Cheng and Hu, 2010, Liu et al., 2012a, 2012b, Yu et al., 2012, Yan et al., 2016, Zhao, Yao et al. 2018).

In the four sampling locations in this study, these metals are not expected to pose a serious ecotoxicological threat. The sediments from SH contained the highest contaminant levels, while the lowest levels were found in JX. This could be because the SH sampling site is located downstream of the Qiangtang River, and JX is upstream (Fig. 1). Smaller size particulate matter settles at downstream locations, and this contains relatively high contaminant concentrations, as has been shown for metals in the downstream coastal area of Pensacola Beach, Florida, USA (Weis and Weis, 1994), and in China for metals downstream of mines and agricultural activities (Yi et al., 2020).

The ERI indicates the combined ecotoxicological risk of the seven metals included in this study, and is presented in Fig. 3. The calculated ERI for individual compounds can be found in the supplementary information, SI. A moderate ecological risk is indicated when an individual compound has an ERI value >40, or the compounds together have an ERI >150 (Hakanson, 1980). As can be seen in Fig. 3, the seven metals have always posed on ecological risk in SH in 2003–2015.

Fig. 3. Ecological risk index (ERI) of the 7 metals; Arsenic, Chromium (no Cr data available for 2003 and 2005), Copper, Zinc, Lead, Cadmium and Mercury in sediments of the JX, NB, SH, ZS locations from 2003 to 2015. An ERI higher than 150 indicates a moderate ecological risk posed by the 7 metals, an ERI less than 150 is considered a low risk. It is important to note that several other toxic compounds could also contribute to the ecological risk.
Unexpectedly, in the sediments of NB and ZS, the ERI increased and reached a moderate risk level in the last few years, from 2013 to 2015. Arsenic, cadmium, and mercury were mostly responsible for the height of the stacked bar charts, thus contributed most to the ecological risk. In the field, the total ecological risk is expected to be significantly greater than the calculated ERI, because only a fraction of the metals and organic toxic compounds present are represented by the seven measured metals. For example, nickel was not included in the monitoring program and has been regularly found in Chinese marine sediments, and is known to cause ecological risks (Zhang et al., 2007, Tao et al., 2015). Furthermore, the possibility that the multiple toxic compounds present may have synergistic effects cannot be excluded, thus enhancing the toxicity more than the ERI indicates (Vu et al., 2017).

The different ecotoxicological risk assessments all clearly indicate that the metals present in the sediments pose ecotoxicological risks to the benthic ecosystem in HZB. The monitoring program also recorded the benthic species present at the sampled locations; therefore, the extent to which the local benthic species composition could be related to the toxic exposures from the metals was determined. Details about the benthic animal species recorded can be found in the supplementary info (S2-S6). Fig. 4 shows the development in benthic species composition over time. The data are presented as numbers of species per benthic animal group, divided into five groups: Pisces, Crustacea, Mollusca, Echinodermata, and Polychaeta. Overall, half of the benthic macrofauna species disappeared in the last decade, and the number in 2003 was probably sub-optimal for the potential richness of these benthic ecosystems because the metal and organic pollutant levels had already reached toxic levels before 2003. This is termed a shifting, or sliding, baseline. In 1994 the average cadmium, lead, and copper concentrations in HZB were as high as 0.7 ± 1.51, 33.3 ± 20, and 31.8 ± 18.7 mg/kg dry weight, respectively (Che et al., 2003); similar, or higher than the concentrations in this study. Pisces, Echinodermata, and Polychaeta were the most affected groups in 2003–2015. At SH, a strong decrease in number of benthic animal species can be seen from 2003 to 2015, and no benthic Pisces were caught after 2009. Also, at NB and ZS, the number of benthic species decreased, and at JX it was already very low in 2003 and this did not change. In 2003, the SH sample contained four types of Polychaeta and six types of Pisces, but in 2015 there was only one type of Polychaeta and no Pisces were found. Also Echinodermata, which was present up to 2007, had completely disappeared from 2009 onwards. In JX no Echinoderms (e.g., star fish, sea urchins, and sea cucumbers) were found in any of the sampling years, which is very extreme for such an abundant phylum. Also significant is the total absence of Molluscs species (e.g., shellfish and snails) in SH, in all samples except 2003 and 2005. The disappearance of Pisces species could be a direct consequence of the presence of toxic compounds in their food chain or from overfishing (Yue et al., 2017), or an indirect effect of the loss of food species, such as Polychaetes and Molluscs. Benthic Pisces, such as Cynoglossus sp. and Odontamblyopus sp. are sensitive to metals in sediment (Ip et al., 2005, Rad, 2016), and were only observed in 2003–2007 at SH and ZS, but were absent during later monitoring. Interestingly, Crustacea, such as the swimming crab (Portunus trituberculatus), shrimps (Portunus palaemon), and prawns (Portunus opalaeon) remained the most abundant group in HZB. This could be because many Crustacea species are tolerant to metals (Jones, 1975) and can accumulate metals in their body without dying (Canli and Furness, 1993; Marsden and Rainbow, 2004). This makes them potential vectors for transferring these toxic compounds into the natural and human food chains.

Other environmental factors can affect benthic communities, such as nutrients, organic pollutants, and the physical properties of sediment. Therefore, all 15 available environmental parameters were analyzed, in combination with the benthic community data, following the t-SNE approach. The parameters include the seven metals, three organic contaminants, organic carbon, N, and P (supplementary information S8). Unfortunately the available dataset was not complete or detailed enough to include sediment particle size. Fig. 5 shows the results for all four sampling locations for all years (except for chromium, lindane, and DDT in 2003 and 2005, and N and P in 2003). The closer the distance in the...
graph, the stronger the correlation between those parameters. Foremost, arsenic and TPH mostly correlated with the number of benthic animal species. Additionally, patterns of combinations of compounds can be observed: mercury and cadmium are strongly associated with organic carbon, and zinc, chromium, and nitrogen are closely associated. These relationships are important, as previous studies have shown that stronger relationships between compounds in aquatic systems may indicate a common source and similar transport behaviour (Suresh et al., 2011, Singh et al., 2017).

The correlation between arsenic and the benthic community is in accordance with the presented ecological risk assessment of metals in HZB. In addition to heavy metals, levels of TPH and other organic contaminants in marine sediments have been shown to be of significant concern for benthic ecosystem safety (Dos Santos et al., 2018, Zoppini et al., 2018). TPH consists of alkanes, olefin, arenes, heterocyclic benzenoids, and many other organic compounds (Li et al., 2019). Based in the current dataset, it cannot be determined which compounds are responsible for the correlation with the benthic community. Although the measured individual organic contaminants in the field are well below SQGs, they only represent a fraction of the organic toxic contaminants present. In the future, a more comprehensive study of organic contaminants is necessary. It is difficult to measure all organic contaminants presents, not only because of the lack of suitable methods and analytical standards, but also because of the costs involved. In vivo and in vitro bioassays are available as a good first step to help analyze marine sediments for the presence of serious levels of organic contaminants. This can guide prioritisation for chemical analyses, and also complement these measures by evaluating the potential impacts on benthic macrofauna. For example, a suite of in vitro assays can be applied to quantify the toxic potency of dioxin-like compounds and PAHs (Murk et al., 1996), efflux pump inhibitors (Georgantzopoulou et al., 2014), or hormone disruptors (Montaño et al., 2013). In vivo bioassays have been successfully used with sea urchins (Echinodermata) and Pisces early life stages to assess whole sediment toxicity or specific organic extracts (Anselmo et al., 2011), and combinations (Murk et al., 1996, Georgantzopoulou et al., 2014, Schipper et al., 2008, Schipper, 2009).

Our research shows that crustacea are the dominant surviving species in the benthic ecosystem of HZB nowadays, such as swimming crab (Portunus trituberculatus), shrimps (Palaemin) and prawns (Exopalaemon). Those benthic species also are common commercial seafood species in the HZB region. In 2016, 550,000 tons of P. trituberculatus were caught in HZB, and ZS is one of the most important regions for swimming crab fisheries (FAO, 2018). Hence, in 2016 we collected swimming crabs and sediment at 4 additional sampling locations at ZS and analyzed metal levels. Fig. 6 shows copper, chromium, arsenic and cadmium concentrations in swimming crab and sediment for these additional sampling locations. As can be seen, copper and especially cadmium clearly bioaccumulate, and cadmium even bioconcentrated between 5 and 17 times in crab compared to the sediment. Strong bioaccumulation of cadmium in aquatic organisms, especially in marine crab species, has been reported before (McPherson and Brown, 2001, Angeletti et al., 2014). This means there is a serious risk that also humans get exposed to cadmium through seafood, and this compound can potentially induce cancer and affect skeletal, urinary, reproductive and other systems in the body (Bernhoft, 2013). Based on our results, with only a small sample size of crabs but further seafood risk assessment in Crustacea from HZB is strongly advised.

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This research illustrates that a moderate benthic ecotoxicological risk existed, and still exists in HZB. This is based on several risk assessment approaches for the seven metals and organic compounds, analyzed in combination with a benthic species composition study.
Specifically, polychaetes and Pisces are the most affected groups, while Crustacea species seem to be quite resistant and merely accumulate the metals. Arsenic poses the main benthic ecological risk, followed by chromium, copper, and mercury. Cadmium did not appear to pose an ecological risk, but potentially poses a seafood safety risk to human consumers of Crustacea. Only a few organic contaminants were analyzed, and these could directly be related to risks in the field. However, the t-SNE analysis related organic contaminants with decreasing benthic species numbers. Although the Chinese Water Pollution Prevention and Control Law of 2007 seems to have slightly decreased the contaminant levels in sediments, in 2015, many contaminants still exceeded safety levels and reductions seem to be limited. No increasing trend of benthic species was found. To enable proper pollution studies in China: a review. Environ. Pollut. 158 (5), 1134–1146.

Declaration of competing interest

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

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Appendix A. Supplementary data

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Fig. 6. Copper, Chromium, Arsenic, Cadmium concentrations in sediments and swimming crab (Portunus trituberculatus) in four additional sampling locations in ZS, Hangzhou bay, sampled in 2016 (in mg/kg dry weight).
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