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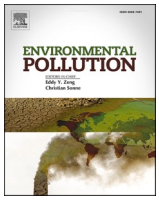

Trace element contamination biomonitoring: A comparative study between the polychaetes *Alitta virens* and *Hediste diversicolor*☆

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ABSTRACT

Trace elements (TEs) remain of significant toxicological concern as many are critical for global decarbonisation. TEs accumulate in sediments so benthic polychaetes (e.g. *Hediste diversicolor* and *Alitta virens*) are highly relevant for ecotoxicology. However, ecological/biological differences could influence TE accumulation and biomonitoring suitability. Exploiting multiple sympatric populations (Solent, UK), we measure sediment and tissue concentrations generating EFs (enrichment factors), AEIs (Adverse Effects Indexes) and tissue bioaccumulation factors. We also assess stable isotope compositions to elucidate diet influences. Despite diverse anthropogenic activity in the Solent, the majority of TEs present low levels of sediment contamination at the sites. For Ni, Pb and As, a combination of mean AEIs >1 and some sediment concentrations exceeding SQVs (Sediment Quality Values) indicate a slight toxicological risk. For Cu and Hg, high EFs and AEI scores confirm they are the greatest risk, thus requiring source identification/control. However, only mean As tissue concentrations reflect contaminated sites, therefore, identifying the As-source(s) is also a priority. Sediment and tissue concentration relationships were generally negative and not significant for both species. Although a significant negative relationship for Cd for *A. virens* requires further investigation, the lack of evidence for TE bioaccumulation from sediment may limit both species' biomonitoring suitability for low-contamination sites. Species differences in tissue concentration were also TE specific: *H. diversicolor* had significantly higher concentrations for Ag, Cu, Hg, Ni and Zn, whilst the reverse was true for Cd, Fe, Cr and As. Whilst ecological differences and that feeding sources are site and species-specific (as evidenced by C, N and S stable isotopes analysis) cannot be ignored, the diverse tissue concentrations strongly suggest different TE regulation strategies per species. Together these data will be important for ecotoxicologists and regulators to select the 'best' polychaete biomonitor and assess TE toxicity under future global decarbonisation trajectories for TE inputs.

1. Introduction

Trace elements (TEs) are often considered pollutants of the past, yet industrial use is still extensive with expanding applications (e.g. nanotechnology, ship emissions, offshore wind farms) causing significant amounts to enter the natural environment (Tourinho et al., 2012; Richir et al., 2021; Watson et al., 2024). In addition, as global energy decarbonisation is dependent on many TEs for cabling (e.g. Cu [copper]), batteries (e.g. Ni [nickel], Fe [iron], Cu) and photovoltaics (e.g. Pb [lead], Cd [cadmium]), the substantial increase in demand without concomitant recycling (currently, only 32% for Cu [ICA-Recycling brief, 2022]) will increase inputs, thus generating substantial future risks for marine biodiversity (Herbert-Read et al., 2022). With recent studies indicating stable or increasing concentrations in benthic systems (Watson et al., 2018; Richir et al., 2021), TEs remain of great toxicological concern.

Understanding the biological effects of contaminants is often through 'model' species. These are used to assess: a) the contamination levels of

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sites (i.e. as biomonitors) and b) the toxicological effects (i.e. using biomarkers at diverse organisational scales). Polychaetes are highly relevant as countless species live within the sediment and so are exposed to sediment-bound contaminants (Pocklington and Wells, 1992). Polychaetes are dominant contributors of infaunal biomass, acting as vectors for the transfer to higher trophic levels since they form the primary food source for many fish and crustaceans (Amara et al., 2001; Serrano et al., 2003). Finally, they can strongly influence sediment characteristics and bioavailability through bioturbation (Banta and Andersen, 2003).

Nereididae (ragworms) have been the focus of much ecotoxicological work (see Reish and Gerlinger, 1997; Lewis and Watson, 2012). In temperate, variable-salinity regions throughout the northern hemisphere, *Hediste diversicolor* is the primary species displaying the characteristics outlined above whereas in muddy sand of littoral/sublittoral zones *Alitta virens* dominates (Bass and Brafield, 1972; Kristensen, 1984). *H. diversicolor* is a surface-deposit feeder, herbivore, predator, filter-feeder and scavenger (Reise, 1979), whereas *A. virens* is an omnivorous macrophagous feeder (Nielsen et al., 1995). *A. virens* is less tolerant of sulphide, and can reach up to 900 mm in length (Wilson Jr et al., 1988) compared to the smaller (~80 mm) *H. diversicolor* (Scaps, 2002). These notable differences are likely to influence the interaction with TE contamination leading Pini et al. (2015) to posit that these two species differ in their responses to Cu and Zn (zinc) exposure via sediments. However, their study was limited by comparing against published *H. diversicolor* data. Although salinity/temperature fluctuations and high porewater sulphide content restrict the landward distribution of *A. virens* and competition restricts the seaward distribution of *H. diversicolor* (Kristensen, 1988; Miron and Kristensen, 1993), populations can still overlap (Miron and Kristensen, 1993; Papaspyrou et al., 2006). Exploiting this sympatry, we collect both species and sediment from the same or close-by sites from multiple locations in the Solent region of the UK. Thus, our objectives are to a) compare the sites and b) the two species for potential accumulation of ten of the most commonly studied TEs (Ag [silver], As [arsenic], Cd, Cr [chromium], Cu, Fe, Hg [mercury], Ni, Pb, and Zn). We then c) compare the relationships between tissue and sediment TE concentrations between the species. Measuring isotope ratios such as C (carbon) ($^{13}C/^{12}C$, hereafter $\delta^{13}C$) can inform the organic carbon source in the diet (Michener and Kaufman, 2007), whilst sulphur ($^{34}S/^{32}S$, hereafter $\delta^{34}S$) can help determine pelagic/benthic food source (Connolly et al., 2004). Finally, nitrogen ratios ($^{15}N/^{14}N$, hereafter $\delta^{15}N$) indicate a species' trophic position and nitrogen source (Michener and Kaufman, 2007; Layman et al., 2012). Understanding each species' trophic and feeding ecology is, therefore, crucial for our final objective of d) comparing the species' bioaccumulation and the routes of TE uptake. The data presented here will be important for ecotoxicologists and regulators to help select the 'best' polychaete biomonitor as well as increasing our understanding of TE accumulation in key benthic species especially as a number of these TEs are going to be of even greater concern in a decarbonised future.

2. Material and methods

2.1. Collection of samples

The Solent region of the UK supports large areas of intertidal mudflats (~6191 ha) (Tubbs, 1999). Whilst of high conservation value, it is heavily utilized both industrially and recreationally by a population of ~1.3 million. Sediments and both species (*H. diversicolor* [HD] and *A. virens* [AV] were sampled from seven sites (Chichester Harbour [CH], Langstone Harbour [LH], Portsmouth Harbour [PH], Isle of Wight [IOW], Western Solent [WS], Itchenor [IT], Warsash [WA] in June 2014 (Fig. 1). For CH, LH and WS sites, the two species were collected from two separate sites, but as close as possible (e.g. ~1 km [CH, LH] and ~10 km [WS]). Sites were selected to cover the expected range in contamination e.g. proximity to ports, large conurbations, industry and a national park (Table 1).

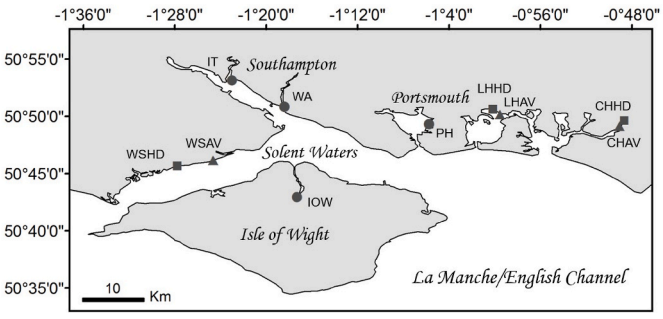

Fig. 1. Map of the site locations sampled for sediment and worm collection. Labels correspond with site descriptions (Table 1). Circles represent sites where both species were collected, squares and triangles represent *H. diversicolor* and *A. virens*, respectively.

At low tide, three sediment cores (15 cm deep, 10 cm diameter, spaced ~2 m apart) were collected from the mid-low shore and were immediately placed in polyethylene plastic bags. GPS coordinates were recorded using a Garmin eTrex with worms collected as close as possible to the sediment sampling location. At each site, an area of approximately 10–25 m^2 was selected, sediment was turned over (to a depth of ~20 cm) with a garden fork before worms were removed by hand. All samples were returned to the laboratory in isothermal containers within 4 h. Sediment cores were then immediately frozen at −20 °C, whilst worms were maintained in flow-through aquaria for 24 h to depurate their guts before freezing at −20 °C.

2.2. Sample processing and analysis

All equipment/benches were decontaminated in 5% HCl or in flow-through seawater for a minimum of 48 h. Defrosted sediment cores were weighed before being lyophilized until constant weight. Dry sediment samples were manually sieved with additional manual grinding before/between each sieving. Three different mesh sieves corresponding to four sedimentary fractions (>2, 0.5–2, 0.0625–0.5 and < 0.0625 mm) were used (Wentworth, 1922). Sediment fractions were weighed and their relative percentage contributions determined. All subsequent chemical analyses were performed with the <63 μm fraction.

Five replicates (five mid/large *A. virens* and five *H. diversicolor* for all TEs excluding Hg and stable isotope analysis which used 5 *H. diversicolor* pooled per replicate) from each site were ground in an agate mortar prior to analysis. Heads were dissected due to the major role of Zn in jaw hardening (Broomell et al., 2006). Weight percent organic matter in sediment was determined by the loss on ignition (Heiri et al., 2001). Prior to TE analysis (except Hg), tissue (100–200 mg) and sediment (100–200 mg) samples were mineralized in Teflon bombs in a closed microwave digester (Ethos D, Milestone Inc.). Digestion of tissue and sediment used HNO_3/H_2O_2 mineralization (Suprapur grade, Merck). For sediment it generates an efficient solubilization of the most important TE-bearing phases, but not the minerals (Bettiol et al., 2008). Samples were diluted to 50 ml prior to being analysed and sediment samples were further centrifuged (10 min at 2000 rpm) and the digestate removed to avoid readsorption processes. TE analyses (except Hg) were performed by Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, PerkinElmer®). An external standardization method was used for calibration (1 g l^{-1} TE standard solutions, Suprapur grade, Merck). For Hg, ground tissue and sediment were weighed and loaded into quartz boats previously conditioned at >400 °C for at least 15 min. Hg concentrations were determined after thermal decomposition of samples (pre-concentration by amalgamation with gold; up to three aliquots) by Atomic Absorption Spectrometry (AAS, Direct Mercury Analyser DMA-80, Milestone Inc.). All quantities of material were adjusted to fall within standard calibration curve concentrations (5–30 ng Hg) prepared from

Table 1
Site details, sediment (n = 3 cores) characteristics (PS: mean particle size, %OC: mean % organic content, % mud: mean % mud particles <63 μm; all with ±SEM and derived physical descriptions [Blott and Pye, 2001]), species sampled including mean wet weight ± SEM. Potential major anthropogenic influences in close proximity to site are also listed, STW: sewage treatment works.

Label	Site name	Coordinates	Species	Mean wet weight	PS (μm)	% OC	% mud	Descriptions (combined)	Potential anthropogenic influences
CHHD	Chichester Harbour	50.826855, −0.810894	HD	0.10 ± 0.01	48.1 ± 3.7	4.9 ± 0.1	24 ± 14	Very coarse silt	Downstream of STW, in agricultural area
CHAV		50.818811, −0.817909	AV	5.3 ± 0.6	486 ± 10	7.9 ± 0.1	3.3 ± 1.9	Medium sand	Downstream of STW, in agricultural area, in front of sailing club
LHHD	Langstone Harbour	50.843517, −1.00224	HD	0.072 ± 0.005	640 ± 76	8.6 ± 0.3	9.0 ± 5.2	Medium/coarse sand	Close to STW and industrial area
LHAV		50.835926, −0.99202	AV	8.9 ± 2	492 ± 11	7.4 ± 0.1	13 ± 7.5	Medium/coarse sand	Close to STW and industrial area
PH	Portsmouth Harbour	50.821563, −1.095615	HD AV	0.11 ± 0.01 6.9 ± 0.5	33.6 ± 2.7	7.6 ± 0.1	35 ± 20	Very coarse/coarse silt	Close to city, ship dismantling area, naval base and port
IT	Itchenor	50.885484, −1.382732	HD AV	0.15 ± 0.02 5.7 ± 0.6	384 ± 74	9.8 ± 0.1	5.2 ± 3.0	Fine/medium sand	Confluence of rivers, close to city and passenger/commercial ports
WA	Warsash	50.847087, −1.306165	HD AV	0.17 ± 0.02 8.6 ± 2	47.9 ± 0.40	5.9 ± 0.1	23 ± 13	Very coarse silt	Confluence of rivers, close to city and opposite oil refinery
IOW	Isle of Wight	50.715634, −1.287941	HD AV	0.15 ± 0.02 6.6 ± 0.6	177 ± 23	11 ± 0.1	8.2 ± 4.7	Fine sand	Close to town and agricultural area
WSHD	Western Solent	50.760593, −1.462553	HD	0.076 ± 0.001	690 ± 100	8.5 ± 0.5	1.2 ± 0.7	Medium/coarse sand	In agricultural area and close to national park
WSAV		50.769018, −1.410469	AV	4.3 ± 0.6	121 ± 23	7.2 ± 0.2	14 ± 8.0	Very fine/fine sand	In agricultural area and close to national park

100 ng l^{-1} standard solutions (Suprapur grade; Merck). Accuracies of analytical methods (ICP-MS and AAS) were evaluated using blank samples and CRMs (Certified Reference Materials): NIST 1566b (oyster tissue), NIST 2976 (mussel tissue), NIST 1577c (bovine liver) and PACS-2 (marine sediment). For each TE, detection decision (L_C), detection limit (L_D) and quantification limit (L_Q) were calculated according to Currie (1999) or Grinzaid et al. (1977), depending on their specific blank distribution. The mean recoveries of certified concentration values were, for ICP-MS analyses (n = 3 by CRM; mean ± standard deviation): 99 ± 11 % for NIST 1566b, 100 ± 15 % for NIST 1577c, 108 ± 7 % for NIST 2976 and 78 ± 36 % for PACS-2 (partial recovery for several PCAS-2 elements because of partial extraction method; see Richir et al., 2013; Townsend et al., 2007); for DMA analyses (n = 10–11 by CRM): 101 ± 8 % for NIST 1566b, 108 ± 6 % for NIST 2976, and 96 ± 12 % for PACS-2. Data were analysed as TE concentrations on a dry weight basis and are expressed in μg g^{-1} (tissue) and mg kg^{-1} (sediment).

For isotope analysis ground worm and sediment were weighed in tin cups and dried at 50 °C (48 h). $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ isotopic tissue composition was determined using an elemental analyser (Vario MICRO Cube, Elementar, Germany) coupled with a continuous-flow isotope-ratio mass spectrometer (Isoprime 100, Elementar, Germany). Data were expressed in the δ notation (‰) (equation (1), where X is the heavy isotope [^{13}C, ^{34}S, ^{15}N], x is the lighter isotope [^{12}C, ^{32}S, ^{14}N] and [X/x]$_{sample}$ and [X/x]$_{standard}$) are the ratios of both stable isotopes in the sample and the international standard: Vienna Pee Dee Belemnite for $\delta^{13}C$, N_2 in atmospheric air for $\delta^{15}N$, and Canyon Diablo troilites for $\delta^{34}S$.

$$\delta X = \frac{X/x_{sample} - X/x_{standard}}{X/x_{standard}} \; x\,1000 \quad (1)$$

CRMs from the IAEA (International Atomic Energy Agency), IAEA-C6 (sucrose; $\delta^{13}C = -10.8 \pm 0.5$‰), IAEA-N2 (Ammonium sulphates, $\delta^{15}N = +20.3 \pm 0.3$‰) and IAEA-S2 (silver sulphide; $\delta^{34}S = +22.7 \pm 0.2$‰), were included in the batch. Laboratory sulfanilic acid (Sigma-Aldrich; $\delta^{13}C = -28.5 \pm 0.3$‰; $\delta^{15}N = -0.4 \pm 0.3$‰; $\delta^{34}S = 1.3 \pm 0.5$‰) was used as quality control every 10 samples. Replicated measurements of worm tissue and sediment pools spread every 10 samples provided standard deviations of ±0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, and ±0.5‰ for $\delta^{34}S$.

2.3. Data processing and statistical analyses

Data were analysed using Minitab v22 and, if required, transformed/ranked to meet parametric assumptions (see supplementary tables). Particle size analysis was performed using Gradistat Ver. 8.0 (Blott and Pye, 2001) with the geometric Folk and Ward (1957) method generating a mean particle size, percentage <63 μm fraction and sediment descriptors. The sieving method did not separate the <63 μm proportion into smaller fractions. Therefore, these were calculated with this size fraction specified at 1 μm; representing the whole fraction. Sites were compared for sediment characteristics using one-way ANOVAs followed by a Tukey HSD pairwise test of means. TE Enrichment Factors (EFs) were generated for each TE/site combination (except Fe and Ag) using the methods of Celis-Hernandez et al. (2020) with the measured Al (aluminium) concentration in each sample taken to normalise the data. Background concentrations were from the North Atlantic region (OSPAR, 2008) with EFs divided into ranks according to the scheme of Birth et al. (2003). AEIs (Adverse Effects Indexes) were calculated according to Muñoz-Barbosa et al. (2012) by dividing the mean TE sediment concentration by the Threshold Effect Level (TEL) of Long et al. (1995). Details of AEI and EF calculations are summarised in Table S1. Mean sediment and tissue TE concentrations were correlated using Spearman's ranks. Comparisons of mean TE tissue concentration were performed using GLMs (General Linear Models) with species and site as fixed factors followed by a Tukey HSD pairwise test of means. If the sediment concentration of the sites that had different sampling locations for each species (CH, LH and WS) were not significantly different (two-sample T tests) then the site was included in the species comparison GLM. Species BCFs (Bioconcentration Factor: mean tissue concentration divided by mean sediment concentration per site [USA EPA, 2010; Regoli et al., 2012]) were compared using two sample T-tests.

3. Results

3.1. Site characteristics, TE concentrations, EFs and AEIs

Sediments from the ten sites were all very or extremely poorly sorted, with diverse skewness (e.g. fine [IOW] to very coarse [PH]) and kurtosis (e.g. very platykurtic [IT] to extremely leptokurtic [WSAV]). Calculated mean particle sizes ranged from 34 μm (PH) to 690 μm (WSHD) with the smaller mean particle sizes generally linked to a higher percentage

organic and mud content (<63 μm). However, this association was not always strong, for example, LHHD had a mean particle size of 640 μm with 9% defined as mud, whereas WSHD had a mean particle size of 690 μm with only 1% mud (Table 1). Unsurprisingly, one-way ANOVAs and subsequent pairwise comparisons confirm that there were significant differences between the sites for all key sediment parameters, see Table S2, that together reflect the key summary descriptions presented: all site descriptions fall between medium/coarse sand and coarse/very coarse silt.

There were significant differences between TE concentrations across sites for sediment as detailed in Table S3. A holistic view using sites ranked by TE concentration indicates that IOW, IT and CHAV had higher contamination levels than WSAV, WSHD and CHHD, but divergence from this broader ordering was observed depending on the TE. For example, WA had the second highest mean sediment concentrations for As, but had the lowest Pb concentration.

Calculated EFs for each site/TE combination except Fe and Ag (no background concentrations are available) are presented in Fig. 2. All sites exhibited no or minor enrichment for Cr and Ni and only IOW was just moderately enriched for Zn. WSHD, WSAV and WA were moderately enriched for As, whilst IOW, CHAV, CHHD were moderately enriched for Pb with WSAV and IT being moderately/severely enriched for Pb. LHHD was distinct from the other nine sites for Cd with an EF score of 6.4, placing it in the moderate/severe enrichment zone compared to minor enrichment or lower (EF: <3) for all other sites. For Cu, all sites had moderate or moderate/severe enrichment with CHAV approaching the very severe threshold (EF: 8.5). Enrichment for Hg was variable between sites, for example the very severe enrichment for IT (EF: 27.4) compared with WSHD (EF: 3.7). Other sites were also enriched: both Chichester sites (CHHD and CHAV) were severely enriched, whilst LHHD, LHAV and IOW were all moderately/severely enriched. None of the sites had a mean AEI >1 for Ag, Cd and Cr, whilst only IOW had an AEI >1 (1.47%)

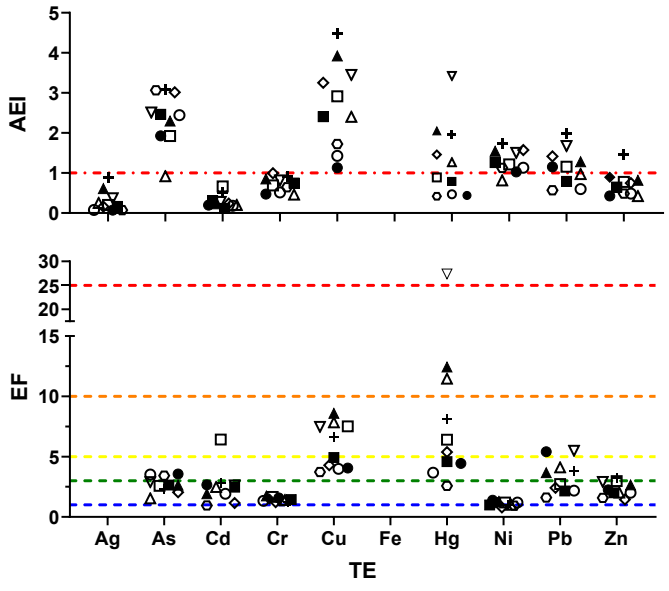

Fig. 2. Top: mean AEI (Adverse Effects Index) score per site for TEs. If AEI >1 (above red dashed line) TE sediment concentrations are high enough to produce adverse effects in biota (Muñoz-Barbosa et al., 2012). Bottom: mean sediment EF (Enrichment Factor) per site for TEs with coloured lines representing degree of enrichment (<1: not enriched [blue], 1–3: minor [green], 3–5: moderate [yellow], 5–10: moderate to severe [orange], 10–25: very severe [red], 25–50: extremely severe [line not shown]). There were no background concentration data for Ag or Fe to generate EFs and no Fe TEL to generate AEI. CHHD: Δ, CHAV: , LHHD: □, LHAV: , PH: ◊, IT: , IOW: +, WSHD: ○, WSAV: ●, WA: . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

for Zn. In contrast, for Cu, As and Ni all sites had AEIs >1 except for CHHD (As and Ni). CHAV, LHHD, PH, IOW, WSAV and IT all had a Pb AEI >1, whilst six sites (CHAV, CHHD, PH, IOW, WSAV and IT) had AEIs >1 for Hg.

3.2. TE tissue concentrations

Using the mean sediment and tissue concentrations per site, the relationships between tissue and sediment concentration for each TE were explored using Spearman's rank correlations. The only significant (strongly negative) correlation (Table 2) identified was Cd for *A. virens*, although there were strong negative correlations for Ag for both species with the relationship for *A. virens* just not significant ($p = 0.052$). Relationships for all TEs were negative for *H. diversicolor* except As, whilst a more diverse response was present for *A. virens* with Cr, Cu, Fe, Hg, Pb and Zn all positive and As and Ni negative.

Mean tissue concentrations (± SEM) of each TE per species and also separated by site are presented in Fig. 3 and supported by Table S4. Mean Ag tissue concentrations for both species per site were between 0.047 μg g^{-1} and 0.28 μg g^{-1} with a maximum individual concentration of 0.39 μg g^{-1} recorded at WA (*H. diversicolor*). Mean tissue concentrations for Cd were in a similar range (0.017–0.19 μg g^{-1}), but were generally less variable with a maximum of 0.25 μg g^{-1} in one *A. virens* from WS. Mean Fe tissue concentrations varied little across sites and species (minimum: 302 μg g^{-1} and maximum: 1100 μg g^{-1}), although one *A. virens* from IT had a tissue concentration of 4200 μg g^{-1}. Mean tissue concentrations in both species for Pb varied between 0.23 μg g^{-1} (*H. diversicolor*, IT) and 2.1 μg g^{-1} (*H. diversicolor*, WA) across sites, whilst mean Cr tissue concentrations were between 0.06 and 1.2 μg g^{-1} for all site/species combinations. Mean tissue concentrations ranged less dramatically for Ni with 2.71 μg g^{-1} (*H. diversicolor*, WA) the highest and 0.78 μg g^{-1} (*A. virens*, IOW) the lowest. For Zn, mean tissue concentrations did not differ greatly between sites and species with a minimum of 56 μg g^{-1} and maximum of 122 μg g^{-1}. Mean Cu tissue concentrations for both species were between 6.9 (lowest) and 47.2 μg g^{-1} (highest) recorded at WA (*H. diversicolor*). Finally, mean As and Hg tissue concentrations did not vary greatly across sites or between species with a range of 7.3–38.7 μg g^{-1} and 0.037–0.088 μg g^{-1}, respectively.

Summaries of the statistical analyses comparing the two species' tissue concentrations and BCFs are shown in Table 3. These confirm that significant differences exist between sites for all TEs except Fe and Zn. Generally, worms from WA and WS had the highest concentrations and those from IOW had the lowest. However, pairwise comparisons for TEs where sites were significantly different as shown in Table S5, confirm that sites clustered in different and more nuanced ways depending on TE.

Zn, As and Ag grouped together; having the highest mean BCFs across both species ranging from 0.618 to 1.284, but these are also joined by Cd for *A. virens*. A second intermediate value group of Cu and

Table 2
Correlation (Corr.) strength and direction (value of coefficient) and statistical significance ([Sig.] value bolded for <0.05) for mean sediment and tissue TE concentrations for each species (HD: *H. diversicolor*, AV: *A. virens*). Correlations performed using Spearman's ranks (all 7 sites included per species).

TE	Species	Corr.	Sig.	Species	Corr.	Sig.
Ag	HD	−0.679	0.094	AV	−0.750	0.052
As	HD	0.429	0.337	AV	−0.607	0.148
Cd	HD	−0.357	0.432	AV	**−0.857**	**0.014**
Cr	HD	−0.286	0.535	AV	0.107	0.819
Cu	HD	−0.500	0.253	AV	0.214	0.645
Fe	HD	−0.179	0.702	AV	0.571	0.180
Hg	HD	−0.607	0.148	AV	0.536	0.215
Ni	HD	−0.250	0.589	AV	−0.429	0.337
Pb	HD	−0.143	0.760	AV	0.286	0.535
Zn	HD	−0.107	0.819	AV	0.393	0.383

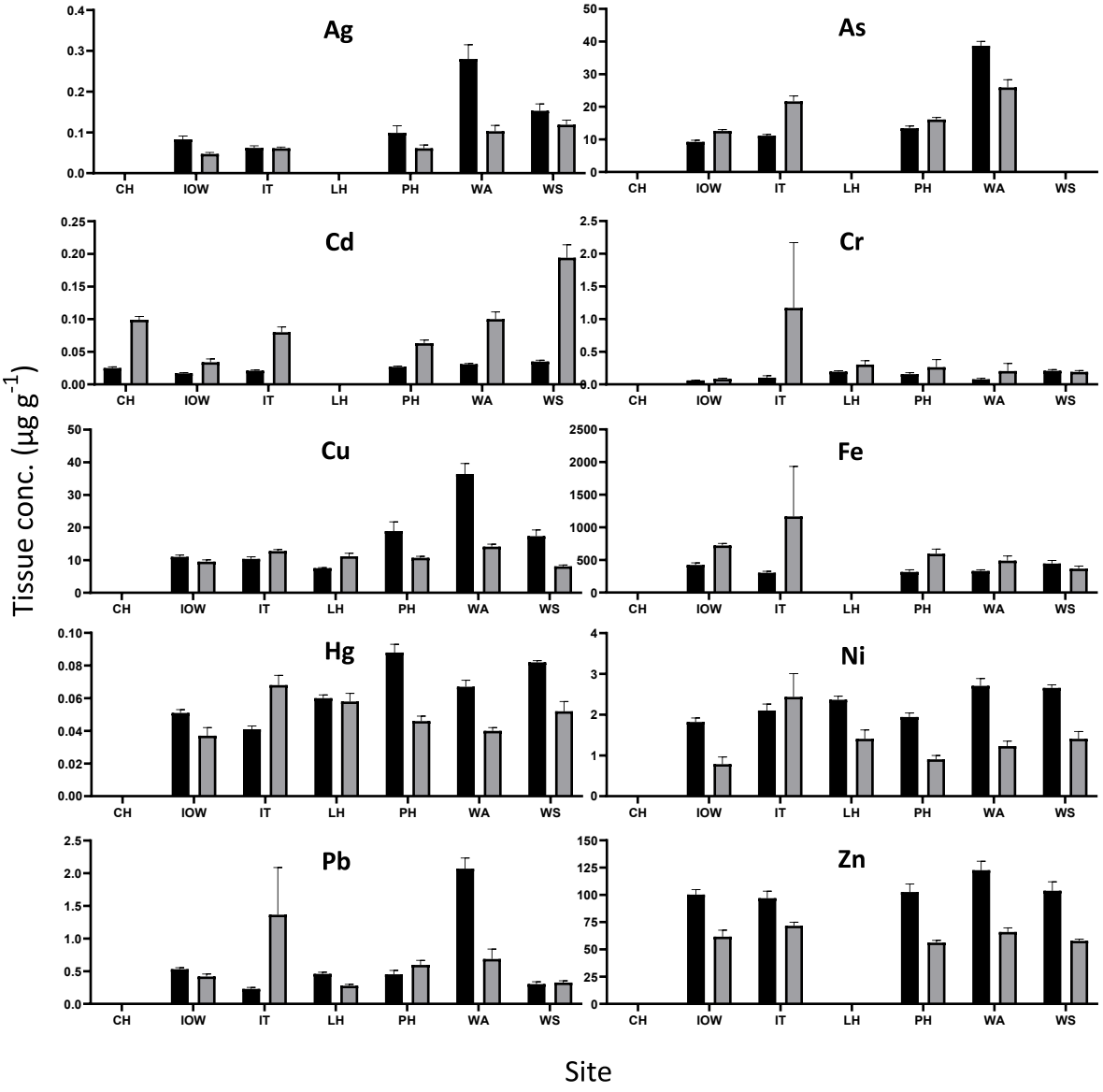

Fig. 3. Mean TE tissue concentration (µg g^{-1} ± SEM) for each species (black bar: *H. diversicolor*, grey bar: *A. virens*) and site (x axis). For CH, LH and WS sites (species collected from two separate sites, but as close as possible), were excluded from the analysis if they had significant differences in sediment TE concentration. Associated summary data and statistical analyses are presented in Table 3.

Table 3
Mean TE tissue concentration (µg g^{-1}) and BCF (Bioconcentration Factor) values for each species (HD: *H. diversicolor*, AV: *A. virens)*. TE tissue comparisons were performed using GLMs; statistically significantly higher ($p < 0.05$) value bolded. For each TE only those close-to-each sites (CH, LH and WS) which did not have significant differences in sediment TE concentration were included in the analysis.

TE	AV conc.	HD conc.	Species sig.	Sites included in analysis	Site sig.	BCF AV	BCF HD	BCF sig.
Ag	0.078	**0.136**	$p < 0.0001$	PH, IT, WA, IOW, WS	$p < 0.0001$	0.618	1.036	$p = 0.583$
As	**19.1**	18.1	$p < 0.0001$	PH, IT, WA, IOW	$p < 0.0001$	1.14	0.854	$p = 0.259$
Cd	**0.095**	0.026	$p < 0.0001$	CH, PH, IT, WA, IOW, WS	$p < 0.0001$	**0.639**	0.176	$p = 0.026$
Cr	**0.369**	0.134	$p < 0.0001$	LH, PH, IT, WA, IOW, WS	$p < 0.0001$	0.011	0.004	$p = 0.080$
Cu	11.1	**16.9**	$p < 0.0001$	LH, PH, IT, WA, IOW, WS	$p < 0.0001$	0.256	0.405	$p = 0.403$
Fe	**667**	364	$p < 0.0001$	PH, IT, WA, IOW, WS	$p = 0.078$	0.021	0.014	$p = 0.084$
Hg	0.050	**0.065**	$p < 0.0001$	LH, PH, IT, WA, IOW, WS	$p = 0.007$	0.425	0.599	$p = 0.445$
Ni	1.36	**2.27**	$p < 0.0001$	LH, PH, IT, WA, IOW, WS	$p < 0.0001$	0.065	**0.112**	$p = 0.014$
Pb	0.612	0.675	$p = 0.583$	LH, PH, IT, WA, IOW, WS	$p < 0.0001$	0.020	0.027	$p = 0.798$
Zn	62.7	**105.2**	$p < 0.0001$	PH, IT, WA, IOW, WS	$p = 0.08$	0.734	**1.284**	$p = 0.041$

Hg (both species) and Ni and Cd (*H. diversicolor*), whilst mean BCFs for Pb, Cr, Fe for both species, and Ni for *A. virens* were at least an order of magnitude lower. The TE-specific nature of tissue concentration differences was also reflected in comparisons between the two species. *H. diversicolor* had significantly higher mean tissue concentrations for Ag, Cu, Hg, Ni and Zn, whilst the reverse was true for As, Cd, Cr and Fe

with no significant difference for Pb (Table 3 and Table S4). The magnitude of the significant differences was TE-specific: the As concentration in *A. virens* was only 6% higher (mean 18.1 μg g^{-1} v. 19.1 μg g^{-1}), whilst the mean Cd concentration ~500% higher in *A. virens* (mean 0.02 μg g^{-1} v. 0.09 μg g^{-1}). Despite these significant differences in mean tissue concentration for eight of the ten TEs, only Cd (*A. virens*), Ni (*H. diversicolor*) and Zn (*H. diversicolor*) had mean BCFs that were significantly higher in one species.

3.3. Isotopic compositions

Fig. 4a and b presents the isotopic compositions for both species. Isotopic compositions (in ‰, n = 35) varied from 8.5 to 13.8 (mean ± SD, 11.1 ± 1.6), from −22.6 to −13.8 (−18.1 ± 2.1) and from 12.5 to 19.0 (16.5 ± 1.5) for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values, respectively for *A. virens*. For *H. diversicolor*, isotopic compositions (in ‰, n = 35) varied similarly: from 7.8 to 13.6 (mean ± SD, 10.9 ± 1.5), from −25.0 to −16.4 (−19.7 ± 2.7) and from 12.0 to 20.5 (16.4 ± 2.5) for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values, respectively. Despite some large ranges for each species per site, the isotopic variability was relatively low (i.e. SD generally below 0.5 for $\delta^{15}N$ and $\delta^{13}C$ and 1.0 ‰ for $\delta^{34}S$ values). There were

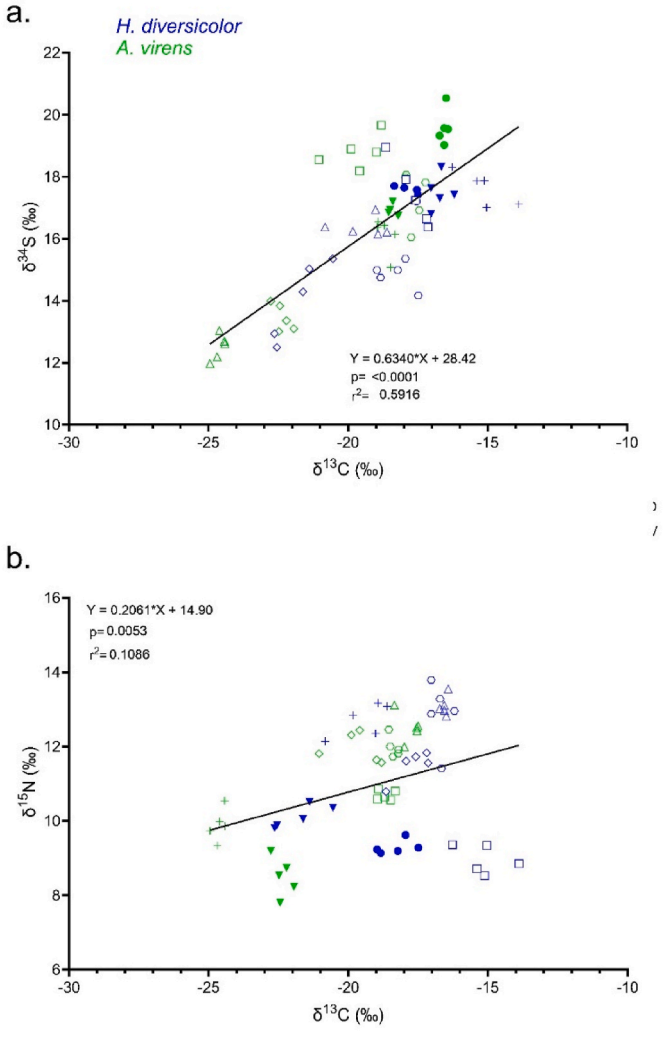

Fig. 4. Isotopic compositions measured (mean ± SD) in *A. virens* and *H. diversicolor* collected in different sites (n = 5 per site and per species). 3a. $\delta^{34}S$ vs. $\delta^{13}C$ values, 3b. $\delta^{15}N$ vs. $\delta^{13}C$ values. Lines represent linear regression model. CH: Δ, LH: □, PH: ◊, IT: , IOW: +, WS: ●, WA: . Green: *A. virens*; Blue: *H. diversicolor*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

significant linear relationships between $\delta^{13}C$ vs. $\delta^{34}S$ values and between $\delta^{13}C$ vs. $\delta^{15}N$ values when the full data set was used (n = 70). Generally, the species did not share the same isotopic space at each site. Nevertheless, there were no obvious trends in the occupation of this isotopic space i.e. a species displaying consistently higher (or lower) $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values than the other and no consistent pattern according to the sampled site for the three investigated isotopic ratios (i.e. the two species did not covary according to site).

4. Discussion

4.1. TE site contamination

The Solent region contains varied coastal habitats and this is reflected in the diverse characteristics of the sites (Table 1). A combination of numerous historic and contemporary TE input sources will inevitably result in significant differences in TE sediment concentrations across sites. Considering the Solent is home to commercial ports, a naval base, many industries and ~1.3 million people it is pleasing to see that, for most TEs, concentrations are relatively low compared to other industrial coastal areas (e.g. Hamdoun et al., 2015; Rainbow, 2018). Although we recognise the limitations of using EFs highlighted by Li et al. (2021), the relatively low concentrations translate to EFs that are moderate or lower for the majority of the sites assessed. For example, LHAV, PH, WA, and IOW showed no evidence of Ni contamination as their EF scores were <1. One site (LHHD) had a noticeably elevated Cd concentration resulting in a mean moderate/severe enrichment score (6.4). LHHD is located close to a STW (Sewage Treatment Works), however, as LHAV (only ~1 km away) had an EF of 2.4, the specific Cd source requires further investigation. Two sites (WSAV and IT) also had elevated mean sediment Pb concentrations leading to moderate/severe enrichment. Pb contamination can be from diverse current and historic inputs (Pacyna et al., 2009), but it is conjecture to implicate the putative sources suggested by Cundy and Croudace (2017) as the sites closer to these sources were not as enriched. Cu is released from many activities (Bhuiyan et al., 2018; Jensen et al., 2016) as well as Solent-specific sources, e.g. scrap metal processing (Cundy and Croudace, 2017). Yet, as all sites were moderately or moderately/severely enriched this would indicate region-wide contamination. The dominance of vessels using Cu-based anti-fouling coatings (inputting 94 t yr^{-1} of Cu into the Solent [Richir et al., 2021]) would seem to be an obvious cause. Given that we posit elevated Cu EFs are driven by the high vessel numbers, it is surprising that Zn EFs were low as Richir et al. (2021) estimated a mean yearly vessel input of 376 t. Declines in UK atmospheric emissions (Defra and BEIS, 2019) may have balanced these inputs to some extent, but the low Zn EFs remain unexplained. Hg also had surprisingly high site EFs with one (IT) representing very severe contamination. Historic sources, for example early anti-fouling paints containing HgO (Yebra et al., 2004) and leaching from coastal municipal waste dumps (Rainbow, 2018) could be responsible. Finally, although Fe and Ag EFs could not be calculated due to a lack of reference data, comparisons with data collated by Rainbow (2018) would not indicate any obvious enrichment for these TEs.

According to Muñoz-Barbosa et al. (2012) AEIs with a score of >1 indicate that a TE concentration is high enough to produce adverse effects in biota. More formalised Sediment Quality Guidelines set SQVs (Sediment Quality Values). Our data can be compared to the recently revised ANZECC/ARMCANZ SQVs (Simpson et al., 2013). Below the ERL (Effects Range Low) adverse effects rarely occur, between the ERL and ERM (Effects Range Median) adverse effects may occasionally occur and above the ERM adverse effects are frequent. As the mean sediment concentrations for Ag, Cd and Cr for all sites are below the ERL and the AEIs were <1, sediments pose a very low ecological risk to biota for these TEs. Mean Zn concentrations for all sites were also < ERL and with all but one having an AEI <1; confirming that Zn contamination levels pose minimal ecological risk. For Ni, Pb and As, the majority of sites had

mean AEIs >1 with some concentrations exceeding the ERL indicating possible biota effects. Nevertheless, for these TE, none of the mean sediment concentrations are close to the ERM indicating a relatively small risk. However, it would still be important to understand the anthropogenic inputs, as Celis-Hernandez et al. (2022) suggested that As and Ni were supplied by lithogenic sources.

Mean Hg sediment concentrations exceed the ERL (0.15 mg kg^{-1}, Simpson et al., 2013) for five sites. None of the mean concentrations are close to the ERM (10 mg kg^{-1}), but as the five sites also had AEIs >1, Hg does pose a potential risk to biota. The mean sediment concentrations of Cu also exceed the ERL (65 mg kg^{-1}) for IOW and CHAV and are very close for IT and PH. Combined with the >1 AEI scores for all sites, Cu, therefore, poses the greatest risk to Solent biota. Compared to other contaminated sites across the UK (Rainbow, 2018) the level of contamination is still low. Nevertheless, Watson et al. (2018) showed that exposure of *A. virens* to sediment spiked with Cu induces DNA damage at concentrations close to or below the ERL, thus reaffirming the need for a multiple screening approach (Simpson et al., 2013). SQVs are also unable to account for cumulative and synergistic effects of multiple TEs.

4.2. Bioaccumulation of TEs

It is of little surprise that mean tissue concentrations were significantly different between sites, driven by sediment characteristics and other factors (discussed below). The mean concentrations recorded for each TE (except Fe) can be compared to other studies for *H. diversicolor* (see Rainbow, 2018) and *A. virens* (Pini et al., 2015) and reflect typical ranges of uncontaminated/low contamination sites for all TEs except As. Mean As tissue concentrations (*H. diversicolor* [WA], *A. virens* [CHAV, WSAV, IT and WA]) place within Rainbow's high range (20–250 mg kg^{-1}). Whilst Rainbow (2018) states that these ranges should be considered working hypotheses, it is concerning that these concentrations are atypically high and indicative of raised local sediment bioavailabilities. Despite the sites having low AEI scores for As, combining the high tissue concentrations with mean sediment concentrations that are close to or exceed the ERL would indicate that As also poses a toxicological risk for both species in these Solent sites. Identifying the source(s) of this TE is, therefore, a priority for further investigation, however, for most polychaete species examined the majority of their arsenic is present as arsenobetaine (see references within Waring and Maher, 2005). It is unlikely that polychaetes accumulate arsenobetaine directly from interstitial water and/or sediments, as it has been reported to be readily degraded in marine sediments (Hanaoka et al., 1992). Instead, arsenobetaine and other organic arsenic compounds are probably accumulated through the ingestion of macroalgae, benthic diatoms and/or phytoplankton as they contain high proportions of phosphate arsenoriboside (Edmonds and Francesconi, 2003). This may also contribute to the lack of significant correlations between sediment and tissue for both species seen here.

Many studies have investigated the relationships between sediment and tissue concentrations for polychaetes showing a positive (e.g. Bryan and Hummerstone, 1979; Muller-Karanassos et al., 2019; Rainbow et al., 2009; Zhou et al., 2003) but also weak or absent relationship (e.g. Amiard et al., 2007; Berthet et al., 2003; Garces and Costa, 2009; Otero et al., 2000; Pini et al., 2015). Rainbow (2018) states that *H. diversicolor* is a TE net accumulator. Nevertheless, our correlation data would indicate the contrary, as only As displayed a positive correlation. Some of these negative relationships are strong (e.g. Ag, −0.679), however, as none of the correlations were significant and the BCFs were low or extremely low we must exercise caution in drawing conclusions. This also applies to the majority of sediment/tissue relationships for *A. virens* (As, Cr, Cu, Fe, Hg, Ni, Pb and Zn), which were more varied in strength and direction, but were also not significant. This lack of support for accumulation of TEs from sediment by *H. diversicolor* and *A. virens* may be because the range of each TE concentration across the Solent sites is narrow, unlike previous studies which have used *H. diversicolor* collected from highly contaminated sediment (up to ~50x the TE concentration recorded here) (Rainbow, 2018). The bioavailability of the TEs within the sediment is also important (Rainbow, 2018) as is the size of worm, as a number of studies have confirmed negative relationships between worm weight and tissue concentration (Poirier et al., 2006; Garces and Costa, 2009; Pini et al., 2015). In contrast, the strong, negative relationship for Cd (highly significant) and Ag (just not significant) for *A. virens* require a detailed investigation into this species' uptake, regulation and detoxification processes for these two TEs. For example, dominant uptake routes for Cd accumulation in polychaetes have been reported to be either the dissolved phase (Zhou et al., 2003) or food (Selck and Forbes, 2004) before utilising Ca transport pathways across the integument (Li et al., 2012). In contrast, Ag does not seem to accumulate in tissues of *H. diversicolor* from sediment spiked with Ag in either aqueous or nanoparticle form (Cong et al., 2011).

4.3. Species-specific TE bioaccumulation

The data summarised in Table 3 confirm that closely related polychaete species diverge in their ability to accumulate/excrete the ten most commonly investigated TEs in coastal habitats. The differences in tissue concentration were also TE specific: *H. diversicolor* had significantly higher mean tissue concentrations for Ag, Cu, Hg, Ni and Zn, whilst the reverse was true for As Cd, Cr and Fe with no significant difference for Pb. Apart from As, all the TE tissue concentrations from the Solent sites reflect areas that are uncontaminated or have low contamination (Rainbow, 2018), however, the magnitude of some of the differences is still large. For example, Cd concentrations were ~500% higher in *A. virens* with the larger the difference generally aligning to a significant difference in TE BCF also.

TE tissue concentrations are dependent on the environmental conditions that help determine bioavailability. Collecting both species from the same site provides, at least superficially, the same TE concentration in the sediment and overlying water. It should also minimise some of the broader environmental influences such as sediment characteristics. Nevertheless, observations confirm that large *A. virens* were always found 20–30 cm below the surface as opposed to *H. diversicolor* which were routinely collected from the top 5–10 cm. This vertical separation may enable *A. virens* to tolerate these ostensibly euryhaline sites (Kristensen, 1988). *A. virens*' larger size and stronger musculature would also enable it to burrow more deeply in sediments. The consequence of this vertical stratification is that even at the same sites, the species are likely to encounter different particle sizes, pore water and organic content levels, all known to drive TE bioavailability (Rainbow, 2018).

The two species are also very different in terms of their ecology and biology, with the most obvious difference being biomass (Table 1). TE polychaete tissue concentrations vary with body mass with Garces and Costa (2009) identifying negative relationships for Pb, Zn and Fe in *Marphysa sanguinea* suggesting this was due to growth dilution. However, due to the pooling of replicates for *H. diversicolor* comparisons between species cannot be explored with our data. Diet is an obvious TE source, but in spite of *H. diversicolor's* diverse feeding methods the majority of bioaccumulated As, Cu and Pb is from ingested sediment (Bryan, 1984) with biodynamic modelling also predicting 99% of Cd and 98% of Zn (Rainbow et al., 2009, 2011). Considering that the correlations between sediment and tissue concentration for *H. diversicolor* were negative for all TEs (except As), worms within the Solent may rely on other feeding methods (Reise, 1979; Riisgard, 1991). Feeding dominated by filter feeding would provide a different TE source (González-Dávila, 1995). Scavenging (Gross, 1921; Copeland and Wieman, 1924), and predation, including eating *H. diversicolor* (Rainbow et al., 2006) are also feeding methods for *A. virens*. Rainbow et al. (2006) showed that *A. virens* bioaccumulated Cu, Zn, Pb and Cd after consuming *H. diversicolor* from sites with elevated TE contamination.

Contrary to our initial belief, the larger *A. virens* did not always

display a higher trophic level than *H. diversicolor*. Indeed, relative position of $\delta^{15}N$ values, an indicator of trophic position (Michener and Kaufman, 2007), between the two species switched according to site (e.g. IT vs. LH; Fig. 4b). More likely, the two species adapt their diet to local conditions, reflecting environments differing in particle sizes and food-web functioning; consistent with plastic trophic ecology (e.g. Reise, 1979; Nielsen et al., 1995). According to their $\delta^{15}N$ values, the two species occupy low (i.e. detritivore, herbivore) to intermediate (i.e. omnivorous) trophic positions in the food web which varied according to site. Whatever their relative trophic position, in a given site, the two species did not generally occupy the same isotopic space, meaning in one given site, they did not share the same trophic resources (Layman et al., 2012). On the other hand, variability of $\delta^{13}C$ and $\delta^{34}S$ values indicate that the resource at the food web basis changed from one site to another (Fig. 4a). Indeed, here, the $\delta^{13}C$ and $\delta^{34}S$ values are highly correlated and can be used as basal source indicators. High $\delta^{13}C$ and $\delta^{34}S$ values could be related to organic matter coming from the water column and/or microphytobenthos, whilst low $\delta^{13}C$ (up to −24‰) and relatively low $\delta^{34}S$ (up to 12‰) values could indicate more terrestrial organic matter input or the use of more reprocessed material from sediment (Fry and Chumchal, 2011). The $\delta^{13}C$ and $\delta^{34}S$ values indicate large differences between sites in terms of resource available for the local fauna. Site trophic positional shifts and resource-use variability further complicate the interpretation of the link between trophic ecology and TE contamination. Further studies should investigate the variation of TE and stable isotopes to explore this site-to-site trophic plasticity.

According to Rainbow et al. (2009) 46–80% of the Ag accumulated in *H. diversicolor* is derived from dissolved sources. The much higher burrow irrigation rate of *H. diversicolor* (Vedel and Riisgård, 1993; Nielsen et al., 1995) would support the significantly higher Ag tissue concentration reported here. Bioturbation is critical for sediment biogeochemical processes and, therefore, TE bioavailability (Gilbert et al., 2021). Although both species are likely to have the same broad bioturbation potential defined by Queirós et al. (2013), differing burrow structure, size, location, lining properties and associated bacterial communities (Esselink and Zwarts, 1989; Miron et al., 1991; Scaps, 2002; Papaspyrou et al., 2006) will all be important for specific TEs.

Although the species' ecology cannot be ignored, the comparison of tissue concentrations and that the direction/magnitude of the differences are TE-specific strongly suggest that the species have dissimilar TE regulation approaches. Uptake mechanisms have not been investigated in *A. virens*, but Ray et al. (1980) showed it was unable to regulate Cd uptake and, therefore, accumulated it rapidly. *H. diversicolor* can tolerate the toxic effects of elevated tissue concentrations by storing TEs as subcellular components within the cytosolic fraction using MTLPs (metallothionein-like proteins) (Berthet et al., 2003; Mouneyrac et al., 2003; Poirier et al., 2006). *H. diversicolor* from highly contaminated sites can also excrete small amounts of accumulated Cu (Zhou et al., 2003; Geffard et al., 2005). For *A. virens,* the lack of a clear MTLP response reported by Watson et al. (2021) indicates that MTLP expression is not a dominant TE response (for Cu/Zn exposure). Recently, Green-Etxabe et al. (2021) showed dramatic upregulation in haemoglobin subunits and linker chains after Cu and Zn exposure for *A. virens* so a direct comparison between the species to investigate physiological mechanisms using molecular markers would be an important next step to confirm if accumulation/regulation/detoxification processes are different. As both species can dominate the infaunal biomass, understanding these processes will be critical to assess the relative risks to their predators, many of which are of commercial and conservation importance (Amara et al., 2001; Serrano et al., 2003).

4.4. Biomonitoring suitability

Biomonitors are species that accumulate TEs and are analysed as a measure of the concentrations for ecotoxicological relevance. *H. diversicolor* has a long study history generating a biomonitoring database of accumulated TE concentrations across habitats with diverse, but often extremely high bioavailabilities/concentrations (see Rainbow, 2018). Yet, our data clearly show that bioaccumulation is species/TE specific even when worms are collected from the same site. For Ag, Cu, Hg, Ni and Zn sediment contamination, the significantly higher mean tissue concentrations in *H. diversicolor* would indicate that this is a more appropriate species. For Cd, Fe, Cr and As *A. virens* would be 'better' with both species accumulating similar levels of Pb. The sites selected in the Solent, although enriched for Cu and Hg and to a lesser extent As, are generally of low contamination. It would, therefore, be an important next step to confirm if the species' differences are retained over a much greater range of sediment concentrations to assess full utility. We would also contest that the lack of any positive correlations between sediment and tissue concentration for *H. diversicolor* (except As) indicate that its biomonitor suitability may need to be re-evaluated. In fact, the generally negative relationships for both species (whether statistically significant or not) would indicate that neither can be used to resolve site differences when contamination levels are low.

5. Conclusion

Although the majority of TEs present low levels of sediment contamination at the Solent sites, elevated AEIs, EFs and some tissue concentrations indicate a renewed, urgent need for source identification/control for some TEs including Cu, Hg and As to minimise the effects on coastal benthic systems. However, as the global transition to decarbonise accelerates, TE release into the marine environment will likely increase concomitantly with the expansion of renewables and associated infrastructure. The risk to coastal habitats from specific TEs (e.g. Cu) is likely to increase further as some coastal areas become service hubs for the offshore industry, but so will the risks to many more diverse habitats. Offshore renewable energy expansion will, therefore, require the selection of appropriate 'model' species to assess site contamination and the toxicological effects of TE inputs. Throughout temperate regions *H. diversicolor* dominates in variable salinities, but is replaced by *A. virens* in fully saline zones (Bass and Brafield, 1972; Kristensen, 1984). The elevated risks to more habitats (e.g. fully saline, subtidal mixed sediments) from renewable energy expansion will, therefore, require the selection of a number of appropriate 'model' benthic species to assess site contamination and the toxicological effects of TE inputs. The 'best' polychaete biomonitor will, therefore, be one that is most closely aligned with the impacted habitat; for example, *A. virens* in sub-tidal/offshore areas and *H. diveriscolor* in estuarine areas. More importantly, as the diverse TE-specific bioaccumulation ability of these closely related species presented here shows, selecting a suite of species that diverge in their ecology, biology and TE regulatory abilities will be critical to understand the *full* impact on the benthic community and associated food webs via trophic transfer.

CRediT authorship contribution statement

G.J. Watson: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **S. White:** Methodology, Investigation, Data curation. **S. Gobert:** Methodology. **G. Lepoint:** Writing – review & editing, Writing – original draft, Methodology. **N. Sturaro:** Methodology. **J. Richir:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Gordon Watson reports financial support was provided by European

Union. Gordon Watson reports a relationship with European Union that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

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