Metal bioavailability and bioaccumulation in the polychaete *Nereis virens* (Sars): the effects of site-specific sediment characteristics

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Highlights

- We studied relationships between Cu and Zn in sediment, pore water and *N. virens*
- Zn in pore water was more readily available than Cu to *N. virens*
- Sediment organic content and grain size were correlated to metal bioavailability
- *N. virens* did not accumulate metals directly from the sediment bioavailable fraction
- *N. virens* may respond in a different way than *N. diversicolor* to metal pollution

Abstract

The present study investigates the relationships between copper (Cu) and zinc (Zn) concentrations in sediment, pore water and their bioaccumulation in the polychaete *Nereis virens*, as well as the importance of site-specific sediment characteristics in that process. Sediment, pore water and *N. virens* were sampled from seven sites with different pollution histories along the English Channel coast. Results showed that site-specific metal levels and sediment characteristics were important in determining the bioavailability of metals to worms. Significant correlations were found between Cu in the sediment and in the pore water and between Zn in the pore water and in *N. virens*. Zn from the pore water was thus more readily available from a dissolved source to *N. virens* than Cu. Data also showed that metal concentrations in *N. virens* were lower than those found in other closely related polychaetes, indicating that it may regulate tissue concentrations of Cu and Zn.

Keywords

Nereis virens, sediment, pore water, copper, zinc

1. Introduction

Even though copper (Cu) and zinc (Zn) are naturally present in the marine environment, they are extensively used in present and past industries e.g. mining, smelting and alloy production leading to high levels of metal in the environment due to their persistence and lack of biodegradability (Walker *et al.*, 2006). Although often considered as ‘old pollutants’ their industrial use is still considerable with new applications such as nanoparticles, ensuring that significant amounts will continue to enter the natural environment (Heggelund *et al.*, 2014, Luoma and Rainbow, 2008, Tourinho *et al.*, 2012). They are, therefore, still a great concern in terms of water quality, risk management and ecotoxicological risk to marine life (Campbell *et al.*, 2006, Fairbrother *et al.*, 2007, Luoma and Rainbow, 2008, Walker *et al.*, 2006).

Cu is known to be one of the most common metals present in coastal marine sediments (Bryan and Langston, 1992). Cu is an essential element of various proteins and enzymes with important functions in cellular respiration and energy metabolism (Greim and Snyder, 2008), but is also one of the most toxic metals to benthic organisms including polychaetes (Reish and Gerlinger, 1997, Watson *et al.*, 2013). Cu plays an important role in redox reactions as it is able to transfer electrons and forms oxygen radicals. Therefore, the toxicity of Cu can also lead to effects on the structure and functions of proteins, membranes, DNA damage and oxidative stress (Schwarz *et al.*, 2013).

High concentrations of Zn have also been recorded in many coastal sediments (Bryan and Langston, 1992). Although an essential metal for aquatic organisms (Walker *et al.*, 2006) playing an important role in the functions of over 150 enzymes (Walker *et al.*, 2006), Zn is also toxic at elevated concentrations to polychaetes (Casado-Martinez *et al.*, 2013, King *et al.*, 2004, Wang and Rainbow, 2005). However, various accumulation patterns have been described regulating the uptake of metals defined by the balance between uptake and excretion rates (Luoma and Rainbow 2008, Rainbow 2002). Metals can be also regulated at a cellular level by detoxification processes usually involving proteins such as metallothioneins (Casado-Martinez *et al.*, 2010, Greim and Snyder, 2008, Walker *et al.*, 2006).

Sediments in coastal areas are commonly considered as sinks for metal pollution with concentrations exceeding three to five times the concentrations found in the overlying water (Bryan and Langston, 1992, Bufflap and Allen, 1995, Davidson *et al.*, 1994, Watson *et al.*, 2013). However, sediments can also be considered as a source for various contaminants (Chon *et al.*, 2012, Ianni *et al.*, 2010, Watson *et al.*, 2013). The resuspension of the sediment by natural or anthropogenic activities can lead to the release of entrapped soluble metals back into the water column and the pore water (Chon *et al.*, 2012). The ecological risk posed by metal-contaminated sediments depends strongly on the sediment characteristics, specific chemical forms of the metals influencing their availability to aquatic organisms (bioavailability) and the ability of these organisms to accumulate (bioaccumulation) or remove metals (Amiard *et al.*, 2007, Casado-Martinez *et al.*, 2010, Pueyo *et al.*, 2001, van Gestel, 2008).

Bryan and Langston (1992) provided the only comprehensive and available list of metal concentrations in sediments, reviewing nineteen estuaries in the UK. Cu concentrations reported by these authors ranged from 7 mg kg^{-1} to 648 mg kg^{-1} dry weight of sediment and Zn concentrations from 46 mg kg^{-1} to 940 mg kg^{-1} in industrial areas. Extreme values were obtained in Restronguet Creek (Cornwall) with 2398 mg kg^{-1} for Cu and 2821 mg kg^{-1} for Zn. These concentrations were expressed as total concentrations and differ from sequential extraction methods where the different fractions of metal partitioning are detailed. Therefore, the total fraction does not reflect the bioavailable fraction (Hseu *et al.*, 2002, Tessier *et al.*, 1979). The utility of sequential methods is still the subject of some debate in the field of geochemistry, but their importance in the fields of environmental chemistry and pollution is recognised (Fernandes, 1997).

Evaluating the bioaccumulation of metals in an ecosystem requires not only the chemical analysis of the metals in the sediment, but also the sediment characteristics (organic content and particle size distribution) and the pore water concentrations in contact with benthic species (Alvarez *et al.*, 2010, King *et al.*, 2004, Ruus *et al.*, 2005). Indeed, pore water is a key exposure route for organisms living within the sediments and feeding from it (Chapman *et al.*, 2002). Studies on pore water concentrations are needed when evaluating the contribution of pollutants trapped within the sediment to the pollution of the overlying water column, the sediment and metal availability to infauna (Bufflap and Allen, 1995, Nayar *et al.*, 2006). Pore water chemistry has been recently included in the framework for metals risk assessment and helps to assess sediment quality and aquatic ecosystem health (Fairbrother *et al.*, 2007).

Polychaetes are the most abundant taxon in benthic communities and, therefore, have been frequently used as representative groups to analyse the health of the benthic ecosystem and measure the effects of various pollutants in the water column and in the sediment. They are especially important as their bioturbation activity in the sediment ensures that they are persistently exposed to pollutants (Dean, 2008, Durou *et al.*, 2008, Lewis and Watson, 2012, Nielsen *et al.*, 1995, Poirier *et al.*, 2006, Rainbow *et al.*, 2009, Reish and Gerlinger, 1997, Watson *et al.*, 2007). *Nereis virens* was selected as the test species in this study for its ecological relevance for marine systems in opposition to the common use of *Nereis diversicolor* (Lewis and Watson, 2012). *N. virens* inhabits muddy sand of the littoral and sublittoral zones of marine and estuarine habitats in boreal temperate regions throughout Europe and the northern hemisphere and is one of the dominant polychaete species by biomass in fully saline areas, replacing *N. diversicolor* (Bass and Brafield, 1972, Kristensen, 1984). *N. diversicolor* is known as a surface-deposit feeder, an herbivore, a predator and a scavenger (Reise, 1979), whereas *N. virens* does not swap between feeding modes; it is an omnivorous macrophages feeder, so the routes of uptake are more certain (Nielsen *et al.*, 1995). It also has lower irrigation rates which may increase the role of pollutants within the sediment and pore water rather than the overlying water (Kristensen and Kostka, 2005). Although it can reach up to 900 mm in length (Wilson *et al.*, 1988), 300 mm is a more usual size which makes it an important predator and prey item for many species (McIntosh, 1908-1910).

The ecological risk and the environmental impact of Cu and Zn is far from being fully evaluated, especially in the context of *N. virens* and sediment quality guidelines (Crane, 2003, Hübner *et al.*, 2009, Luoma and Rainbow, 2008). In addition, the relationship between the ability of *N. virens* to bioaccumulate these metals and their sediment/pore water environmental bioavailability is essential to understand Cu and Zn toxicity in the sediment and the greater impact on ecological function for this species, *e.g.* trophic transfer (Amiard *et al.*, 2007, King *et al.*, 2004, Rainbow *et al.*, 2006, Wang and Rainbow, 2005). This study aims to examine for the first time the relationship between metal concentrations in the sediment, pore water and *N. virens* and the impact of site-specific sediment characteristics. Seven sites with different pollution histories have been selected along the English Channel coast to: (1) fully assess the Cu and Zn bioavailable concentrations in the sediment and the pore water; (2) understand how sediment characteristics (particle size and organic content) play a role in determining these concentrations; (3) understand the relationship between Cu and Zn concentrations in the sediment and the pore water and tissue concentrations in *N. virens.* Finally, (4) the relationships between *N. virens* size and their tissue concentrations for both metals have also been examined.

2. Materials and methods

2.1. Collection of samples

Seven sites located along the English Channel south coast of the UK (Devon, Cornwall, Dorset & Hampshire counties) were sampled for sediments, pore water and *N. virens* from summer 2011 to winter 2012: 1) Mylor, Fal Estuary; 2) Saltash, Tamar Estuary; 3) Holes Bay, Poole Harbour; 4) Tipner, Portsmouth Harbour; 5) Broadmarsh, Langstone Harbour; 6) The Conservancy and 7) Dell Quay, Chichester Harbour (Fig. 1, Table 1). Sites were selected based on their pollution history (Bryan and Langston, 1992, Hübner, 2009) and the presumed or confirmed presence of *N virens*. Site GPS coordinates were recorded using a Garmin eTrex device (Garmin International, Inc.). All sites except~~,~~ Mylor and The Conservancy are known to be used for bait collection (Watson *et al.*, 2007; Watson and bait shops, Pers. Comm.).

At low tide, three sediment cores (15 cm deep, 10 cm diameter) were collected from the mid-shore area and were immediately placed in polyethylene Minigrip plastic re-sealable bags. Next to each sediment core, pore water samples were collected using the appropriate porextractor device developed by Nayar *et al.*, (2006) for the field extraction of pore water from muddy sediments. The pore water samples, collected from a depth of approximately 10 cm, were directly transferred into 15 ml centrifuge tubes. At each site, except Mylor where no worms were available, 10 to 15 worms were collected by turning over the sediment to a depth of about 20 cm and removed by hand. All samples were transported from the field to the laboratory in isothermal containers before being refrigerated at 4 °C for sediment cores or frozen at -20 °C for pore water until further analysis. All worms were kept in sediments from the corresponding site in a flow through system for 24 hours to depurate their guts before being frozen (Watson *et al.*, 2013).

2.2. Processing of samples

All chemicals used during the sample processing were purchased from Sigma-Aldrich Co. and the materials such as centrifuge tubes were obtained from Fisher Scientific Inc. All muddy sediments were homogenised before being processed. Thirty grams of wet sediment (number of replicates n = 3 per site) was dried at 105 °C until constant weight, placed in a muffle furnace at 475 °C for 4.5 hours and then reweighed to give the mean percentage of organic content (Greiser and Faubel, 1988). For the particles less than 63 µm, 20 ml of a solution made by dissolving 33 g of sodium hexametaphosphate with 7 g of sodium carbonate in 1 L distilled water was added to each sample and left overnight to settle (British Standards Institution., 2009). The samples were then transferred into a sonicator bath for 2 h before being assessed by laser diffraction (Malvern Mastersizer 2000). The grain size distribution was described geometrically using the Folk and Ward (1957) method, giving 4 descriptors: the average size, the spread of the sizes around the average (sorting), the symmetry or preferential spread to one side of the average (skewness), and the degree of concentration of the grains relative to the average (kurtosis) (Blott and Pye, 2001).

Concisely, 0.5 g of less than 63 µm dried fine sediment were further analysed for Cu and Zn using the BCR three-step sequential extraction procedure developed by the Standards, Measurements and Testing Programme (formerly BCR) of the European Commission (Mäkelä *et al.*, 2011, Pueyo *et al.*, 2001). This procedure is based on acetic acid (step 1), hydroxylammonium chloride (step 2) and hydrogen peroxide/ammonium acetate (step 3) extractions and assesses the distribution of metals in the following fractions: (a) exchangeable; (b) reducible and (c) oxidizable. The exchangeable, water and acid soluble fraction, targets the extraction of metals bound to soluble species, carbonates and cation exchange sites. Metals bound to iron and manganese oxyhydroxides are extracted in the reducible fraction and metals associated to organic matter and sulphides are part of the oxidizable fraction (Davidson *et al.*, 2004, Filgueiras *et al.*, 2002, Ianni *et al.*, 2010, Mossop and Davidson, 2003). The sum of the three steps is described as the presumed bioavailable concentration of Cu and Zn as they target the most mobile and most available phases of the metals (Davidson *et al.*, 1994, Filgueiras *et al.*, 2002, Kwon *et al.*, 2001, Ramos *et al.*, 1999, Zimmerman and Weindorf, 2010). BCR-701 reference sediment was used to monitor recovery and efficiency levels (Pueyo *et al.*, 2001, Sutherland, 2010). Percentage recoveries (mean ± SD) for Cu for steps 1, 2 and 3 were 110.64 ± 2.14, 97.58 ± 1.66 and 102.20 ± 0.80, respectively. Percentage recoveries for Zn for steps 1, 2 and 3 were 99.02 ± 0.86, 92.5 ± 4.51 and 103.10 ± 0.21, respectively.

Ten ml of the pore water samples (n = 3 per site) were acidified by adding 25 µl of concentrated nitric acid (HNO_3 69-71%) to the samples and left to digest for 30 min. To obtain a final digestion solution of 0.14 mmol L^{-1} HNO_3 (pH < 2), 2.5 ml of distilled water was finally added to the samples (Rausch *et al.*, 2005).

Sampled *N. virens* (n = 10-15 per site) were defrosted and their heads removed due to the major role of Zn in hardening of Nereid jaws (Broomell *et al.*, 2006, Bryan and Gibbs, 1979). They were then dried individually at 60 °C for 24 hours and ground using a pestle and mortar before being digested according to Berthet *et al.* (2003) and Rainbow *et al.* (2006). Approximately 0.3 g of each dried worm was ultrasonicated for 1 h before slowly adding 0.5 ml of nitric acid (HNO_3 69-71%). All tubes were then heated in a water bath at 75 °C for 5 to 6 hours, but checked every hour and more acid was added if needed to prevent them from drying out. Distilled water (20 ml) was added to each tube and the final volumes were recorded before being refrigerated at 4 °C until the samples were analysed. The analysis was verified by the use of the reference material TORT-2 from the National Research Council Canada giving a recovery percentage (mean ± SD) of 91.63 ± 1.93 for Cu and 99.52 ± 2.84 for Zn.

Sediment, pore water and *N. virens* samples were all analysed using a Varian Spectra AA 220FS Flame Atomic Absorption Spectrophotometer FAAS (Varian Medical Systems, Inc.). For the FAAS, standard solutions of copper and zinc (5 mg L^{-1}) were used to calibrate the spectrophotometer. For each of the assays, all glassware was rinsed in 10 % analytical grade HCl and then 3 times in distilled water before being left to dry.

2.3. Statistical analysis

All data were analysed using Minitab (version 15) and were tested to meet the assumptions for parametric tests. When the data did not meet these assumptions they were transformed using either arcsine transformation, square root transformation or Johnson transformation. To assess which of the square root or the Johnson transformation provided the best normality, the Individual Distribution Identification function from Minitab was used.

Differences between mean transformed data for percentage organic content and particle size were analysed using a one-way ANOVA followed by a Tukey HSD pairwise comparison test of means with equal sample numbers. Differences between mean transformed metal concentrations in sediment, pore water, or *N. virens* and differences between mean transformed worm weights were analysed using one-way ANOVAs followed by Tukey HSD pairwise comparison test of means with equal (sediment and pore water metal concentrations) or unequal (*N. virens* metal concentrations and weights) sample numbers. Particle size distribution descriptors were produced using Gradistat (version 8.0) (Blott and Pye, 2001) and then analysed as above.

Pearson's correlation coefficient was applied on raw or transformed data to identify correlations between sediments characteristics (organic content and particle size) and metal concentrations in sediments and pore water and between metal concentrations in tissues, sediments and pore water.

Relationships between individual worm weight and metal concentrations in *N. virens* were modelled using power function according to previous studies (Mubiana et al., 2006, Richir and Gobert, 2014) where tissue metal concentration (Y) is a power function of the individual worm weight (W) [Eq. (1)] :

$$Y=aW^b. \tag{1}$$

Relationship of Eq. (1) was double logarithmically transformed to yield one linear function (Eq. (2)] :

$$\mathrm{Log}_{10}(Y) = \log_{10}a + b\log_{10}(X) \tag{2}$$

where $\log_{10}a$ is the Y-intercept and b is the slope.

3. Results

3.1. Sediment characteristics

All sites were characterised as very poorly sorted, very coarse skewed muddy sediment composed mainly of fine and very coarse silt (Table 2). The majority of the sediment from Mylor was composed of very coarse and fine silt whereas all the other site sediment was mainly composed of fine silt. The organic content of the sediment ranged from 2.32 % for Tipner to 8.90 % for Mylor (Table 2) with an overall significant difference between sites when compared using a one-way ANOVA ($F_{6, 14} = 63.82$, $p = 0.000$). Pairwise comparisons confirmed that all sites were significantly different from each other except Mylor and Holes Bay; Dell Quay and The Conservancy; and Dell Quay and Tipner.

According to the four descriptors of the silt grain size distribution, statistical analyses of the mean particle size showed significant differences between all sites ($F_{6, 14} = 44.24$, $p = 0.000$), with the average grain sizes ranging from 14.2 µm for The Conservancy to 28.2 µm for Mylor (Table 2). Mylor was significantly different from all the other sites, whilst Saltash was significantly different from all sites except Broadmarsh and Dell Quay. The Conservancy was not significantly different from Holes Bay and Tipner. One-way ANOVAs for sorting ($F_{6, 14}$ = 27.56, $p = 0.000$), skewness ($F_{6, 14}$ = 16.75, $p = 0.000$) and kurtosis ($F_{6, 14}$ = 22.06, $p = 0.000$) of the particle size all revealed an overall significant difference between sites. Pairwise comparisons revealed that Mylor and The Conservancy were significantly different from each other for all three parameters. For sorting, Mylor was also significantly different from all sites, whilst Saltash was significantly different from all sites except Broadmarsh and Dell Quay; and Tipner was significantly different from all sites except Holes Bay and The Conservancy. Pairwise comparisons of the skewness revealed that Mylor was significantly different from all sites except Broadmarsh. Pairwise comparisons of kurtosis showed that Mylor was significantly different from all sites except Saltash and Dell Quay, whilst The Conservancy was significantly different from all sites except Tipner.

3.2. Metal bioavailability in the sediment

The concentrations of Cu and Zn from the BCR three-step sequential extraction procedure are presented in Table 3. The presumed bioavailable fraction of metals in the sediment was obtained by summing the three steps of the BCR procedure (Davidson *et al.*, 1994, Filgueiras *et al.*, 2002, Kwon *et al.*, 2001, Pueyo *et al.*, 2001, Zimmerman and Weindorf, 2010).

Concentrations of presumed bioavailable metals ranged from 10.8 mg kg^{-1} dry weight (Broadmarsh) to 422 mg kg^{-1} dry weight (Mylor) for Cu and 36.4 mg kg^{-1} dry weight (Broadmarsh) to 671 mg kg^{-1} dry weight (Mylor) for Zn. There were significant differences between all sites for Zn ($F_{6, 14}$ = 14.66, $p = 0.000$) and copper ($F_{6, 14}$ = 105.89, $p = 0.000$) using one-way ANOVAs. The pairwise comparisons for Cu presumed bioavailable concentrations revealed that Mylor, Tipner and Broadmarsh were significantly different from all other sites. Saltash and Holes Bay were not significantly different from each other, but were significantly different from all other sites. Dell Quay and The Conservancy were not significantly different from each other but were significantly different from all other sites. Presumed bioavailable concentrations of Zn showed that the sites separated into two groups. Mylor, Saltash and Holes Bay had high levels of Zn, ranging from 159 mg kg^{-1} to 671 mg kg^{-1}. These were not significantly different from each other, but were significantly different from the other group of Tipner, Broadmarsh, Dell Quay and The Conservancy, which had lower concentrations ranging from 36.4 mg kg^{-1} to 54.0 mg kg^{-1}.

Although sites strongly differ regarding their absolute metal concentrations in the different fractions of the BCR three-step sequential extraction method, similarities were observed regarding their proportional distribution between the different fractions. Cu was mainly present in the oxidizable fraction with an average for all sites of 49 %, with lower percentages in the reducible (30 %) and exchangeable (21 %) fractions. For Zn, 63 % of its bioavailability was present in the exchangeable fraction, 19 % in the reducible and 18 % in the oxidizable fraction. The Conservancy had the highest percentage of Cu present in the oxidizable fraction (63 %) and the lowest percentage of Zn present in the exchangeable fraction (44 %). With 77 % of Zn present in the exchangeable fraction, Saltash had the highest percentage of Zn present in this extraction step over all the sites.

3.3. Metal concentrations in the pore water

Cu concentrations in the pore water ranged from 0.68 µg L^{-1} for Broadmarsh to 1.85 µg L^{-1} for Mylor (Table 4). Mylor had significantly higher Cu concentrations than Broadmarsh or Holes Bay ($F_{6,\,14} = 4.94$, $p = 0.007$); all other sites were not significantly different from each other. Even though pore water concentrations of Zn ranged from 0.49 µg L^{-1} for Holes Bay to 3.03 µg L^{-1} for Broadmarsh (Table 4), no significant differences were present between sites ($F_{6,\,14} = 2.19$, $p = 0.117$).

3.4. Metal bioaccumulation in *N. virens*

Mean tissue concentrations of both Cu and Zn differed considerably between sites (Table 5). Tissue concentrations of Cu varied from 7.36 μg g^{-1} dry weight for Broadmarsh to 17.4 μg g^{-1} dry weight for The Conservancy. Variability in Zn concentrations was higher than for Cu, with a mean of 69.0 μg g^{-1} dry weight for worms from Saltash to 182 μg g^{-1} dry weight for those collected from Broadmarsh, even though those from Broadmarsh had the lowest Cu concentrations. One-way ANOVAs revealed significant differences between sites for Cu ($F_{5, 80} = 8.18$, $p = 0.000$) only. Worms from Broadmarsh had significantly lower Cu tissue concentrations than worms from all other sites except Holes Bay and Saltash. Worms from the Conservancy also had significantly higher Cu levels than Saltash.

3.5. Relationships between sediment characteristics and metal concentrations in the sediment and the pore water

The organic content for the sediment showed a significant correlation with presumed bioavailable Cu ($r = 0.596$; $p = 0.004$) and Zn ($r = 0.658$; $p = 0.001$) concentrations in the sediment as well as Cu concentrations in the pore water ($r = 0.475$; $p = 0.030$). The correlation between the organic content and Zn concentrations in the pore water was not significant ($r = 0.082$; $p = 0.722$). Particle size showed a significant correlation with both Cu ($r = 0.791$; $p < 0.001$) and Zn ($r = 0.740$; $p < 0.001$) presumed bioavailable concentrations in the sediment. The correlation between particle size and metal concentrations in the pore water showed a significant correlation for Cu only ($r = 0.579$; $p = 0.006$).

3.6. Relationships between tissue metal concentrations and weight

One-way ANOVA revealed significant differences between sites in the mean wet weight of collected worms ($F_{5, 80} = 17.83$, $p = 0.000$). Worms from Saltash and Broadmarsh were significantly heavier than worms from all other sites, but were not different from each other. Across all sites, Cu concentrations and worm weight had a significant negative relationship ($r^2 = 0.204$, $p < 0.001$), suggesting that heavier worms accumulate less Cu in their tissues or that worms that accumulate less Cu in their tissues are heavier (Fig. 2a). For Zn concentrations and worm weight, the relationship was still negative but not significant ($r^2 = 0.004$, $p > 0.05$; Fig. 2b). As it is possible that these relationships were confounded by site-specific differences, the relationships between *N. virens* individual metal concentrations and weight were also modelled separately for each site using power function (Table 6). Sites analysis revealed that the relationships between *N. virens* individual copper concentrations and weight were significant for Saltash ($r^2 = 0.382$, $p < 0.05$), Holes Bay ($r^2 = 0.299$, $p < 0.05$) and Dell Quay ($r^2 = 0.365$, $p < 0.05$). Relationships for Zn revealed significance for Broadmarsh ($r^2 = 0.671$, $p < 0.05$) and The Conservancy ($r^2 = 0.770$, $p < 0.001$). For all the other sites, the relationships were not significant ($p > 0.05$).

3.7. Relationships between metal concentrations in tissues, the sediment and the pore water

No significant correlation was obtained between Cu concentrations in tissues and Cu presumed bioavailable concentrations in the sediment ($r = -0.054$, $p = 0.919$) or Cu concentrations in the pore water ($r = -0.054$, $p = 0.958$). However, Cu concentrations in the pore water and Cu presumed bioavailable concentrations in the sediment had a significant positive correlation ($r = 0.855$, $p = 0.014$; Fig. 3a).

In opposition to Cu, Zn concentrations in tissues and Zn concentrations in the pore water had a significant positive correlation ($r = 0.837$, $p = 0.038$; Fig. 3b). No significant correlation was shown between Zn presumed bioavailable concentrations in the sediment and Zn concentration in the pore water ($r = 0.321$, $p = 0.482$) or Zn concentrations in *N. virens* ($r = -0.471$, $p = 0.346$).

4. Discussion

4.1. Pollution history and site characteristics

The seven sites were chosen because of their diverse pollution histories in relation to Cu and Zn (Bryan and Langston, 1992). Although sediment at all sites was described as very poorly sorted, very coarse skewed and muddy composed mainly of fine and very coarse silt, significant site-specific differences in the characteristics of the sediment were observed. These differences in organic content and particle size were mainly reflected in the presumed bioavailable concentrations of metals in the sediment. Indeed, organic content and particle grain size are important factor in metal binding (Amiard *et al.*, 2007, Richir *et al.*, 2012, Standley, 1997, Tsai *et al.*, 2003). Taking into account the site characteristics (mainly organic content) and presumed bioavailable concentrations in the sediment, our data reveals that Mylor, Saltash and Holes Bay were very different from Tipner, Broadmarsh, Dell Quay and The Conservancy. Mylor and Saltash also had high Cu and Zn concentrations in the pore water compared to the other sites. The Fal and Tamar Estuaries and Poole Harbour are known to be impacted by present and past industrial activities such as mining activities and records of high metal pollution at these sites have been published (Bryan and Langston, 1992, Hübner, 2009). Even though it is well recognised that particle size has a key role in metal bioavailability in the sediment and the metal concentrations in the pore water (Amiard *et al.*, 2007), the low variability in particle size between all sites would indicate that it is the diverse metal pollution history of these sites driving the observed differences (Bryan and Langston, 1992; Hübner, 2009). Therefore, it is clear that historic pollution (often over long timescales) and new inputs such as metal nanoparticles are critical factors in determining site conditions (Walker *et al.*, 2006).

4.2. Metal toxicity guidelines

Environmental quality standards (EQS) are used as guidelines to evaluate the potential risk of various pollutants to marine biota (Matthiessen *et al.*, 1999). The revised UK annual average EQSs for dissolved Cu and Zn are 5 µg L^{-1} and 10 µg L^{-1} in seawater, respectively, whilst the Ecotoxicological Assessment Criterion (EACs) was proposed by the Oslo and Paris Commission (OSPAR) with 0.1 - 1.0 µg L^{-1} for Cu and 0.5 - 5.0 µg L^{-1} for Zn (Matthiessen *et al.*, 1999). Teasdale *et al.*,(1995) argued that the seawater EQSs cannot be quantitatively compared to the values obtained for the pore water samples, unless the fluxes between pore water and overlying seawater for a site are known. However, a simple comparison does reveal that pore water Zn and Cu concentrations from the present study were well within these values for the UK EQS, but close to or exceeding the upper thresholds for Cu EACs for all sites except Broadmarsh. This indicates that at these sites Cu can represent a potential ecotoxicological risk for aquatic species.

Sediment Quality Guidelines (SQGs) were developed to assess risk for metal concentrations in the sediments (Luoma and Rainbow, 2008) with a number of methods developed to estimate the potential adverse effects of pollutants to aquatic life (Crane, 2003, Hübner *et al.*, 2009, Luoma and Rainbow, 2008). Four threshold values were selected to compare with our data: ERL (Effects Range-Low; 34 for Cu and 150 for Zn), ERM (Effects Range-Medium; 270 for Cu and 410 for Zn) TEL (Threshold Effect Levels; 18.7 for Cu and 124 for Zn) and PEL (Probable Effect Levels; 108 for Cu and 271 for Zn) (CCME, 1998, Long and Morgan, 1990). Comparing presumed bioavailable metal concentrations to SQGs (taking in to account that these values are based on total concentrations of metals in sediments and our values are presumed bioavailable levels), only Broadmarsh was below all four SQGs for both Cu and Zn confirming that this site was at minimal risk for toxic effects on aquatic organisms. Tipner, Dell Quay and The Conservancy were also below the standards for Zn making these sites at low risk for adverse effects. In contrast, Holes Bay, Saltash and Mylor had presumed bioavailable Cu concentrations that exceeded the TEL and ERL values for Cu and Zn and in the case of Saltash and Mylor exceeded the PEL for Cu and the PEL and ERM for Zn. The majority of the sites sampled, therefore, exhibit presumed bioavailable concentrations of metals that have the potential for adverse effects on the benthos, requiring further investigation (Miller *et al.*, 2000).

4.3. Metal bioavailability

Critical to the interpretation of SQGs is bioavailability. The concentration of presumed bioavailable metals is not directly proportional to the total concentration in water or sediment (Borgmann, 2000). Operationally defined sequential extraction is widely used and provides valuable information on bioavailability (Kersten, 2002), but is not without its limitations (Nirel and Morel, 1990). To provide a more complete picture of the link between the sediment and the biota, concentrations in the pore water were also measured as it is a key exposure route for macrofauna (Chapman *et al.*, 2002). These concentrations varied significantly between sites for Cu, but were also significantly positively correlated with the presumed bioavailable fraction in the sediment. This would indicate that the presumed bioavailable fraction in the sediment directly influences the levels in the pore water and is related to the ‘historical’ pollution of a site (Lee and Lee, 2005). However, the concentrations in the pore water were also significantly correlated with the overlying water concentrations ($r = 0.868$, $p = 0.025$; additional data – not shown – for the overlying water were provided by the UK Environmental Agency). A number of authors (Chapman, 2002, Wang, 2001, Warren *et al.*, 1998) have shown that the overlying water is the main contaminant exposure route for the infauna living within a burrow, especially for those organisms that irrigate their burrows. *N. virens* is capable of significant irrigation rates estimated by Kristensen and Kostka (2005) to be 100 L m^{-2} d^{-1}. This level of irrigation could mean that the overlying water is the dominant source of Cu exposure.

The affinity for Cu to the oxidizable phase (associated to organic materials and sulphides) is well known and, in accordance to previous studies (Koretsky *et al.*, 2006, Kwon *et al.*, 2001, Luoma, 1986), metals in this fraction are assumed to remain in the sediment-for long periods. Although it did vary between sites, the majority of the Cu was present in the oxidizable phase (49 %) giving weight to the theory that most of the Cu present in the pore water came from the overlying water. A detailed geochemical assessment for each site would be required to understand the fluxes and influence of bioturbating species like *N. virens* to confirm this. It is also concerning that continuous ‘new’ sources of Cu within the water column as well as resuspension of the sediment and release of Cu back into the water column may be driving the pore water concentrations, although this did not seem to be positively correlated with Cu concentrations in *N. virens*. Identifying these alternative sources for Cu and Zn should be a priority for regulatory agencies especially as there has been a tendency to consider the sediment contamination to be historical and, therefore, outside current control.

In contrast, pore water concentrations of Zn did not vary significantly between sites and were not significantly positively correlated with the presumed bioavailable fraction in either the sediment or the overlying water (r = 0.137, p = 0.795). Zn like Cu is a chalcophile metal and under anoxic conditions has a strong affinity for sulphide phases and carbonates (Koretsky *et al.*, 2006). However, our data revealed that across all sites only a small amount of Zn was affiliated with this phase (18 %); instead most was in the exchangeable phase (63 %). Thus, Zn seemed to be more readily bioavailable than Cu.

It is not clear why the site variability was so high regarding Cu and Zn concentrations in the sediment, specifically the high concentrations in one of the sediment cores at Broadmarsh. Sediment is well known for its spatial heterogeneity and this may have been exacerbated by bait collection activities. The majority of the sites sampled are regularly used for bait collection, which involves repeated turning over the sediment to a depth of approximately 30 cm (Watson *et al.*, 2007). Not only does this disturb the sediment resulting in significant and repeated mixing of the redox layers (Mclusky *et al.*, 1983), but the bioavailability of metals for sediment-dwelling organism increases (Howell, 1985). It is, therefore, highly likely that in areas where digging is common (e.g. Broadmarsh) the local sediment conditions may result in a much higher level of availability of Cu and Zn (as well as other pollutants) to the benthos. An urgent assessment of the impacts of this process is, therefore, required.

4.4 Metal bioaccumulation in polychaetes

Many studies have investigated the relationships between metal bioavailability and tissue concentrations for the closely related species *N. diversicolor* (Amiard *et al.*, 2007, Berthet *et al.*, 2003, Bird *et al.*, 2011, Bryan and Hummerstone, 1971, García-Alonso *et al.*, 2010, Geffard *et al.*, 2005, Mouneyrac *et al.*, 2003, Otero *et al.*, 2000, Poirier *et al.*, 2006, Rainbow and Smith, 2013, Rainbow *et al.*, 2009, Zhou *et al.*, 2003). However, the present study is, to our knowledge, the first to investigate these relationships for Cu and Zn in *N. virens*. Some of these *N. diversicolor* studies have shown a positive relationship between concentrations in worms and levels in the sediment (*e.g.* Bryan and Hummerstone, 1971, Rainbow *et al.*, 2009, Zhou *et al.*, 2003) whereas others have shown this relationship to be weaker or absent (*e.g.* Amiard *et al.*, 2007, Berthet *et al.*, 2003, Otero *et al.*, 2000). Nevertheless, the majority of these studies have recorded much higher tissue concentrations in *N. diversicolor* than in *N. virens* (Table 5) even from sites where sediment Cu concentrations are lower than those reported here (Amiard *et al.*, 2007, Bryan and Hummerstone, 1971, Geffard *et al.*, 2005, Poirier *et al.*, 2006, Rainbow *et al.*, 2009, Zhou *et al.*, 2003). Although total metal concentrations are long recognized as not having a predictive ecotoxicological value (Allen and Janssen, 2006, Meyer, 2002, Morel, 1983, Pueyo *et al.*, 2001, Tessier and Turner, 1995), it is interesting that presumed bioavailable fractions of Cu in the sediment and in the pore water were not reflected in the tissues of *N. virens* as this species may be capable of regulating metals even at high concentrations. It is also interesting that worms from sites with low concentrations of presumed bioavailable Cu in the sediment (*e.g.* The Conservancy) had significantly higher tissue concentrations (nearly twice the mean value) than those of Saltash, even though this site had a much higher presumed bioavailable concentration in the sediment (Table 3). There are a number of possible explanations for these results. Firstly, the sediment presumed bioavailable fractions defined by the BCR sequential extraction procedure may not actually be truly bioavailable to this species (Howard and Shu, 1996, Tessier and Campbell, 1987). Secondly, alternative routes and other possible influences on accumulation rates *e.g.* bioturbation processes, feeding methods and size etc., are all known to differ between these species (Kristensen and Kostka, 2005, Nielsen *et al.*, 1995, Scaps, 2002). Thirdly, and most likely, is that *N. virens* has a different metal accumulation pattern compared to *N. diversicolor* and is able to regulate the concentration of Cu much more effectively by reducing the uptake rate or by enhancing detoxification processes when facing high metal concentrations in the environment.

Metal uptake mechanisms have not been investigated in this species, but Ray *et al.*,(1980) showed *N. virens* was unable to regulate cadmium uptake under controlled conditions and, therefore, accumulated it rapidly. *N. diversicolor* seems to be able to tolerate the toxic effects of elevated tissue concentrations by storing it as detoxified subcellular components within the cytosolic fraction using metallothionein-like proteins (Berthet *et al.*, 2003, Mouneyrac *et al.*, 2003). However, even if this process were to occur in *N. virens* sampled here, the metals would still be released during the extraction process. *N. diversicolor* from highly contaminated sites do appear to excrete accumulated Cu, but only insignificant amounts possibly by shedding their epicuticle which contains Cu-rich deposits (Geffard *et al.*, 2005, Zhou *et al.*, 2003). This process may occur in *N. virens*, but is unlikely to account for such low tissue concentrations presented here. Investigating the physiological mechanisms that may maintain these very low levels is an important next step in understanding the response to elevated concentrations.

A lack of any significant relationship between the presumed bioavailable concentrations of Zn in the sediment and concentrations in *N. virens* may mean that it is more likely to be affected by metal concentrations via solution (from pore water or the overlying water) than via the sediment based on its feeding method and burrowing activity (Chapman, 2002, Kristensen and Kostka, 2005, Wang, 2001, Warren *et al.*, 1998). *N. virens* as well as *N. diversicolor* are known to be omnivorous and feed on the surface sediment (Kristensen, 2001, Woff, 1973). However, quantitative evidence on sediment ingestion is missing and where it is present the level of intake is low (only 17.6 % of digestive contents was sediment) (Costa et al., 2006). This new information shows that the lack of significant relationship between the presumed bioavailable concentrations of metals in the sediment and concentrations in *N. virens* in not unexpected. However, the fact that pore water concentrations were positively correlated with tissue concentrations indicates that this species can accumulate Zn within its tissues. This was confirmed by the elevated Zn concentrations that were much higher than Cu. This has further been reflected in other studies with nereids. Indeed, Zn concentrations in *N. virens* compared to those of *N. diversicolor* are much more comparable (Amiard *et al.*, 2007, Berthet *et al.*, 2003, Bryan and Hummerstone, 1971) or lower (Bird *et al.*, 2011, Otero *et al.*, 2000, Poirier *et al.*, 2006, Zhou *et al.*, 2003). The reasons given above would also explain the lower concentrations of Zn in *N. virens*, although nereids (and many other species) are able to regulate their internal Zn levels (Amiard *et al.*, 1987, Bryan and Hummerstone, 1973).

It is not surprising that the mean weight of worms collected differed between sites as population structures are always likely to be inherently different, driven by bait collection (Watson *et al.*, 2007) and collecting worms using a fork is also biased for size and (Blake, 1979). Worm weight is important in determining tissue metal concentrations, with negative relationships for many metals in *N. diversicolor* (Poirier *et al.*, 2006) and the eunicid *Marphysa sanguinea* (Garces and Costa, 2009). The lack of bioaccumulation in *N. virens* for Zn and Cu was supported by the overall (all sites combined) negative relationships between body weight and tissue concentrations. The relationship for Zn was not significant and weaker than Cu's, but it may be that the ability to regulate Zn reduced the strength of this relationship. Sites relationships between metal concentrations and weight revealed differences between sites. Significant relationships ($p < 0.05$) were reported for Saltash, Holes Bay and Dell Quay for Cu and for Broadmarsh and The Conservancy for -Zn. These differences in relationships clearly indicate the importance of sites and further investigations taking into account a wider selection of sizes and a greater number of worms should be considered. In addition, all significant relationships were negative with the exception of Saltash for Cu. It is not clear why this site had a significant positive correlation for Cu, but clearly requires further investigation. Negative relationships between worm weight and metal concentrations have been suggested to be due to growth dilution combined with the sequestration of metals into the hard structures and epithelial surfaces (Garces and Costa, 2009). Bryan and Gibbs (1979) showed that hard structures such as jaws have very high levels of Zn (up to 40 % of the total Zn tissue concentration). However, they also demonstrated that Zn levels in nereid jaws are high regardless of the environment and Cu is also a minor component of jaws. Differential uptake routes as the worms get larger (*e.g.* a change of feeding guild); changes in cellular regulation capacity or underlying physiological/metabolic changes may all play a role.

The data presented here have provided the first evidence that *N. virens* may respond in a different way to *N. diversicolor* when exposed to sediments contaminated with Cu and Zn. Both these species are classed by Lewis and Watson (2012) as ‘model’ species of polychaetes for ecotoxicology, but these differences highlight that a suite of species is required to investigate the full diversity of responses to pollution. The present data have also highlighted the need to investigate the mechanistic diversity of responses (and, therefore, tolerance) in closely related species.

Metal concentrations in organisms always show individual variability (Poirier *et al.*, 2006) and it is clear from Fig. 2 that this was considerable for *N. virens*. Some of this may be due to the collection site and worm weight, but some worms of similar weight and from the same site had nearly a 10-fold difference in Zn tissue concentrations and nearly a 3-fold difference for Cu. As suggested by Berthet *et al.*, (2003), this variability could result from the different strategies for accumulation and excretion in sensitive and tolerant individuals. Identifying the underlying drivers for this inter-individual variability (tolerance) is an important area for ecotoxicology as developing metal resistance capacities leads to an energy cost that may have significant implications at the population level (Durou, 2005, Pook *et al.*, 2009). Teasing apart the drivers of this response-variability using just field-collected populations is difficult considering the inherent inter-site variability in the populations and environmental conditions. To fully understand the effects of long term exposure, the processes of tolerance and inter-individual response, experimental studies are required to control these confounding factors.

5. Conclusion

The outcome of this study reveals the importance of site characteristics and metal levels in the understanding of metal bioaccumulation in *N. virens*. The seven sites studied differed taking into account the presumed bioavailable metal concentrations in the sediment and site characteristics such as organic content. While comparing metal concentrations obtained from the pore water samples and from the presumed bioavailable metal fraction in the sediment, only one site (Broadmarsh) was below the EQS and SQG revealing that this site was at low risk for adverse effects on the benthos in opposition to all the other sites studied. This information revel the urge to proceed to more investigation regarding the potential toxicity of Cu and Zn on benthos species in the UK. The significant correlation obtained between Zn in the pore water and in *N. virens* revealed that not only the sediment has to be considered as a metal uptake site but more importantly that *N. virens* seemed to present a preferential metal uptake from the dissolved sources such as pore water. This study also underlined the importance and the difference in metal uptake and metal tissue concentrations between *N. virens* and other closely related polychaetes, most probably explained by its metal regulation processes, its bioturbation and feeding activity. It is recommended that the addition of sites along with a wider selection of sizes and a greater number of worms would give a considerable insight in the relationships between metal uptake, worm weight and metal concentrations in the environment.

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Sites		GPS coordinates		Dates
Name	Location (County)	North	West	
Mylor	Fal Estuary (Cornwall)	50°10'38.20"N	5° 2'54.69"W	02/23/2012
Saltash	Tamar Estuary (Devon)	50°24'52.96"N	4°12'40.17"W	02/24/2012
Holes Bay	Poole Harbour (Dorset)	50°43'36.91"N	2° 0'22.03"W	10/27/2011
Tipner	Portsmouth Harbour (Hampshire)	50°49'23.16"N	1° 5'42.26"W	10/26/2011
Broadmarsh	Langstone Harbour (Hampshire)	50°50'36.26"N	1° 0'31.65"W	07/22/2011
The Conservancy	Chichester Harbour (Hampshire)	50°49'7.06"N	0°48'59.36"W	10/28/2011
Dell Quay	Chichester Harbour (Hampshire)	50°48'29.00"N	0°52'2.81"W	10/28/2011

Table 1

Name and location of the seven sites along the English Channel coast sampled for sediments (n = 3), pore-water (n = 3) and *N. virens* (n = 10-15) from summer 2011 to winter 2012.

Sites	Organic content (%)	Silt particle size distribution (%)				Silt particle size descriptors			
		Very coarse	Medium	Fine	Very fine	x_a (µm)	σ_a	Sk_a	K_a
		(63-31 µm)	(31-8 µm)	(8-4 µm)	(4-2 µm)				
Mylor	8.90 ± 0.35	40.9 ± 0.28	10.8 ± 0.10	37.4 ± 0.47	0.09 ± 0.00	28.2 ± 0.34	5.58 ± 0.01	0.41 ± 0.01	0.71 ± 0.00
Saltash	6.68 ± 0.48	33.9 ± 0.59	9.55 ± 0.24	46.9 ± 1.01	0.11 ± 0.00	20.4 ± 0.69	5.17 ± 0.04	0.64 ± 0.03	0.82 ± 0.01
Holes Bay	8.83 ± 0.18	28.8 ± 0.72	8.39 ± 0.38	54.3 ± 1.44	0.13 ± 0.00	16.6 ± 0.37	4.75 ± 0.06	0.75 ± 0.00	0.96 ± 0.02
Tipner	2.32 ± 0.19	26.3 ± 0.97	7.62 ± 0.06	58.3 ± 1.08	0.13 ± 0.00	15.3 ± 0.55	4.50 ± 0.12	0.76 ± 0.00	1.04 ± 0.04
Broadmarsh	5.20 ± 0.31	34.1 ± 1.01	11.0 ± 0.28	43.9 ± 1.54	0.10 ± 0.00	21.4 ± 1.08	5.09 ± 0.07	0.57 ± 0.04	0.84 ± 0.02
The Conservancy	3.96 ± 0.05	24.5 ± 1.35	7.78 ± 0.45	59.8 ± 2.24	0.14 ± 0.01	14.2 ± 0.76	4.24 ± 0.16	0.75 ± 0.00	1.12 ± 0.06
Dell Quay	3.28 ± 0.42	33.0 ± 0.80	9.28 ± 0.19	48.3 ± 1.16	0.11 ± 0.00	19.5 ± 0.87	5.12 ± 0.06	0.69 ± 0.04	0.83 ± 0.02

Table 2

Characteristics of homogenised 15 cm deep sediment cores (n = 3) sampled from seven sites located along the English Channel coast from summer 2011 to winter 2012. All data are given as mean ± SEM. Organic content and silt particle size distribution are expressed in %. The four particle size descriptors: average grain size (x_a, in µm), spread of the sizes around the average (sorting, σ_a), symmetry or preferential spread to one side of the average (skewness, Sk_a), and degree of concentration of the grains relative to the average (kurtosis, K_a), were calculated according to the geometrically method of Folk and Ward (1957). Silt particle size distribution and silt particle size descriptors were determined using the statistical package Gradistat (v.8.0; Blott and Pye, 2001).

Cu

Sites	Step 1		Step 2		Step 3		Bioavailable conc.
Mylor	83.1 ± 24.3	(23%)	71.2 ± 27.9	(35%)	268 ± 20.9	(41%)	422 ± 63.6
Saltash	17.3 ± 2.66	(24%)	30.8 ± 5.91	(27%)	39.8 ± 0.94	(50%)	87.8 ± 6.55
Holes Bay	7.33 ± 0.84	(25%)	16.0 ± 4.39	(37%)	24.7 ± 3.82	(39%)	48.0 ± 5.00
Tipner	5.59 ± 0.77	(24%)	6.20 ± 0.47	(25%)	11.6 ± 1.71	(52%)	23.4 ± 1.90
Broadmarsh	2.53 ± 0.81	(15%)	3.81 ± 0.53	(33%)	4.48 ± 0.52	(51%)	10.8 ± 0.57
The Conservancy	7.79 ± 0.56	(20%)	11.7 ± 0.93	(17%)	12.3 ± 1.55	(63%)	31.8 ± 1.41
Dell Quay	7.79 ± 0.71	(20%)	8.09 ± 1.83	(35%)	16.9 ± 0.98	(45%)	32.8 ± 2.98

Zn

Sites	Step 1		Step 2		Step 3		Bioavailable conc.
Mylor	299 ± 39.4	(58%)	140 ± 38.8	(23%)	232 ± 69.0	(19%)	671 ± 46.0
Saltash	106 ± 14.6	(77%)	35.7 ± 6.50	(15%)	33.4 ± 6.47	(9%)	175 ± 23.8
Holes Bay	113 ± 6.08	(59%)	36.7 ± 8.29	(19%)	9.60 ± 1.77	(22%)	159 ± 31.0
Tipner	32.6 ± 4.90	(70%)	6.19 ± 2.54	(13%)	3.67 ± 1.75	(17%)	42.5 ± 9.25
Broadmarsh	21.2 ± 1.71	(71%)	8.25 ± 0.51	(23%)	6.95 ± 0.40	(6%)	36.4 ± 4.56
The Conservancy	31.9 ± 2.14	(44%)	10.3 ± 0.72	(21%)	11.8 ± 0.86	(35%)	54.0 ± 6.98
Dell Quay	29.2 ± 1.63	(60%)	5.40 ± 1.28	(20%)	7.17 ± 1.72	(19%)	41.8 ± 7.65

Table 3

Cu and Zn concentrations (mean ± SEM, in mg kg^{-1} dry weight) in homogenised 15 cm deep sediment cores (n = 3) sampled from seven sites located along the English Channel coast from summer 2011 to winter 2012. The BCR three-step sequential extraction protocol was performed on sediment less than 63 µm. This procedure assesses the distribution of metals in the following fractions: exchangeable (*i.e.* water and acid soluble; step 1), reducible (*i.e .* iron/manganese oxides; step 2) and oxidizable (*i.e .* organic matter and sulphides; step 3; Pueyo *et al.* , 2001). The sum of the three steps is described as the presumed bioavailable concentration of Cu and Zn. Cu and Zn concentrations for the three steps are also expressed in percentage of their respective bioavailable concentration.

Table4

Sites	Cu	Zn
Mylor	1.85 ± 0.23	2.14 ± 0.55
Saltash	1.57 ± 0.30	1.40 ± 0.48
Holes Bay	0.83 ± 0.02	0.49 ± 0.04
Tipner	0.84 ± 0.04	0.90 ± 0.25
Broadmarsh	0.68 ± 0.14	3.03 ± 1.90
The Conservancy	0.93 ± 0.15	0.65 ± 0.01
Dell Quay	0.93 ± 0.04	0.53 ± 0.06

Table 4

Cu and Zn concentrations (mean ± SEM, in $\mu g\ L^{-1}$) in pore water (n = 3) sampled from seven sites located along the English Channel coast from summer 2011 to winter 2012.

Sites	Cu	Zn	Wet weight
Mylor	n/a	n/a	n/a
Saltash	10.7 ± 0.68	69.0 ± 53.7	11.7 ± 0.79
Holes Bay	12.4 ± 0.91	95.8 ± 16.7	3.93 ± 0.77
Tipner	13.5 ± 0.83	99.7 ± 15.6	4.92 ± 0.64
Broadmarsh	7.36 ± 0.51	182 ± 54.8	11.6 ± 2.50
The Conservancy	17.4 ± 1.47	80.8 ± 18.5	3.26 ± 0.76
Dell Quay	15.4 ± 1.18	92.1 ± 8.17	3.27 ± 0.53

Table 5

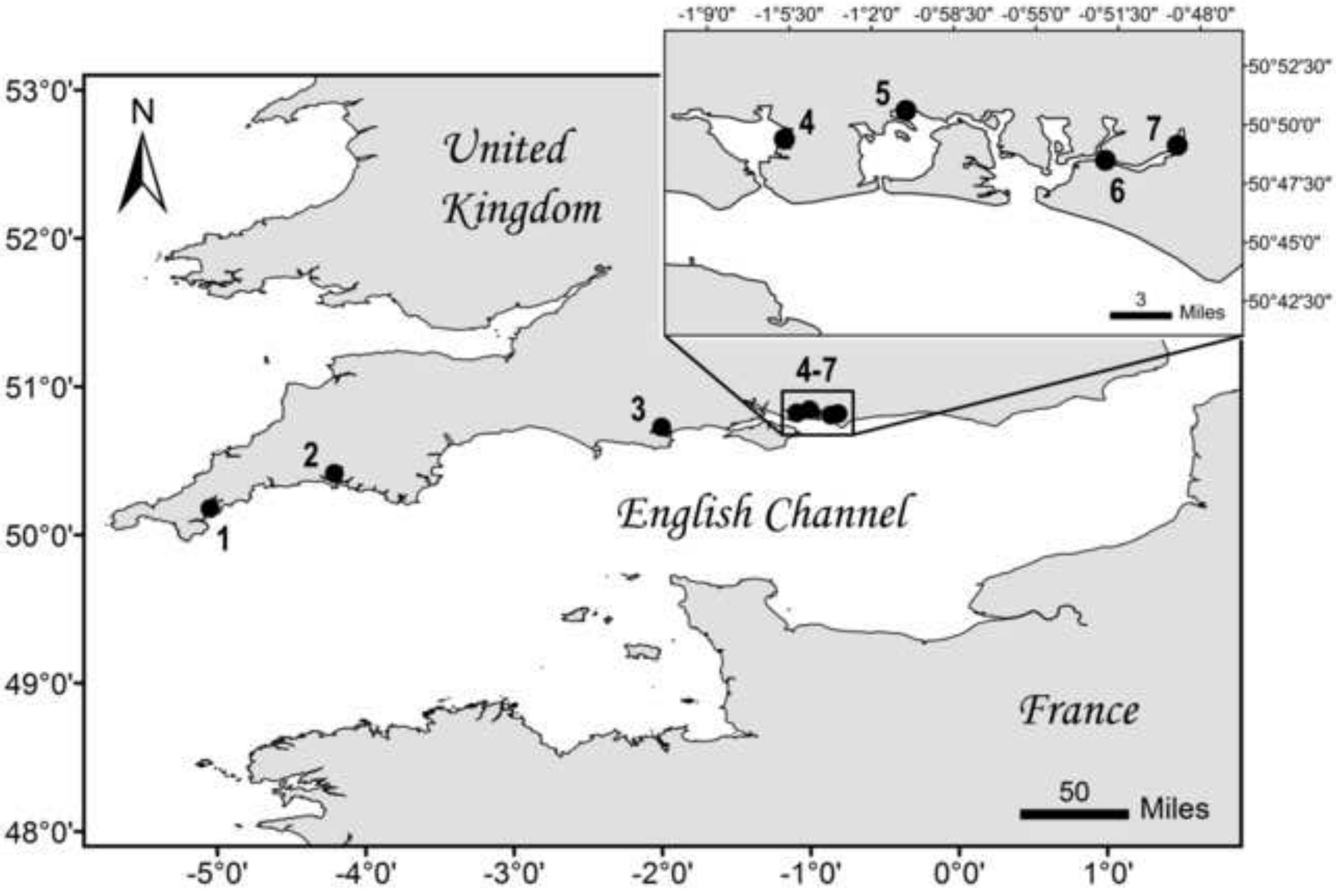
Cu and Zn tissue concentrations (mean ± SEM, in µg g^{-1} dry weight) and wet weight (mean ± SEM, in g) of *N. virens* (n = 10-15) sampled from six sites located along the English Channel coast from summer 2011 to winter 2012. No worms were sampled at Mylor (n/a: not available). Worm heads were removed before metal analyses due to the major role of Zn in hardening of Nereid jaws.

	N. virens individual concentrations x weight							
Sites	Cu				Zn			
	b	r^2	*p*	dev.	*b*	r^2	*p*	dev.
Mylor		n/a				n/a		
Saltash	1.562	0.382	< 0.05	s.	0.135	0.048	> 0.05	n.s.
Holes Bay	-0.185	0.299	< 0.05	s.	-0.111	0.023	> 0.05	n.s.
Tipner	-0.068	0.034	> 0.05	n.s.	-0.067	0.006	> 0.05	n.s.
Broadmarsh	-0.150	0.146	> 0.05	n.s.	-1.728	0.671	< 0.05	s.
The Conservancy	-0.078	0.078	> 0.05	n.s.	-0.368	0.770	< 0.001	s.
Dell Quay	-0.377	0.366	< 0.05	s.	0.414	0.105	> 0.05	n.s.

Table 6

Double log transformed power functions modelling relationships between *N. virens* individual metal concentrations and their respective weight. Worms (n = 10-15) were sampled from six sites located along the English Channel coast sampled from summer 2011 to winter 2012. No worms were sampled at Mylor (n/a: not available). *b* is the slope of linear functions and r^2, *p*-levels and deviation (dev.) from the model: s. = significant, n.s. = non-significant are the

Figure1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-0°51'30"
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50°52'30"
50°50'0"
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50°42'30"
3
Miles
4
5
6
7
53°0'
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United Kingdom
English Channel
France
1
2
3
4-7
50
Miles
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-4°0'
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-1°0'
0°0'
1°0'

Figure2

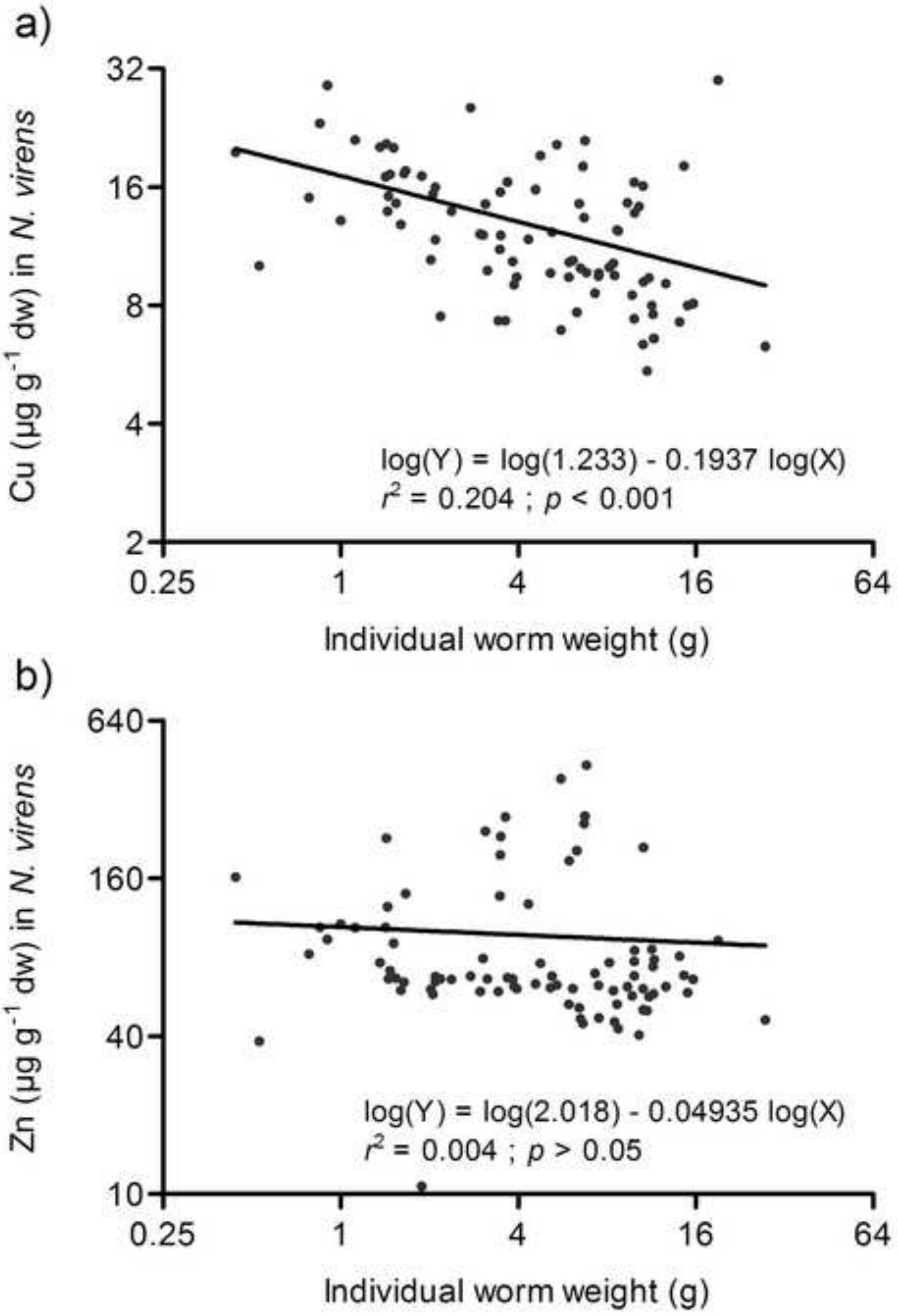
a)
32
16
8
4
2
Cu (µg g-1 dw) in N. virens
log(Y) = log(1.233) - 0.1937 log(X)
r2 = 0.204 ; p < 0.001
0.25
1
4
16
64
Individual worm weight (g)
b)
640
160
40
10
Zn (µg g-1 dw) in N. virens
log(Y) = log(2.018) - 0.04935 log(X)
r2 = 0.004 ; p > 0.05
0.25
1
4
16
64
Individual worm weight (g)

Figure3

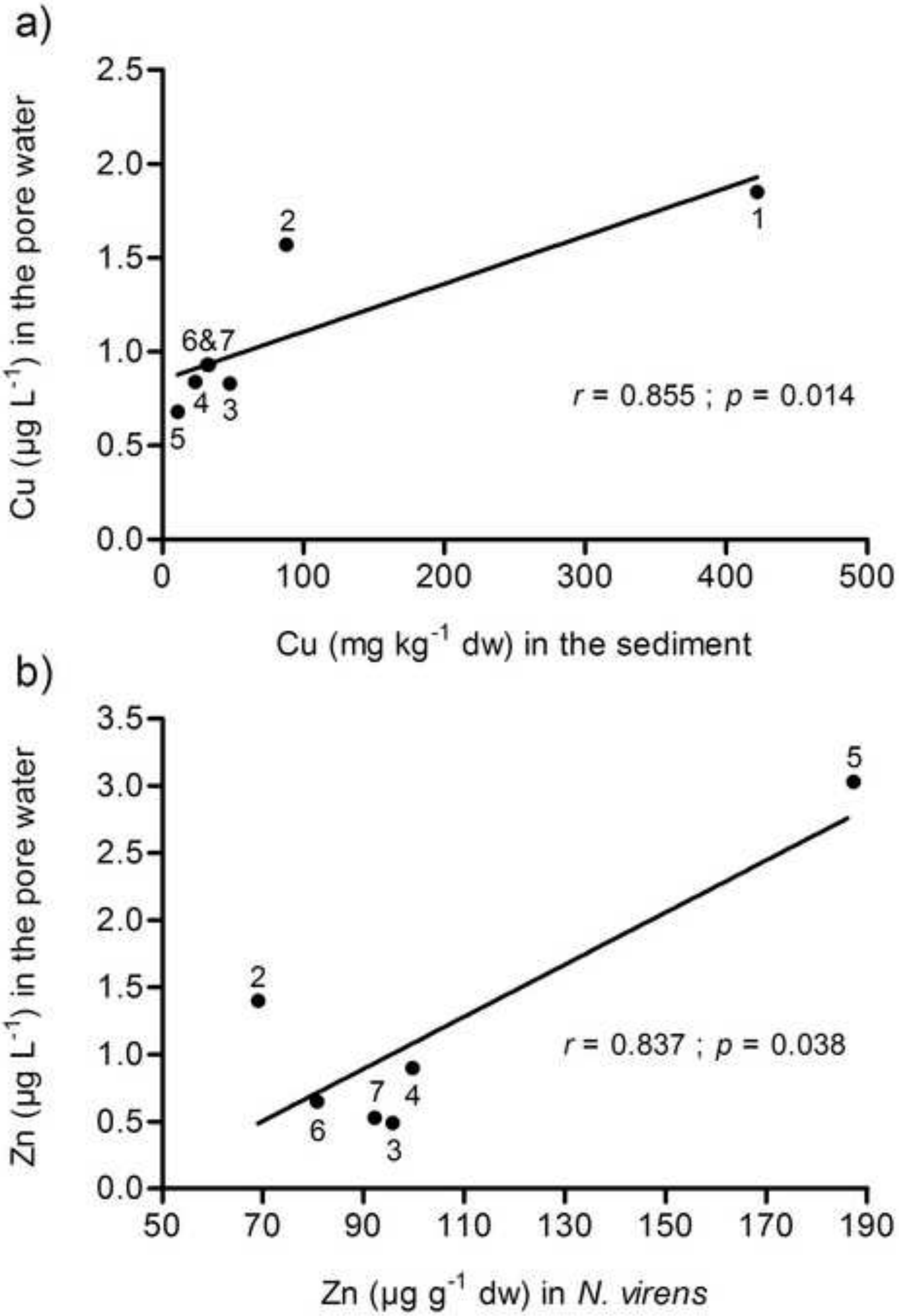
a)
Cu (µg L⁻¹) in the pore water
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
Cu (mg kg⁻¹ dw) in the sediment
1
2
3
4
5
6&7
r = 0.855 ; p = 0.014
b)
Zn (µg L⁻¹) in the pore water
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
50
70
90
110
130
150
170
190
Zn (µg g⁻¹ dw) in N. virens
2
3
4
5
6
7
r = 0.837 ; p = 0.038

Figure legends

Fig. 1.

Map showing the location of the seven sites located along the English Channel coast (Devon, Cornwall, Dorset & Hampshire counties) and sampled for sediments (n = 3), pore-water (n = 3) and *N. virens* (n = 10-15) from summer 2011 to winter 2012. Sampled sites are numbered on graphs: 1) Mylor, Fal Estuary; 2) Saltash, Tamar Estuary; 3) Holes Bay, Poole Harbour; 4) Tipner, Portsmouth Harbour; 5) Broadmarsh, Langstone Harbour; 6) The Conservancy and 7) Dell Quay, Chichester Harbour.

Fig. 2.

Double log transformed power functions modelling relationships between individual *N. virens* a) Cu or b) Zn concentrations (µg g^{-1} dry weight) and their respective wet weight (g). Worms (N = 86) were sampled from six sites located along the English Channel coast sampled from summer 2011 to winter 2012. Worm heads were removed before metal analyses due to the major role of Zn in hardening of Nereid jaws. Equations and their corresponding fitting parameters (r^2 and *p*-levels) are reported on the graphs.

Fig. 3.

Relationships between a) mean Cu concentrations in the pore water (µg L^{-1}) and mean Cu bioavailable concentrations in the sediment (mg kg^{-1} dry weight) and between b) mean Zn concentrations in the pore water (µg L^{-1}) and in *N. virens* (µg g^{-1} dry weight) sampled from seven sites located along the English Channel coast sampled from summer 2011 to winter 2012. Sampled sites are numbered on graphs: 1) Mylor, Fal Estuary; 2) Saltash, Tamar Estuary; 3) Holes Bay, Poole Harbour; 4) Tipner, Portsmouth Harbour; 5) Broadmarsh, Langstone Harbour; 6) The Conservancy and 7) Dell Quay, Chichester Harbour. Pearson's correlation coefficients (*r*) and their *p*-values are reported on the graphs. Mylor is not reported on graph b) as no worms were collected on this site. Worm heads were removed before metal analyses due to the major role of Zn in hardening of Nereid jaws. The BCR three-step sequential extraction procedure was used to determine the bioavailable concentrations of Cu and Zn in the sediment (Pueyo *et al.*, 2001).