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Evaluation of ecotoxicological effects associated with coastal sediments of the Yellow Sea large marine ecosystem using the marine copepod *Tigriopus japonicus*

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ABSTRACT

A copepod bioassay with *Tigriopus japonicus* was applied to evaluate the relative ecotoxicity of sediments in the Yellow and Bohai seas, and contributions of individual PAHs to copepod toxicity were evaluated. Mean toxicity was greatest in the Yellow Sea of China, followed by the Bohai Sea and Yellow Sea of Korea. Elevated concentrations of sedimentary PAHs, alkylphenols, and styrene oligomers back-supported the significant toxicities observed in bioassay. Copepod toxicity in relation to PAHs indicated the greatest contribution by indeno[1,2,3-*c*,*d*]pyrene. However, lacked contribution by PAHs, viz., 2.4 and 3.0 % for the total immobilization and mortality, respectively, indicated a large proportion of unknown toxicants being widely distributed along the Yellow Sea Large Marine Ecosystem (YSLME) coastline. Overall, the present study provides useful baseline information for evaluating the potential sedimentary toxicants, with emphasizing further investigation to identify the unknown toxicants at an LME scale, and elsewhere.

1. Introduction

Industrialization and urbanization in the coastal areas of South Korea and China adjoining the Yellow and Bohai seas are rapidly growing (Meng et al., 2017; Khim et al., 2018a; Kim et al., 2020). Many contaminants have been introduced into the Yellow and Bohai seas through >100 large and small rivers, including the Han, Yeongsan, and Geum rivers in South Korea and the Yangtze, Yellow, Liao, and Jiahe rivers in China (Wang et al., 2015; Jeon et al., 2017). Various sources of pollution (e.g., domestic sewage and industrial wastewater) exist around these rivers, generating various environmental concerns (Xiao et al., 2017; Shi et al., 2021). Since the late 2000s, many studies have performed various coastal pollution assessments in the Yellow and Bohai seas, documenting the contamination status of sediment, soil, seawater, and freshwater (Hong et al., 2012; Khim et al., 2018a, 2018b; Tian et al., 2020; Yoon et al., 2020; Shi et al., 2021). These studies focused on the distribution and potential sources of persistent toxic substances (PTSs) and metals to diagnose the input of anthropogenic activities in marine ecosystems and environmental health. However, because of a wide variety of compounds in the sediments, it is difficult to identify the key toxicants through targeted chemical analysis (Hu et al., 2015).

While contaminants in sediment can be analyzed with various instruments to measure the concentration of target chemicals, chemical analysis cannot determine biological potencies and interactions among individual compounds (Hong et al., 2016). In particular, when concentrations are below sediment quality guidelines or the limits of detection, these chemicals can act independently or interact with agonists or antagonists, impacting the biological potency of certain mixtures of contaminants in the marine environment (Cha et al., 2019; Kim et al., 2019). Many studies have reported that bioassays can compensate for this limitation (Burton et al., 2002; Manzo et al., 2008). Ecotoxicological risks are caused by the myriad of known and unknown toxic substances

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in the environment; thus, it is necessary to consider the diverse biological effects of organisms with different trophic levels. Previous sediment toxicity studies in the Yellow and Bohai seas have generally utilized in vitro assays using transactivation cells and luminescence bacteria (Table S1 of the Supplementary Materials). In vivo assays using microalgae and crustaceans (e.g., Ostracoda) were performed only for local sites, and could not cover a large scale (Table S1).

The harpacticoid copepod, *Tigriopus japonicus*, is a benthic species that mainly inhabits the intertidal zone of Korea, Japan, and China (Lee et al., 2007). *T. japonicus* is a primary consumer of phytoplankton, and it is preyed on by fish in the marine ecosystem (Ohman and Hirche, 2001; Kwok and Leung, 2005). *T. japonicus* has a short life cycle, can be collected in large quantities in the field, and has the advantage of being easy to culture in the laboratory (Lee and Taga, 1985; Lee, 1991). In addition, the *Tigriopus* genus exhibits relatively high sensitivity to toxicity, with high reproducibility in repeated experiments. Consequently, some countries use it as a test organism to evaluate ecological toxicity in marine environments (Barka et al., 2001; Forget et al., 1998).

To elucidate the mechanisms causing the toxicity of pollutants in copepods, various approaches are used, including gene expression profiling (Hayes and Bradfield, 2005), proteomic and transcriptome (Hong et al., 2017), and aryl hydrocarbon receptors (AhR) (Kim et al., 2015). AhR is activated by dioxin-like chemicals, such as polycyclic aromatic hydrocarbons (PAHs), halogenated aromatic hydrocarbons, and styrene oligomers (SOs) (Hong et al., 2012; Hong et al., 2016).

Here, we used the copepod *T. japonicus* bioassay to evaluate the toxic effects of PTSs in sediments of the Yellow and Bohai seas. The main objectives included: (1) evaluating toxicity in copepods on organic extracts of sediments collected from the Yellow and Bohai seas; (2) determining the relationship between toxicity and chemical data; (3) assessing the toxicity of individual 16 PAHs on copepods; and (4) quantifying the extent to which PAHs contribute toward inducing overall toxicity in sediments; and (5) comparing our data with those of previous studies to clarify the sensitivity of copepod assessment. The results of this study are expected to demonstrate the toxic effects of contaminants on copepods in the Yellow and Bohai seas, providing baseline information on how PTSs contribute to the contamination of the marine ecosystem. To the best of our knowledge, this study is the first to determine sediment ecotoxicity to copepods in the extensive Yellow and Bohai seas.

2. Materials and methods

2.1. Study area and sampling

A previous study reported the concentrations of PTSs in 125 coastal sediments of the Yellow Sea Large Marine Ecosystem (YSLME), which includes the Yellow and Bohai seas (Yoon et al., 2020). In the present study, we selected 25 sites, which had low to high concentrations, to assess the ecotoxicity of PTSs in both the Yellow and Bohai seas. The 25

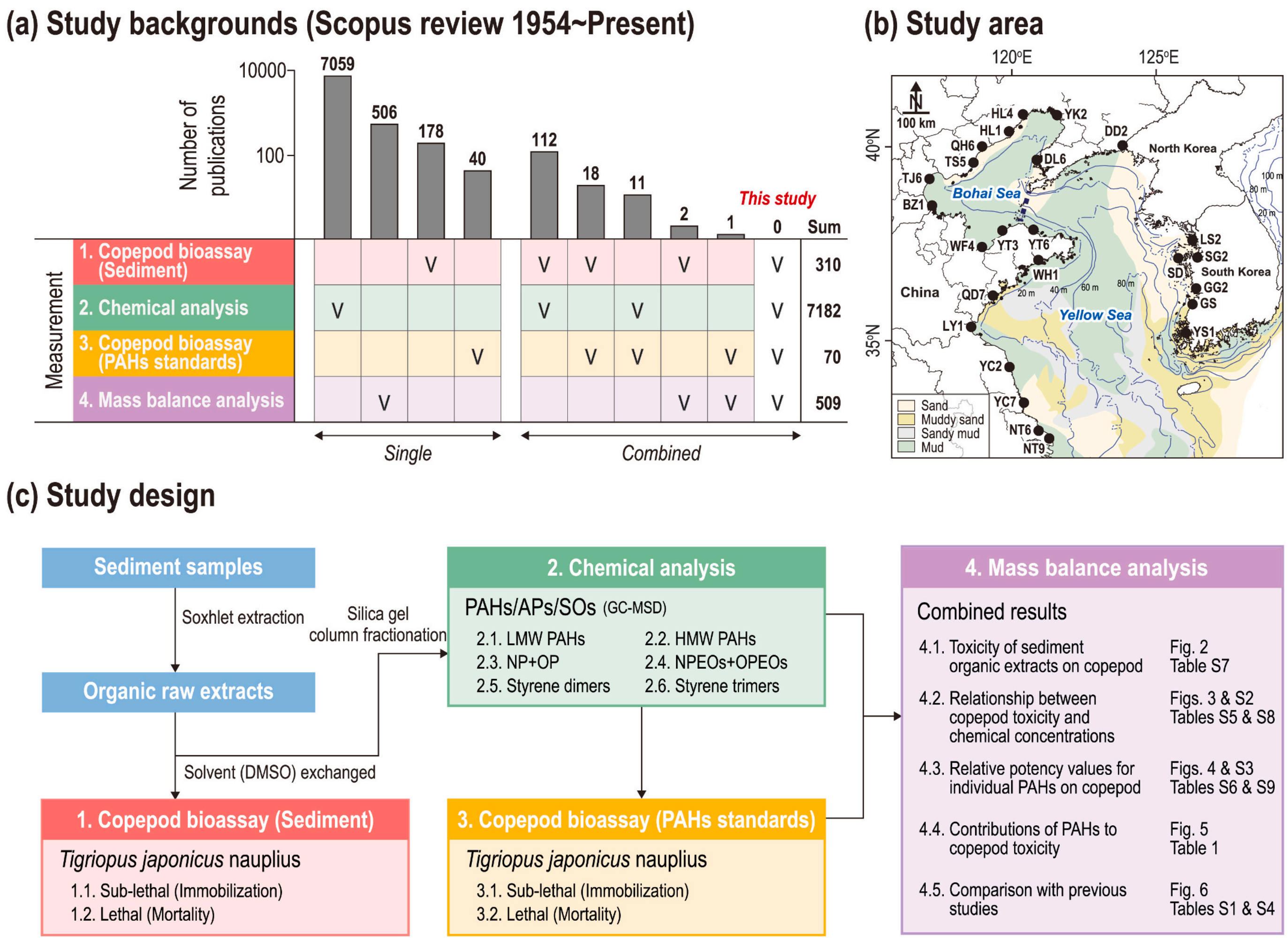


Fig. 1. (a) Study backgrounds (Scopus review 1954–present), (b) study area, and (c) study design for bioassay, chemical analyses, and potency balance analysis used to identify toxicity contributions of polycyclic aromatic hydrocarbons in the sediments from the Yellow and Bohai seas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sites included several metropolitan cities in Korea (Seoul, Incheon, Asan, Gunsan, and Mokpo) and China (Beijing, Tianjin, Dalian, Huludao, Qinhuangdao, Weifang, Yantai, Qingdao, and Nantong). The sites encompassed freshwater ($n = 2$), brackish water ($n = 13$), and seawater ($n = 10$) areas. Details on the sampling area are provided in Table S2, including geographic location, habitat, and land-use type (Yoon et al., 2020). Land-use types near the sites were classified, including agriculture, aquaculture, barren, beach, industry, and municipality.

In June–July 2018, the top 2 cm of sediments were collected from the coasts of the Yellow and Bohai seas (Fig. 1). The collected samples were placed in a glass bottle and stored in an icebox, transported to the laboratory, and stored at −20 °C until analysis (Yoon et al., 2020).

2.2. Sample preparation

Details on sample preparation procedures for the bioassay are provided in previous studies (Hong et al., 2016; Lee et al., 2017). Briefly, the sediments were freeze-dried, passed through a 1-mm sieve, and homogenized. Approximately 10 g of homogenized sediment was placed in a glass thimble and extracted on a Soxhlet extractor with 300 mL dichloromethane (DCM, Burdick & Jackson, Muskegon, MI) for 16 h. An aliquot of the organic extract was used to assess the toxicity of PTSs in sediments. The solvent of organic extracts for bioassay was exchanged for dimethyl sulfoxide (DMSO, Sigma Aldrich, Saint Louis, MO). The organic extracts of sediments in DMSO were diluted to concentrations equivalent to 0.001, 0.005, 0.01, 0.05, and 0.1 % DMSO v/v (0.1, 0.5, 1, 5, 10 g sediment equivalent L^{-1}). The maximum DMSO concentration was set to 0.1 %, at which background DMSO toxicity was negligible (Li et al., 2014).

Sixteen PAH standards were obtained from Sigma-Aldrich, including naphthalene (Na), acenaphthene (Ace), acenaphthylene (Acl), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fl), pyrene (Py), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benz[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DahA), benzo[*g,h,i*]perylene (BghiP), and indeno[1,2,3-*c,d*]pyrene (IcdP). The solvent of 16 PAH standards for bioassay was exchanged to DMSO. Exposure concentrations for PAH standards were described in Table S3.

2.3. Culture of *Tigriopus japonicus*

The benthic copepod *T. japonicus* was reared in the Laboratory of Marine Benthic Ecology at Seoul National University (Seoul, Republic of Korea). The copepods were initially donated by EH R&C Inc. (Incheon, Republic of Korea). *T. japonicus* was cultured using 30 psu filtered (GF/F, Whatman, Kent, UK) seawater at 25 ± 1 °C under a 12:12-h light and dark cycle. Copepods were fed with the haptophyte *Isochrysis galbana* (Table S3). Only *T. japonicus* of nauplius stage from 1 to 3 (N1–N3 nauplius stages) were used in the exposure experiments on sediment extracts and PAH standards.

2.4. *Tigriopus japonicus* bioassay

Organic extracts of sediment and PAH standards were exposed to *T. japonicus* to measure its immobilization and mortality. The *T. japonicus* bioassay was performed following a previous method (Lee et al., 2008; Table S3). In brief, copepods were collected by sieving them through a 0.3–0.5-mm mesh. The nauplius was collected using a glass pipette and stereomicroscope (Olympus model SZX16, Olympus, Tokyo, Japan), and was then transferred to 6-well plates. Five concentrations were used for each organic extract and PAH standards, and blank (natural seawater) was also tested. There were three replicates for each concentration and ten animals in each well. Tests were conducted in 4 mL solution in each well. Tests were conducted at 25 ± 1 °C under a 12:12-h light and dark cycle. Test duration and age of test organisms for bioassay were determined as more sensitive conditions in the toxicity test using BaP and DbahA (Fig. S1). After 96 h, the number of dead and immobile nauplii were counted under a stereomicroscope. The number of live nauplii, immobile nauplii (nauplii that did not shift their barycenter but moved their appendages), and dead nauplii (nauplii that did not swim nor move any appendages for 10 s of observation were deemed dead) was counted (Moeris et al., 2021). Mortality is the most common endpoint (Table S4), which might be useful in comparison with previous studies. Sánchez-Bayo and Goka (2006) reported that the immobilization of copepods could put them at serious risk because they could make them vulnerable to predator attack and could cause difficulties in feeding. Thus, these two endpoints (i.e., mortality and immobilization) were chosen in this study. Effective concentration (EC50) and lethal concentration (LC50) were defined as sample concentrations that cause immobilization and mortality in 50 % of exposed copepods, respectively, and were calculated through probit analysis. The measured toxicity of the organic extracts of sediment and PAH standards was expressed as the toxic unit (TU) (Manzo et al., 2008) (Eq. (1)).

$$\text{Toxic unit (TU)} = \frac{100}{\text{EC50 or LC50 of sample extracts}} \tag{1}$$

A previous study reported the concentrations of PTSs (PAHs, SOs, and alkylphenols (APs)) in sediments at the same 25 stations in the Yellow and Bohai seas (Yoon et al., 2020). Concentrations of PTSs are presented in Table S5 and were compared with the observed toxicities in the present study. Low molecular weight (LMW) PAHs were defined as PAHs having 2 to 4 benzene rings, and high molecular weight (HMW) PAHs were defined as PAHs having 5 to 6 benzene rings. APs are included 4-tert-octylphenol (t-OP), 4-tert-octylphenol monoethoxylate (t-OP1EO), 4-tert-octylphenol diethoxylate (t-OP2EO), nonylphenols (NPs), nonylphenol-monoethoxylate (NP1EO), and nonylphenol diethoxylate (NP2EO). SOs include styrene dimers and trimers, such as 1,3-diphenylpropane, *cis*-1,2-diphenylcyclobutane, 2,4-diphenyl-1-butene, and *trans*-1,2-diphenylcyclobutane (SD4), 2,4,6-triphenyl-1-hexene, 1e-phenyl-4e-(1-phenylethyl)-tetralin, 1a-phenyl-4e-(1-phenylethyl)-tetralin, 1a-phenyl-4a-(1-phenylethyl)-tetralin, 1e-phenyl-4a-(1-phenylethyl)-tetralin, and 1,3,5-triphenylcyclohexane.

2.5. Potency balance analysis

To determine the contribution of PAHs to toxicity in *T. japonicus*, the relative toxic potencies of tested samples and potency balance analysis were implemented based on a previous study, with minor modifications (Hu et al., 2015). BaP was selected as the positive standard to quantify the toxic potency of tested samples in the present study. Bioassay-derived toxicity equivalents of sediment were calculated relative to BaP (BEQ) by dividing the concentration of BaP that caused 50 % immobilization or mortality of *T. japonicus* by the volume of sampled extracts that produced equivalent immobilization and mortality (50 %) of *T. japonicus* (Eq. (2)).

$$\text{BEQ} = \frac{\text{EC50 or LC50 of BaP}}{\text{EC50 or LC50 of sample extracts}} \tag{2}$$

The relative potency values (RePs) of individual compounds were calculated by dividing the concentration of BaP that produced 50 % immobilization or mortality of *T. japonicus* by the concentrations of individual compounds that caused 50 % immobilization or mortality (Eq. (3)) (Hu et al., 2015). EC50s or LC50s for BaP and individual compounds to *T. japonicus* are listed in Table S6.

$$\text{REP}_i = \frac{\text{EC50 or LC50 of BaP}}{\text{EC50 or LC50 of compound}_i} \tag{3}$$

Calculated toxicity equivalents of individual compounds (CEQ) were calculated as the sum of their measured concentrations for individual compounds in samples multiplied by their respective ReP values. Total CEQs (ΣCEQs) of tested samples were the sum of CEQs of all individual compounds (Eq. (4)).

$$\sum CEQ = \sum_{i=1}^{n} ReP_i \times \text{concentrations of individual compound}_i \qquad (4)$$

To determine whether the BEQs of the tested samples were accounted for, the potency balance analysis was conducted to compare BEQs and CEQs. Relative contributions of individual PAHs to the total equivalents determined in the bioassay in each sample were determined by the ratio of CEQs for individual PAHs and BEQs of the tested sample.

2.6. *Data analysis and statistics*

EC50 and LC50 of organic extracts and PAH standards were calculated using SPSS 24.0 (SPSS Inc., Chicago, IL). Significant differences in toxicity with respect to region, habitat, and land-use type were identified by the Kruskal-Wallis test and Mann-Whitney test using SPSS 24.0 (SPSS Inc.). The relationships between toxicity and concentrations of PTSs were determined using Pearson correlation analysis in SPSS 24.0 (SPSS Inc.). Principal components analysis (PCA) was performed to interpret and visualize the correlation analysis results using PRIMER v6 software (PRIMER-E Ltd., Plymouth, UK).

3. Results and discussion

3.1. *Toxicity of sediment organic extracts*

The mean toxicity of copepods with respect to spatial distribution, habitat, and land-use type showed no significant difference ($p > 0.05$; Fig. 2; Table S7). Mean copepod toxicity was the greatest in the Yellow Sea of China (YSC), followed by the Bohai Sea (BS) and Yellow Sea of Korea (YSK) (Fig. 2a; Table S7). The great toxicity in the YSC was attributed to there being several large and economically prosperous port cities with millions of inhabitants along with the Yangtze, Huaihe, Liaohe, and Yalu River systems (Tian et al., 2020). The sites NT6 ($TU_{immobilization} = 30.3$; $TU_{mortality} = 20.4$) and NT9 ($TU_{immobilization} = 21.3$; $TU_{mortality} = 18.9$) had the greatest toxicity in the YSC. NT6 and NT9 are located in Nantong City, which has a great number of industries, including chemical, electricity, manufacturing, and textiles (Zhang et al., 2011), with great concentrations of PAHs and metals in this area (Wang et al., 2014; Liu et al., 2016). The greater toxicity in this area seems to be related to the large inputs of land-derived PTSs (Han et al., 2017; Yoon et al., 2020). HL4 had the greatest toxicity of all sampling sites, and is a brackish and industrial area in BS ($TU_{immobilization} = 43.5$, $TU_{mortality} = 37.0$). HL4 is located in Huludao City, which flows into the Wuli River and is assumed to be toxic due to direct pollution from the surrounding environment. In HL4, there are various industrial activities, including a Zn mine with metal contamination (Wang et al., 2012; Zhu et al., 2020), petrochemical, ship-building, and power plants (Zheng et al., 2007). YSK had the lowest average toxicity compared to China (YSC and BS); however, a hotspot (LS2) was found. Although the water quality of Lake Sihwa has improved somewhat compared to the past, unknown PTSs that induce toxic effects on in vitro assays remain (Jeon et al., 2017; Lee et al., 2017). The continuous monitoring and management of unknown PTSs are necessary for this area.

Our results showed that the toxicity of copepods was greater in brackish areas compared to seawater and freshwater areas (Fig. 2b). Although *T. japonicus* has an optimal salinity (range 27.1–34.3 psu) for survival (Lee and Hu, 1981), it generally inhabits brackish areas, and salinity in seawater areas may act as a stressor (Kwok and Leung, 2005). Nevertheless, the greater toxicity of copepods in brackish areas might be because of their closer proximity to the inputs of land-driven contaminants, not the effects of salinity (Hwang et al., 2021). With respect to land-use type, the mean copepod toxicities were ordered as follows: industry > barren > beach > aquaculture > municipality > agriculture (Fig. 2b). Industrial areas had the greatest copepod toxicity due to their being located in hotspots of toxicity (e.g., HL4 and LS2 located in BS and YSK, respectively). Great concentrations of PAHs have been detected in sediments of Huludao in recent years, with the source has been

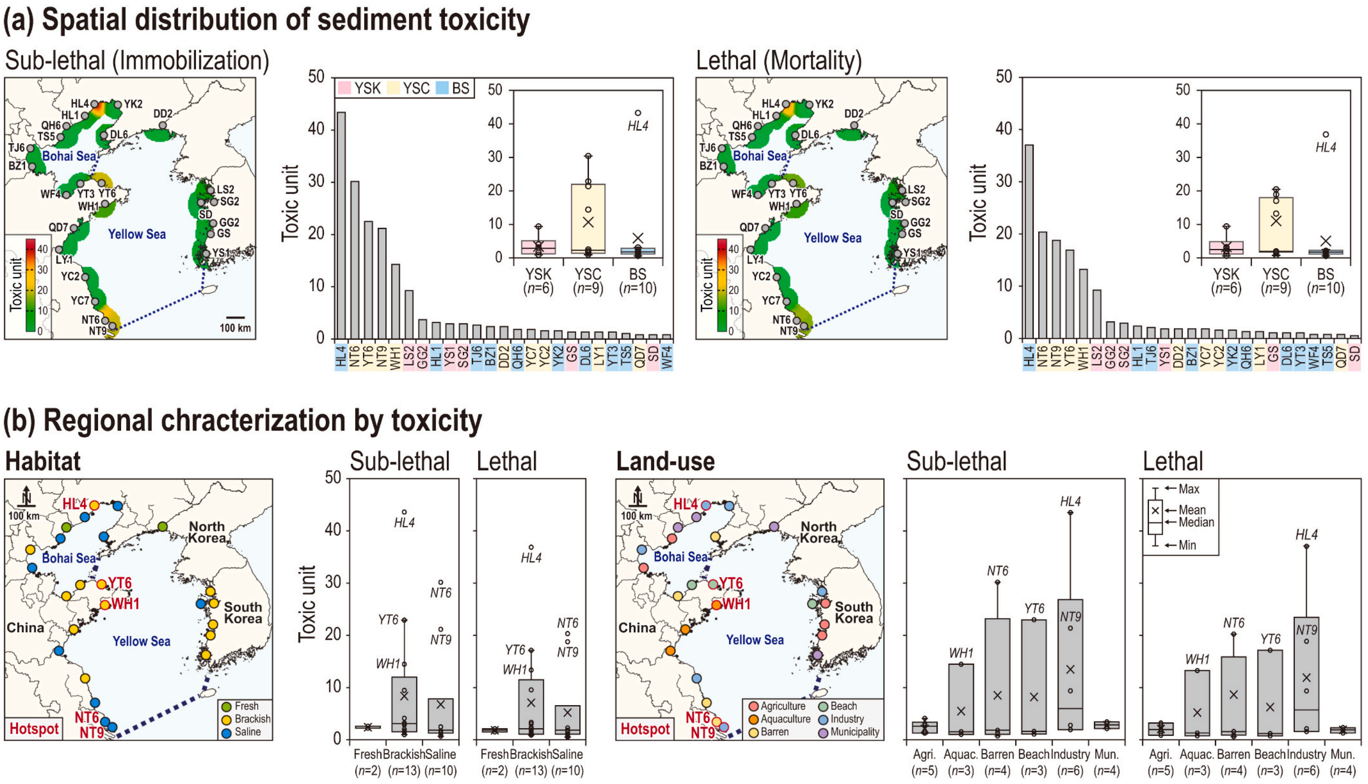


Fig. 2. (a) Spatial distribution of toxic units (immobilization and mortality) for *Tigriopus japonicus* in the organic extracts of sediments in the Yellow and Bohai seas and (b) toxicity characterization with respect to habitat and land-use type. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

identified as the input of PTSs produced by various industrial activities (Yoon et al., 2020). Lake Sihwa has also been reported as an area affected by industrial waste, including the combustion of heavy-duty gasoline engines and diesel engines in nearby cities (Lee et al., 2017). Thus, industrial activities represent a major source of PTSs in HL4 and LS2.

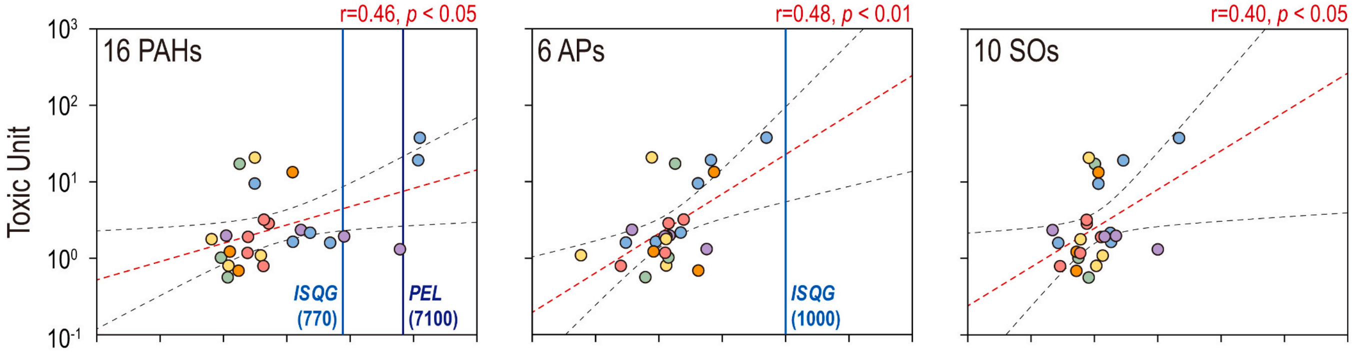
3.2. Relationship between copepod toxicity and chemical concentrations

Between copepod toxicity and the measured concentrations of PTSs in the sediments showed a statistically significant relationships (immobilization: PAHs, r = 0.46, $p < 0.05$; APs, r = 0.49, $p < 0.01$; SOs, r = 0.41, $p < 0.05$; mortality: PAHs, r = 0.46, $p < 0.05$; APs, r = 0.48, $p < 0.01$; SOs, r = 0.40, $p < 0.05$) (Figs. 3; S2; Table S8). Not surprisingly, concentrations of PAHs, APs, and SOs were also greatest at HL4, which exhibited the greatest toxicity. The concentrations of PAHs in HL4 exceeded the thresholds of various sediment quality guidelines (SQGs), including the Canadian interim sediment quality guideline (ISQG; 770 ng g^{-1} dw) (Canadian Council of Ministers of the Environment (CCME), 2001, 2002) and USA probable effect level (PEL; 7100 ng g^{-1} dw) (Solberg et al., 2003). Among the PAHs examined, immobilization and mortality were both significantly and positively correlated to concentrations of Phe, Ant, Fl, Py, BaA, Chr, BbF, and BghiP ($p < 0.05$) (Table S8). In previous studies, the effects of these chemicals on other copepod species had been assessed. For example, in the copepod *Oithona davisae*, Phe has been reported to cause immobilization and mortality, and Fl and Py influence mortality (Barata et al., 2005). In addition, it has been reported that Phe in sediments affects specific reproductive endpoints, such as total offspring (nauplii) and clutch size of the copepod *Amphiascus tenuiremis* (Bejarano et al., 2004).

Among the APs, immobilization was significantly and positively correlated to the concentrations of t-OP, t-OP1EO, t-OP2EO, NP, and NP2EO ($p < 0.05$), while mortality was significantly and positively correlated to concentrations of t-OP1EO, t-OP2EO, NP, and NP2EO ($p < 0.05$) (Table S8). When t-OP, an endocrine disruptor, is exposed to the copepod *T. japonicus*, the growth rate of nauplii and copepods is inhibited, with the onset of incubation (Bang et al., 2008), and growth being delayed (Marcial et al., 2003). The present study showed no

(a) Land use type (Photo at selected locations)

(b) Relationship between PTSs and TU



(c) Relationship between individual chemicals and TU

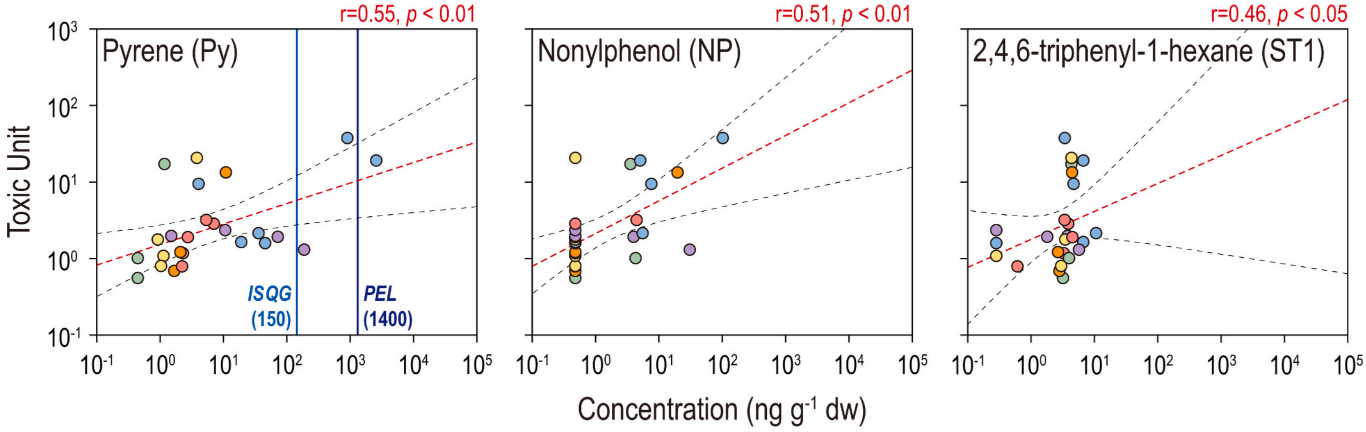


Fig. 3. (a) Field images of land use types in study area. Spearman rank correlation between the concentrations of (b) total chemicals (16 PAHs, 6 APs, and 10 SOs) and (c) individual compounds (Py, NP, and ST1) in the sediments and toxic unit (mortality) for organic extracts. A concentration below the limit of detection was replaced with a value of half of the limit of detection before statistical analyses were performed.

significant correlation between NP1EO concentration and copepod toxicity ($p > 0.05$) (Table S8). Although a previous study with similar results showed that NP1EO did not affect the fecundity, sex ratio, and survival rate of the copepod *Eurytemora affinis*, a direct comparison was difficult with *T. japonicus* due to different life cycles and characteristics (Marcial et al., 2003). Among the examined SOs, immobilization and mortality were both significantly and positively correlated to the concentrations of SD3 and ST1 ($p < 0.05$) (Table S8). However, to the best of our knowledge, no studies have confirmed that SOs affect copepods, with such studies being required in the future to provide more relevant data for the management of these compounds and their risks in sediments.

Overall, results showed that PAHs, APs, and SOs in sediments could affect copepod toxicity. However, it was difficult to identify the effect of individual chemicals on copepod toxicity. The concentrations of only PAHs exceeded the Canadian ISQG (770 ng g^{-1} dw) at 4 out of 25 locations and the USA PEL (7100 ng g^{-1} dw) at 2 out of 25 locations; thus, potency balance was conducted to assess the toxic effects of individual PAHs that were expected to have contributed to total induced copepod toxicity in sediments significantly.

3.3. *Relative potency values for PAHs on copepod*

To determine the contribution of individual PAHs to total induced copepod toxicity in sediments, EC50, LC50, and ReP of 16 PAHs were determined (Figs. 4; S3; Table S6). IcdP, BghiP, BbF, BkF, Ace, Py, and Na had greater ReP values for mortality than BaP. Regarding mortality and immobilization, IcdP had the greatest toxicity (ReP = 9.8 and 9.9, respectively), followed by BghiP (ReP = 6.3 and 5.7, respectively). The mean ReP values for mortality and immobilization to HMW PAHs were 3.3 and 3.4, respectively. For LMW PAHs, the toxicity for mortality and immobilization were both 1.3. This result showed that the copepod toxicity to HMW PAHs was greater than that for LMW PAHs. However, only a few studies have reported the LC50 or EC50 of copepod toxicity for HMW PAHs compared to LMW PAHs. To the best of our knowledge, the present study is the first to confirm the ReP values of 16 individual PAHs for copepod toxicity.

The copepod toxicity results for the 16 PAHs showed that there was a positive correlation between the number of benzene rings and toxicity (TU) ($p < 0.05$) (Fig. 4c). In general, PAHs with a larger number of benzene rings have a higher octanol-water partitioning coefficient (K_{ow}). K_{ow} indicates the tendency of organic compounds to adsorb to sediment and/or living organisms and is generally expressed as log K_{ow}. Thus, HMW PAHs with high log K_{ow} values are more resistant to oxidation, reduction, and evaporation processes, leading to greater persistence in the environment (Edokpayi et al., 2016). Because of these properties, HMW PAHs appear to be more toxic to copepods. Similarly, experiments on algae showed that PAHs with higher log K_{ow} values have greater toxicity (Hutchinson et al., 1979). In addition, it was reported that the toxicity of individual PAHs to 33 species, including fish, amphibians, arthropods, mollusks, and polychaetes, was positively correlated to the log K_{ow} of each compound by use of the quantitative structure-activity relationship (QSAR) (Di Toro et al., 2000).

We compared our results with those of previous studies on other species to evaluate the sensitivity of *T. japonicus* to PAHs over the same exposure time (48 h) (Table S9). Previous studies showed that the LC50 of *O. davisae* adult was 7.19 and 12.29 mg L^{-1} for Na and Py, respectively (Barata et al., 2005). In comparison, our results showed that the LC50 of nauplii *T. japonicus* was 3.18 and 4.56 mg L^{-1} for Na and Py, respectively. The LC50 of *T. japonicus* nauplii was 16.9 mg L^{-1} for Flu, whereas the LC50 for *O. davisae* and *Acartia tonsa* adults was 1.80 mg L^{-1} (Barata et al., 2005) and 0.02 mg L^{-1} (Bellas and Thor, 2007), respectively. *T. japonicus* was relatively sensitive to Na and Py compared to *O. davisae* adult. The ecotoxicity results for the contaminants showed species-specific and compound-specific properties. Consequently, it is important to compile a database on multiple compounds for each species. Thus, it is needed to assess the degree of sediment contamination and toxicity of contaminants with various test species (Giesy and Hoke, 1989; Chapman et al., 2002).

3.4. *Contributions of PAHs to copepod toxicity*

Potency balance between bioassay-derived toxicity equivalents (BEQs) and chemical (instrument)-derived equivalent concentrations (CEQs) was conducted to assess the chemical-specific contribution to total induced copepod toxicity of the organic extracts of sediments (Fig. 5a; Table 1). The mean BEQs for copepod immobilization and mortality in sediments were 226,500 and 207,700 ng g^{-1} dw, respectively. The mean CEQs for copepod immobilization and mortality by PAHs were 4675 and 4630 ng g^{-1} dw, respectively. The mean

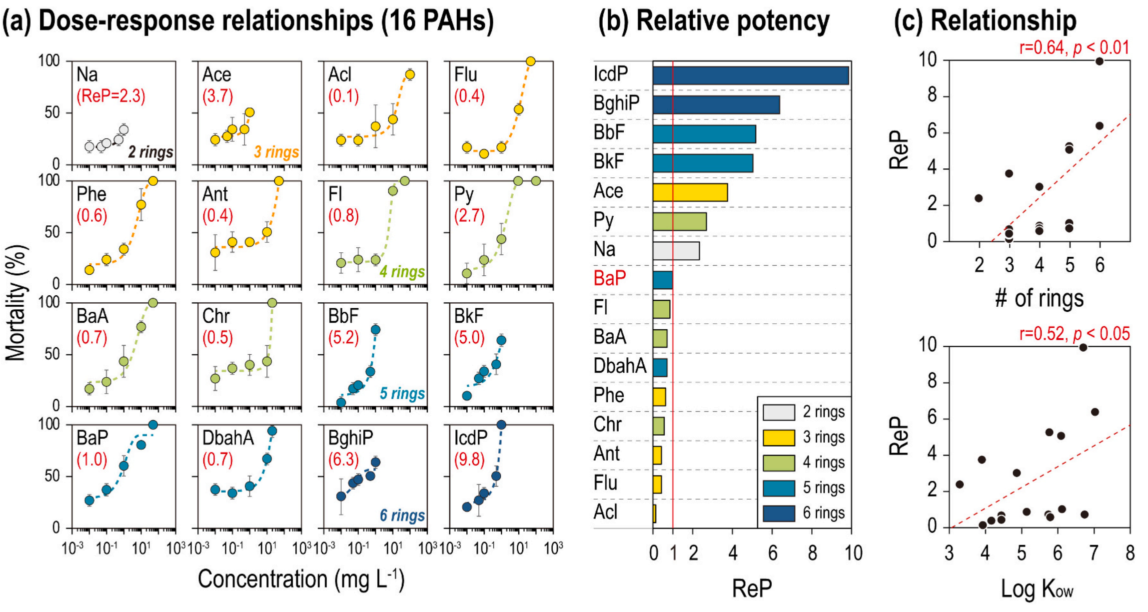


Fig. 4. (a) Dose-response relationships for the mortality of 16 PAHs in the *Tigriopus japonicus* bioassay (Error bar: mean ± SD), (b) relative potency (ReP) values of 16 PAHs for the copepod toxicity, and (c) relationship between the toxic units (mortality) and benzene rings and log K_{ow} of PAHs.

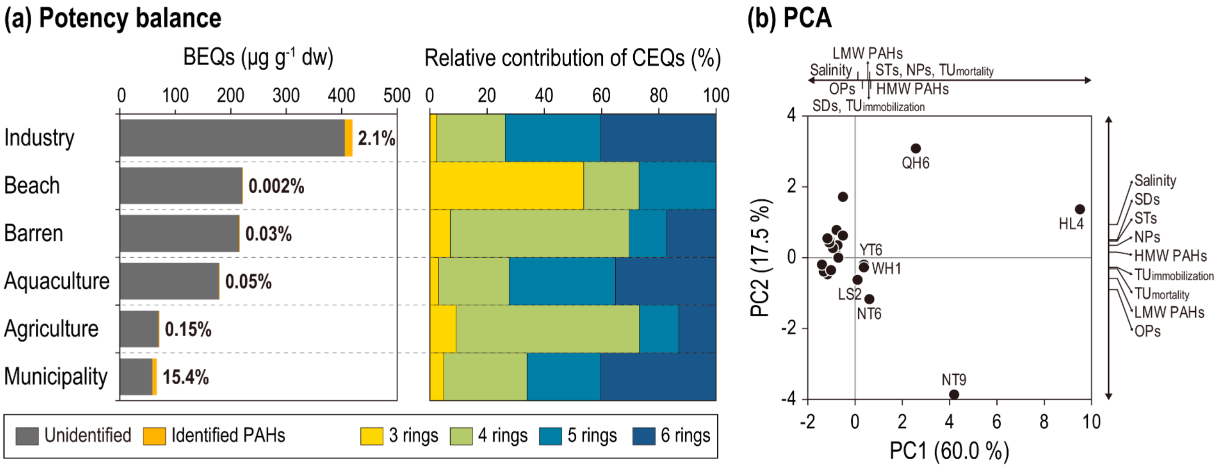


Fig. 5. (a) Identification and relative contribution of copepod toxicity in sediments from the Yellow and Bohai seas. (b) Principal component analysis (PCA) ordination of concentrations of high molecular weight polycyclic aromatic hydrocarbons (HMW PAHs), low molecular weight PAHs (LMW PAHs), nonylphenols (NPs), octylphenols (OPs), styrene dimers (SDs) and styrene trimers (STs), and copepod immobilization and mortality for organic extracts of sediments ($TU_{immobilization}$ and $TU_{mortality}$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Bioassay-derived toxicity equivalents (BEQs) of sediment extracts, calculated toxicity equivalents (CEQs) for PAHs in samples and contributions of total CEQs to their corresponding BEQs.

Site information	Immobilization			Mortality		
	BEQs[a] (ng g^{-1} dw)	CEQs[b] (ng g^{-1} dw)	Contribution (%)	BEQs (ng g^{-1} dw)	CEQs (ng g^{-1} dw)	Contribution (%)
Region						
Yellow Sea-Korea (YSK)	112,900	56	0.1	112,800	53	0.1
Yellow Sea-China (YSC)	341,400	4373	1.1	303,800	4210	1.1
Bohai Sea (BS)	191,400	7718	4.9	178,100	7760	6.5
Habitat						
Freshwater (<5 psu)	69,500	14,400	22.3	57,000	14,490	30.5
Brackish water (5–30 psu)	264,700	3839	0.4	252,100	3850	0.4
Seawater (>30 psu)	208,300	3818	0.9	180,000	3670	0.9
Land use type						
Agriculture	75,600	89	0.1	69,800	85	0.1
Aquaculture	174,000	124	0.1	179,000	119	0.0
Barren	272,300	21	0.0	214,500	19	0.0
Beach	262,300	6	0.0	220,300	6	0.0
Industry	424,700	14,400	2.1	420,100	14,230	2.1
Municipality	84,790	7324	11.3	66,600	7370	15.4
All sites	226,500	4675	2.4	207,700	4630	3.0

[a] BEQs: Bioassay-derived toxicity equivalents calculated relative to BaP.

[b] CEQs: Calculated toxicity equivalents of individual compounds calculated relative to BaP.

contribution of PAHs for total copepod immobilization and mortality in sediments was 2.4 % and 3.0 %, respectively. The contribution of PAHs for overall toxicity was weak (<6 %), except for QH6. 16 PAHs explained 41.1 and 57.1 % of the BEQs for immobilization and mortality, respectively, in site QH6. For QH6, the major contributor of copepod immobilization and mortality was IcdP (45.7 % and 45.2 %, respectively), followed by BbF (21.5 % and 22.2 %) and BghiP (15.2 % and 16.7 %). This result was attributed to great sediment concentration and high ReP. IcdP, BbF, and BghiP are HMW PAHs, which are generated by the combustion of gasoline and diesel in vehicles (Li and Duan, 2015). Considering that QH6 is a municipal area located in Qinhuangdao City, these results indicate that transportation is a major source of PAHs in this area.

We also attempted to identify the major chemicals affecting the sediment toxicity of *T. japonicus* (Fig. 5b). PCA was performed using toxicity data ($TU_{immobilization}$ and $TU_{mortality}$ for organic extracts) from the present study and concentrations of chemicals (HMW PAHs, LMW PAHs, NPs, OPs, SDs, and STs) and environmental parameters (salinity) from the previous study (Yoon et al., 2020). The two principal components, PC1, and PC2, accounted for 60.0 % and 17.5 % of the total variance, respectively. When sites were ordinated based on PC1, they were classified into two groups. The first group included HL4, NT9, and QH6 with greater concentrations of PAHs, while the second group included all other sites. Even within the first group, NT9 and QH6 were classified by the PC2. This result might be due to the composition of PAHs being different, resulting in the high copepod toxicity only shown in NT9. It was confirmed that the ecotoxicological effects were different depending on the ratio of individual PAHs, although the total concentrations of 16 PAHs were great.

3.5. Comparison with previous studies

We compared the data of the current study with a previous study conducted on bacterial toxicity at the same sites (Fig. 6). All data were divided into two groups: (1) group A ($n = 10$), sites with greater copepod toxicity; and (2) group B ($n = 15$), sites with greater bacterial toxicity

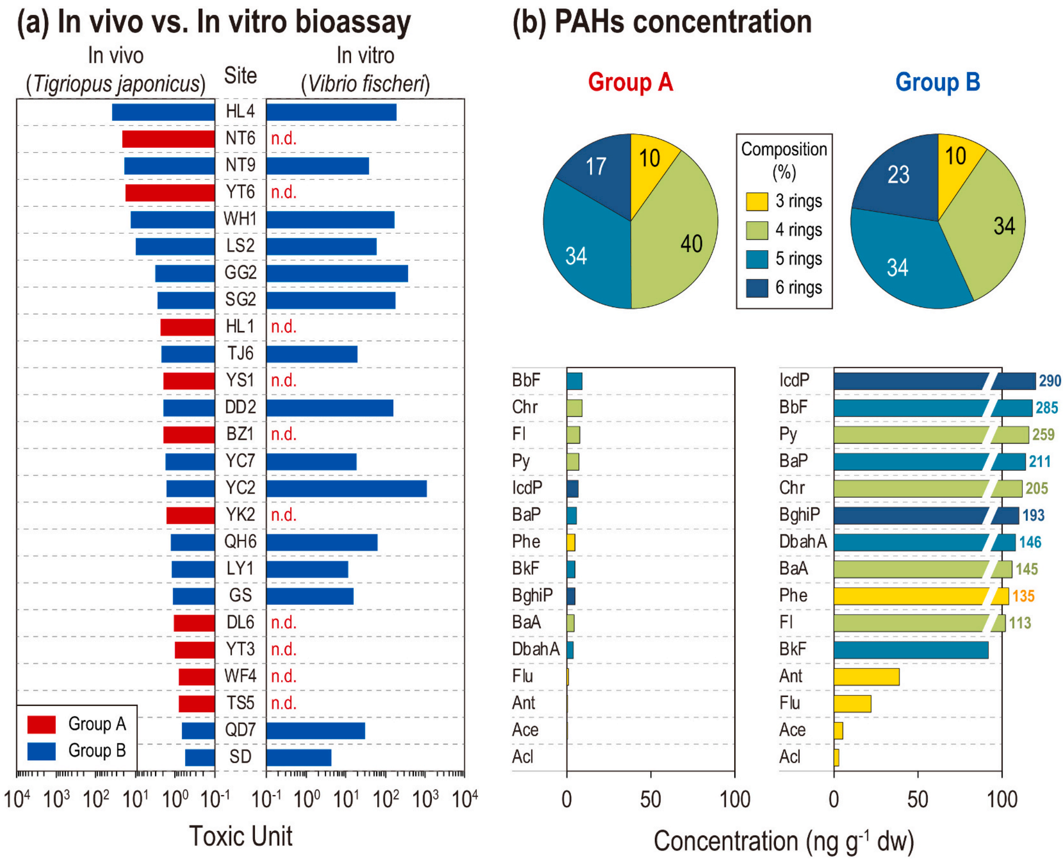


Fig. 6. Comparison of (a) copepod toxicity and bacterial toxicity to the organic extracts of sediments in the Yellow and Bohai seas and (b) PAHs composition within each group (n.d: Not detected). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 6a). At the 25 sites, copepod toxicity ranged from 0.6 to 37 TU (mean 5.8 TU), while bacterial toxicity ranged from 0.1 to 1087 TU (mean 94.9 TU). Interestingly, TU values were greater in bacterial toxicity, but none of the toxicity was detected in group A sites. The composition of both groups based on the number of benzene rings was ordered as: 4 rings >5 rings >6 rings >3 rings. However, the concentrations of individual compounds for 16 PAHs ranged from 0 to 9 ng g^{-1} dw in group A sites, and 5 to 290 ng g^{-1} dw in group B sites. Group B had much greater PAH concentrations than group A. Because copepod toxicity did not differ between group A (with low PAH concentrations) and group B (with high PAH concentrations) ($p > 0.05$), copepod toxicity is sensitive, even to low PAHs concentrations. In contrast, bacterial toxicity was not detected in group A (with low PAH concentrations), whereas relative toxicity was observed in group B (with high PAH concentrations).

From these results, it can be inferred that the copepod assay is more suitable for sensitive comparison of the toxicity difference between the sites. In comparison, the bacterial assay is more appropriate as a screening technique for determining the presence or absence of toxicity in study areas. Since a 1:1 comparison with bacterial toxicity values might not be appropriate, TU toxicity criteria for copepods needs to be developed by assimilating more copepod toxicity data.

4. Conclusions

The copepod *T. japonicus* bioassay has been successfully applied to assess the level of ecotoxicological contamination in the Yellow and Bohai seas, highlighting the primary contribution of PAHs to the total sediment toxicity. This study also confirmed that other PTSs, such as APs and SOs had also induced significant toxicological effects on copepod. To the best of our knowledge, this study was the first to determine the toxicity of all 16 PAHs with respect to a single copepod species. Our results clearly showed a positive correlation between the number of benzene rings and PAHs toxicity; thus, to manage PAHs, the focus should be given to the HMW PAHs. It should also be noted that the contribution of PAHs to total sediment toxicity in the Yellow and Bohai seas was not high. However, the results of the present study will be useful in selecting priority pollutants based on their contribution to copepod toxicity in coastal sediments in the Yellow and Bohai seas. Further studies would be necessary to identify and select unknown toxicants that could explain the overall toxicity of sediments by the use of effect-directed analysis toward multiple lines of evidence approach.

CRediT authorship contribution statement

Shin Yeong Park: Conceptualization, Formal analysis, Statistical analyses, Visualization, Writing – original draft.

Junghyun Lee: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

Seongjin Hong: Formal analysis, Writing – review & editing,

Taewoo Kim: Investigation.

Seo Joon Yoon: Investigation.

Changkeun Lee: Formal analysis.

Bong-Oh Kwon: Writing – review & editing, Project administration.

Wenyou Hu: Investigation, Project administration, Funding acquisition.

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Jong Seong Khim: Conceptualization, Investigation, Statistical analyses, Visualization, Writing – review & editing, Project administration, Funding acquisition, Supervision.

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.113937.

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<Marine Pollution Bulletin>

Supplementary materials for

Evaluation of ecotoxicological effects associated with coastal sediments of the Yellow Sea Large Marine Ecosystem using the marine copepod *Tigriopus japonicus*

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References

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Supplementary Tables

Table S1. Summary of ecotoxicological studies in Yellow and Bohai seas.

Region	**Year**	**Matrix**	**Compound**	**Concentration** [a]	**Unit**	**Bioassay**	**Species**	**Endpoint**	**Exposure time**	**Reference**
Bohai sea	2008	Sediment extracts	DL-PAHs	36–1100 (260)	ng g^{-1} dw	Cell bioassay	H4IIE-*luc*		72 h	Hong et al. (2012)
West sea	2008	Sediment extracts	DL-PAHs	30–210 (86)	ng g^{-1} dw	Cell bioassay	H4IIE-*luc*		72 h	Hong et al. (2012)
	2018	Sediment extracts	16 PAHs	6.36–57 (22.3)	ng g^{-1} dw	Marine bacteria	*Vibrio fischeri*	Bioluminescence inhibition	15 min	Hwang et al. (2021)
Nanyang and Lianjiang River	2006	Sediment elutriate	16 PAHs	410–1632	ug kg^{-1}	Marine bacteria	*Vibrio fischeri*	Bioluminescence inhibition	15 min	Wang et al. (2009)
	2006	Sediment elutriate	16 PAHs	410–1632	ug kg^{-1}	Microalgae	*Selenastrum capricornutum*	Growth rate	96 h	Wang et al. (2009)
	2006	Whole sediment	16 PAHs	410–1632	ug kg^{-1}	Ostracoda	*Heterocypris incongruens*	Mortality	6 d	Wang et al. (2009)

[a] Min–max (mean).

Table S2. Description of the sampling sites and parameters of sediments in the Yellow and Bohai seas.

Sampling sites	Abb. [a]	Latitude (°N)	Longitude (°E)	Land-use type	Salinity (psu)	MC [b] (%)	TN [c] (%)	TOC [d] (%)
Yellow Sea of Korea (YSK)								
Lake Sihwa	LS2	37.3257	126.6571	Industry	28.6	78.64	0.128	1.38
Sapgyo	SG2	36.8951	126.8191	Agriculture	27.2	86.09	0.145	1.00
Sinduri	SD	36.8385	126.1834	Beach	31.3	1.80	0.028	0.10
Geumgang	GG2	36.0085	126.7353	Agriculture	12.7	89.44	0.104	1.60
Gomso	GS	35.5728	126.6636	Agriculture	24.9	84.97	0.063	0.71
Yeongsan	YS1	34.7821	126.4441	Municipality	26.7	95.72	0.050	0.98
Yellow Sea of China (YSC)								
Dandong	DD2	39.9436	124.2828	Municipality	0.4	89.70	0.137	1.80
Yantai	YT6	37.5753	121.2966	Beach	28.0	8.09	0.007	0.06
Weihai	WH1	36.8266	121.4636	Aquaculture	19.9	86.30	0.161	1.40
Qingdao	QD7	35.7405	119.9111	Aquaculture	14.2	1.54	0.014	0.32
Lianyungang	LY1	34.9023	119.1961	Aquaculture	46.6	60.09	0.039	0.39
Yancheng	YC2	33.8160	120.4768	Industry	0.8	90.71	0.075	0.63
	YC7	32.8821	120.9646	Barren	41.7	73.65	0.063	0.78
Nantong	NT6	32.1535	121.4562	Barren	43.1	93.29	0.077	1.12
	NT9	31.9337	121.8257	Industry	33.3	92.41	0.066	1.53
Bohai Sea (BS)								
Dalian	DL6	39.5058	121.4033	Barren	36.0	46.58	0.015	0.16
Yingkou	YK2	40.6900	122.1292	Industry	35.3	76.98	0.071	0.86
Huludao	HL4	40.7469	120.9347	Industry	9.1	90.16	0.341	3.12
	HL1	40.2697	120.4622	Municipality	33.7	76.60	0.134	2.40
Qinhuangdao	QH6	39.9203	119.5667	Municipality	0.4	78.20	0.274	4.67
Tangshan	TS5	39.4308	119.2800	Agriculture	35.3	32.52	0.066	0.43
Tianjin	TJ6	38.7667	117.5694	Industry	29.7	80.35	0.058	0.82
Binzhou	BZ1	38.2637	117.8511	Agriculture	31.6	74.66	0.041	0.49
Weifang	WF4	37.0765	119.4793	Barren	25.6	71.59	0.386	0.35
Yantai	YT3	37.5518	120.2482	Beach	17.4	0.00	0.002	0.09

[a] Abbreviation. [b] Mud contents (MC). [c] Total nitrogen (TN). [d] Total organic carbon (TOC).

Table S3. Final culture conditions and copepod bioassay protocol with *Tigriopus japonicus*.

Parameters	Conditions	
Culture/test incubation conditions		
Temperature	25 ± 1 ℃	
pH	7–9	
Salinity	25–35 psu	
Dissolved oxygen	≥ 80% saturation	
Photoperiod	12 h light:12 h dark	
Toxicity testing parameters		
Test type	Static non-renewal	
Test duration	96 h	
Test chamber	6-well plate	
Test solution volume	4 mL	
Age of test organisms	≥ 24 h old	
No. of organisms per test chamber	10	
No. of replicate chambers per concentration	3	
No. of organisms per concentration	30	
Feeding regime	None	
Test chamber aeration	None	
Dilution water	0.7-µm filtered seawater	
Test endpoint	Immobilization, mortality	
Test acceptability	≥ 90% survival rate in controls, water quality parameters within acceptable limits, Cd-LC50 within cusum chart limits	
Test concentrations	Na, Ace, BbF, BkF, BghiP, IcdP	0.01, 0.05, 0.1, 0.5, and 1 mg L^{-1}
	Acl, Py	0.01, 0.1, 1, 10 and 100 mg L^{-1}
	Flu, Phe, Ant, Fl, BaA, BaP	0.01, 0.1, 1, 10 and 50 mg L^{-1}
	Chr, DbahA	0.01, 0.1, 1, 10 and 20 mg L^{-1}

Table S4. Summary of ecotoxicological studies using copepod bioassay.

Compound	**Bioassay**	**Species**	**Development stage**	**Endpoint**	**Exposure time**	**Reference**
Bunker A and C refined oil	Copepod	*Tigriopus japonicus*	Adult	Mortality	96h	Ara et al. (2002)
Cu, Irgarol 1051, Zinc pyrithione	Copepod	*Tigriopus japonicus*	Adult	Mortality	96h	Bao and Leung (2006)
Cd, Cu, Hg, Ni, Ag, Zn	Copepod	*Tigriopus brevicornis*	Adult	Metallothionein induction	96h	Barka et al. (2001)
	Copepod	*Tigriopus brevicornis*	Adult	Mortality	96h	
Nonylphenol	Copepod	*Tisbe battagliai*	Nauplius	Population growth rate	8week	Bechmann (1999)
Polyethylene microplastics	Copepod	*Acartia clausi*	Nauplius	Immobility	48h	Beiras et al. (2018)
	Copepod	*Acartia clausi*	Nauplius	Mortality	48h	
	Copepod	*Tigriopus fulvus*	Nauplius	Immobility	48h	
	Copepod	*Tigriopus fulvus*	Nauplius	Mortality	48h	
Atrazine	Copepod	*Amphiascus tenuiremis*	Juvenile	Malformation	7d	Bejarano and Chandler (2003)
	Copepod	*Amphiascus tenuiremis*	Juvenile	Reproduction rate	7d	
	Copepod	*Amphiascus tenuiremis*	Nauplius	Population growth rate	7d	
Cu	Copepod	*Nitocra spinipes*	Adult	Mortality	96h	Bengtsson (1978)
17-Esteradiol, 17-thinylestradiol, diethylstilbestrol	Copepod	*Nitocra spinipes*	Adult	Mortality	18d	Breitholtz and Bengtsson (2001)
	Copepod	*Nitocra spinipes*	Adult	Reproductive success	18d	
17-Esteradiol, 17-thinylestradiol, diethylstilbestrol	Copepod	*Nitocra spinipes*	Nauplius	Development	18d	
Polybrominated diphenyl ether	Copepod	*Nitocra spinipes*	Nauplius	Development	26d	Breitholtz and Wollenberger (2003)
	Copepod	*Nitocra spinipes*	Nauplius	Population growth rate	26d	
Oestrogens	Copepod	*Nitocra spinipes*	Adult	Fecundity	24d	Breitholtz et al. (2001)
	Copepod	*Nitocra spinipes*	Adult	Mortality	96h	
	Copepod	*Nitocra spinipes*	nauplius	Development	96h	
Synthetic musks	Copepod	*Nitocra spinipes*	Adult	Mortality	96h	Breitholtz et al. (2003)
	Copepod	*Nitocra spinipes*	Adult	Population growth rate	96h	
	Copepod	*Nitocra spinipes*	Egg	Larval development rate	96h	
tributyltin (TBT)	Copepod	*Acartia tonsa*	Adult	Mortality	48h	Bushong et al. (1988)
	Copepod	*Eurytemora affinis*	Adult	Mortality	72h	
Cd, Cu	Copepod	*Tigriopus japonicus*	Nauplius	Development	21d	D'Agostino and Finney (1974)
	Copepod	*Tigriopus japonicus*	Nauplius	Mortality	21d	
	Copepod	*Tigriopus japonicus*	Nauplius	Multi-generation effect	21d	
Dieldrin	Cladoceran	*Daphnia pulex*	Adult	Mortality	48h	Daniels and Allan (1981)
	Cladoceran	*Daphnia pulex*	Nauplius	Population growth rate	48h	

Table S4. (continued).

Compound	Bioassay	Species	Development stage	Endpoint	Exposure time	Reference
Dieldrin	Copepod	*Eurytemora affinis*	Adult	Mortality	48h	Daniels and Allan (1981)
	Copepod	*Eurytemora affinis*	Nauplius	Population growth rate	48h	
Carbofuran, dichlorvos, malathion, atrazine	Copepod	*Tigriopus brevicornis*	Nauplius	Mortality	96h	Forget et al. (1998)
As, Cd	Copepod	*Tigriopus brevicornis*	Nauplius	Mortality	96h	
Cd, Cu, atrazine, carbofuran, dichlorvos, malathion	Copepod	*Tigriopus brevicornis*	Adult	Acetylcholinesterase (AChE) activity	96h	Forget et al. (1999)
	Copepod	*Tigriopus brevicornis*	Adult	Mortality	96h	
Sediment leachate	Copepod	*Tigriopus brevicornis*	Juvenile	Mortality	7d	Geffard et al. (2005)
Cu, Pb, Ni, Zn	Copepod	*Amphiascus tenuiremis*	Adult	Mortality	96h	Hagopian-Schlekat et al. (2001)
Cd, Cu	Copepod	*Tisbe battagliai*	Adult	Mortality	8d	Hutchinson et al. (1994)
	Copepod	*Tisbe battagliai*	Adult	Reproduction rate	8d	
20-Hydroxyecdysone, diethylstilbestrol	Copepod	*Tisbe battagliai*	Nauplius	Development	21d	Hutchinson et al. (1999a)
	Copepod	*Tisbe battagliai*	Nauplius	Mortality	21d	
	Copepod	*Tisbe battagliai*	Nauplius	Reproduction rate	21d	
Estrone, 17-esteradiol, 17-ethinylestradiol	Copepod	*Tisbe battagliai*	Nauplius	Development	21d	Hutchinson et al. (1999b)
	Copepod	*Tisbe battagliai*	Nauplius	Mortality	21d	
	Copepod	*Tisbe battagliai*	Nauplius	Reproduction rate	21d	
TBT	Copepod	*Acartia tonsa*	Larvae	Development	8d	Kusk and Petersen (1997)
	Copepod	*Acartia tonsa*	Larvae	Mortality	8d	
Cu, TBT	Copepod	*Tigriopus japonicus*	Adult	Mortality	96h	Kwok and Leung (2005)
4,4-Octylphenol (4,4-OP)	Copepod	*Tigriopus japonicus*	Adult	Expression of glutathione S-transferase (GST) gene	2h	Lee et al. (2006)
Polychlorinated biphenyl (PCB)	Copepod	*Tigriopus japonicus*	Adult	Expression of glutathione S-transferase (GST) gene	2d	Lee et al. (2006)
17-Estradiol, bisphenol A, 4-nonylphenol, p-t-octylphenol, 20-hydroxyecdysone, dimethyl sulphoxide	Copepod	*Tigriopus japonicus*	Nauplius	Development	24h	Marcial et al. (2002)
	Copepod	*Tigriopus japonicus*	Nauplius	Mortality	24h	

Table S4. (continued).

Compound	Bioassay	Species	Development stage	Endpoint	Exposure time	Reference
Sodium dodecyl sulphate (SDS)	Algae	*Dunaliella tertiolecta*	-	Growth rate	72h	Mariani et al. (2006)
	Bacteria	*Vibrio fischeri*	-	Light reduction	15min	
	Copepod	*Tigriopus fulvus*	Nauplius	Mortality	96h	
	Fish	*Dicentrarchus labrax*	Juvenile	Mortality	96h	
	Sea urchin	*Paracentrotus lividus*	Egg	Fertilization rate	1h	
Cu	Copepod	*Tigriopus californicus*	Adult	Mortality	96h	O'Brien et al. (1988)
fullerene (C_{60})	Copepod	-	Adult	Mortality	96h	Oberdörster et al. (2006)
	Daphnia	*Daphnia magna*	Egg	Life-cycle	21d	
	Fish	*Oryzias latipes*	Adult	Cytochrome P450 isozyme	96h	
	Fish	*Pimephales promelas*	Adult	Cytochrome P450 isozyme	96h	
	Hyalella	*Hyalella azteca*	Adult	Mortality	96h	
17-Estradiol (E2), di(2-ethylhexyl)phthalate (DEHP), 4-(tert-octyl)phenol (OP), 4-nonyphenol (NP), bisphenol A (BisA) and benzo[α]pyrene (B[α]P)	Copepod	*Tigriopus japonicus*	Adult	Expression of small heat shock protein gene (HSP20)	96h	Seo et al. (2006b)
Cd, Cu	Copepod	*Eurytemora affinis*	Nauplius	Abnormal molting	96h	Sullivan et al. (1983)
	Copepod	*Eurytemora affinis*	Nauplius	Mortality	96h	
Cd, Cu	Copepod	*Eurytemora affinis*	Nauplius	Predator avoidance	96h	Sullivan et al. (1983)
	Copepod	*Eurytemora affinis*	Nauplius	Swimming speed	96h	
Ni	Copepod	*Acartia tonsa*	Adult	Mortality	96h	Taylor (1981a)
Zn	Copepod	*Tigriopus japonicus*	Adult	Mortality	96h	
	Copepod	*Acartia tonsa*	Adult	Mortality	96h	Taylor (1981b)
Single-Walled Carbon Nanotubes (SWNTs)	Copepod	*Amphiascus tenuiremis*	Adult	Mortality	96h	Templeton et al. (2006)
	Copepod	*Amphiascus tenuiremis*	Egg	Larval development rate	35d	
Diflubenzuron	Copepod	*Acartia tonsa*	Adult	Mortality	5d	Tester and Costlow (1981)
	Copepod	*Acartia tonsa*	Adult	Reproduction rate	5d	
	Copepod	*Acartia tonsa*	Egg	Egg viability	24h	
	Copepod	*Acartia tonsa*	Nauplius	Development	5d	

Table S4. (continued).

Compound	**Bioassay**	**Species**	**Development stage**	**Endpoint**	**Exposure time**	**Reference**
Synthetic musks	Copepod	*Acartia tonsa*	Adult	Mortality	48h	Wollenberger et al. (2003)
	Copepod	*Acartia tonsa*	Nauplius	Development	5d	
Polybrominated diphenyl ether, 2,4,6-tribromophenol, 20-hydroxyecdysone, tetrabromobisphenol A	Copepod	*Acartia tonsa*	Adult	Mortality	48h	Wollenberger et al. (2005)
	Copepod	*Acartia tonsa*	Nauplius	Development	5d	
Trinitrotoluene (TNT)	Algae	*Selenastrum capricornutum*	-	Growth rate	7d	Won et al. (1976)
	Copepod	*Tigriopus californicus*	Adult	Mortality	72h	
	Oyster	*Crassostrea gigas*	Larvae	Mortality	96h	

Table S5. Concentrations of persistent toxic substances in the sediments of the Yellow and Bohai seas.

Site information	**Abb.** [a]	**Concentration (ng g^{-1} dry weight)** [b]		
		16 PAHs	**6 APs**	**10 SOs**
Yellow Sea of Korea (YSK)				
Lake Shihwa	LS2	31.5	41.8	11.6
Sapgyo	SG2	52.4	14.3	7.6
Sinduri	SD	11.8	6.2	8.2
Geumgang	GG2	44.1	24.8	7.6
Gomso	GS	24.3	12.7	6.0
Yeongsan	YS1	11.2	14.8	22.0
Yellow Sea of China (YSC)				
Dandong	DD2	813.2	12.4	14.5
Yantai	YT6	18.2	18.2	10.3
Weihai	WH1	124.8	75.0	11.7
Qingdao	QD7	17.5	42.2	5.2
Lianyungang	LY1	12.8	8.3	5.3
Yancheng	YC2	126.3	9.0	18.4
	YC7	6.6	13.1	6.1
Nantong	NT6	31.7	7.8	8.2
	NT9	12029.8	66.9	28.8
Bohai Sea (BS)				
Dalian	DL6	39.4	0.6	13.6
Yingkou	YK2	489.9	3.0	2.7
Huludao	HL4	12735.6	503.7	218.1
	HL1	169.2	3.8	2.2
Qinhuangdao	QH6	6202.7	57.2	100.8
Tangshan	TS5	43.3	2.5	2.9
Tianjin	TJ6	236.5	22.3	18.0
Binzhou	BZ1	24.7	13.3	12.8
Weifang	WF4	12.2	13.2	10.8
Yantai	YT3	9.2	14.3	5.6

[a] Abbreviation. [b] Yoon et al. (2020).

Table S6. EC50 and LC50 values after 96 h exposure of the copepod *Tigriopus japonicus* to individual PAHs.

Compounds	**Abb.** [a]	**EC50 (mg L^{-1})**	**LC50 (mg L^{-1})**
Naphthalene	Na	1.3	1.5
Acenaphthene	Ace	0.9	1.0
Acenaphthylene	Acl	26.1	36.0
Fluorene	Flu	7.2	9.3
Phenanthrene	Phe	3.7	5.5
Anthracene	Ant	8.5	8.5
Fluoranthene	Fl	2.2	4.3
Pyrene	Py	1.0	1.2
Benz[*a*]anthracene	BaA	2.6	5.0
Chrysene	Chr	5.8	6.5
Benzo[*b*]fluoranthene	BbF	0.6	0.7
Benzo[*k*]fluoranthene	BkF	0.6	0.7
Benz[*a*]pyrene	BaP	3.2	3.6
Dibenz[*a*,*h*]anthracene	DbahA	4.7	5.2
Benzo[*g*,*h*,*i*]perylene	BghiP	0.6	0.6
Indeno[1,2,3-*c*,*d*]pyrene	IcdP	0.3	0.4

[a] Abbreviation.

Table S7. Summary of toxic units for organic extracts of sediments in the Yellow and Bohai seas.

Site information		**Immobilization (Toxic unit)**			**Mortality (Toxic unit)**		
	n	**Min.–Max.**	**Median**	**Mean**	**Min.–Max.**	**Median**	**Mean**
Regions							
Yellow Sea-Korea (YSK)	6	0.8–9.3	3.0	3.6	0.6–9.3	2.4	3.2
Yellow Sea-China (YSC)	9	0.8–30.3	2.4	10.8	0.7–20.4	1.9	8.5
Bohai Sea (BS)	10	0.8–43.5	1.9	6.0	0.8–37.0	1.4	5.0
Salinity							
Freshwater (<5 psu)	2	2.0–2.4	2.2	2.2	1.3–1.9	1.6	1.6
Brackish water (5–30 psu)	13	0.8–43.5	2.9	8.3	0.7–37.0	2.1	7.1
Seawater (>30 psu)	10	0.8–30.3	1.8	6.6	0.6–20.4	1.7	5.0
Land use type							
Agriculture	5	1.0–3.9	2.6	2.4	0.8–3.1	1.9	2.0
Aquaculture	3	0.8–14.3	1.3	5.5	0.7–13.2	1.2	5.0
Barren	4	0.8–30.3	1.6	8.6	0.8–20.4	1.4	6.0
Beach	3	0.8–22.7	1.3	8.3	0.6–16.9	1.0	6.2
Industry	6	1.8–43.5	6.0	13.4	1.6–37.0	5.7	11.8
Municipality	4	2.0–3.3	2.7	2.7	1.3–2.3	1.9	1.9
All sites	25	0.8–43.5	2.4	5.8	0.6–37.0	1.9	3.4

Table S8. Spearman rank correlation between the concentrations of 16 PAHs, 6 APs, and 10 SOs in the sediments and toxic units of organic extracts from the sediments.

Compounds	**Abb.** [a]	**Immobilization**		**Mortality**	
		r	***p*-value**	**r**	***p*-value**
16 Polycyclic aromatic hydrocarbons	**16 PAHs**	**0.46**	**<0.05**	**0.46**	**<0.05**
Naphthalene	Na	0.29	0.17	0.24	0.25
Acenaphthene	Ace	0.26	0.20	0.26	0.22
Acenaphthylene	Acl	0.24	0.26	0.18	0.40
Fluorene	Flu	0.36	0.08	0.36	0.08
Phenanthrene	**Phe**	**0.46**	**<0.05**	**0.46**	**<0.05**
Anthracene	**Ant**	**0.51**	**<0.01**	**0.53**	**<0.01**
Fluoranthene	**Fl**	**0.49**	**<0.05**	**0.51**	**<0.01**
Pyrene	**Py**	**0.54**	**<0.01**	**0.55**	**<0.01**
Benz[*a*]anthracene	**BaA**	**0.48**	**<0.05**	**0.50**	**<0.05**
Chrysene	**Chr**	**0.49**	**<0.05**	**0.49**	**<0.05**
Benzo[*b*]fluoranthene	**BbF**	**0.54**	**<0.01**	**0.54**	**<0.01**
Benzo[*k*]fluoranthene	BkF	0.38	0.06	0.39	0.05
Benz[*a*]pyrene	BaP	0.35	0.09	0.36	0.08
Dibenz[*a*,*h*]anthracene	DbahA	0.19	0.36	0.16	0.43
Benzo[*g*,*h*,*i*]perylene	**BghiP**	**0.44**	**<0.05**	**0.45**	**<0.05**
Indeno[1,2,3-*c*,*d*]pyrene	IcdP	0.33	0.11	0.32	0.12
6 Alkyl phenols	**6 APs**	**0.49**	**<0.01**	**0.48**	**<0.01**
4-tert-octylphenol	**t-OP**	**0.41**	**<0.05**	0.40	0.05
4-tert-octylphenol monoethoxylate	**t-OP1EO**	**0.46**	**<0.05**	**0.42**	**<0.05**
4-tert-octylphenol diethoxylate	**t-OP2EO**	**0.40**	**<0.05**	**0.44**	**<0.05**
Nonylphenol	**NP**	**0.53**	**<0.01**	**0.51**	**<0.01**
Nonylphenol-monoethoxylate	NP1EO	0.39	0.05	0.39	0.05
Nonylphenol diethoxylate	**NP2EO**	**0.43**	**<0.05**	**0.42**	**<0.05**
10 Styrene oligomers	**10 SOs**	**0.41**	**<0.05**	**0.40**	**<0.05**
1,3-diphenylpropane	SD1	0.23	0.28	0.26	0.21
cis-1,2-diphenylcyclobutane	SD2	0.33	0.11	0.32	0.12
2,4-diphenyl-1-butene	**SD3**	**0.42**	**<0.05**	**0.40**	**<0.05**
trans-1,2-diphenylcyclobutane	SD4	0.16	0.45	0.16	0.44
2,4,6-triphenyl-1-hexene	**ST1**	**0.46**	**<0.05**	**0.46**	**<0.05**
1e-phenyl-4e-(1-phenylethyl)-tetralin	ST2	0.28	0.18	0.25	0.22
1a-phenyl-4e-(1-phenylethyl)-tetralin	ST3	0.29	0.16	0.26	0.21
1a-phenyl-4a-(1-phenylethyl)-tetralin	ST4	0.29	0.17	0.24	0.24
1e-phenyl-4a-(1-phenylethyl)-tetralin	ST5	0.26	0.21	0.23	0.28
1,3,5-triphenylcyclohexane (isomer mix)	ST6	0.24	0.26	0.18	0.40

[a] Abbreviation.

Table S9. Comparison of toxicity for individual PAHs between the copepod *Tigriopus japonicus* and other marine copepod species.

Compounds	Endpoint	Copepod species	Stage	Exposure time (h)	EC50 or LC50 (mg L^{-1})	Reference
Na	Immobilization	*Tigriopus japonicus*	Nauplius	48	1.92	***This study***
				96	1.33	***This study***
		Oithona davisae	Adult	48	4.47	Barata et al. (2005)
	Mortality	*Tigriopus japonicus*	Nauplius		3.18	***This study***
				96	1.52	***This study***
		Eurytemora affinis	Nauplius	24	3.84	Ott et al. (1978)
		Oithona davisae	Adult	48	7.19	Barata et al. (2005)
Ace	Immobilization	*Tigriopus japonicus*	Nauplius	48	2.20	***This study***
				96	0.90	***This study***
	Mortality			48	2.27	***This study***
				96	0.96	***This study***
Acl	Immobilization	*Tigriopus japonicus*	Nauplius	48	46.33	***This study***
				96	26.09	***This study***
	Mortality			48	65.12	***This study***
				96	35.99	***This study***
Flu	Immobilization	*Tigriopus japonicus*	Nauplius	48	8.50	***This study***
				96	7.15	***This study***
		Oithona davisae	Adult	48	11.63	Barata et al. (2005)
	Hatching	*Acartia tonsa*	Eggs		0.01	Bellas and Thor (2007)
	Recruitment				0.01	Bellas and Thor (2007)
	Mortality	*Tigriopus japonicus*	Nauplius		16.90	***This study***
				96	9.30	***This study***
		Oithona davisae	Adult	48	1.80	Barata et al. (2005)
		Acartia tonsa			0.02	Bellas and Thor (2007)
				96	0.02	Bellas and Thor (2007)
Phe	Immobilization	*Tigriopus japonicus*	Nauplius	48	4.89	***This study***
				96	3.66	***This study***
		Oithona davisae	Adult	48	0.64	Barata et al. (2005)
	Mortality	*Tigriopus japonicus*	Nauplius		8.40	***This study***
				96	5.50	***This study***
		Quinquelaophonte sp.	Adult		0.75	Stringer et al. (2012)
		Robertsonia propinqua			0.89	Stringer et al. (2012)
		Oithona davisae		48	0.52	Barata et al. (2005)
Ant	Immobilization	*Tigriopus japonicus*	Nauplius	48	9.32	***This study***
				96	8.53	***This study***
	Mortality	*Tigriopus japonicus*	Nauplius	48	9.68	***This study***

Table S9. (continued).

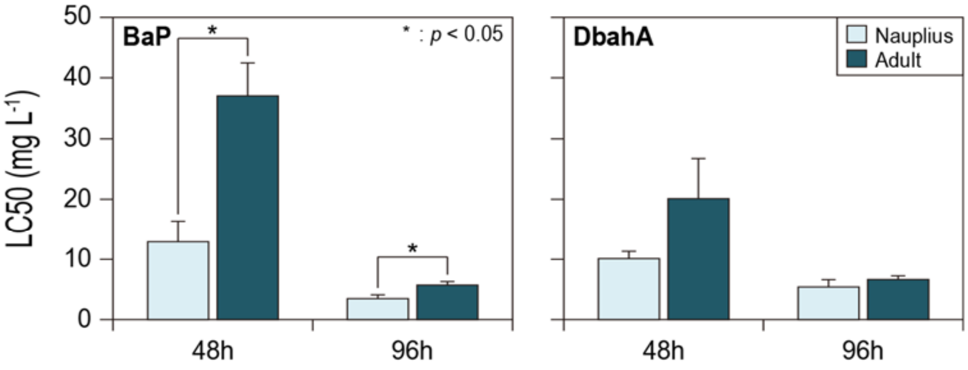
Compounds	Endpoint	Copepod species	Stage	Exposure time (h)	EC50 or LC50 (mg L^{-1})	Reference
Ant	Mortality	*Tigriopus japonicus*	Nauplius	96	8.53	***This study***
Fl	Immobilization	*Tigriopus japonicus*	Nauplius	48	4.69	***This study***
				96	2.15	***This study***
	Immobilization	*Oithona davisae*	Adult	48	0.13	Barata et al. (2005)
	Feeding rate	*Tisbe battagliai*		144	0.03	Barata et al. (2002)
	Clutch size				0.07	Barata et al. (2002)
	Reproduction				0.06	Barata et al. (2002)
	Mortality	*Tigriopus japonicus*	Nauplius	48	5.64	***This study***
				96	4.30	***This study***
		Oithona davisae	Adult	48	0.20	Barata et al. (2005)
		Tisbe battagliai	Nauplius	96	0.07	Barata et al. (2002)
			Adult	144	0.10	Barata et al. (2002)
			Eggs		0.09	Barata et al. (2002)
Py	Immobilization	*Tigriopus japonicus*	Nauplius	48	3.56	***This study***
				96	0.97	***This study***
		Oithona davisae	Adult	48	0.11	Barata et al. (2005)
	Mortality	*Tigriopus japonicus*	Nauplius		4.56	***This study***
				96	1.20	***This study***
		Oithona davisae	Adult	48	12.29	Barata et al. (2005)
BaA	Immobilization	*Tigriopus japonicus*	Nauplius	48	6.41	***This study***
				96	2.59	***This study***
	Mortality		Nauplius	48	10.64	***This study***
				96	5.02	***This study***
Chr	Immobilization	*Tigriopus japonicus*	Nauplius	48	7.79	***This study***
				96	5.79	***This study***
	Mortality		Nauplius	48	8.56	***This study***
				96	6.52	***This study***
BbF	Immobilization	*Tigriopus japonicus*	Nauplius	48	0.98	***This study***
				96	0.63	***This study***
	Mortality		Nauplius	48	1.23	***This study***
				96	0.69	***This study***
BkF	Immobilization	*Tigriopus japonicus*	Nauplius	48	0.93	***This study***
				96	0.63	***This study***
	Mortality		Nauplius	48	1.11	***This study***
				96	0.71	***This study***
BaP	Immobilization	*Tigriopus japonicus*	Nauplius	48	12.33	***This study***

Table S9. (continued).

Compounds	Endpoint	Copepod species	Stage	Exposure time (h)	EC50 or LC50 (mg L^{-1})	Reference
BaP	Immobilization	*Tigriopus japonicus*	Nauplius	96	3.18	***This study***
	Mortality			48	13.19	***This study***
				96	3.57	***This study***
			Adult	48	36.46	***This study***
				96	5.60	***This study***
		Eurytemora affinis	Nauplius		0.06	Forget-Leray et al. (2005)
DbahA	Immobilization	*Tigriopus japonicus*	Nauplius	48	8.97	***This study***
				96	4.67	***This study***
	Mortality			48	9.86	***This study***
				96	5.18	***This study***
			Adult	48	18.34	***This study***
				96	6.40	***This study***
BghiP	Immobilization	*Tigriopus japonicus*	Nauplius	48	0.67	***This study***
				96	0.56	***This study***
	Mortality			48	0.73	***This study***
				96	0.56	***This study***
IcdP	Immobilization	*Tigriopus japonicus*	Nauplius	48	0.56	***This study***
				96	0.32	***This study***
	Mortality			48	0.72	***This study***
				96	0.36	***This study***

Supplementary Figures

(a) Life cycle



(b) Exposure time

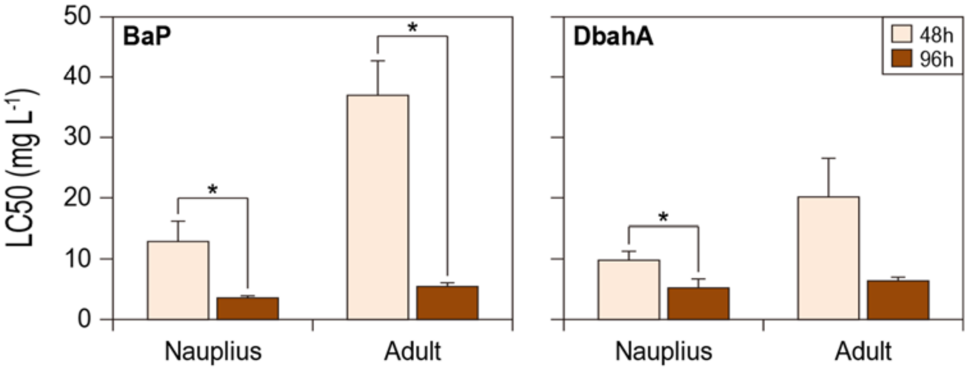
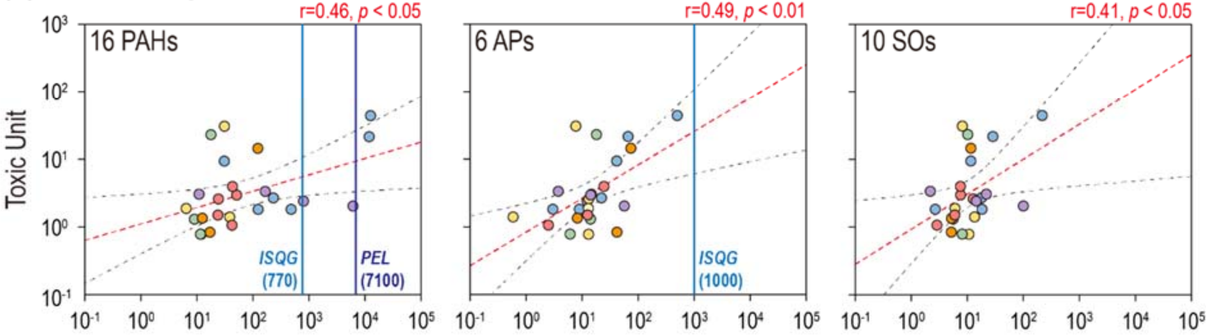


Fig. S1. Results of copepod toxicity for benz[*a*]pyrene (BaP) and dibenz[*a*,*h*]anthracene (DbahA), assessed (a) after 48 h and 96 h exposure and (b) in the nauplius and adult forms to determine choice sensitive exposure time and life cycle.

(a) Land use type (Photo at selected locations)

(b) Relationship between PTSs and TU



(c) Relationship between individual chemicals and TU

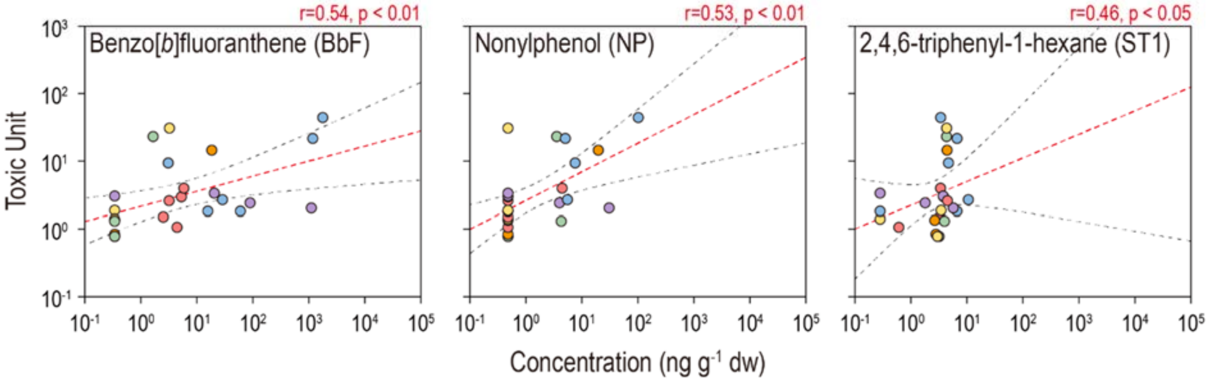


Fig. S2. (a) Field images of land use types in study area. Spearman rank correlation between the concentrations of (b) total chemicals (16 PAHs, 6 APs, and 10 SOs) and (c) individual compounds (BbF, NP, and ST1) in the sediments and toxic unit (immobilization) for organic extracts. A concentration below the limit of detection was replaced with a value of half of the limit of detection before statistical analyses were performed.

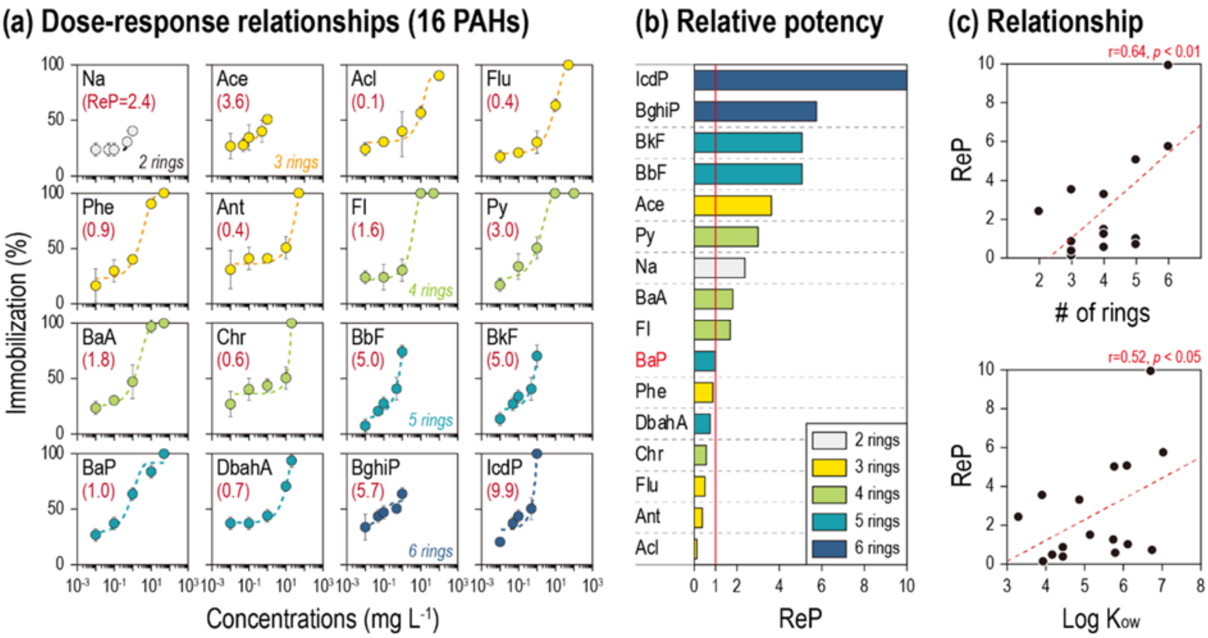


Fig. S3. (a) Dose-response relationships for the immobilization of 16 PAHs in the *Tigriopus japonicus* bioassay (Error bar: mean±SD), (b) relative potency (ReP) for the immobilization of 16 PAHs and (c) relationship between the ReP (immobilization) and benzene rings and Log K_{ow} of PAHs.

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