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Seasonal dynamics of trace elements in sediment and seagrass tissues in the largest *Zostera japonica* habitat, the Yellow River Estuary, northern China

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ABSTRACT

Trace element accumulation is an anthropogenic threat to seagrass ecosystems, which in turn may affect the health of humans who depend on these ecosystems. Trace element accumulation in seagrass meadows may vary temporally due to, e.g., seasonal patterns in sediment discharge from upstream areas. In addition, when several trace elements are present in sufficiently high concentrations, the risk of seagrass loss due to the cumulative impact of these trace elements is increased. To assess the seasonal variation and cumulative risk of trace element contamination to seagrass meadows, trace element (As, Cd, Cr, Cu, Pb, Hg, Mn and Zn) levels in surface sediment and seagrass tissues were measured in the largest Chinese *Zostera japonica* habitat, located in the Yellow River Estuary, at three sites and three seasons (fall, spring and summer) in 2014–2015. In all three seasons, trace element accumulation in the sediment exceeded background levels for Cd and Hg. Cumulative risk to *Z. japonica habitat* in the Yellow River Estuary, from all trace elements together, was assessed as "moderate" in all three seasons examined. Bioaccumulation of trace elements by seagrass tissues was highly variable between seasons and between above-ground and below-ground biomass. The variation in trace element concentration of seagrass tissues was much higher than the variation in trace element concentration of the sediment. In addition, for trace elements which tended to accumulate more in above-ground biomass than below-ground biomass (Cd and Mn), the ratio of above-ground to below-ground trace element concentration peaked at times corresponding to high water discharge and high sediment loads in the Yellow River Estuary. Overall, our results suggest that trace element accumulation in the sediment may not vary between seasons, but bioaccumulation in seagrass tissues is highly variable and may respond directly to trace elements in the water column.

1. Introduction

Seagrass is regarded as a coupled social–ecological system (Cullen-Unsworth et al., 2014), and thus the condition of seagrass ecosystems affects human health (Lamb et al., 2017). However, seagrass habitats are degrading worldwide (Waycott et al., 2009). For example, large-scale decline of the seagrass species *Zostera japonica* has been reported at various locations within Asia (Abe et al., 2009; Hodoki et al., 2013; Zhang et al., 2015). Seagrass decline has been attributed to many factors that include natural causes, but in > 70% of the cases anthropogenic factors are thought to be responsible (Hemminga and Duarte, 2000). Seagrass meadows are sensitive ecosystems that show variations in their distribution both seasonally and spatially (Boudouresque et al., 2009).

With rapid urbanization and industrialization, coastal areas in China are now facing great challenges in regard to trace metal contamination. Large volumes of suspended sediment comprising particulate trace elements (TEs), organic matter, nutrients, and minerals are transported to the ocean by rivers every year (Milliman and Meade, 1983). TEs from both natural and human activities have been shown to accumulate in marine habitats through atmospheric and terrestrial contributions (Halpern et al., 2008), and can negatively impact the health of seagrasses (Macinnis-Ng and Ralph, 2002).

For example, the Yellow River Estuary in northern China is well known to be subjected to high sediment loads but relatively low water discharge to the sea (Milliman and Meade, 1983). According to the most recently available data (Lijin station, 2014–2015), the sediment load is approximately 3×10^7 tons per year, and the water discharge is

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only 1.14×10^{10} m^3 (http://www.yellowriver.gov.cn/nishagonggao). Hence, the Yellow River Estuary is characterized by a sediment to water ratio of 0.0026. This value is almost 20 times higher than the sediment to water ratio in the Yangtze River (Qiao et al., 2007), the third largest river in the world which itself is a huge sediment source and is heavily polluted (Dong et al., 2014). However, sediment loads and water discharges differ in magnitude throughout the year, and hence there may be seasonal patterns in the threat to coastal areas posed by sediments and the TEs they carry.

Contamination of aquatic ecosystems by TEs has been a focus of study in recent years because TEs tend to concentrate, via bioaccumulation and sedimentation, in coastal vegetated ecosystems such as mangroves, salt marshes (Weis and Weis, 2004) and seagrasses. Some elements play a key role in biological processes in living organisms (Cu, Fe, Mn, Zn and Ni), but others such as As, Pb, Hg, Cr and Cd are toxic non-essential elements (e.g. Chang et al., 1996). Thus, at sufficiently high concentrations, certain TEs can act as environmental stressors for the local ecology. Environmental stress due to individual TEs depends on their individual concentrations, but this stress can become cumulative when multiple TEs are present in sufficiently high concentrations. TE accumulation and impact on physiological and biochemical processes is known for some seagrass species (Malea and Kevrekidis, 2013). Previous studies indicate that TEs act on CO_2 fixation and therefore can negatively impact seagrass photosynthetic physiology (Macinnis-Ng et al., 2004). However, TEs may also impact on seagrass physiology in several other ways (reviewed in Richir and Gobert, 2016).

Z. japonica is one of the most widely distributed seagrass species in the world, and occurs in temperate and subtropical coastal regions (Fan et al., 2011). The distribution of *Z. japonica* in China, based on a nationwide survey, has been recently reported (Fan et al., 2011; Zheng et al., 2013; Zhang et al., 2015). However, larger *Z. japonica* beds are very rare, due to rapid declines resulting from increasingly severe habitat destruction (Lee, 1997; Lee et al., 2005; Abe et al., 2003, 2009; Fan et al., 2011; Mach et al., 2014; Zhang et al., 2015). In 2015, a large and continuous *Z. japonica* bed with an area ca. 1000 ha was found in the Yellow River Estuary of Shandong province in China (Zhou et al., 2016). Whilst the sediment and seagrass accumulation of TEs in this *Z. japonica* bed has been previously investigated (Lin et al., 2016a), the seasonal distribution of this accumulation, and the biological risk to this seagrass habitat due to cumulative contributions from all TEs together, has not yet been established.

Thus, the main aims of this study were to (i) assess the seasonal variation in sediment TE concentration, and seasonal variation in ecological risk due to sediment TEs (As, Cd, Cr, Cu, Pb, Hg, Mn and Zn) in the Yellow River Estuary; (ii) assess the seasonal variation in seagrass tissue TE concentrations, for above-ground biomass, below-ground biomass, and their ratio; and (iii) develop hypotheses for how seasonal variation in seagrass TE concentration is affected by TE concentrations in the environment.

2. Materials and methods

2.1. Study area

The *Z. japonica* habitat is located in the Yellow River Estuary, within the northeastern Gulf of China (Fig. 1). This intertidal seagrass bed extends along the coast, from lines A to B shown in Fig. 1, with a length ca. 30 km and an area ca. 1000 ha. The seagrass bed is adjacent to a *Spartina alterniflora* habitat, forming a unique ecological landscape. The main direction of the current along this coastal habitat is southeast, due to anticlockwise circulation in the southern part of the Bohai Sea (Zhang, 2015).

Although we do not have data for TE loading in the Yellow River during our study period (Bi et al., 2014), monitoring of sediment load and water discharge is carried out at a nearby hydrometric station (Lijin station; Xu et al., 2016; data available online at: http://www.yellowriver.gov.cn/nishagonggao). During summer months, large volumes of suspended sediment, which are highly likely to comprise particulate TEs, are transported along the Yellow River to the ocean every year (Fig. 2).

2.2. Field sampling and analyses

Three seagrass sampling sites S1, S2 and S3 were chosen in a single linear transect running northwest to southeast along the coast (from N 38°00′, E 119°15′ to N 37°43′, E 119°30′). For the seagrass sites, S1 was located in the coastal area north and furthest from the mouth of the Yellow River, S2 was located north of the river mouth and midway along the transect, and S3 was located south and closest to the river mouth. The *Z. japonica* meadow inhabits intertidal sandy or muddy bottoms at depths of approximately 1–2 m.

Z. japonica samples were collected from the three seagrass sites (4 replicates per site, replicates separated by a distance of 50 m), in three seasons: fall (August to September 2014), spring (April 2015) and summer (July 2015). As shown in Fig. 2, these sampling times coincide with times of high, low and high sediment discharge in the Yellow River, respectively. The *Z. japonica* samples were washed, cleaned of sediments, and separated into above-ground and below-ground plant compartments. Epiphytes, where present, were removed from leaves by wiping them off with a paper towel, or scraping them off with a razor blade. Samples of seagrass were dried at 60 °C for 24 h, manually ground to a coarse powder, and freeze-stored before being transferred to the laboratory for TE analysis. In addition, surficial sediment samples were collected from the top 5 cm of the *Z. japonica* stands ($n = 4$ per site), using an acrylic plastic corer to avoid contamination. The samples were dried and then freeze-stored until TE analyses were conducted. All containers were new and had been washed with acid.

Seagrass and sediment samples were analyzed for eight TEs: As, Cd, Cr, Cu, Pb, Hg, Mn and Zn. Individual TE concentrations were measured and analyzed at the Institute of Geology, Chinese Academy of Sciences (Langfang, Hebei, China), following the protocol described in Lin et al. (2016b). In this paper, when we discuss TE concentration, we are always referring to total concentration. When performing statistical analyses, we considered each TE separately rather than cumulatively, because any statistically significant effects identified in all eight TEs pooled together would be more difficult to interpret. Prior to all statistical analyses, the distribution of the data was tested using the Shapiro-Wilk test.

2.3. Assessing trace element contamination in the sediment

Sediments are a suitable indicator of TE pollution in estuarine and coastal areas (Batley, 1989). Assessing seasonal variation in ecological risk in the Yellow River Estuary due to sediment TEs, which is the first aim of our study, was accomplished by:

(1) Statistical tests of the differences in TE concentration between sites and seasons using ANOVA and repeated measures ANOVA respectively, and post-hoc Tukey tests, all with a significance of $p < 0.05$, and.

(2) Calculation of two indices of TE contamination – the geoaccumulation index (I_{geo}) and the ecological risk index *(RI)* – for each trace element and season, averaged over the three sites. The second index, *RI*, was also used to assess cumulative risk from all trace elements together, averaged over the three sites, for each of the three seasons tested.

The first index – the geoaccumulation index (I_{geo}) – quantifies TE contamination in the sediment according to (Muller, 1981)

$$I_{geo} = \log_2\left[\frac{[\mathrm{TE}]_{\mathrm{sediment}}}{1.5[\mathrm{TE}]_{\mathrm{background}}}\right], \quad (1)$$

where $[\mathrm{TE}]_{\mathrm{sediment}}$ is the TE concentration in the sediment (mg/kg), $[\mathrm{TE}]_{\mathrm{background}}$ is the geochemical background concentration of the

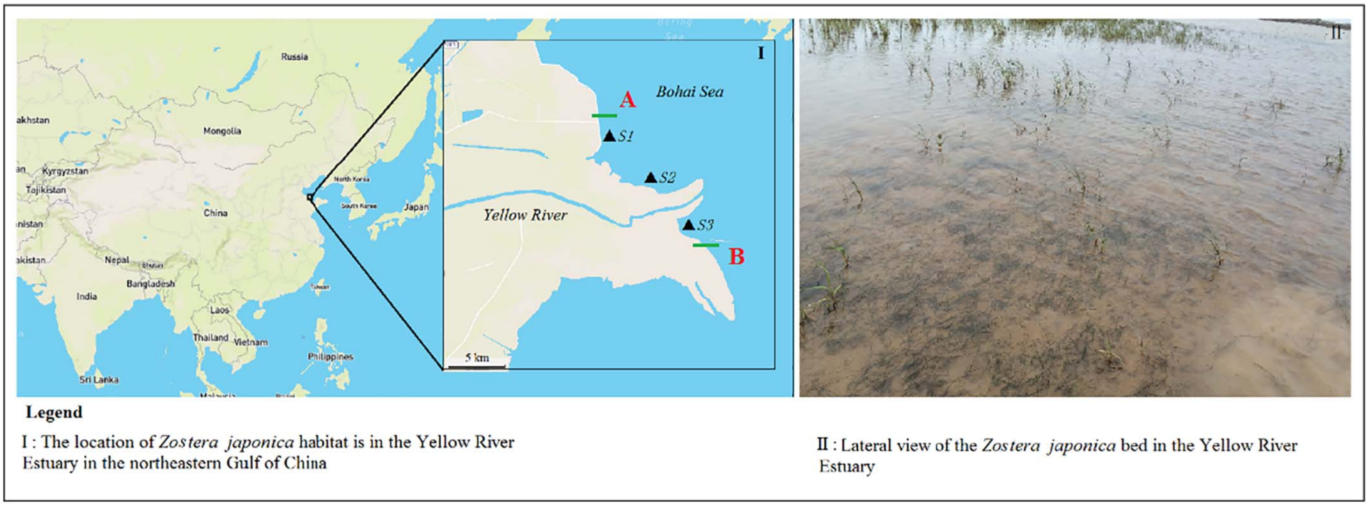

Fig. 1. The location of *Z. japonica* habitat in the Yellow River Estuary, China. The horizontal red lines marked A and B indicate the northern and southern ends of the *Z. japonica* meadow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

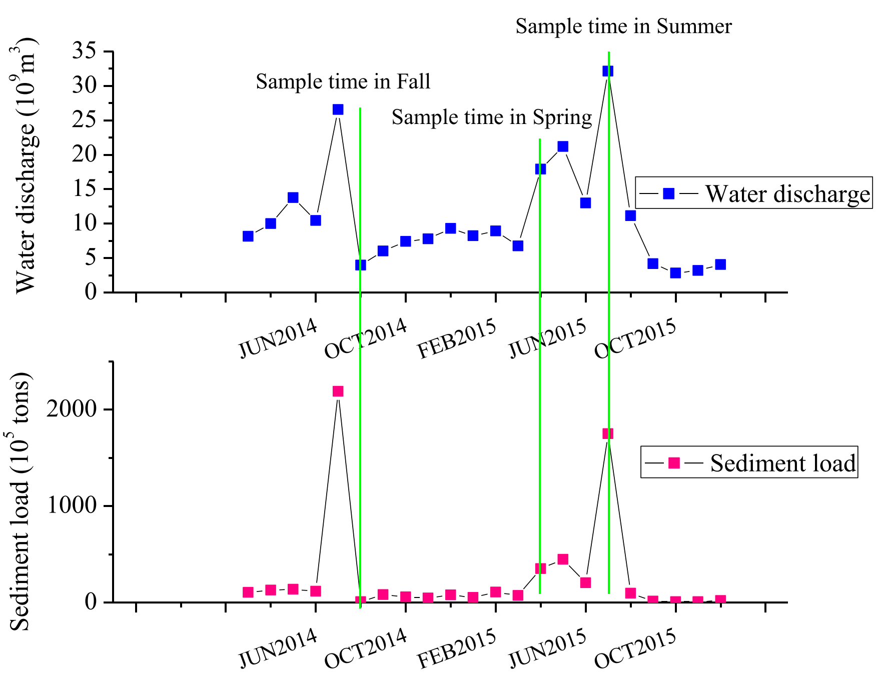

Fig. 2. Monthly sediment load (red dots) and water discharge (blue dots) in the Yellow River Estuary, and the three times when seagrass and sediment were sampled for the present study (vertical green lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

element (mg/kg) in the study area, which we obtained from Zhao and Nan (1992), and 1.5 is the background matrix correction factor due to lithogenic influences which is a requirement for calculation of this index.

The second index – the ecological risk index (*RI*) – estimates the cumulative ecological risk, based on concentrations of all known TE concentrations in the sediment, and the toxic response factor of each TE (Hakanson, 1980). The *RI* is calculated as

$$RI = \sum_{i}^{n} E_r{}^i = \sum_{i}^{n} T_r{}^i \left(\frac{[\mathrm{TE}]_{\mathrm{sediment}}{}^i}{[\mathrm{TE}]_{\mathrm{background}}{}^i} \right), \quad (2)$$

where $E_r{}^i$ is the ecological risk due to an individual TE *i*, and $T_r{}^i$ is the toxic response factor of an individual TE *i*.

2.4. Assessing trace element accumulation in seagrass tissues

To address the second aim of our study which is to assess TE contamination in seagrass tissues, we first performed statistical tests of the difference in TE concentration within seagrass tissues, between seasons, using repeated measures ANOVA and post-hoc Tukey tests at a significance of $p < 0.05$. Thereafter, the ability of seagrass to bioaccumulate individual TEs was calculated using the bioconcentration factor (BCF) (Kilminster, 2013; Lewis et al., 2007),

$$\mathrm{BCF} = \frac{[TE]_{\mathrm{seagrass}}}{[TE]_{\mathrm{sediment}}}, \quad (3)$$

where $[\mathrm{TE}]_{\mathrm{seagrass}}$ is the total TE concentration in the relevant seagrass tissues (mg/kg). BCFs were calculated for both the above-ground and below-ground seagrass tissues.

Ratios of TE concentrations between the above-ground and below-ground seagrass tissues, $[\mathrm{TE}]_{\mathrm{above\text{-}ground}}/[\mathrm{TE}]_{\mathrm{below\text{-}ground}}$, were calculated and plotted for each TE and season. The accumulation properties of above-ground and below-ground tissues, for each TE, were also compared using ANOVA and post-hoc Tukey tests at a significance of $p < 0.05$.

2.5. Assessing relationships between trace element accumulation in seagrass and the environment

For the third aim of our study, we investigated how trace element concentrations in seagrass tissues are affected by trace element concentrations in the external environment. To accomplish this, we plotted $[TE]_{seagrass}$ vs $[TE]_{sediment}$, and compared the seasonal dynamics of seagrass TE content with the seasonal dynamics of the sediment load and water discharge in the Yellow River Estuary (Fig. 2). Due to the high variability observed in this data, no statistical tests were used. To investigate the variability further, the variability in $[TE]_{seagrass}$ and the variability in $[TE]_{sediment}$ was also compared for each of the eight TEs, by use of the formula

$$Variability\ (\%) = \frac{\max([TE]) - \min([TE])}{\frac{1}{2}(\max([TE]) + \min([TE]))} \times 100\%, \quad (4)$$

where max([TE]) and min([TE]) are the maximum and minimum concentrations respectively of the trace element from measurements at all sites and seasons, either in the seagrass tissues or sediment.

3. Results

3.1. TE concentrations and distributions in sediment

For five of the eight TEs (Cd, Cu, Hg, Mn and Pb), there were statistically significant differences in TE concentration of the sediment between the three seasons tested (repeated measures ANOVA, $p < 0.05$, Table 1). For these five TEs, the highest concentrations were observed in either fall or spring, not in summer, but this pattern may not represent a real effect. Sampling sites closest to the mouth of the Yellow River Estuary tended to be more contaminated, as follows. For four of the eight TEs (Cd, Hg, Pb and Zn), there were statistically significant differences between the three sites tested (ANOVA, $p < 0.05$, Table 1). For these four TEs, the concentrations at the site furthest from the river mouth (S1) were always significantly less than the concentrations at the other two sites (sites S2 and S3, see Table 1). Averaged over all sites and seasons, [Mn] was the most abundant element in the sediment, and sediment TE concentration followed the sequence [Mn] > [Zn] > [Cr] > [Pb] > [Cu] > [As] > [Cd] > [Hg] (Table 1).

Trace elements Cd and Hg were predicted to be of greatest ecological concern in the Yellow River Estuary, based on TE contamination index calculations from sediment concentrations of these elements. This result was consistent between both indices of TE sediment contamination tested (I_{geo} and *RI*), as follows. Based on geoaccumulation index,

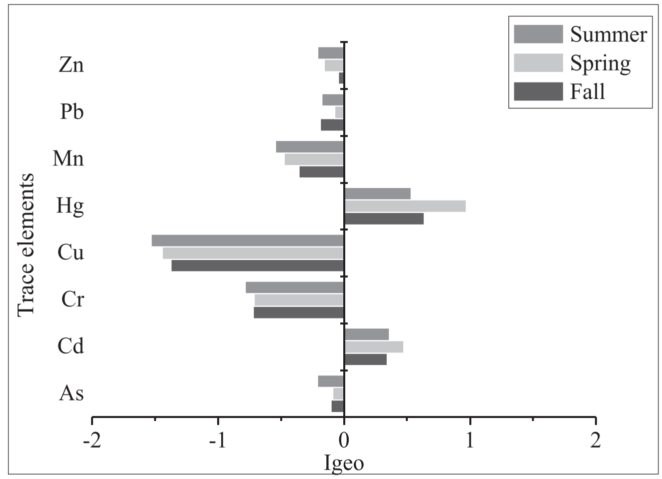

Fig. 3. Seasonal variation of geoaccumulation index in the *Z. japonica* habitat in the Yellow River Estuary, averaged across all sites. Sediment pollution by TEs is classified from the value of I_{geo} according to the following scale: < 0 = none, 0–1 = none to moderate, 1–2 = moderate.

sediment in the Yellow River Estuary is polluted by two of the eight TEs, Cd and Hg ($I_{geo} > 0$, Fig. 3). According to this index, the pollution by Hg and Cd is classified as "none to moderate", in all three seasons. Similarly, Cd and Hg were the TEs which posed the greatest individual ecological risk based on *RI*, in all three seasons, presenting "medium" and/or "moderate" risks based on this index (Table 2). Cumulatively, the risk from all TEs together, was between 150 and 300 in all seasons. Therefore, in all three seasons, the ecological risk due to the cumulative effect of the eight TEs measured is classified as "moderate".

3.2. TE concentrations and distributions in seagrass tissues

Although most of the seagrass tissue TE concentrations varied significantly with season, there was no consistent trend in these variations. For all eight TEs, there were statistically significant differences between seasons for TE concentrations in below-ground seagrass biomass, when averaged across all sites (repeated measures ANOVA, $p < 0.05$, Table 3). For all TEs excluding Cu and Zn, there were statistically significant differences between seasons for TE concentrations in above-ground seagrass biomass, when averaged across all sites (repeated measures ANOVA, $p < 0.05$). For four of the eight TEs (As, Cr, Hg and Pb), the highest TE concentrations were observed in spring, for both above-ground and below-ground biomass. However, the lowest concentrations of seagrass tissue Cd and Mn were also observed in spring, for both above-ground and below-ground biomass (Table 3).

Bioconcentration of TEs within above-ground and below-ground tissues of *Z. japonica* showed highly variable responses between seasons and TEs (Fig. 4). The highest bioaccumulation was observed for Cd in

Table 1
Seasonal and site variation of sediment TE concentrations, in the Yellow River Estuary (all elements in μg g^{-1} DW, except Hg and Cd in ng g^{-1} DW; four replicates per site and season). Results are presented as mean ± SD. Significant differences in TE concentrations between seasons for a particular TE (calculated using RM ANOVA), and between sites for a particular TE (calculated using ANOVA), are indicated by different superscript letters.

Trace elements	Fall	Spring	Summer	S1 (Coastal area)	S2 (North estuary area)	S3 (South estuary area)
As	10.14 ± 1.16	10.57 ± 2.65	9.72 ± 1.14	10.87 ± 2.57	9.95 ± 1.30	9.60 ± 0.76
Cd	152.23 ± 10.80 ^{b}	171.56 ± 11.05^{a}	148.12 ± 11.75^{b}	143.22 ± 10.25^{c}	154.51 ± 7.88^{b}	163.05 ± 14.21^{a}
Cr	53.74 ± 3.73	54.86 ± 2.47	52.22 ± 4.26	53.11 ± 2.77	53.79 ± 4.62	55.23 ± 2.33
Cu	20.09 ± 2.62^{a}	18.70 ± 1.40^{b}	17.59 ± 0.94^{c}	17.50 ± 0.57	19.07 ± 2.19	19.04 ± 1.38
Hg	36.98 ± 5.52^{b}	44.05 ± 9.85^{a}	32.55 ± 9.69^{c}	28.95 ± 7.73^{c}	43.80 ± 6.90^{a}	40.83 ± 7.27^{b}
Mn	527.53 ± 38.49^{a}	485.27 ± 40.27^{b}	462.87 ± 13.44^{c}	482.59 ± 43.52	493.40 ± 51.33	499.64 ± 30.71
Pb	20.18 ± 1.56^{b}	21.36 ± 1.22^{a}	19.92 ± 1.10^{c}	19.46 ± 1.53^{c}	20.90 ± 1.02^{b}	21.09 ± 1.14^{a}
Zn	59.41 ± 4.66	53.81 ± 4.18	51.89 ± 2.86	51.72 ± 3.76^{c}	55.74 ± 5.90^{b}	57.64 ± 3.45^{a}

Table 2
Ecological risk index for each of the three seasons within the Yellow River Estuary, based on TE sediment concentrations averaged over all sites. Ecological risk based on an individual TE, E_r^i, is classified according to the following scale: < 40 = low, 40–80 = medium, 80–160 = moderate. Ecological risk based on cumulative impacts of all TEs, *RI*, is classified according to the following scale: < 150 = low, 150–300 = moderate. Background values $[TE]_{background}^i$ are in units of μg g^{-1} DW.

Trace elements	Fall		Spring		Summer		T_r^i	Background values $[TE]_{background}^i$
	$\frac{[TE]_{sediment}^i}{[TE]_{background}^i}$	E_r^i	$\frac{[TE]_{sediment}^i}{[TE]_{background}^i}$	E_r^i	$\frac{[TE]_{sediment}^i}{[TE]_{background}^i}$	E_r^i		
As	1.40	13.96	1.41	14.09	1.30	12.95	10	7.5
Cd	1.90	57.08	2.08	62.51	1.92	57.71	30	0.077
Cr	0.91	1.82	0.91	1.83	0.87	1.74	2	60
Cu	0.58	2.89	0.55	2.76	0.52	2.59	5	33.9
Hg	2.33	93.24	2.94	117.46	2.17	86.80	40	0.015
Mn	1.17	1.17	1.08	1.08	1.03	1.03	1	450
Pb	1.32	6.58	1.42	7.12	1.33	6.64	5	15
Zn	1.45	1.45	1.35	1.35	1.30	1.30	1	40
RI	178.20		208.18		170.77			
Classification	Moderate		Moderate		Moderate			

both above-ground and below-ground tissues, and this result was only true in one season (fall: September 2014 in Fig. 4).

Based on the ratio of TE concentration between above-ground and below-ground seagrass tissues, $[TE]_{above\text{-}ground}/[TE]_{below\text{-}ground}$ (Fig. 5), three TEs (Mn, Cu and Cd) accumulated more strongly in the above-ground than the below-ground seagrass tissue and three TEs (Hg, As and Cr) accumulated more strongly in the below-ground than the above-ground seagrass tissue (ANOVA, $p < 0.05$).

3.3. Comparing TE concentrations between seagrass tissues and sediment

For two of the TEs (Cd and Mn) our data suggested the possibility that bioaccumulation of these TEs by above-ground seagrass biomass might respond directly to TE concentrations in the water column. These two TEs had generally the highest ratios of concentration between above-ground and below-ground seagrass tissues, $[TE]_{above\text{-}ground}/[TE]_{below\text{-}ground}$ (Fig. 5), and the seasonal pattern of these ratios qualitatively followed the temporal pattern of sediment load and water discharge (Fig. 2). Sediment load and water discharge were high just prior to/during sampling in fall and summer; this coincided with peaks in $[TE]_{above\text{-}ground}/[TE]_{below\text{-}ground}$ for Cd and Mn. For all other TEs, the ratio of TE concentration between above-ground and below-ground seagrass tissues did not vary substantially between seasons. Hence, this result should be treated cautiously as a hypothesis for future work and may also be specific only to the TEs Cd and Mn.

In contrast, there was no clear trend between $[TE]_{seagrass}$ and $[TE]_{sediment}$ (Fig. 6). On first glance, this might indicate that seagrass TE content is not a good bioindicator for TE contamination in the sediment. However, for most of the TEs, the TE concentration in the sediment varied by < 30% across all sites and seasons (Table 4), suggesting that there may not be sufficient environmental variability in sediment TE concentration to identify whether there is a possible trend between $[TE]_{seagrass}$ and $[TE]_{sediment}$ or not. In addition, the variability in $[TE]_{seagrass}$ was far greater, typically 100–200% (Table 4), indicating that either seagrass uptake of TE is highly variable due to its complex physiology or seagrass tissue TE concentration is also responding to other environmental stimuli (e.g. TE uptake directly from the water column).

4. Discussion

In response to the three aims of our experiment, the conclusions were: (i) sediment TE concentration and cumulative ecological risk due to sediment TEs did not vary substantially between seasons (Fig. 3 and Table 2); (ii) seasonal variation in seagrass TE concentration was highly variable (Fig. 4) but the preference of TE accumulation between above-ground and below-ground biomass compartment was less seasonally variable (Fig. 5), and for six of the eight TEs we could statistically identify a biomass compartment that these TEs were more greatly bioaccumulated (Table 5); and (iii) seagrass TE concentration may be responding directly both to TE concentrations in the sediment and the water column, as described further below.

4.1. Seasonal dynamics of TE concentrations in seagrass and sediment

Seasonal patterns of TE accumulation were highly variable (Fig. 6), but the variability in TE concentration differed markedly between seagrass tissue and sediment (Table 4). This suggested that either (1) there was insufficient environmental variability in sediment TE concentration to identify correlations between seagrass tissue TE and sediment TE, or (2) seagrass TE responds to environmental cues other than sediment TE, such as TE concentrations in the water column. The

Table 3
Seasonal variation of TE concentrations in *Z. japonica*, averaged over the three sites, in the Yellow River Estuary (all elements in μg g^{-1} DW, except Hg and Cd in ng g^{-1} DW; four replicates per site and season). Results are presented as mean ± SD. Statistical significance in TE concentrations between seasons were tested using repeated measures ANOVA and post-hoc Tukey tests at a significance level of $p < 0.05$. For a particular TE and seagrass biomass compartment, statistically significant differences are indicated by different superscript letters.

Trace elements	Below-ground tissue of *Z. japonica* (Mean ± SD)			Above-ground tissue of *Z. japonica* (Mean ± SD)		
	Fall	Spring	Summer	Fall	Spring	Summer
As	31.81 ± 2.01^c	82.90 ± 17.42^a	48.46 ± 14.59^b	5.20 ± 0.91^b	8.54 ± 4.18^a	4.27 ± 1.47^b
Cd	1691.57 ± 357.33^a	1009.33 ± 258.99^b	1323.26 ± 200.67^b	4578.75 ± 931.93^a	1344.94 ± 145.71^b	4151.56 ± 1600.64^a
Cr	5.91 ± 2.49^b	12.69 ± 1.39^a	7.13 ± 1.92 ^{b}	4.53 ± 1.27^b	6.79 ± 1.25^a	2.90 ± 0.68^c
Cu	9.02 ± 1.97^b	15.46 ± 3.16^a	11.45 ± 3.47^b	15.31 ± 5.22	17.33 ± 4.30	15.19 ± 2.88
Hg	18.56 ± 3.50^b	28.49 ± 1.70^a	18.42 ± 1.46^b	14.54 ± 5.26^b	25.67 ± 4.37^a	12.14 ± 3.21^b
Mn	594.86 ± 133.44^a	394.41 ± 122.42^b	456.67 ± 132.46^b	2722.99 ± 1124.48^a	746.47 ± 370.15^b	2006.41 ± 804.86^a
Pb	4.07 ± 0.77^b	10.01 ± 1.98^a	4.35 ± 0.93^b	3.20 ± 0.32^b	5.35 ± 1.36^a	2.87 ± 1.09^b
Zn	32.74 ± 4.99^b	39.72 ± 1.73^a	38.30 ± 7.07^a	37.67 ± 15.77	41.21 ± 5.88	39.45 ± 6.45

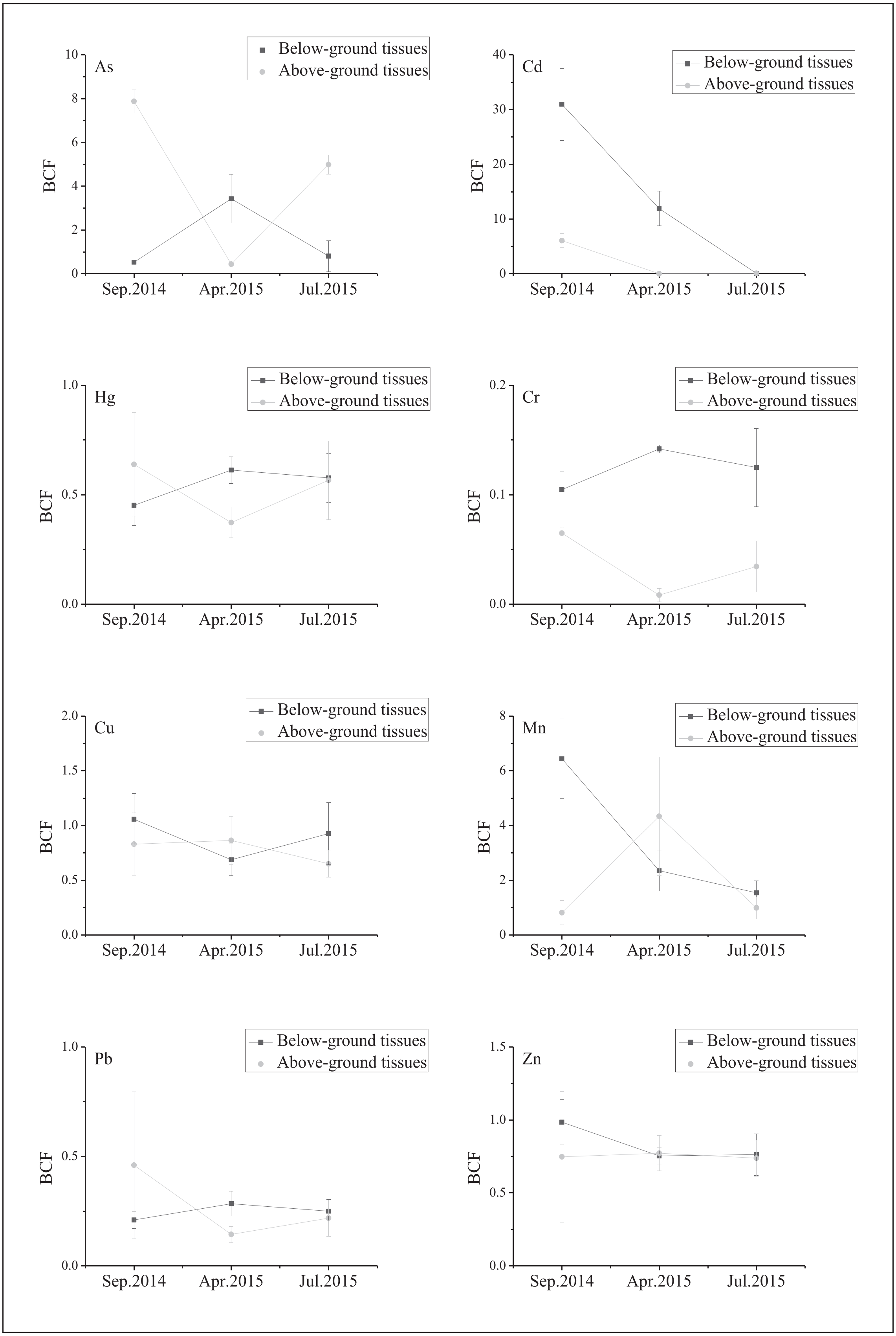

Fig. 4. Seasonal variation in the bioconcentration factors (BCF), for the eight TEs, in the above-ground and below-ground tissues of *Z. japonica*, averaged across the three sites. Results are presented as mean ± SD ($n = 4$).

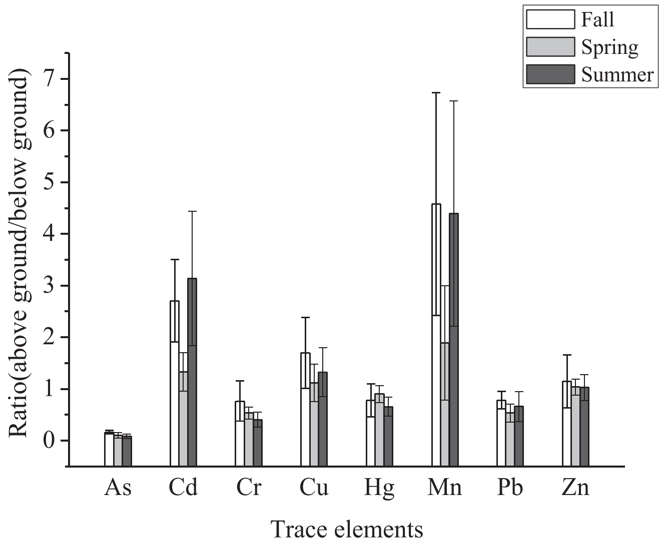

Fig. 5. Ratio of TE concentration in the above-ground seagrass biomass to TE concentration in the below-ground seagrass biomass, for each TE and season, averaged over the three sites. Results are presented as mean ± SD (n = 4).

latter hypothesis is partially supported by our observations of higher ratios of TE between above-ground to below-ground seagrass tissues for Cd and Mn (Fig. 5) when sediment load and water discharges are high (Fig. 2), as well as previous studies that showed that seagrass tissue can accumulate water column TEs within 4 weeks (Tupan and Azrianingsih, 2016). A clear next step is therefore to experimentally identify the temporal dynamics of seagrass TE bioaccumulation responses to TE in the sediment and the water column, as well as the subsequent translocation of these TEs between seagrass leaves, roots and rhizomes (Ahmad et al., 2015).

Table 4
Variability of TE concentration in the sediment, above-ground and below-ground seagrass tissues, for data from all sites and seasons measured, in *Z. japonica* in the Yellow River Estuary. Variability was calculated using Eq. (4).

Trace element	Variability of TE concentration		
	Sediment	Above-ground tissue	Below-ground tissue
As	37%	186%	146%
Cd	28%	188%	105%
Cr	15%	128%	148%
Hg	84%	137%	116%
Cu	25%	168%	124%
Mn	21%	163%	167%
Pb	24%	198%	139%
Zn	25%	127%	99%

Total TE concentration in sediment is not always directly related to that available in the sediment. Hence, the inconclusive relationship between sediment TE and seagrass TE might also be due to the fact that the available TE fraction is not always the same over the season (e.g. fresh deposited sediments with contaminants can have a different ratio of total TE to available TE than older deposits).

The present research also expands upon our previous study (Lin et al., 2016a) by demonstrating how seasonal dynamics affect conclusions about TE accumulation in seagrass tissue and sediment. Similarly to the previous study, sediment at sites closest to the mouth of Yellow River Estuary were more contaminated with TEs, and averaged over all sites and seasons the relative abundance of the eight TEs tested in the sediment was unchanged. However, the present study newly reveals that there is large seasonal variability in bioaccumulation of TEs by seagrass tissue. Therefore, it may not always be straightforward to determine *a priori* which TE will be most highly accumulated by

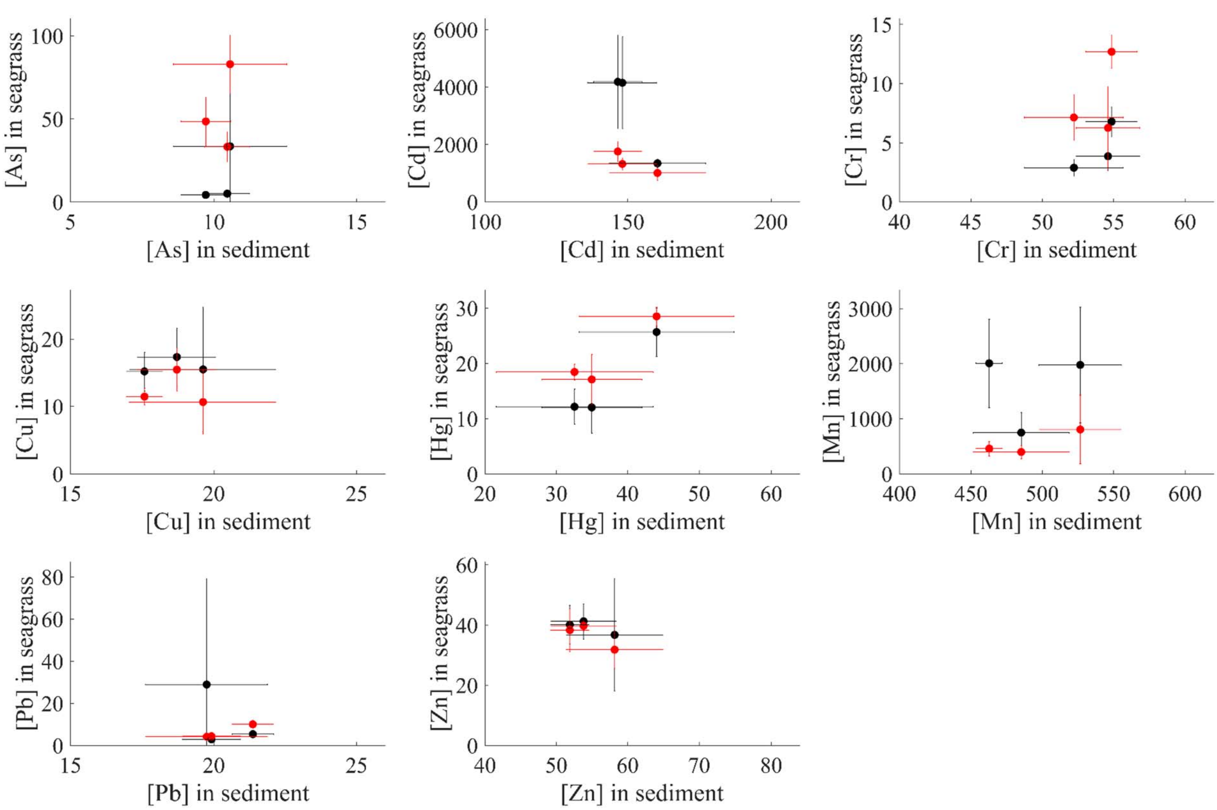

Fig. 6. Relationship between TE concentration in seagrass tissues (black = above-ground biomass, red = below-ground biomass) and TE concentration in the sediment. Each data point represents a different season, averaged across the three sites. All elements in μg g^{-1} DW, except Hg and Cd in ng g^{-1} DW. Results are presented as mean ± SD (n = 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Bioaccumulation properties of As, Pb, Cr, Cu, Mn, Hg, Zn and Cd in *Z. japonica* (this study) compared to studies in other seagrass species. A: Greater accumulation in above-ground tissue than below-ground tissue; B: Greater accumulation in below-ground tissue than above-ground tissue below-ground parts; A/B: no significant difference between above-ground and below-ground ($p > 0.05$) in our study, or no significant difference / unclear in other studies. Where no capital letter is presented, this indicates that this species/TE combination was not investigated.

Trace elements	This work	Other studies	
	Zostera japonica	*Zostera capricorni*	*Cymodocea nodosa*
Mn	A	A[a]	A[b]
Cu	A	A[a]	A/B[b]
Cd	A	B[a]	A[b]
Pb	A/B	B[a]	A/B[b]
Zn	A/B	B[a]	A[b]
Hg	B	–	–
As	B	B[a]	A/B[c]
Cr	B	–	B[c]

[a] Howley et al. (2006)
[b] Llagostera et al. (2011)
[c] Malea and Kevrekidis (2013)

seagrass.

4.2. Do TEs accumulate more in the shoots or roots of seagrass?

Taken together with other studies (Table 5), our results suggest that seagrass, regardless of species, may preferentially accumulate Mn in the above-ground tissue, and *Zostera* species may preferentially accumulate Cu and As in the above-ground and below-ground tissues respectively. Based on the seasonal patterns of Cd and Mn ratio between above-ground and below-ground compartments that we observed in *Z. japonica* (Fig. 5), one hypothesis to test for future work is: Does preferential uptake of TEs by above-ground or below-ground tissue indicate that seagrass TE bioaccumulation is more sensitive to TEs in the water column or sediment, respectively?

4.3. Cumulative risk of TEs to seagrass

In the Yellow River Estuary, the cumulative ecological risk posed by all sediment TEs considered together was assessed as "moderate" in all three seasons, due to the low variability in sediment TEs between seasons. Trace elements Cd and Hg were predicted to be of greatest ecological concern, and this conclusion was predicted from, and consistent between, two calculated indices of ecological risk. Of the eight TEs measured, Cd and Hg are also the TEs with the highest estimated toxicity (quantified by the factor T_r^i, see Table 2), so the concentrations of these two TEs in the Yellow River Estuary need to be carefully monitored in the future. When increasing amounts of TEs are imported into estuaries (Nath et al., 2014; Deycard et al., 2014), intertidal sediments may become seriously contaminated if effective measures are not taken to control this issue. Future work may include identification of the sources of these TEs, for example by additional spatial measurements of TEs further upstream in the Yellow River, to prevent TEs from causing future loss of the largest Chinese *Z. japonica* habitat.

However, our identification of Cd and Hg as posing the greatest ecological risk, is based on their concentration in the sediment, not their concentration in seagrass tissue. Future work could therefore also involve development of ecological risk indices based on bioaccumulation of TEs by the seagrass itself, as this may generate different conclusions regarding the ecological risk posed by environment TEs. More generally, different conclusions may be reached about which TEs are of most concern, depending on the criteria used to assess ecological risk (Lin et al., 2016a).

5. Conclusion

The variability we observed in the seasonal dynamics of TE concentrations, in the sediment and seagrass of the Yellow River Estuary, highlights the physiological complexity of seagrass as well as the temporally varying conditions in TE exposure faced by seagrass meadows in the natural environment. Certain TEs accumulate more greatly in above-ground than below-ground seagrass tissues, and vice versa, so identifying the risk to seagrass health of these accumulations is a necessary step to assist in protection of these aquatic habitats for the future.

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