An evaluation of the bioavailability and bioaccumulation of copper and zinc in the Polychaete *Nereis virens* from intertidal mudflats with different pollution histories.

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Abstract

The king ragworm (*Nereis virens*) is an ecologically and commercially important polychaete species of soft sediment inter-tidal communities of the northern hemisphere and is known to be impacted by various pollutants. More specifically in many coastal locations, *N. virens* is exposed to a range of heavy metals including copper (Cu) and zinc (Zn) which are known to be highly toxic to polychaetes. This species is also collected for bait which may change the bioavailability of these metals in the sediment, and those available to the worms. To assess the relationship between copper and zinc concentrations in the sediment, pore-water and associated worms, samples were collected from a number of sites on the south coast of the UK, with different levels of pollution. All with all sites characterised as very poorly sorted, very coarse skewed muddy sediment composed mainly of fine and very coarse silt. sites presented some differences in sediment characteristics which reflected different levels of metal pollution. The percentage organic matter of the sediment ranged from 2.32 % (Tipner) to 8.90 % for Mylor Interestingly, data from Langstone Harbour in the Solent show relatively low bioavailable metal concentrations in sediments despite being a heavily dug site. In opposition, Mylor situated in the Fal Estuary, which suffers from a heavily metal history, presented the highest metal pollution. For all sites, concentrations in the tissues also suggest that *N. virens* bioaccumulates more zinc than copper although inter-individual variability was high. However, no relationship was found between the metal tissue concentrations and the weight of the worms. Surprisingly, no correlation was observed between metal concentrations in the worms and their weight but strong correlations were found between copper concentrations in the sediment and in the pore water and between zinc concentrations in the pore water and in *N. virens*. Theses correlations revealed a strong link between copper concentration in the sediment and in the pore water and also that pore water is a main source for accumulation of zinc in *N. virens*. Finally, our data also suggest that, although sites can be dug areas, metal bioavailability and bioaccumulation can vary considerably.

1. Introduction

Copper (Cu) and zinc (Zn) are naturally present in the marine environment, but are also legacies of past contamination due their persistence and lack of biodegradability (Walker *et al.*, 2006). Although often considered as 'old pollutants' their industrial use is still significant with new applications such as nanoparticles. These new fields are likely to ensure that significant amounts will enter the natural environment (Heggelund *et al.*, 2014, Luoma, 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, 2008, Walker *et al.*, 2006). Copper is known to be one of the most common metals present in coastal marine sediments and is also one of the most toxic to many benthic organisms including polychaetes, especially early life stages (Reish and Gerlinger, 1997, Watson *et al.*, 2013). Although zinc is an essential metal for aquatic organisms (Walker *et al.*, 2006), high concentrations have been recorded in many coastal sediments (Bryan and Langston, 1992) and it is also toxic at elevated concentrations to polychaetes (Casado-Martinez *et al.*, 2013, King *et al.*, 2004, Wang and Rainbow, 2005). The toxic effects of these metals depend principally on their absorption, concentration, persistence and their mode of action (Shanker, 2008). Copper is an essential element of various proteins and enzymes with important functions in cellular respiration and cellular energy metabolism (Greim and Snyder, 2008). It plays an important role in redox reactions as it is able to transfer electrons and forms oxygen radicals. Therefore, the toxicity of copper can lead to effects on the structure and functions of proteins, membranes, DNA damage and oxidative stress (Schwarz *et al.*, 2013). While zinc plays an essential role in the functions of over 150 enzymes (Walker *et al.*, 2006), it has to be regulated to avoid toxic effects to the organisms (Rainbow and Smith, 2013). Levels of zinc in organisms are regulated 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, 1995b, 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 in to 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 or remove metals (bioaccumulation) (Amiard *et al.*, 2007, Casado-Martinez *et al.*, 2010, Pueyo *et al.*, 2001, van Gestel, 2008). Bryan and Langston (1992) provided a comprehensive list of metal concentrations in sediments; reviewing nineteen estuaries in the UK. Copper concentrations ranged from 7 mg kg^{-1} to 648 mg kg^{-1} dry weight of sediment and zinc 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 copper and 2821 mg kg^{-1} for zinc. However, these concentrations were expressed as total concentrations, obtained by nitric acid digestion. Methods for total metal concentrations evaluate the overall degree of contamination in an environment and differ from sequential extraction methods where the different fractions of metal partitioning are detailed so the total fraction does not reflect the bioavailable fraction (Hseu *et al.*, 2002, Tessier *et al.*, 1979). The utility of these 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).

To assess metal bioavailability in the sediment the revised three-step extraction procedure developed by the Community Bureau of Reference (BCR, now called Standards Measurements and Testing [SM&T] Programme) has been routinely used (Pueyo *et al.*, 2001). The sequential extraction was designed to target three fractions of the metals, the exchangeable, water and acid soluble (step 1); the reducible (step 2) and the oxidisable (step 3). 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 oxidisable fraction (Davidson *et al.*, 2004, Filgueiras *et al.*, 2002, Ianni *et al.*, 2010, Mossop and Davidson, 2003).

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 intertidal 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 (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, 1995b, Nayar *et al.*, 2006). Pore water chemistry has been recently included in the metals risk assessment framework and helps to assess sediment quality and aquatic ecosystem health (Fairbrother *et al.*, 2007). A number of *ex-situ* or *in-situ* methods have been developed over the years in order to study pore water chemistry (Bufflap and Allen, 1995a, Bufflap and Allen, 1995b, Chapman *et al.*, 2002), but we have used the most appropriate for field extraction of pore water from muddy sediments (Nayar *et al.*, in 2006).

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, 2011, 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 compared to *Nereis diversicolor* (Lewis and Gordon, 2011). It 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. virens*, unlike *N. diversicolor*, does not swap between feeding modes; it's omnivorous, so the routes of uptake are more certain (Nielsen *et al.*, 1995). It is 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 still makes it an important predator and prey item for many species (McIntosh, 1908-1910). Bait digging activities turnover the sediment which can release metals and increase metal bioavailability in the sediment (Howell, 1985, Watson *et al.*, 2007). Therefore, the relationship between the ability to bioaccumulate metals and the sediment/pore water environment ability is essential to understand toxicity of metals in the sediment and the greater impact on ecological function e.g. trophic transfer (Amiard *et al.*, 2007, King *et al.*, 2004, Rainbow *et al.*, 2006, Wang and Rainbow, 2005).

Even though metals are considered 'old pollutants', the ecological risk to and impact on the environment is far from being fully evaluated especially in the context of the relevance of

sediment quality guidelines (Crane, 2003, Hubner *et al.*, 2009, Luoma, 2008). For the first time this study aims to examine the relationship between metal concentrations in the sediment, pore water and tissues of *N. virens* and the impact of site-specific sediment characteristics. Seven sites with different pollution histories have been selected to (1) fully assess the copper and zinc 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 the relationship between tissue concentrations in *N. virens*. Finally, (3) the relationship between size and tissue concentrations for both metals has also been examined.

2. Materials and methods

2.1 Description of the sampling sites

Seven sites along the English Channel coast were selected based on their pollution history (Bryan and Langston, 1992, Hübner, 2009) and the presumed presence of nereids. These sites: Mylor, Fal Estuary; Saltash, Tamar Estuary; Holes Bay, Poole Harbour; Tipner, Portsmouth Harbour; Broadmarsh, Langstone Harbour; The Conservancy and Dell Quay, Chichester Harbour were sampled from summer 2011 to winter 2012 to determine metal concentrations in the sediment, the pore water and in the tissues of *N. virens*. A number of these sites are protected under various levels of European and national legislation and many are also used for the collection bait specifically for *N. virens* (Figure 1 and Table 1).

Fig. 1.

Table 1

2.2 Collection of samples

At low tide three cores of sediment (15 cm deep, 10 cm diameter) were collected from the mid-shore area and were immediately placed in polyethylene Minigrip plastic re-sealable bags. The GPS coordinates were recorded using a Garmin eTrex device. Next to each

sediment core, pore water samples were also collected using a porextractor device (Nayar *et al.*, 2006). The pore water samples, collected from a depth of approximately 10 cm, were directly transferred to 15 ml centrifuge tubes s. At each site, except Mylor, ten to fifteen worms were collected by turning over the sediment to a depth of about 20 cm and removing by hand. All samples were transported from the field to the laboratory in isothermal containers before being refrigerated at 4 °C until further analysis for sediment cores or frozen at -20 °C for pore water. All worms were kept in sediment-free conditions for 24 hours to depurate their guts before being frozen.

2.3 Sample processing

All chemicals used during the sample processing were purchased from Sigma-Aldrich and the materials such as centrifuge tubes were obtained from Fisher Scientific. Sediment samples were dried at 105 °C and then placed in a muffle furnace at 475 °C for 4.5 hours and samples were then reweighed to give the mean percentage organic content. For the particle size analysis a wet sieving method was performed by sieving one scoop (25 ml) of wet sediment through a 63 micron mesh. For the particles less than 63 μm, 20 ml of a solution containing 33 g of sodium hexametaphosphate with 7 g of sodium carbonate dissolved into a beaker with 1 l distilled water was added to each sample and left overnight to settle. The samples were then transferred into a sonication bath for 2 hours before being assessed by laser diffraction (Malvern Mastersizer 2000).

Sediment samples were analysed using the 3-step BCR method sequential extraction developed by the Standards, Measurements and Testing Programme (formerly BCR) of the European Commission (Mäkelä *et al.*, 2011, Pueyo *et al.*, 2001) . This method assesses the distribution of metals in the following fractions: (a) exchangeable (e.g. water and acid soluble); (b) reducible (e.g. iron/manganese oxides) and (c) oxidizable (e.g. organic matter and sulphides) and are described as the fraction of the metals available to the environment (Davidson *et al.*, 1994, Filgueiras *et al.*, 2002, Kwon *et al.*, 2001, Ramos *et al.*, 1999, Zimmerman and Weindorf, 2010). Briefly, 0.5 g of the (<63 μm) dried sediment was used to obtain the 3 steps of the BCR sequential extraction according to Pueyo *et al.*, (2001). BCR-701 reference sediment was used to monitor recovery and efficiency levels (Pueyo *et al.*, 2001, Sutherland, 2010). Percentage recoveries for copper for steps one, two and three were 110.64 ± 2.14, 97.58 ± 1.66, 102.20 ± 0.80, respectively. Recoveries for zinc steps one, two and three were 99.02 ± 0.86, 92.5 ± 4.51, 103.1 ± 0.21, respectively. The pore water samples

were acidified by adding 25 µl of concentrated nitric acid (HNO_3) to the samples and left to digest for 30 minutes. 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).

Worms were defrosted and the heads removed due to the major role of zinc in hardening of Nereid jaws (Broomell *et al.*, 2006, Bryan and Gibbs, 1979). They were then dried individually at 60 °C for 24 hours before digesting according to (Berthet *et al.*, 2003, 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 (15.8 mol l^{-1}). All tubes were then heated in a water bath at 75 °C for 5 to 6 hours, but checked every hour with more acid added 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 for copper of 91.63 ± 1.93 and 99.52 ± 2.84 for zinc. Sediment, pore water and the tissue samples were all analysed using a Varian Spectra AA 220FS Flame Atomic Absorption Spectrophotometer FAAS. For each of the assays, all glassware was rinsed in 10 % analytical grade HCL and then three times in distilled water before being left to dry.

2.4 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 the Individual Distribution Identification function from Minitab to assess which transformation provided the best normality, either a square root transformation or the Johnson transformation. Arcsine transformed data for percentage organic content and particle size were analysed using a one-way ANOVA and a subsequent Tukey's pairwise comparisons. Data for metal concentrations in sediments, pore water, tissues and worm weights were all transformed using either a square root transformation or a Johnson transformation. These transformations were all followed by a one-way ANOVA and a subsequent Tukey's pairwise comparisons. Particle size distribution descriptors were produced using Gradistat (version 8.0) (Blott and Pye, 2001) and then analysed as above.

3. Results

3.1 Sediment characteristics

Sediment site characteristics are presented in Table 2 with all sites characterised as very poorly sorted, very coarse skewed muddy sediment composed mainly of fine and very coarse silt. The majority of sediment from Mylor was composed of very coarse silt whereas The Conservancy's sediment was mainly composed of fine silt. All the other sites were similar to The Conservancy with the majority of the sediment being fine silt. Subsequent analyses did reveal other differences between key descriptors. The percentage organic matter of the sediment ranged from 2.32 % (Tipner) to 8.90 % for Mylor with an overall significance difference between sites when compared using a one-way ANOVA ($F_{6, 14} = 63.82$, $p = 0.000$). Pairwise comparisons confirm that all sites were significantly different from each other except Mylor and Holes Bay; Dell Quay and The Conservancy; and Dell Quay and Tipner. The grain size distribution is described geometrically using Folk and Ward giving four parameters: (a) the average size, (b) the spread of the sizes around the average (sorting), (c) the symmetry or preferential spread to one side of the average (skewness), and (d) the degree of concentration of the grains relative to the average (kurtosis) (Blott and Pye, 2001). Statistical analysis of the mean particle size showed significant differences between all sites ($F_{6, 14} = 44.24$, $p = 0.000$), with the means ranging from 14.16 μm for The Conservancy to 28.17 μm for Mylor. Mylor was significantly different from all the other sites. Saltash was significantly different from all sites except Broadmarsh and Dell Quay. With the lower mean particle size, The Conservancy was not significantly different from Holes Bay and Tipner. One-way ANOVA 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. Mylor was also significantly different from all sites for level of sorting. Saltash was significantly different from all sites except Broadmarsh and Dell Quay. Tipner was also 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 with the exception of 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. Indeed, The Conservancy and Tipner had a mesokurtic (normal) distribution whereas all sites had a platykurtic (relatively flat) distribution.

3.2 Metal bioavailability in the sediment

The concentrations of zinc and copper from the different BCR steps are presented in Table 3. The bioavailable fraction of metals in the sediment was obtained by summing the three steps of the BCR method (Davidson *et al.*, 1994, Filgueiras *et al.*, 2002, Kwon *et al.*, 2001, Pueyo *et al.*, 2001, Zimmerman and Weindorf, 2010). Metal bioavailability ranged from 10.83 mg kg^{-1} dw (Broadmarsh) to 421.83 mg kg^{-1} dw (Mylor) for copper and 36.43 mg kg^{-1} dw (Broadmarsh) to 670.99 mg kg^{-1} dw (Mylor) for zinc. As expected there were significant differences between all sites for zinc ($F_{6, 14} = 14.66$, $p = 0.000$) and copper ($F_{6, 14} = 105.89$, $p = 0.000$) using one-way ANOVAs after a Johnson transformation. The pairwise comparisons for copper bioavailability 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. Bioavailability of zinc showed that the sites separated in to two groups. Mylor, Saltash and Holes Bay had high levels of zinc (concentrations ranged from 159.35 mg kg^{-1} (Holes Bay) to 670.99 mg kg^{-1} (Mylor). 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 36.43 mg kg^{-1} to 54.02 mg kg^{-1}. Copper was mainly present in the oxidisable fraction with an average of 49 %, then in the reducible fraction with 30 % and in the exchangeable fraction with 21%. For zinc, 63% of its bioavailability was expressed in the exchangeable fraction, then 19 % in the reducible and 18 % in the oxidisable fraction. The Conservancy had the highest percentage of copper (63%) present in the oxidisable fraction and the lowest percentage of zinc present in the exchangeable fraction (44%). With 77% of zinc present in the exchangeable fraction, Saltash had the highest percentage of zinc present in this step over all the sites.

Table 3

3.3 Metal concentrations in the pore water

Metal concentrations in the pore water (Table 4) showed a highly significant difference between sites for copper ($F_{6, 14} = 4.94$, $p=0.007$). Even though pore water concentrations of zinc ranged from 0.49 µg l^{-1} for Holes Bay to 2.58 µg l^{-1} for Broadmarsh, no significant differences were present between sites ($F_{6, 14} = 2.19$, $p=0.117$). Copper concentrations in the pore water ranged from 0.59 µg l^{-1} for Broadmarsh to 1.85 µg g^{-1} for Mylor with this site having significantly higher concentrations than Broadmarsh or Holes Bay. All other sites were not significantly different from each other.

Table 4

3.4 Metal accumulation in *N. virens*

Mean tissues concentrations of both zinc and copper differed considerably between sites (Table 5) with tissue concentrations of copper varying from 7.36 µg g^{-1} dw for Broadmarsh to 17.42 µg g^{-1} dw for The Conservancy. Variability in zinc concentrations was even higher with a mean of 68.98 µg g^{-1} dw for worms from Saltash to 182.21 µg g^{-1} dw for those collected from Broadmarsh even though worms from Broadmarsh had the lowest copper accumulation. One-way ANOVAs using a square root transformation revealed significant differences between sites for copper ($F_{5, 80} = 8.18$, $p = 0.000$) only. Worms from Broadmarsh had significantly lower tissue concentrations than worms from all other sites except Holes Bay and Saltash. Worms from the Conservancy also had significantly higher levels than Saltash.

Table 5

3.5 Tissue concentration and weight

The analysis of the wet weights of those worms collected confirms that their mean weight also differed between sites ($F_{5, 80} = 17.83$, $p = 0.000$). Worms from Saltash and Broadmarsh were significantly heavier than worms from all other sites, but were not different from each other. As weight of the worm is an obvious variable that might be important in determining the tissue concentrations of both metals so correlations of each worm's weight and tissue concentration were performed (Fig. 2 and 3). Across all sites copper concentrations and

worm weight had a weak, but significant negative correlation (Pearson correlation = -0.337, p = 0.002). For zinc concentrations and worm weight, the correlation was still negative but not significant and very weak (Pearson correlation = -0.132, p = 0.226). These results suggest that heavier worms have accumulated less copper in their tissues. It is possible that these relationships are confounded by site-specific differences so correlations between copper concentrations and weight for each site (Table 6) were also performed. Analysis revealed that only Saltash had a significant positive modest correlation. For all the other sites, the correlation was weak, negative and not significant. Data for zinc revealed a mixture of positive (Saltash and Dell Quay) and negative correlations, but all, were weak and non-significant.

3.6 Tissue concentration and metal concentrations in the sediment and pore water

No strong or significant correlation was obtained between copper accumulation in the tissues and copper concentrations in the sediment (Pearson correlation = -0.054, p = 0.919) or in the pore water (Pearson correlation = -0.054, p = 0.958). However, copper concentrations in the pore water and copper bioavailability in the sediment had a significant strong positive correlation (Pearson correlation = 0.855, p = 0.014) (Figure 4) with zinc accumulation and zinc concentration in the pore water also having a significant strong positive correlation (Pearson correlation = 0.837, p = 0.038) (Figure 5). No strong or significant correlation was shown between zinc bioavailability in the sediment and zinc in the pore water (Pearson correlation = 0.321, p = 0.482) or zinc accumulation in the tissues (Pearson correlation = -0.471, p = 0.346).

4. Discussion

The seven sites were chosen because of their diverse pollution histories in relation to copper and zinc (Bryan and Langston, 1992). Although there are significant site-specific differences in the characteristics of the sediment (especially organic content) the percentage fractions of silts and other descriptors do show that they are they are all generally very poorly sorted, muddy sediment with similar proportions of silt types. levels of zinc and copper and so it is no surprise to see the differences in concentrations of zinc and copper. . The Fal Estuary is known to have been impacted for centuries by mining activity making it one of the most metal polluted estuaries in the UK with values reaching 2398 µg g^{-1} (dry weight) for copper

and 2821 µg g^{-1} (dry weight) for zinc in Restronguet Creek (Bryan and Langston, 1992). In January 1992, occurred a devastating event, the Wheal Jane incident. The Wheal Jane mine accidentally released between 25,000 to 50,000 m^3 of metal contaminated mine water in a 24-h period via Carnon River into Restronguet Creek and affected the entire Fal Estuary (Younger *et al.*, 2005). This mine water contained in excess of 3500 mg l^{-1} of dissolved metals (principally iron, zinc, cadmium, copper, arsenic, aluminium, plus other traces of toxic metals) and lead to an increase of zinc and copper concentrations respectively 880 and 250 times higher than the quality standards (EQS) for the Carnon River (EQS : 500 µg l^{-1} for zinc and 28 µg l^{-1} for copper) (Younger *et al.*, 2005). Recent data for Mylor still show high levels of metal pollution in the Fal Estuary with 852 ppm for copper and 856 ppm for zinc based on total acid digestion (pers. com., 2012, Dr Bill Langston, Marine Biological Association, Plymouth). From our study, sediment from Mylor had high organic content and high mean particle size revealing a high probability of metal binding in the environment. Sites with high microbial and benthic community are more likely to be affected by metal contamination in the sediment and the pore water through uptake routes and bioaccumulation (Standley, 1997). In addition, particle grain size is an important factor in metal binding (Amiard *et al.*, 2007). From our data, Mylor had the highest metal bioavailable concentration in the sediment and high metal concentrations in the pore water. Historical data combined with the latest data from our study reflect a persistence of metal concentrations in the Fal Estuary. In 1992, Bryan and Langston listed the Tamar Estuary as the 3rd contaminated sites in the UK with 330 µg g^{-1} (dry weight) for copper and 452 µg g^{-1} (dry weight) for zinc based on total metal concentrations in the sediment. With metal bioavailable concentrations of 87.84 mg kg^{-1} dw for copper and 175.10 mg kg^{-1} dw for zinc combined with high organic content and high particle size obtained in this study, Saltash (Tamar Estuary) seems to be a site where metal pollution is relatively high. The same study from 1992 revealed high concentrations of copper (50 µg g^{-1} dw) and zinc (165 µg g^{-1} dw) in Holes bay (Poole Harbour) sediments (Bryan and Langston, 1992). Seventeen years later, another study revealed high metal concentrations with 52 mg kg^{-1} for copper and 215 mg kg^{-1} for zinc showing metals longevity in sediments (Hübner, 2009). From our sediment characteristics data, Holes Bay had lower metal concentrations than Mylor and Saltash but high organic content showing still high probability of metal binding in the environment. Portsmouth, Langstone and Chichester Harbours are three adjacent Harbours situated in Hampshire less impacted by metal pollution history. Interestingly, Broadmarsh (Langstone Harbour) had the lowest metal concentration in the sediment, the lowest copper concentration in the pore water but the highest zinc concentration

in the pore water. This high concentration in the pore water for zinc could be explained by the high value obtained for one from the core but it could also be explained by seasonal variability. Seasonal changes in pore water and sediment redox geochemistry can strongly influence metal distribution and therefore lead to fluctuations in metal bioavailability (Koretsky *et al.*, 2006). Sampling for this study was undertaken over the same season with the exception of Broadmarsh sampled in summer. The difference in sampling time could explain the high level of zinc concentration in the pore water while oxidisable copper concentrations have been proven to be similar in winter and spring and is expected to be similar in summer (Koretsky *et al.*, 2006). Therefore, seasonality at the exception of Broadmarsh for zinc concentration in the pore is not a confound factor for our data. Tipner (Portsmouth Harbour), Dell Quay and The Conservancy (Chichester Harbour) presented similar sediment characteristics and therefore similar metal concentrations. All four sites had low metal bioavailability in the sediment compared to Mylor, Saltash and Holes Bay. In addition, The Conservancy presented the lowest mean particle size indicating a lower probability in metal binding. Regarding the grain size distribution of the sediment, The Conservancy and Tipner had a mesokurtic (normal) distribution whereas all sites had a platykurtic (relatively flat) distribution revealing that most sites had a wider range of distribution and are most likely to be affected by metal binding than The Conservancy and Tipner. Chichester and Langstone Harbours are both site designated as Site of Special Scientific Interest (SSSI) and are of marine biological importance (Natural England). Therefore, at these sites the water quality is routinely monitored by the Environment Agency (EA).

The link between metal concentrations in the environment and their availability for bioaccumulation in different species is very complex and involves a number of parameters such as salinity, temperature, acid volatile sulphide (AVS) (Bryan and Langston, 1992, Ogendi *et al.*, 2007), which were not recorded in this study.

Environmental quality standards (EQS) are used as guidelines to evaluate the potential risk of various pollutants to marine biota. The revised UK annual average EQS for dissolved copper and zinc are 5 µg l^{-1} and 10 µg l^{-1} in seawater, respectively (Matthiessen *et al.*, 1999), whilst the Ecotoxicological Assessment Criterion was proposed by the Oslo and Paris Commission (OSPAR) with 0.1 - 1.0 µg l^{-1} for copper and 0.5 - 5.0 µg l^{-1} for zinc (OSPAR, 1994). Pore water data has been compared to EQS seawater guidelines, but for organic contaminants only (Dueri *et al.*, 2008). We do not believe that the seawater EQSs can be

quantitatively compared to the value obtained for the pore water samples, unless the fluxes between pore water and overlying seawater for a site is known (Teasdale et al., 1995). However, a direct comparison does reveal that porewater concentrations for both zinc and copper are well within these values for the UK EQS, but close to or exceeding the upper thresholds for EACs for copper for all sites except Broadmarsh suggesting that at these sites copper represents a potential ecotoxicological risk for aquatic species.

Sediment Quality Guidelines (SQGs) were developed to assess risk for metal concentrations in the sediments. Even though no uniform guidelines exist, a number of methods have been developed to estimate the potential adverse effects of pollutants to aquatic life (Crane, 2003, Hubner *et al.*, 2009, Luoma, 2008). Four threshold values have been selected to compare with our data: ERL (Effects Range-Low), ERM (Effects Range-Medium) TEL (Threshold Effect Levels) and PEL (Probable Effect Levels) (Table 7). However, it should be noted that theses values are based on total concentrations of metals in sediments (strong acid digestion). Our study is based on bioavailable copper and zinc concentrations which in previous work represented an average of 84.5% of total copper (Watson *et al.*, 2013) and 70% of zinc concentrations (Kwon *et al.*, 2001, Watson *et al.*, 2013). In addition, the certified concentrations for the BCR - 701 showed that bioavailable copper and zinc concentrations represented between 80.74 % - 85.50% (average 83.12%) and 78.26 % - 82.50 % (average 80.38%) of the total copper and zinc concentrations respectively (Sutherland, 2010). Comparing bioavailable metal concentrations to SQGs is therefore possible when taking into account the bioavailable fraction of total concentration. Using these data, only Broadmarsh was below all four SQGs for copper and zinc confirming that Broadmarsh is at minimal risk for toxic effects on aquatic organisms. Tipner, Dell Quay and The Conservancy were also below the standards for zinc making these sites at low risk for adverse effects. In contrast, Holes Bay, Saltash and Mylor have bioavailable copper concentrations that exceed the TEL and ERL values for copper and zinc and in the case of Saltash and Mylor exceed the PEL for copper and the PEL and ERM for zinc. The majority of these sites, therefore, exhibit bioavailable concentrations of these metals that have the potential for adverse effects on the benthos (Miller et al., 2000).

Critical to the interpretation of SQGs is the level of bioavailability. Total extractable metal analysis provides information concerning possible enrichment of sediment, but the concentrations of bioavailable metals are not directly proportional to the concentrations 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 sediment and the biota, concentrations in the pore water were also measured as it is a key exposure route for contaminants (Chapman et al., 2002). Pore water concentrations varied significantly between sites for copper, but were also significantly positively correlated with the bioavailable fraction in the sediment. This would indicate that the bioavailable fraction in the sediment directly influences the levels in the pore water. However, the concentrations in the pore water are also significantly strongly correlated with overlying water concentrations (Pearson correlation 0.868 p= 0.025) (EA). A number of authors (Chapman, 2002; Warren et al., 1998; Wang et al., 2001) have shown that for infauna living within a burrow that the overlying water is the main exposure route for contaminants 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 copper. The affinity for copper 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 sediments for long periods. Although it did vary between sites the majority of the copper was present in the oxidisable phase giving weight to the theory that most of the copper present in the pore water comes 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.

In contrast pore water concentrations of zinc did not vary significantly between sites and were not significantly positively correlated with the bioavailable fraction in either the sediment or the overlying water (Pearson correlation 0.137 p= 0.795). Zinc like copper is a chalcophile metal and under anoxic condition has a strong affinity to sulphide phases and carbonates (Koretsky *et al.*, 2006), However, our data revealed across all sites that only a small amount of zinc is affiliated with this phase; most is in the exchangeable phase. Thus, zinc seems to be more readily available than copper. It is not clear why the site variability was so high, specifically the high concentration at Broadmarsh. The majority of sites chosen are utilised regularly for bait collection, specifically for *N. virens* which involves repeated turning of the sediment to a depth of approximately 30 cm to extract bait (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 the local sediment conditions may result in a much higher level of

availability of copper and zinc (as well as other pollutants) to the benthos. An urgent assessment of the impacts of this process is, therefore, required.

Many studies have investigated the relationship between metal bioavailability and tissue concentrations for *N. diversicolor* (Berthet et al., 2003; Mouneyrac et al., 2003; Zhou et al., 2003; Poirier et al., 2006; Amiard et al., 2007; Rainbow et al., 2009; Bird et al., 2011; Garcia-Alonso et al., 2012; Rainbow and Smith, 2013), but none have investigated *N. virens*. These studies, broadly speaking, have reported tissue concentrations of copper in *N. diversicolor* from equivalent sites to be higher than those of *N. virens*, even in sites with high sediment concentrations (Mylor, Saltash and Holes Bay). This is confirmed by the lack of a correlation between bioavailable copper concentration and tissue concentration. Although total metal concentrations are long recognized as not having a predictive ecotoxicological value it is surprising to that bioavailable fractions of copper in the sediment are also not reflected in the tissues. Zinc tissue concentrations are more comparable, but do seem to be more variable between sites in our study.

it is clear that there is considerable inert-individual variation.

Although, no *N. virens* were found while sampling at Mylor, personal communication with bait shops assured the presence of *N. diversicolor* and *N. virens* in the Fal Estuary. *N. diversicolor* can be found at highly contaminated site and is known to be tolerant to copper and zinc contaminated sediments (Bryan and Langston, 1992). Developing metal resistance capacities is expected to lead to an energy cost or a cost of tolerance (Durou, 2005, Pook *et al.*, 2009). Here we reported, different levels of copper and zinc bioaccumulation in *N. virens* suggesting that this species like *N. diversicolor*, is also tolerant to copper and zinc. The study from Poirier et al. (2006) compared levels of copper and zinc in *N. diversicolor* from a contaminated site (Seine Estuary) and a reference site (Authie Estuary) on the French coast of the English Channel. Our bioaccumulation data can be compared to levels obtained from the reference site, Authie Estuary, revealing that *N. virens* accumulated lower levels of metals than *N. diversicolor*. This observation can be confirmed with a more recent study where metal accumulation in *N. diversicolor* was monitored from three sites in the UK, Blackwater (Essex), Tavy (Cornwall) and Restronguet Creek (Cornwall) (Rainbow and Smith, 2013). These sites sampled in September 2010 revealed that copper ranged from 23.1 $\mu g\ g^{-1}$ to 662 $\mu g\ g^{-1}$ and zinc from 132 $\mu g\ g^{-1}$ to 214 $\mu g\ g^{-1}$ in *N. diversicolor*. All our data for *N. virens* are lower than theses concentrations at the exception of Broadmarsh where *N. virens* accumulated higher levels of zinc. However, highest concentrations of metals in *N. virens*

compared to *N. diversicolor* are not unusual as *N. diversicolor* is a prey of *N. virens*. Previous studies of trophic transfer from prey *N. diversicolor* to predator *N. virens* revealed net accumulation of metals from the prey (Rainbow *et al.*, 2006) and differences in internal distributions of copper and zinc that could explain the regulation of metals in *N. virens* and the trophic availabilities of theses metals (Rainbow and Smith, 2013).

Mylor, Saltash and Holes Bay were the sites with the highest metal concentrations in the sediment but this did not reflect high metal accumulation in the tissues. Even though, copper concentrations in the sediment were strongly linked to copper concentration in the pore water, no correlation was obtained for copper concentration in the environment with copper accumulation in the worms. Therefore, our study showed that *N. virens* is tolerant to copper independently of metal bioavailability in the sediment and metal concentrations in the pore water. This suggests that *N. virens* can survive in highly contaminated site and has therefore developed metal resistance. For zinc, our data revealed that zinc accumulation in *N. virens* was strongly correlated to the concentration of zinc in the pore water but not to zinc bioavailability in the sediment. Therefore, zinc accumulation in the worms doesn't seem to be directly impacted by the metal history of the site or the sediment characteristics or the zinc bioavailable concentrations in the sediment but is strongly influenced by the level of zinc concentrations in the pore water.

Monitoring biological responses to a pollutant requires the use of biomarkers. Over the years more and more studies have been conducted looking at the effect of metals in aquatic invertebrates using a range of different biomarkers such as behavioural responses (Buffet *et al.*, 2011, Lead *et al.*, 2011), lysosomes (Ballan-Dufrantais *et al.*, 2001, Mouneyrac *et al.*, 2003, Ringwood *et al.*, 1999), metallothioneins (Amiard *et al.*, 2006, Berthet *et al.*, 2005, Won *et al.*, 2008) or genotoxic responses (Lewis and Galloway, 2008). The addition of biomarkers to this study would give a full investigation of the effects of copper and zinc on an individual and a population level of the polychaete *N. virens* using environmentally relevant concentrations.
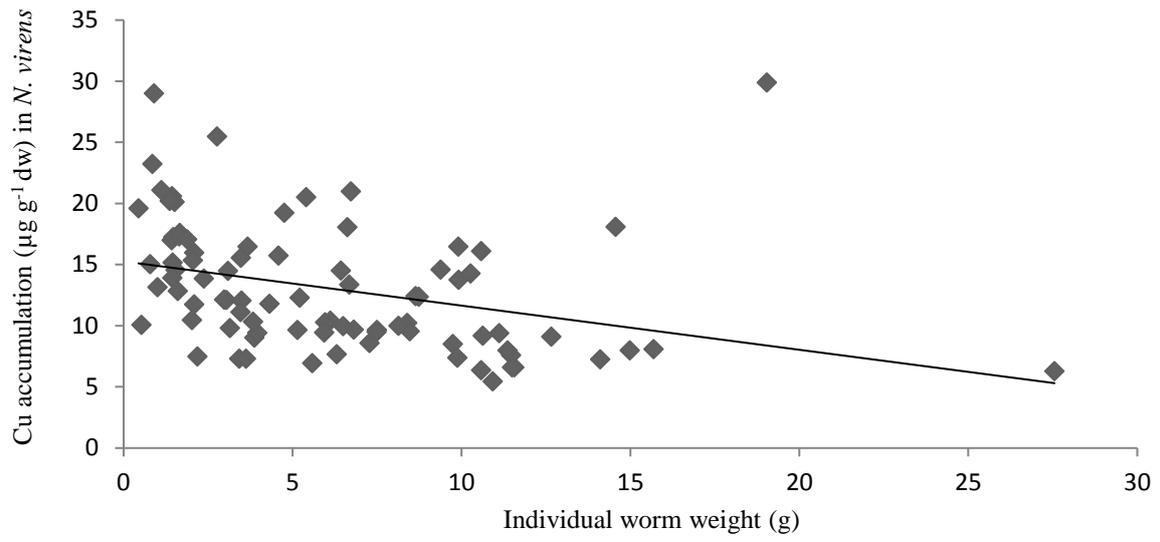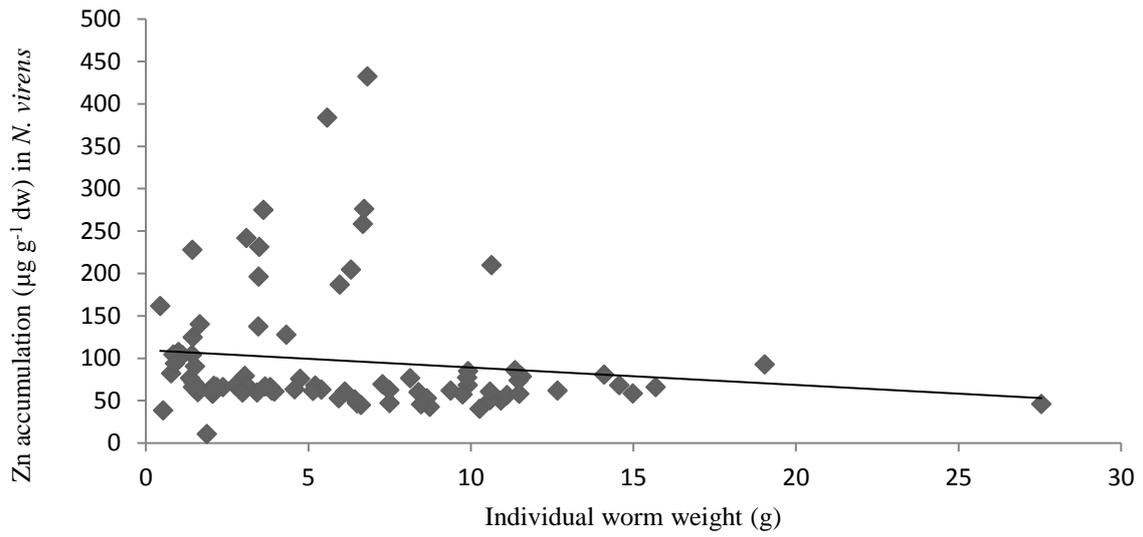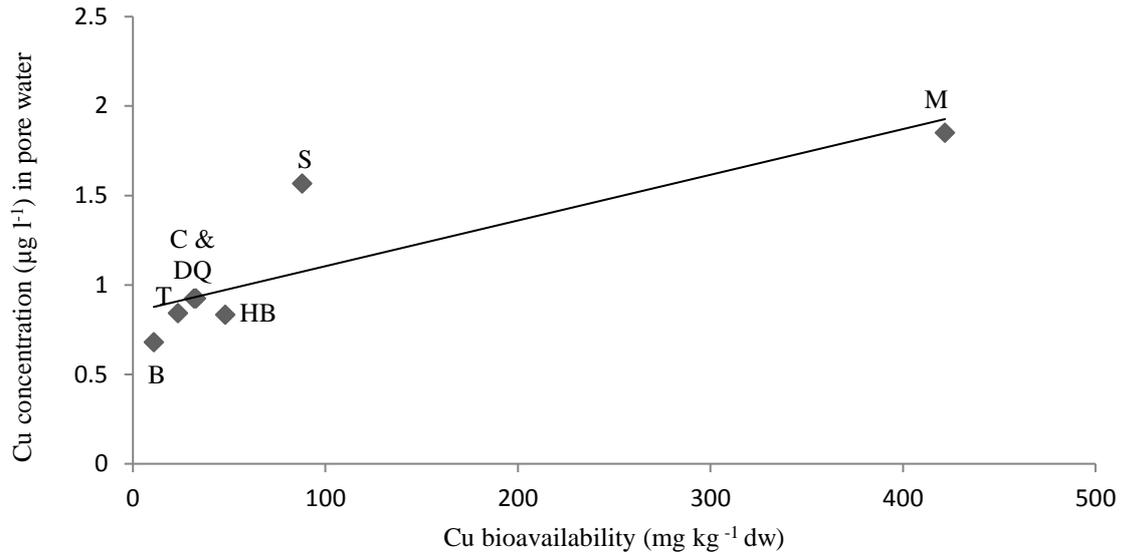
Fig. 1.

Fig. 2.

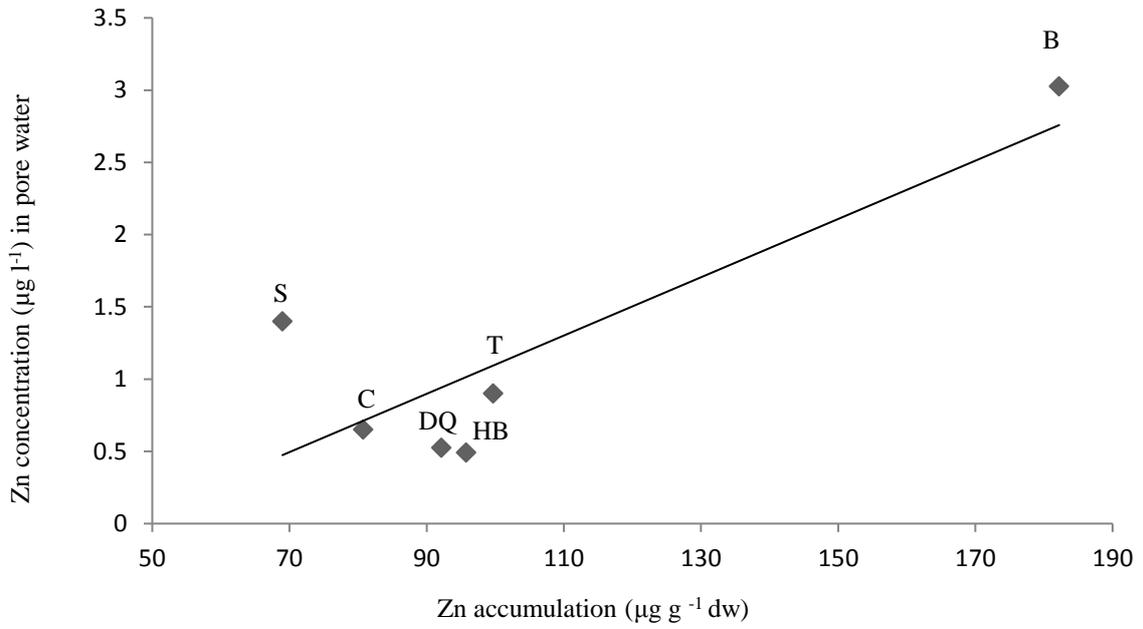**Fig.3.**

Fig. 4.

Fig. 5.

Table 2

Sampling sites	GPS coordinates	Sampling time	Conservation designations	Site used for bait collection evidence from (Watson *et al.*, 2007) and Pers. Comm. from Dr Watson and bait shops
Mylor Fal Estuary (Cornwall)	50°10'42"N,5°04'046"W; 50°10'40.83"N,5°3'27.48"W; 50°10'37.27"N,5° 2'57.91"W	23.02.2012	SAC SSSI	no
Saltash Tamar Estuary (Devon)	50°24'58.12"N, 4°12'38.97"W; 50°24'558"N, 4°12'409"W; 50°24'47.51"N, 4°12'34.71"W	24.02.2012	SAC SPA SSSI	yes
Holes Bay Poole Harbour (Dorset)	50°43'617"N, 2°00'448"W; 50°43'619"N, 2°00'451"W; 50°43'619"N, 2°00'455"W	27.10.2011	SPA	yes
Tipner Portsmouth Harbour (Hampshire)	50°49'310"N, 1°05'744"W; 50°49'312"N, 1°05'736"W; 50°49'312"N, 1°05'754"W	26.10.2011	SPA	yes
Broadmarsh Langstone Harbour (Hampshire)	50°50'223"N, 0°59'702"W; 50°50'215"N, 0°59'698"W; 50°50'207"N, 0°59'694"W	22.07.2011	SAC SEMS SPA SSSI	yes
Dell Quay Chichester Harbour (Hampshire)	50°49'086"N, 0°48'941"W; 50°49'04"N, 0°48'56"W; 50°49'03"N, 0°48'56W	28.10.2011	SAC SPA SSSI	yes
The Conservancy Chichester Harbour (Hampshire)	50°48'470'"N, 0°51'938"W; 50°48'28"N, 0°52'00"W; 50°48'29"N, 0°52'01"W			no

Table 3

Study sites	Organic content (mean % ± SEM)	Mean (x_a) ± SEM	Sorting (σ_a) ± SEM	Skewness (Sk_a) ± SEM	Kurtosis (K_a) ± SEM	% Mud	Very coarse silt (%) (x) ± SEM	Medium Silt (%) (x) ± SEM	Fine Silt (%) (x) ± SEM	Very fine silt (%) (x) ± SEM
Mylor	8.90 ± 0.35	28.17 ± 0.34	5.58 ± 0.01	0.41 ± 0.01	0.71 ± 0.00	100	40.92 ± 0.28	10.81 ± 0.10	37.37 ± 0.47	0.09 ± 0.00
Saltash	6.68 ± 0.48	20.42 ± 0.69	5.17 ± 0.04	0.64 ± 0.03	0.82 ± 0.01	100	33.85 ± 0.59	9.55 ± 0.24	46.94 ± 1.01	0.11 ± 0.00
Holes Bay	8.83 ± 0.18	16.60 ± 0.37	4.75 ± 0.06	0.75 ± 0.00	0.96 ± 0.02	100	28.75 ± 0.72	8.39 ± 0.38	54.34 ± 1.44	0.13 ± 0.00
Tipner	2.32 ± 0.19	15.27 ± 0.55	4.50 ± 0.12	0.76 ± 0.00	1.04 ± 0.04	100	26.29 ± 0.97	7.62 ± 0.06	58.34 ± 1.08	0.13 ± 0.00
Broadmarsh	5.20 ± 0.31	21.43 ± 1.08	5.09 ± 0.07	0.57 ± 0.04	0.84 ± 0.02	100	34.05 ± 1.01	11.00 ± 0.28	43.90 ± 1.54	0.10 ± 0.00
Dell Quay	3.28 ± 0.42	19.45 ± 0.87	5.12 ± 0.06	0.69 ± 0.04	0.83 ± 0.02	100	33.01 ± 0.80	9.28 ± 0.19	48.32 ± 1.16	0.11 ± 0.00
The Conservancy	3.96 ± 0.05	14.16 ± 0.76	4.24 ± 0.16	0.75 ± 0.00	1.12 ± 0.06	100	24.50 ± 1.35	7.78 ± 0.45	59.80 ± 2.24	0.14 ± 0.01

Table 4

Sites	Copper concentrations				Zinc concentrations			
	BCR Step 1	BCR Step 2	BCR Step 3	Bioavailable concentrations	BCR Step 1	BCR Step 2	BCR Step 3	Bioavailable concentrations
Mylor	83.08 ± 24.32 (23%)	71.21 ± 27.93 (35%)	267.53 ± 20.88 (41%)	421.83 ± 63.55	298.59 ± 39.35 (58%)	139.97 ± 38.83 (23%)	232.43 ± 68.95 (19%)	670.99 ± 46.00
Saltash	17.25 ± 2.66 (24%)	30.80 ± 5.91 (27%)	39.79 ± 0.94 (50%)	87.84 ± 6.55	105.93 ± 14.58 (77%)	35.73 ± 6.50 (15%)	33.43 ± 6.47 (9%)	175.10 ± 23.79
Holes Bay	7.33 ± 0.84 (25%)	15.99 ± 4.39 (37%)	24.67 ± 3.82 (39%)	47.99 ± 5.00	113.04 ± 6.08 (59%)	36.71 ± 8.29 (19%)	9.60 ± 1.77 (22%)	159.35 ± 30.97
Tipner	5.59 ± 0.77 (24%)	6.20 ± 0.47 (25%)	11.57 ± 1.71 (52%)	23.35 ± 1.90	32.60 ± 4.90 (70%)	6.19 ± 2.54 (13%)	3.67 ± 1.75 (17%)	42.45 ± 9.25
Broadmarsh	2.53 ± 0.81 (15%)	3.81 ± 0.53 (33%)	4.48 ± 0.52 (51%)	10.83 ± 0.57	21.23 ± 1.71 (71%)	8.25 ± 0.51 (23%)	6.95 ± 0.40 (6%)	36.43 ± 4.56
Dell Quay	7.79 ± 0.71 (20%)	8.09 ± 1.83 (35%)	16.88 ± 0.98 (45%)	32.76 ± 2.98	29.19 ± 1.63 (60%)	5.40 ± 1.28 (20%)	7.17 ± 1.72 (19%)	41.75 ± 7.65
The Conservancy	7.79 ± 0.56 (20%)	11.67 ± 0.93 (17%)	12.30 ± 1.55 (63%)	31.75 ± 1.41	31.93 ± 2.14 (44%)	10.32 ± 0.72 (21%)	11.77 ± 0.86 (35%)	54.02 ± 6.98

Table 5

Sites	Cu concentrations ± SEM	Zn concentrations ± SEM
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
Dell Quay	0.93 ± 0.04	0.53 ± 0.06
The Conservancy	0.93 ± 0.15	0.65 ± 0.01

Table 6

Study sites	Mean Cu accumulation ± SEM	Mean Zn accumulation ± SEM	Average worms wet weight ± SEM
Saltash	10.72 ± 0.68	68.98 ± 53.67	11.69 ± 0.79
Holes Bay	12.41 ± 0.91	95.76 ± 16.66	3.93 ± 0.77
Tipner	13.46 ± 0.83	99.66 ± 15.56	4.92 ± 0.64
Broadmarsh	7.36 ± 0.51	182.21 ± 54.78	11.56 ± 2.50
Dell Quay	15.39 ± 1.18	92.14 ± 8.17	3.27 ± 0.53
The Conservancy	17.42 ± 1.47	80.75 ± 18.52	3.26 ± 0.76

Table 7

Sites	Correlation between copper accumulation and individual weight		Correlation between zinc accumulation and individual weight	
	Pearson correlation	p-value (*p*)	Pearson correlation	p-value (*p*)
Saltash	0.585	0.017	0.268	0.315
Holes Bay	-0.354	0.179	-0.271	0.310
Tipner	-0.224	0.341	-0.203	0.390
Broadmarsh	-0.374	0.361	-0.620	0.101
Dell Quay	-0.345	0.227	0.348	0.222
The Conservancy	-0.120	0.710	-0.496	0.101

Table 8

	SQGs			
	ERL	ERM	TEL	PEL
copper	34	270	18.7	108
zinc	150	410	124	271

Fig. 4. Sampling sites on the south coast of the UK in Devon, Cornwall, Dorset & Hampshire labelled 1 to 7. Site 1: Mylor; Site 2: Saltash; Site 3: Holes Bay; Site 4: Tipner; Site 5: Broadmarsh; Site 6: Dell Quay; Site 7: The Conservancy.

Fig. 5. Correlation between copper (Cu) accumulation and the individual weight (g) of the worms for all sites. Pearson correlation = -0.337, $p = 0.002$ (n=86). Each point represents one worm.

Fig.6. Correlation between zinc (Zn) accumulation and their individual weight (g) of the worms for all sites. Pearson correlation = -0.132, $p = 0.226$ (n=86). Each point represents one worm.

Fig. 4. Correlation between mean copper (Cu) concentration in the pore water and copper (Cu) bioavailability in sediment. (M: Mylor, S: Saltash; HB: Holes Bay; T: Tipner; B: Broadmarsh; DQ: Dell Quay; C: The Conservancy). Note : The Conservancy and Dell Quay are represented as a single point as their concentrations are very similar. Pearson correlation = 0.855, $p = 0.014$.

Fig. 5. Correlation between zinc (Zn) accumulation in *N. virens* and zinc (Zn) concentration in the pore water. (S: Saltash; HB: Holes Bay; T: Tipner; B: Broadmarsh; DQ: Dell Quay; C: The Conservancy). Note: Mylor isn't represented in the graph as no ragworms were collected on this site. Pearson correlation = 0.837, $p = 0.038$.

Table 9

Description of the sampling sites and times. SAC: Special Area of Conservation, SEMS: Solent European Marine Site, SPA: Special Protection Area and SSSI: Site of Special Scientific Interest.

Table 10

Study sites characteristics. Data for the particle size distribution is detailed using the statistical package, Gradistat (v.8.0). (a) the average size, (b) the spread of the sizes around the average (sorting), (c) the symmetry or preferential spread to one side of the average (skewness), and (d) the degree of concentration of the grains relative to the average (kurtosis) based on logarithmic (original) Folk and Ward (1957) graphical measures (Blott and Pye, 2001, Watson *et al.*, 2013).

Table 11

Bioavailable metal concentrations for copper and zinc (sum of steps 1-3) expressed in mg kg$^-$^{1} dry weight ± SEM and in percentages, obtained in the sediment from the study sites detailing the concentrations in the three phases: BCR Step 1: Exchangeable phase; BCR Step 2: Reducible phase and BCR Step 3: Oxidisable phase.

Table 12

Copper (Cu) and zinc (Zn) concentrations expressed in (μg l^{-1}) sampled in the pore water.

Table 13

Copper (Cu) and zinc (Zn) tissue concentration (minus the head) of *N. virens* expressed in

(μg g^{-1} dry weight) with the mean worm weight (g) from each site. No ragworms were collected at the site Mylor.

Table 14

Site specific correlations between metal accumulations in *N. virens* tissues and the individual weight. Pearson correlation of weight and copper concentrations in *N. virens* tissues = -0.337, $p = 0.002$. Pearson correlation of weight and zinc concentrations in *N. virens* tissues = -0.132, $p = 0.226$.

Table 15

Sediment quality guidelines (SQGs) for metals: ERL (Effects Range-Low), ERM (Effects Range-Medium), TEL (Threshold Effect Levels) and PEL (Probable Effect Levels), concentrations are expressed in mg kg^{-1} dry weight (CCME, 1998, Hubner *et al.*, 2009, Long *et al.*, 1995)

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