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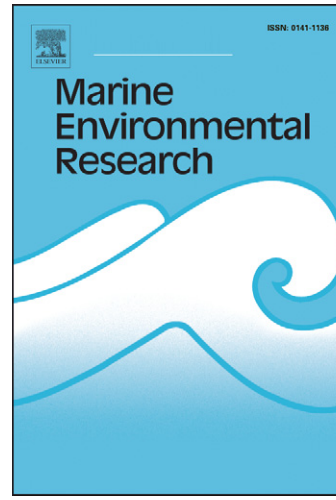

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Biodynamic modelling of the accumulation of Ag, Cd and Zn by the deposit-feeding polychaete *Nereis diversicolor*: inter-population variability and a generalised predictive model

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Abstract

Biodynamic parameters of the ragworm *Nereis diversicolor* from southern Spain and south England were experimentally derived to assess the inter-population variability of physiological parameters of the bioaccumulation of Ag, Cd and Zn from water and sediment. Although there were some limited variations, these were not consistent with the local metal bioavailability nor with temperature changes. Incorporating the biodynamic parameters into a defined biodynamic model, confirmed that sediment is the predominant source of Cd and Zn accumulated by the worms, accounting in each case for 99% of the overall accumulated metals, whereas the contribution of dissolved Ag to the total accumulated by the worm increased from about 27 to about 53 % with increasing dissolved Ag concentration. Standardized values of metal-specific parameters were chosen to generate a generalised model to be extended to *N. diversicolor* populations across a wide geographical range from western Europe to North Africa. According to the assumptions of this model, predicted steady state concentrations of Cd and Zn in *N. diversicolor* were overestimated, those of Ag underestimated, but still comparable to independent field measurements. We conclude that species-specific physiological metal bioaccumulation parameters are relatively constant over large geographical distances, and a single generalised biodynamic model does have potential to predict accumulated Ag, Cd and Zn concentrations in this polychaete from a single sediment metal concentration.

Keywords: *Nereis diversicolor*, cadmium, zinc, silver, biodynamic modelling, sediment

1. Introduction

Estuarine sediments act as sinks for contaminant trace metals of anthropogenic origin and these toxic metals may be taken up by deposit-feeding invertebrates ingesting the sediment (Luoma and Rainbow, 2008). These sediment metals not only have the capacity to cause direct ecotoxicological effects on this invertebrate but may also enter coastal food chains leading to vertebrates such as fish, birds and even man, with the potential to cause indirect ecotoxicological effects. An understanding of the uptake and accumulation of toxic metals by estuarine deposit-feeding invertebrates is therefore key to understanding the environmental risk associated with the metal contamination of estuarine sediments.

Biodynamic modelling is now being used to deconstruct the routes of uptake and subsequent accumulation of trace metals by marine animals, and accumulated concentrations predicted by biodynamic modelling generally match field concentrations very well (Luoma and Rainbow, 2005, 2008). The principal advantages of the biodynamic modelling approach are that (i) geochemical and biological processes affecting the metal bioaccumulation are considered simultaneously, (ii) it accounts separately for the metal uptake from distinct exposure routes, (iii) trace metal accumulation biodynamics has the potential to link total uptake rate with expected toxicity in a given species (Simpson and King, 2005; Wang and Rainbow, 2008). Use of biodynamic modelling includes geochemical analysis of environmental metal concentrations and measurement of key physiological parameters for a particular species from a site, and explains the great variability of accumulated metal concentrations found in organisms (Luoma and Rainbow, 2005). The biodynamic model describes the steady state concentration of metal in an organism as the sum of the net accumulation from solution and from the diet, the latter now appreciated as being a major source of metal accumulation in many aquatic invertebrates (Luoma and Rainbow, 2005; Wang, 2002).

Biodynamic modelling has recently been applied to two estuarine deposit-feeding polychaetes, defining the relative importance of dissolved and dietary uptake routes and successfully predicting field accumulated concentrations – the lugworm *Arenicola marina* (Casado-Martinez et al., 2009a,b) and the common ragworm *Nereis diversicolor* (Rainbow et al., 2009a,b). In both polychaetes ingested sediment supplies almost all accumulated cadmium and zinc, while for silver dissolved metal is a significant source for uptake (Casado-Martinez et al., 2009a,b; Rainbow et al., 2009a,b).

As the application of biodynamic modelling becomes more widespread, further questions become relevant. The first such question asks how variable are the physiological parameters defining metal accumulation between different populations of a species. These parameters are the uptake rate constant from the dissolved phase, the assimilation efficiency from the ingested particulate phase, and the efflux rate constants following uptake from water and diet (all measured in the laboratory typically using tracers), as well as the ingestion rate, and the growth rate constant of the species (often taken from the literature). Incorporation of these physiological parameters into the model, together with the dissolved and dietary metal concentrations determined in the field, provides site-specific predictions of metal accumulation in the relevant species at that site. So Rainbow et al. (2009a) explored variability in the above physiological parameters in populations of *N. diversicolor* from south England, concluding that, although there was some variability, the variation was limited and correlation with changes in local metal bioavailabilities were absent. In this study we investigate whether such conclusions still hold if populations of the worm from southern Spain are included in the comparison, with particular attention to the effect of raised temperature. We have therefore measured the physiological bioaccumulation parameters for Ag, Cd and Zn in *N. diversicolor* from Río San Pedro, Puente Zuazo and Trocadero (a reduced set of parameters in the latter case owing to limited numbers of worms available) from the region of Cadiz, Spain at 18ºC, and in a population from the Blackwater estuary in England (Table 1), again at 18ºC for comparison against the equivalent data collected for this latter population at 10ºC by Rainbow et al. (2009a).

The subsequent question addressed is whether it is possible to choose standardised values of the relevant parameters that can be applied in a generalised biodynamic model of metal accumulation by *N. diversicolor* and allow accurate prediction of bioaccumulated concentrations in the worm across a wide range of western European and Moroccan estuaries of different degrees of contamination. Such a generalised model would allow prediction on a mechanistic basis of accumulated metal concentrations in the worm from a single sediment concentration, and greatly facilitate any modelling of metal uptake, accumulation and potentially ecotoxicity in a key estuarine species (Gillet et al., 2008; Mouneyrac et al., 2009).

2. Materials and methods

2.1 Collection

Ragworms, *Nereis diversicolor,* (minimum length 3 cm) were collected by hand from the intertidal zones of three sites located in the Río San Pedro and Caño Sanctri-Petri saltmarsh environment (SW Spain) and from the Blackwater estuary (SE England) in September 2007 (Table 1). Populations of *N. diversicolor* were selected from sites characterized by different degrees of contamination; Río San Pedro is a shallow tidal inlet with no known elevated metal concentrations; Puente Zuazo receives domestic effluents from a town of about 88,000 habitants; and Trocadero is situated near a shipyard. The Blackwater estuary is considered by Rainbow et al. (2009a,b) to be uncontaminated in comparison with the SW England sites used in their comparisons. Ragworms were transported to the laboratory in cool boxes in wet sediment from the collection site. In the laboratory worms were kept in sediment from the site covered by artificial seawater (TM: Tropic Marin, Tropicarium Buchschlag, Dreieich, Germany) at a salinity of 16 at 18˚C. The artificial seawater was used to provide physico-chemical stability and replication for trace metal uptake studies (Rainbow, 1997).

2.2 Metal analyses

Organism and sediment samples were also collected for stable metal analyses. Ten worms from each site were placed in artificial seawater to depurate the digestive tract for 2 days. Worms were dried to constant weight at 60˚C (Table 1) and digested in concentrated nitric acid (Suprapur, Merck) at 100˚C, and made up to a known volume with double distilled water. The accuracy of the analyses for Cd and Zn (certified Ag concentrations not available) was checked by analysis of certified reference material (TORT-2, Lobster Hepatopancreas, National Research Council, Canada) and indicated good agreement (the recovery was 92-98%) between measured concentrations and the reference values. Sediment samples were dried at 60˚C, digested in concentrated nitric acid at 100˚C and made up to a known volume with double distilled water. Concentrations of Zn were determined by inductively coupled plasma optical emission spectrometer (Optima 2000 DV, Perkin Elmer), while the analyses of Cd and Ag were carried out by ICP-MS (Serie X7, Thermo elemental).

2.3 Organic matter

The organic matter content in the oxic surface sediment was determined by the loss of weight on ignition at 450˚C for 48 hours.

2.4 Radioisotopes

Radioactive tracers ^{109}Cd and ^{65}Zn were obtained from Brookhaven National Laboratory, New York, USA, and ^{110m}Ag from Riso National Laboratory, Denmark. Radioisotopes in live worms and faecal pellets in experiments were counted on an LKB Wallac 1480 Wizard gamma counter. A standard was used with each analysis to establish the background radiation level and activity changes in samples due to radioactive decay during the experimental periods.

2.5 Uptake from solution

Nereis diversicolor from all four sites were exposed individually to radiolabelled Cd, while three populations (Blackwater estuary, Río San Pedro and Puente Zuazo) were also exposed to radiolabelled Ag and Zn, separately in acid-washed 100 ml beakers with 50 ml TM at 16 salinity at 18˚C. Exposure concentrations were 0.17, 0.5, 1, 5 μg l^{-1} for Cd; 0.92, 2, 4, 6 μg l^{-1} for Ag; and 10, 20, 40, 60 μg l^{-1} for Zn. These concentrations were labelled with 0.25 μCi ^{109}Cd, ^{110m}Ag l^{-1} or 2.5 μCi ^{65}Zn l^{-1}. All experimental beakers were soaked in the corresponding experimental solution for 3 days before experiments to saturate all adsorption sites. Worms were acclimated to experimental conditions in 50 ml of clean TM water individually for 3 days then exposed to fresh labelled metal solution. Counts were determined after 3 h (assessment of adsorption onto body surface) and on days 1, 2, 3 and 4 of the uptake phase. After each count worms were replaced to the corresponding beaker filled with the renewed metal solution in order to maintain the initial exposure concentration. After 4 days worms were replaced to 50 ml clean TM and counted after 8 h (loss of weakly bound metals) and on days 3, 5, 7, 10, 12, 14 of the depuration phase. At the end of the experiments worms were dried individually at 60˚C to a constant weight.

2.6 Uptake from ingested sediment

In order to examine the dietary uptake, radiolabelled sediment as a food source was offered to each worm. To avoid any exposure to radiolabelled metal in the porewater, worms were fed on labelled sediment packaged in gelatine as described by Rainbow et al. (2009a). For each metal separately, approximately 0.25 g of wet sediment from the site of collection in the Blackwater estuary was labelled with 2 µCi of radiotracer and left to stand at 4˚C for at least 1 month. A gelatine solution was prepared (2.4 g gelatine (Sigma), in 15 ml TM at salinity of 16) under gentle heat with 0.25 ml cod liver oil (Seven Seas Health Care, Hull, UK) added as feeding stimulant. This solution was mixed with the labelled sediment and the resulting mixture solidified by cooling at 4 ˚C.

Six worms from three different sites (Blackwater estuary, Río San Pedro and Puente Zuazo) were transferred individually to small plastic boxes containing 50 ml of artificial sea water (salinity 16, 18˚C) a few days before the start of experiments. During the acclimation worms were trained to accept packaged food by feeding unlabelled sediment treated as described above. Worms were then allowed to feed on a package of labelled sediment overnight. Sixteen hours after feeding each worm was counted for 30 seconds, this count representing 100% of ingested labelled metal content before defaecation. Worms were then placed individually in TM (salinity 16, 18˚C) and were fed unlabelled sediment following the same procedure. Radioactivity contents in live worms and casts were determined at 8, 24, 32, 48, 56 and 72h. After then worms were allowed to continue depuration of any metal and counted every two days during the following week. Worms were placed individually in plastic boxes containing acid-washed sand covered by the artificial sea water and counted every 2 or 3 days for another week. The medium (TM water or acid-washed sand) was renewed after every count.

2.7 Data analysis

Parametric tests, such as regression analyses and analysis of variance (ANOVA) followed by Tukey's HSD *post hoc* methods were performed by using STATISTICA (Statsoft, version 6). Data expressed as percentages have been arcsine-transformed before using statistics. Level of significance was established at p=0.05.

2.8 Biodynamic modelling of trace metal accumulation

The biodynamic model assumes that net bioaccumulation at steady state (C_{ss}) in an animal can be described by the following equation:

$$C_{ss}= (K_u \times C_w)/(K_{ew} + g) + (AE \times IR \times C_f)/(K_{ef} + g)$$

where K_u is the metal uptake rate constant from solution (l g^{-1} d^{-1}), C_w is the metal concentration in solution (µg l^{-1}), AE is the assimilation efficiency (%) of the metal from ingested food, C_f is the metal concentration in food (µg g^{-1}), IR is the ingestion rate (g $g^{-1}d^{-1}$), g is the growth rate constant (d^{-1}), and K_{ew} and K_{ef} are the efflux rate constants (d^{-1}) after uptake from solution and food respectively. In this study the ingested food is sediment and K_{ef} is expressed as K_{esed}.

Although living in burrows in the sediment, *Nereis diversicolor* strongly irrigates its burrow with a current of oxygenated water from the overlying water column (Banta et al., 1999, Kristensen, 2001), and water bathing the worm is from the water column and not pore water in chemical equilibrium with the sediment particles. We have, therefore, followed Casado-Martinez et al. (2009b) and Rainbow et al. (2009b) by not considering dissolved concentrations of metals in pore water in equilibrium with sediment particles but used overlying water dissolved metal concentrations in our modelling. Dissolved metal concentrations are not available for the water column in the estuaries sampled and we have therefore fallen back on ranges of dissolved concentrations reported in the literature (Luoma and Rainbow, 2008). Dissolved concentration ranges extending from low concentrations typical of uncontaminated coastal waters to concentrations recognised as atypically high and indicative of significant anthropogenic contamination in estuaries are: 0.006 to 0.03 μg Ag l^{-1} (Smith and Flegal, 1993; Ranville and Flegal, 2005), and 0.01 to 0.10 μg Cd l^{-1} and 0.3 to 5 μg Zn l^{-1} (both from Table 5.5, Luoma and Rainbow, 2008). We have no reason to consider that local dissolved concentrations in the water column at our sites are atypically high, and in our modelling we have used 0.01 μg Ag l^{-1}, 0.01 μg Cd l^{-1} and 0.3 μg Zn l^{-1}.

The growth rate constant of *Nereis diversicolor* is mainly influenced by the type of available food sources, and an artificial food supply normally results in a greater value. Kristensen (1984) reported an average weight-specific growth rate constant of 0.005 d^{-1} for a population of *N. diversicolor* in Norsminde Fjord, Denmark. Riisgard et al. (1996) found the growth rate constant to be in the range of 0.002-0.025 d^{-1} in populations from Kertinge Nor, Denmark, and 0.049-0.056 d^{-1} when the worms were fed on algal cells or shrimp. Olivier et al. (1996) recorded a growth rate constant of 0.0056 to 0.019 d^{-1} for this species fed on plant material. In agreement with Rainbow et al. (2009b) who carried out a sensitivity analysis of the effects on a biodynamic model of varying the growth rate constant of *N. diversicolor* from 0.005 to 0.05 d^{-1}, we have chosen to use a growth rate constant of 0.02 d^{-1} in the models to be employed here.

Cammen (1980) has shown that the assimilation processes of deposit-feeding invertebrates, including polychaetes like *Arenicola marina* and *Nereis diversicolor*, are focused on the organic matter component of the sediment. Indeed deposit-feeding invertebrates in general appear to maintain a rate of ingestion of organic matter which is dependent on body weight but independent of the organic content of the ingested sediment, following the relationship $C = 0.381W^{0.742}$ where C is the organic matter (mg) ingested per organism per day and W the body dry weight (mg) of the organism (Cammen, 1980). Thus there appears to be a constant organic matter ingestion rate for a given size animal, even though the rate of ingestion of total sediment will vary substantially. We have therefore followed the arguments of Casado-Martinez et al. (2009b) and Rainbow et al. (2009b), and used the mean body weight of the worms to calculate the rate of ingestion of sediment-associated organic matter using the above equation of Cammen (1980), also presenting the total sediment ingestion rate (Table 2)

We need also to estimate the relevant measure of metal concentration in the ingested sediment. Since we are using an ingestion rate of $g_{OM}\ g_{organism}^{-1}\ d^{-1}$ in the determination of uptake rates from food, then the concentration of metal in the sediment must be in similar units, and the most relevant measure is the metal concentration in the organic component of the sediment. There is no direct method to separate metal associated with the organic components of sediment from that associated with inorganic ligands. One way to obtain the correct units would be to divide the mass of metal in a gram of sediment by the mass of organic matter. But this makes the false assumption that all sediment metal is associated with the organic matter, while it is well known that there will necessarily be metals associated with inorganic components of oxidised sediment, not least manganese and iron oxide components (Luoma and Rainbow, 2008). Since Ag, Zn and Cd in the oxidised sediment are distributed between organic and inorganic components (Luoma and Rainbow, 2008), it is not valid to assume that all metal in the sediment is associated with organic matter, nor is it valid to assume that no metal is associated with sedimentary organic matter. The default assumption made by Casado-Martinez et al. (2009b) and Rainbow et al. (2009b) and followed here is that organic and inorganic sedimentary components have a generally similar affinity for the metals, and thus the same metal concentration (content per unit weight of that component);

i.e. the metal is distributed relatively evenly among organic and inorganic binding sites in the sediment. We accept that this is unlikely to be exactly correct (e.g. Luoma and Bryan, 1982), but consider this to be a more valid generalization than the alternatives. Under such conditions, the metal concentration per unit mass of organic material is necessarily the same as the metal concentration per unit mass of total sediment. Our estimate of the metal concentration of the organic component of the sediment is therefore the total metal sediment concentration.

3. Results

3.1 Trace metal concentrations

The concentrations of Cd, Ag and Zn measured in oxic surface sediments and in *Nereis diversicolor* originating from the four sites are summarized in Table 1. Sediment from the Blackwater estuary had the highest Cd concentration, whereas the Spanish sediments exhibited similar concentrations. The bioaccumulated concentrations of Cd in the ragworms were significantly correlated with the local sediment concentrations ($p<0.001$). The highest Ag concentration was found in sediment from Puente Zuazo and the highest Zn concentration in sediment from Trocadero, but in neither case was this reflected in the highest bioaccumulated concentration in the local ragworms.

3.2 Uptake and depuration from solution

The uptake of labelled metal from solution into the body was plotted against the exposure period for each exposure concentration (see Rainbow et al. (2009a) for example) and the slopes of these regression lines (uptake rates) were then expressed as a function of the dissolved exposure concentrations. The slope of this fitted line is defined as the uptake rate constant (K_u) of the radiolabelled metal (Table 3). Typically the uptake rate showed a significant linear increase ($p<0.05$) with increasing exposure concentrations except in the case of worms from the Blackwater estuary for Ag; the latter result may simply be an effect of chance when working at the $p=0.05$ level of significance. In general, the highest absolute uptake rates at a given dissolved molar concentration were recorded for dissolved Ag and the lowest for dissolved Cd, showing one order of magnitude difference.

As K_u is a unified measure of the uptake rate by a particular population of worms under the physicochemical conditions used in the tests (salinity 16, 18˚C), it can be compared between the populations (Rainbow et al., 2009a). Analysis of variance of Cd K_u showed significant differences between the populations studied (Table 3); the Cd K_u of the Puente Zuazo population was significantly higher than those of the Blackwater and Trocadero populations, and the latter population showed a significantly lower K_u than that observed in the worms from Río San Pedro. The uptake rate constant of Ag did not vary significantly between the populations (Table 3). Only one comparison showed a significant difference between populations in the case of Zn Ku, between worms from the Blackwater estuary and Puente Zuazo (Table 3).

The change in temperature from 10ºC to 18ºC did not significantly affect the uptake rate constants of any of the three metals in worms from the Blackwater estuary (Table 3).

In the depuration phase, counts expressed as percentages of the final count after the uptake phase were ln-transformed, and then plotted against the efflux time (see Rainbow et al. (2009a) for example). The slope of the regression line is the efflux rate constant (K_{ew} in d^{-1}) of the metal, and the biological retention half-life of the metal is calculated as 0.693 divided by the K_{ew} (Table 3). Data points of the depuration phase were examined carefully and when two compartments were detected, only the 'slow pool' was considered for the calculation of the efflux rate constant.

According to the efflux rate constants of radiolabelled metal after uptake from solution, Ag and Zn were excreted more rapidly than Cd by worms from each site (Table 3). The slower depuration rate of Cd resulted in longer retention in the tissue (biological half-lives varied between 31.2 and 76.5 d). Significant intersite differences in these efflux rate constants were only found in the case of Zn, with worms from Puente Zuazo exhibiting a significantly higher efflux rate constant than those from the Blackwater estuary (Table 3).

For the Blackwater population, only in the case of silver did exposure to 18ºC as opposed to 10ºC result in a significantly changed (in this case increased) efflux rate constant (Table 3).

3.3 Assimilation and depuration from food

The radioactive counts of the live animal after ingestion of the radiolabelled meal were plotted against the time (see Rainbow et al. (2009) for example). The assimilation efficiency (AE) was considered as the percentage of ingested labelled metal retained in the worm after complete defaecation. After this initial rapid loss resulting from defaecation, the percentage of the remaining radioactive metal declined very slowly or stayed at a constant level, representing the 'slow pool' of assimilated metal. In most cases, unassimilated labelled metals were eliminated by worms 3 to 4 days after the pulse-chase feeding. As above, counts expressed as percentages of the initial count after feeding were then ln-transformed, and plotted against the efflux time. The efflux rate constant of radiolabelled metal after uptake from the labelled sediment (K_{esed}) was calculated as the slope of the regression line of the 'slow pool'. The AE percentage was estimated by back extrapolation of this regression line to intercept the y axis.

Table 4 shows that assimilation efficiencies of *Nereis diversicolor* for Cd were highest in the population from Río San Pedro, significantly above that of the Blackwater estuary population. In the case of Ag no significant intersite differences in the AEs were recorded, a result influenced by the low number of samples available (Table 4). No significant differences in assimilation efficiencies for Zn were found between the populations (Table 4).

The change in temperature from 10ºC to 18ºC had no significant effect on the AE of Cd and Zn of the Blackwater population, but there was a significant difference in the case of the Ag AE (Table 4).

The efflux rate constants of each metal by *Nereis diversicolor* did not differ significantly between the populations (Table 4). Retention of Cd after uptake from food was greater than observed for Zn in all populations, with a corresponding longer biological half life of Cd (31.1-39.2 d) than of Zn (17.0-21.8 d) (Table 4).

The change in temperature from 10ºC to 18ºC had no significant effect on the efflux rate constants of Cd and Ag for the Blackwater worms after uptake from ingested sediment, but there was a significant difference in the case of Zn (Table 4).

3.4 Comparison of efflux rate constants after uptake from solution or ingested sediment

Efflux rate constants of radiolabelled Cd, Ag and Zn after uptake from solution or ingested sediment were compared for each metal, and no significant difference was found in each population at 18˚C.

3.5 Biodynamic modelling

The physiological parameters measured for each population (Tables 3 and 4) have been entered into a biodynamic model, together with the associated sediment metal concentration (Table 1), and the estimated ingestion rate of the worms from each site expressed in terms of sedimentary organic matter (Table 2). As explained above, single values have been entered for the growth rate constant ($0.02 d^{-1}$) and for the dissolved concentrations of Cd ($0.01 \mu g\ l^{-1}$), Ag ($0.01 \mu g\ l^{-1}$) and Zn ($0.3 \mu g\ l^{-1}$) which can be considered low. Steady state bioaccumulated concentrations predicted by the biodynamic model for each population of worms are shown in Table 5.

The model shows that the sediment is the vastly predominant source of Cd and Zn, accounting for more than 99% of the total Cd and Zn accumulated in the ragworms. In the case of Ag, uptake from water contributed a significant proportion (24 to 33%) of the metal bioaccumulated.

The predicted steady state metal concentrations in the worms from the different sites are compared with the measured concentrations in Table 6. The model with the present assumptions overpredicted in all cases the accumulated concentration of Cd; it underpredicted the Ag concentration of the Blackwater worms but gave good correspondence for Río San Pedro and Puente Zuazo worms. Predictions for Zn bioaccumulation were also good.

4. Discussion

4.1 Biodynamic parameters

In this study, biodynamic accumulation parameters of *Nereis diversicolor* from the different estuaries have been determined as population-specific coefficients, addressing the question of how variable are the physiological parameters defining metal accumulation between different populations of a species.

One possible cause of variation may be the effect of previous field contamination history on the uptake and elimination processes of the animals. Field measurements, however, showed no extraordinary high concentrations of Cd, Ag and Zn either in sediment or in ragworm at each site in this study, and we did not find any relationship between metal bioavailability (as measured by accumulated concentration) and uptake rate constant in ragworms from the four sites. Nevertheless, some intersite differences in some biodynamic parameters were found.

In the case of the mean of K_u of the three metals, the highest uptake rate constants from solution were recorded for Ag followed by Zn and Cd in ragworms from the three populations, similarly to the observations in the same species by Rainbow et al. (2009a). Our results also agree with those observed in other marine polychaetes, such as in *Nereis succinea* by Wang et al. (1999) or in the lugworm *Arenicola marina* by Casado-Martínez et al. (2009a). Trace metals are typically taken up from solution across the apical cell membrane of a surface epithelial cell of an aquatic invertebrate via facilitated diffusion carried by a transporter protein, the dissolved free metal ion representing the best model for the dissolved metal form that binds to the protein (Luoma and Rainbow, 2008). The free cadmium ion may also cross the membrane via calcium ion channels; zinc may similarly be transported by calcium channels in vertebrates but not invertebrates (Luoma and Rainbow, 2008). In addition to the possible binding of free silver ions to a transporter protein for facilitated diffusion, some of these silver ions may also cross the membrane by trespassing in sodium channels. One possible reason for the high uptake rate of dissolved silver by aquatic invertebrates is the fact that the predominant form of Ag in brackish and marine water is the zero-charged, non-polar, chloro-complex AgCl which can diffuse through the membrane, in addition to any other routes of uptake (Luoma et al., 1995).

There were no significant inter-population differences in the efflux rate constants of Cd and Ag after uptake from solution, but Puente Zuazo exhibited significantly enhanced efflux of Zn compared to worms from the Blackwater estuary. The regulation of total Zn body burden by *N. diversicolor* has been documented (Amiard et al., 1987; Bryan and Hummerstone, 1973; Bryan et al., 1980), as the worms have developed an efficient efflux system to remove excess metal. It appears to be the case that in the worms from Puente Zuazo, the higher uptake rate constant (Table 3) is compensated by the higher efflux rate constant (Table 3), to result in a final regulated body concentration that is not elevated (Table 1).

According to Cammen (1980) deposit feeders ingest sediments at a rate of about twice their body weight per day. Thus to model bioaccumulation it is essential to assess metal uptake from the food. A direct link has been demonstrated between AE and accumulated metal concentration in mussels (Fisher et al., 1996), and a population of the clam, *Ruditapes philippinarum*, from a metal-contaminated site exhibited a higher Cd AE than did clams originating from uncontaminated sites (Shi and Wang, 2004). In our study, the assimilation efficiency for Cd from sediment differed significantly between the population from Río San Pedro (high) and the Blackwater (low), but it did not follow the rank order of sediment or bioaccumulated Cd concentrations. Rainbow et al. (2009a) showed some limited inter-population differences in metal AE between populations of *N. diversicolor* from six UK estuaries, but these differences were also not consistent with the local metal exposure history. Similarly, no inter-population differences in Cd and Zn AE in mussel, *Perna viridis* collected from sites differentially enriched with trace metals were recorded by Blackmore and Wang (2003).

In our study, the efflux rate constants after dietary metal uptake showed no significant differences between the populations, similarly to the results for Ag, Cd and Zn Ke observed in the barnacle *Balanus amphitrite* (Rainbow et al., 2003) or in the Cd and Zn Ke of marine clams, *Ruditapes philippinarum* and *Mactra veneriformis* (Shi and Wang, 2004). No differences between the two efflux rate constants of each metal after uptake from water or ingested sediment (K_{ew} and K_{esed}) suggest that the same physiological handling process is in action irrespective of the route of metal uptake.

Temperature generally enhances many physiological processes that determine the rates of metal uptake, elimination or detoxification (Heugens et al., 2003). As increased temperature may affect both influx and efflux rates of metals, net bioaccumulation may or may not increase (Luoma, 1983). Increase in temperature resulted in increased uptake rates of dissolved Cd, Cr and Zn in the clam, *Corbicula fluminea*, and also their assimilation efficiency of Cd from sediment (Lee and Lee, 2005). Heugens et al. (2003) reported enhanced Cd uptake rate at elevated temperature as well as some alterations in Cd elimination in *Daphnia magna*. Despite the numerous studies indicating higher metal uptake at higher temperature due to the higher rate of metabolism, Wang and Fisher (1997) found a negative relationship between Ag assimilation efficiency and temperature in *Mytilus edulis*. The influence of the temperature on the kinetic parameters was not consistent in our study; Ag uptake from solution and Zn efflux rate after uptake from solution showed significant positive correlation with the temperature, but Cd assimilation from sediment was significantly lower at 18˚C.

We conclude that there was no consistent significant effect of the change in temperature between 10°C and 18°C on the biodynamic accumulation parameters measured. Thus we consider that it is valid to include British and Spanish populations of *N. diversicolor* (and by extension populations of the worm from western Europe and north Africa) in any attempt to produce a generalised biodynamic model of Ag, Cd and Zn bioaccumulation in *N. diversicolor*.

4.2 Biodynamic modelling of trace metals in Nereis diversicolor

We incorporated the population-specific biodynamic accumulation parameters of *Nereis diversicolor* from the different estuaries determined here into a biodynamic model to predict the accumulated Cd, Ag and Zn concentrations in ragworms from each site (Table 5). There was generally good agreement (Table 6, Figure 1a), although the model overpredicted in all cases the accumulated concentration of Cd, and underpredicted the Ag concentration of the Blackwater worms. Predictions of Ag bioaccumulation for two Spanish sites and for Zn bioaccumulation at all sites were excellent.

The model also provides information about the contribution of the sediment or solution as metal source to the overall metal accumulation (Wang and Rainbow, 2008). Our results are consistent with those previously recorded in the deposit feeding polychaetes studied, in that more than 99% of Cd and Zn, and about 70% of Ag, are derived from sediment ingestion at low dissolved metal concentrations (*Arenicola marina* – Casado-Martinez et al. (2009a) and *Nereis diversicolor* (Rainbow et al., 2009a).

We have then gone on to conduct sensitivity analyses to check how the changes in certain parameters -namely, the growth rate constant and dissolved metal concentration- affect the accumulated metal concentration. The growth rate constant was varied from 0.005 to 0.05 d^{-1}, and the dissolved metal concentrations from 0.01 to 0.10 µg Cd l^{-1}, 0.01 to 0.03 µg Ag l^{-1} and 0.03 to 5 µg Zn l^{-1}.

Independently of the variation in growth rate constant and dissolved metal concentration over these ranges, the worms always accumulated more than 90% of the total

Cd and Zn from sediment. The choice of a high or low Cw essentially had no effect on predicted concentrations of Cd and Zn in the worms (compare Figs 1a and 1b at growth rate constant 0.02 d^{-1}). The effect of changes in the growth rate constant on predicted accumulated metal concentrations using the low Cw is shown in Figure 2. Any effect becomes more considerable only when growth rate constant is below 0.02 d^{-1}. The modelled Cd bioaccumulation was overpredicted at all growth rate constants modelled, with the best fit between predicted and measured concentrations using the maximum growth rate constant. In contrast, at higher growth rate constants the Zn bioaccumulation predicted by the model was below the measured concentration (about 60%), while at lower growth rate constants the prediction was more similar to the field observation.

Ag showed a different pattern, as at high dissolved concentrations the contribution of dissolved and sediment Ag was equal at all growth rate constants. For instance, using the maximum dissolved concentration, approximately 53 % of total Ag was accumulated from the water (mean of the three populations), while this value decreased to 27 % at low Cw, This input of Ag from solution is much greater than in the case of Cd or Zn (Table 5). It has to be mentioned that the atypically high seawater Ag concentrations used here for the sensitivity analysis were recorded in estuaries contaminated by mining activities in the 1990s and nowadays are not common in the estuarine environment (Rainbow et al., 2009b). For Ag the predicted concentration at both low and high Cw accurately matched the observed concentration in the worms, except in the case of the Blackwater population (Table 6, Figs. 1a, 1b).

4.3 Generalized model

The second question addressed is whether it is possible to choose standardised values of the relevant parameters that can be applied in a generalised biodynamic model of Ag, Cd and Zn accumulation by *N. diversicolor* and allow accurate prediction of bioaccumulated concentrations in the worm across a wide range of western European and even north African (Moroccan) estuaries of different degrees of contamination. To generate this model, we have chosen approximately median values of the physiological parameters measured for each population, together with a similar median value for the ingestion rate of sedimentary organic matter, as measured or calculated in this study and by Rainbow et al. (2009b). These have been incorporated, together with the values of growth rate constant and dissolved metal concentrations used in the model detailed in Table 5, in the list of model parameters given in Table 7.

The comparisons of the steady state concentrations predicted by this generalised model against measured concentrations from our studies and in the literature are given in Table 8. The predicted (y) and measured (x) concentrations have been entered into regression models (Figures 3a-c) and the regression coefficients compared against unity which would indicate perfect agreement.

The regression model for the full data set for cadmium (Figure 3a) had a regression coefficient of 1.763 (95% CL 0.650), significantly above 1, confirming overprediction of bioaccumulated concentrations by the model with the assumptions given in Table 7. In most populations the concentrations of Cd predicted by modelling were approximately two-fold higher than the field measurements. The bioavailability of metals to *Nereis diversicolor* may be affected by factors controlling the local partitioning of sediment-bound metals between different sediment constituents (Luoma and Bryan, 1982). For instance, the enhanced metal concentration observed in the sediment did not result in raised Cd concentration in ragworms from Boulogne harbour in the study of Berthet et al. (2003), as reflected here in the underprediction by our model (Table 8). Thus atypical local factors affecting Cd bioavailability in sediments will cause specific differences between predicted and observed concentrations in the worms, over and above any general trend to overpredict or underpredict by the model. The reason for the general overprediction by the model of the accumulated Cd concentrations in *N. diversicolor* will presumably not lie in a wrong choice of growth rate constant, for this would affect all predicted accumulated metal concentrations equally – not the case here. A possible cause is the chosen assimilation efficiency for Cd from the sediment. It may be that the technique used here overestimates the typical assimilation efficiencies of sediment cadmium in the field. A revised AE of 0.25 in the model would have given a very accurate prediction of the measured accumulated concentrations in the worm. Alternatively (or additionally), the error may lie in the assumption that the Cd concentration of the organic component of the sediment is modelled by the total metal concentration rather than a factor of it perhaps related to the percentage organic content of the sediment.

The full data set for Ag is greatly affected by the large underprediction of the Ag concentration in the Seine worms (Table 8), almost certainly caused by an underestimation of the dissolved Ag concentration in this contaminated estuary (Amiard et al., 2009). The regression coefficient for the full data set (0.234, 95% CL 0.156) comes closer to unity after removal of this data point (0.662, 95% CL 0.138) (Figure 3b), but the model does still underpredict observed Ag bioaccumulation. Luoma and Bryan (1982) concluded that extraction of sediment with HCl appeared to be the best technique to model the bioavailable fraction of many sedimentary metals. The Ag concentrations in most of the sediments here were determined after stronger acid digestion (“total” metal) that probably did not reflect the biologically available quantity of metal, and HCl-extracted Ag concentrations in the sediments may be more appropriate for use in this model. The model predictions when using the nitric acid-extracted sediment concentrations was very close to the measured concentrations for the worms from the Gannel, Tressilian and Bidasoa River, perhaps in reflection of local factors affecting local sediment Ag bioavailability. The physicochemical characteristics of any sediment will affect the metal bioavailability and consequent uptake by deposit feeders. Silver tends to form strong organo- or sulphide complexes that are of low bioavailability, but some organic complexes increase rather than decrease the bioavailability (Bryan and Langston, 1992). The presence of other trace metals also influences metal uptake; the Ag concentration in deposit feeding clam, *Scrobicularia plana* was inversely correlated with Cu concentration in sediment (Luoma and Bryan, 1982).

The very high Restronguet Creek data point (Table 8) dominates any regression of the zinc data, single handedly bringing about a very significant regression with a regression coefficient significantly greater than 1 (2.510, 95% CL 0.275). Elimination of this point eliminates any significance of the regression of the remaining data set (reg. coeff. 0.551, 95% CL 0.509). Furthermore the data seem to fall into two categories, perhaps partly discriminating between worms in which regulation of Zn body concentration has broken down or not (Rainbow et al. 2009b). Thus, we present two regression lines for zinc after exclusion of the Restronguet Creek point (Figure 3c). The regression coefficient for one of the lines is significantly raised above 1 (1.527, 95% CL 0.208) indicating the overprediction of accumulated Zn in populations that probably are better able to regulate their body Zn concentrations, while the coefficient of the other line fell below unity (0.726, 95% CL 0.560), with these latter worms containing higher Zn concentrations than predicted by the model.

In the absence of mean dry weight values of each population we used a mean (21.2 mg) of mean dry weights of populations from UK estuaries for the generalised model. The ingestion rate is related to animal body weight; thus the sensitivity analysis was extended to a range of animal sizes (16.2-57.1 mg) as well. The IR is 0.175 g OM g^{-1} d^{-1} when using the average dry weight, whereas this value varies over the range of 0.134 to 0.186 g OM g^{-1} d^{-1} at the chosen maximum and the minimum animal size, respectively. Considering that sediment is the main source of various metal accumulations, the use of a specific dry weight for the calculation of IR could improve the accuracy of the model prediction.

5. Conclusion

Physiological metal bioaccumulation parameters in populations of ragworms from southern Spain and south England did not show any consistent inter-population variations relating to the local metal bioavailability or the effect of raised temperature. These findings allowed us to define metal-specific parameters of *N. diversicolor* and apply them in a generalized model with some success. According to the assumptions of this model, predicted steady state concentrations of Cd and Zn in *N. diversicolor* were overestimated, those of Ag underestimated, but still of the same order of, and comparable to, independent field measurements. We conclude that species-specific physiological metal bioaccumulation parameters are relatively constant over large geographical distances and climatic zones, and a single generalised biodynamic model does have the potential to predict accumulated Ag, Cd and Zn concentrations in this polychaete from a single sediment metal concentration.

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Figure captions

Figure 1. Ag, Cd and Zn concentrations (µg g^{-1}) measured (± 95% CL) in *Nereis diversicolor* from one UK and two Spanish estuaries compared to metal concentrations predicted from independently derived biodynamic constants and sediment metal concentrations (see Table 5) using (a) low or (b) high dissolved metal concentrations expected in metal contaminated estuaries.

Figure 2. The effect of changes in growth rate constant from 0.005 to 0.05 d^{-1} on the predicted concentrations of Cd, Ag and Zn in polychaetes from one UK and two Spanish estuaries. Other parameters are as in Table 5.

Figure 3. The predicted and measured concentrations of (a) Cd, (b) Ag and (c) Zn in *Nereis diversicolor* from western European and Moroccan estuaries using the biodynamic parameters presented in Table 8. In Figure 3c, open circles: populations considered able to regulate their body Zn concentrations; filled circles: populations less able to regulate body Zn concentrations.

Table 1. Collection details and concentrations of Cd, Ag and Zn (µg g^{-1} ± sd dry weight) in oxic surface sediment and the polychaete worm *Nereis diversicolor*.

Site	Geographic coordinates	Dates of collection		Cd	Ag	Zn
Blackwater	051˚44.08'N,	13/09/07	Sediment	1.95 ± 0.26	0.46 ± 0.09	93 ± 17
	000˚41.34'E,		*N. diversicolor*	<0.2 - <0.8	1.38 ± 0.61	237 ± 37
Río San Pedro	036˚32.77'N,	08/09/07	Sediment	0.60 ± 0.09	0.37 ± 0.01	101 ± 6
	006˚12.39W		*N. diversicolor*	0.22 ± 0.00	0.30 ± 0.00	152 ± 6
Puente Zuazo	036˚28.35'N,	08/09/07	Sediment	0.56 ± 0.02	0.98 ± 0.28	121 ± 18
	006˚11.71'W		*N. diversicolor*	0.21 ± 0.01	0.30 ± 0.00	160 ± 9
Trocadero	036˚30.90'N,	08/09/07	Sediment	0.58 ± 0.07	0.45 ± 0.04	145 ± 8
	006˚14.36'W		*N. diversicolor*	0.19 ± 0.02	0.26 ± 0.00	126 ± 5

Table 2. Ingestion rates of *Nereis diversicolor* from 3 Spanish and 1 UK estuaries following Cammen (1980). See text for details.

	Mean ± sd dry wt of worms g	Organic matter consumption per worm mg OM d^{-1}	Ingestion rate of organic matter g OM g^{-1} d^{-1}	% organic matter in sediment	Ingestion rate of sediment g g^{-1} d^{-1}
Blackwater	0.0172 ± 0.0084	3.15	0.183	12.1	1.51
Río San Pedro	0.0255 ± 0.0223	4.21	0.171	12.7	1.34
Puente Zuazo	0.0180 ± 0.0165	3.25	0.181	8.6	2.10
Trocadero	0.0571 ± 0.0239	7.66	0.134	11.1	1.21

Table 3. *Nereis diversicolor*: Uptake rate constants (K_u: mean, SE, l g^{-1} d^{-1}) and efflux rate constants (K_{ew}: mean, SE, d^{-1})of different populations for Cd, Ag and Zn from solution at 18ºC, and a comparison of constants between Blackwater populations at 18ºC (Blackwater 18) and 10ºC (Blackwater10, from Rainbow et al. 2009a). Constants sharing a common letter in any comparison are not significantly different (P>0.05) by Tukey's *post hoc* test.

	Uptake				**Efflux**						
	K_u				**K_{ew}**				% 'slow' pool		
	Mean	SE	n	Tukey's	Mean	SE	Tukey's	Half life (d)	Mean	SE	n
Cadmium											
Puente Zuazo	0.0410	0.0057	20	A	0.0184	0.0039	A	41.8	62.8		15
Río San Pedro	0.0243	0.0040	19	A, B	0.0277	0.0037	A	31.2	34.3		12
Blackwater	0.0117	0.0016	21	B, C	0.0175	0.0036	A	50.0			16
Trocadero	0.0100	0.0015	22	C	0.0163	0.0036	A	76.5			17
Blackwater 10	0.0134	0.0015	15	A	0.0184	0.0018	A				14
Blackwater 18	0.0117	0.0016	21	A	0.0175	0.0036	A				16
Silver											
Puente Zuazo	0.7775	0.3514	18	A	0.0519	0.0072	A	18.1	63.6		17
Blackwater	0.6717	0.4427	14	A	0.0553	0.0032	A	13.7			22
Río San Pedro	0.4971	0.1664	9	A	0.0683	0.0117	A	15.0			12
Blackwater 10	2.7856	0.6887	15	A	0.0436	0.0058	A				10
Blackwater 18	0.6717	0.4427	14	A	0.0553	0.0032	B				22
Zinc											
Blackwater	0.0769	0.0137	22	A	0.0422	0.0036	B	19.1	73.6	12.9	24
Río San Pedro	0.0557	0.0211	10	A, B	0.0469	0.0040	A,B	15.2	73.5		5
Puente Zuazo	0.1748	0.0416	20	B	0.0728	0.0065	A	11.6	59.1		20

Blackwater 10	0.1021	0.0346	17	A	0.0359	0.0024	A	17
Blackwater 18	0.0769	0.0137	22	A	0.0422	0.0036	A	24

Table 4. *Nereis diversicolor*: Assimilation efficiencies (AE: mean, SE) and efflux rate constants (K_{esed}: mean, SE, d^{-1}) of different populations for Cd, Ag and Zn at 18ºC, and a comparison of constants between Blackwater populations at 18ºC (Blackwater 18) and 10ºC (Blackwater10, from Rainbow et al. 2009a). Other details as for Table 3.

	Assimilation				**Efflux**				
	AE (%)	SE	n	Tukey's	K_{esed}	SE	Half time	n	Tukey's
Cadmium									
Río San Pedro	60.9	7.8	6	A	0.0229	0.0042	39.2	6	A
Puente Zuazo	47.1	14.2	4	A,B	0.0186	0.0079	33.5	4	A
Blackwater	20.9	5.9	4	B	0.0280	0.0080	31.1	4	A
Blackwater 10	50.7	5.1	7	A	0.0232	0.0029		7	A
Blackwater 18	20.9	5.9	4	B	0.0280	0.0080		4	A
Silver									
Río San Pedro	28.2	3.4	2	A	0.0917	0.0481	5.2	3	A
Puente Zuazo	26.6	2.6	2	A	0.1909	0.1168	5.8	2	A
Blackwater	13.4	0.4	2	A	0.0193		36.0	1	A
Blackwater 10	34.4	7.2	10	A	0.0835	0.0165		9	A
Blackwater 18	13.4	0.4	2	A	0.0193			1	A
Zinc									
Blackwater	67.8	9.2	6	A	0.0331	0.0033	21.8	6	A
Río San Pedro	54.5	12.7	5	A	0.0512	0.0099	18.9	5	A
Puente Zuazo	40.9	6.5	4	A	0.0467	0.0106	17.0	4	A
Blackwater 10	45.4	14.8	4	A	0.0059	0.0098		4	A
Blackwater 18	67.8	9.2	6	A	0.0331	0.0033		6	B

Table 5. Biodynamic modelling of accumulated Ag, Cd and Zn concentrations in *Nereis diversicolor* collected from 2 Spanish and 1 UK estuaries in 2007. (g: growth rate constant (0.02 d^{-1}); AE: assimilation efficiency; IR: ingestion rate; OM: organic matter).

		Accumulation from water				Accumulation from ingested sediment					Contribution to accumulation		Predicted steady state concn
	mean dry wt g	K_u L g^{-1} d^{-1}	Concn µg L^{-1}	K_{ew} d^{-1}	K_{ew} + g d^{-1}	AE	IR of OM g OM/g/d	Concn in sediment µg g^{-1}	K_{esed} d^{-1}	K_{esed} + g d^{-1}	water %	sediment %	µg g^{-1}
CADMIUM													
Blackwater	0.0172	0.0117	0.01	0.0175	0.0375	0.209	0.183	1.95	0.0280	0.048	0.2	99.8	1.56
Río San Pedro	0.0255	0.0243	0.01	0.0277	0.0477	0.609	0.171	0.60	0.0229	0.0429	0.3	99.7	1.46
Puente Zuazo	0.0180	0.0410	0.01	0.0184	0.0384	0.471	0.181	0.56	0.0186	0.0386	0.9	99.1	1.25
SILVER													
Blackwater	0.0172	0.6717	0.01	0.0553	0.0753	0.134	0.183	0.46	0.0193	0.0393	23.7	76.3	0.38
Río San Pedro	0.0255	0.4971	0.01	0.0683	0.0883	0.282	0.171	0.37	0.0917	0.1117	26.1	73.9	0.22
Puente Zuazo	0.0180	0.7775	0.01	0.0519	0.0719	0.266	0.181	0.98	0.1909	0.2109	32.6	67.4	0.33
ZINC													
Blackwater	0.0172	0.0769	0.3	0.0422	0.0622	0.678	0.183	93	0.0331	0.0531	0.2	99.8	218
Río San Pedro	0.0255	0.0557	0.3	0.0469	0.0669	0.545	0.171	101	0.0512	0.0712	0.2	99.8	132
Puente Zuazo	0.0180	0.1748	0.3	0.0728	0.0928	0.409	0.181	121	0.0467	0.0667	0.4	99.6	135

Table 6. *Nereis diversicolor*: Comparisons of predicted (from Table 5) and measured concentrations of Cd, Ag and Zn (µg g^{-1} ± 95% CL dry weight) in worms from sampling sites.

	Cd		Ag		Zn	
	Predicted	Measured	Predicted	Measured	Predicted	Measured
Nereis diversicolor						
Blackwater	1.56	<0.2 - <0.8	0.38	1.38 ± 0.44	218	237 ± 26
Río San Pedro	1.46	0.22 ± 0.00	0.22	0.30 ± 0.04	132	152 ± 15
Puente Zuazo	1.25	0.21 ± 0.03	0.33	0.30 ± 0.00	135	160 ± 22

Table 7. Selected values of the accumulation biodynamic parameters used to generate model predictions of accumulated metal concentrations in *Nereis diversicolor*.

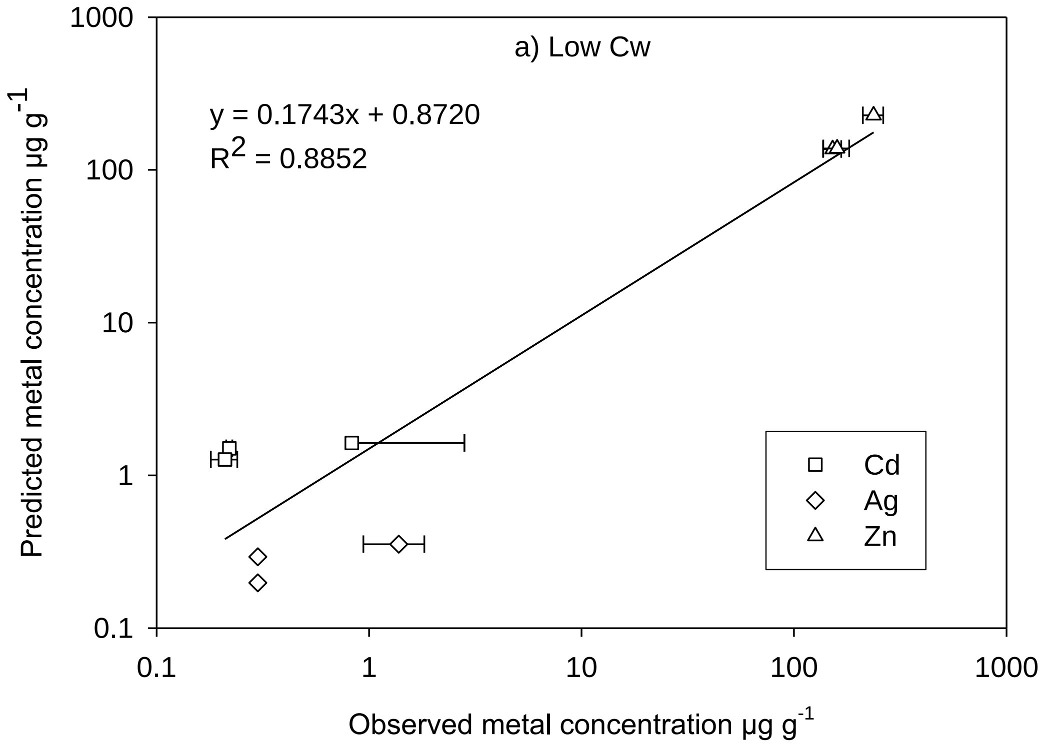
Parameter	Cd	Ag	Zn
K_u (l $g^{-1}d^{-1}$)	0.015	3.0	0.07
K_{ew} (d^{-1})	0.025	0.05	0.04
AE	0.50	0.35	0.50
K_{esed} (d^{-1})	0.02	0.06	0.04
g (d^{-1})	0.02	0.02	0.02
IR (g OM $g^{-1}d^{-1}$)	0.175	0.175	0.175
C_w (µg l^{-1})	0.01	0.01	0.3

Table 8. *Nereis diversicolor*: Model-predicted metal concentrations using the chosen values of the parameters listed in Table 7, compared with measured concentrations in worms from western European and Moroccan estuaries (μg g^{-1} dry body weight).

	Cd		Ag		Zn	
	Predicted	Measured	Predicted	Measured	Predicted	Measured
Rio San Pedro	1.32	0.22	0.71	0.3	148	152
Puente Zuazo	1.23	0.21	1.18	0.3	177	121
Blackwater[a]	4.27	<0.2-<0.8	0.78	1.38	136	237
East Looe[a]	1.58	<1.6-<2.7	0.78	2.48	97	294
Gannel[a]	3.04	<0.8-<1.9	0.72	0.68	366	258
Restronguet Creek[a]	6.24	2.0	1.50	4.82	4769	1925
Tavy[a]	4.12	2.6	0.82	1.32	308	472
West Looe[a]	3.09	<1.1-<4.2	1.31	2.9	151	366
Bay of Somme[b]	0.11	0.05			153	105
Boulogne harbour[b]	0.13	0.06			129	88
Seine estuary[c]	1.10	1.6	1.50	16	123	334
Authie estuary[c]	0.53	0.1	1.04	1.3	60	212
Mylor Creek[d]	1.99	0.91	2.57	2.8	304	208
Cowlands Creek[d]	0.38	0.17	1.12	0.9	318	218
Truro River[d]	3.15	1.44	7.70	9.5	568	389
Tressilian River[d]	2.85	1.3	1.58	1.5	435	298
St Just Creek[d]	1.56	0.71	0.66	0.3	228	156
Hayle[d]	1.03	0.47	4.49	5.3	380	260
Tamar[d]	1.16	0.53	0.89	0.6	261	179
Avon[d]	0.31	0.14	0.51	0.1	288	197
Ría de Plencia[e]			0.96	0.30	177	181
Oualidia lagoon[f]			0.89	0.24	152	115
Khniffis lagoon[f]	0.81	1.00	0.66	0.11	102	94
Bidasoa River[g] st.2			1.42	1.40		
Bidasoa River[g] st.4			0.96	1.30	320	186

[a] Rainbow et al. (2009a)
[b] Berthet et al. (2003)
[c] Amiard et al. (2009)
[d] Bryan and Gibbs (1983)
[e] Saiz-Salinas & Francés-Zubillaga (1997)
[f] Idardare et al. (2008)
[g] Saiz-Salinas et al. (1996)

Figure 1.


a) Low Cw
y = 0.1743x + 0.8720
R2 = 0.8852
Predicted metal concentration μg g-1
Observed metal concentration μg g-1
1000
100
10
1
0.1
0.1
1
10
100
1000
Cd
Ag
Zn

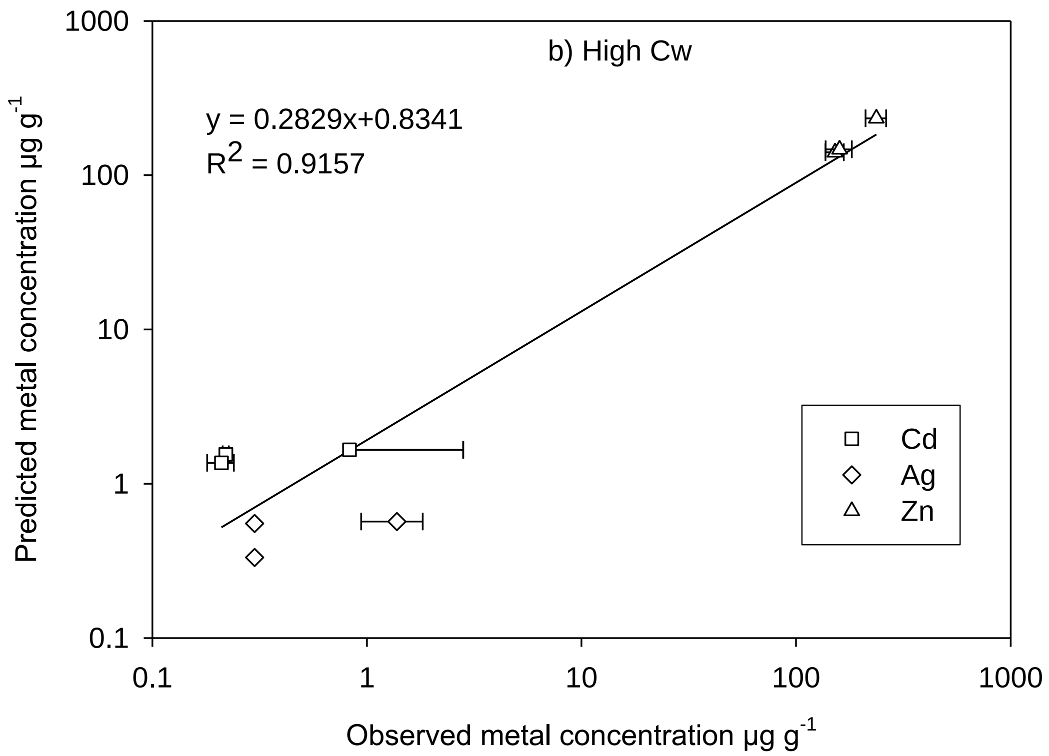
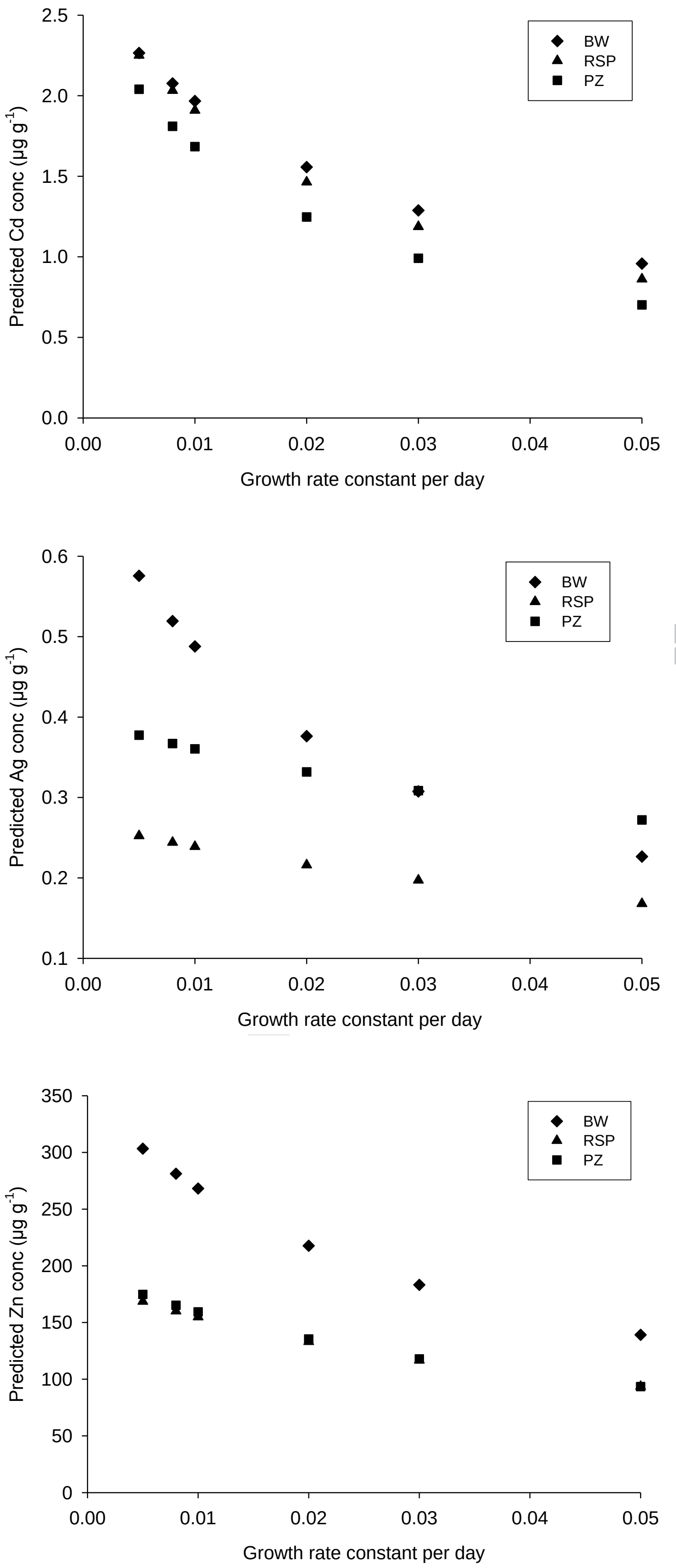
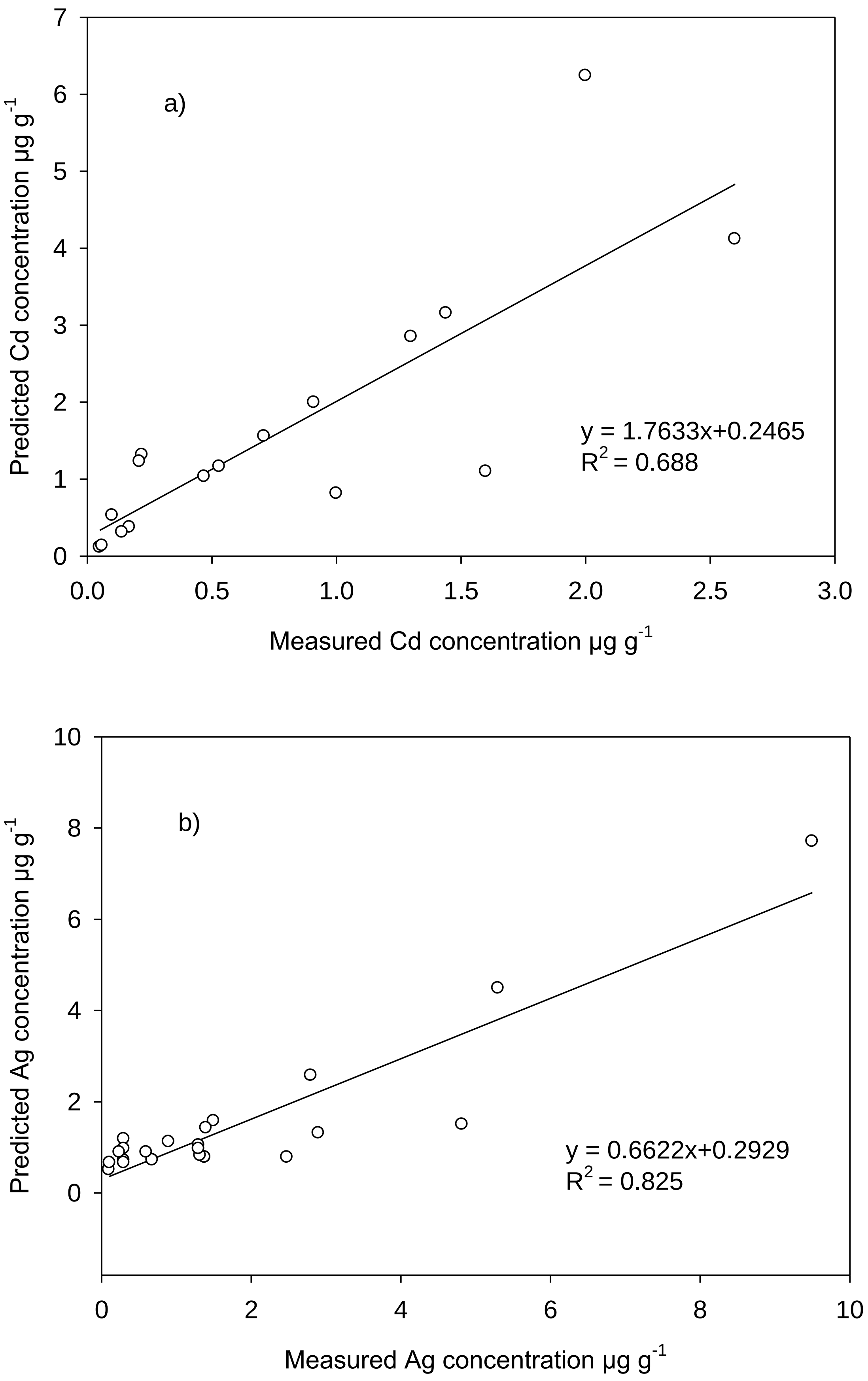

b) High Cw
y = 0.2829x+0.8341
R2 = 0.9157
Predicted metal concentration μg g-1
Observed metal concentration μg g-1
1000
100
10
1
0.1
0.1
1
10
100
1000
Cd
Ag
Zn

Figure 2.

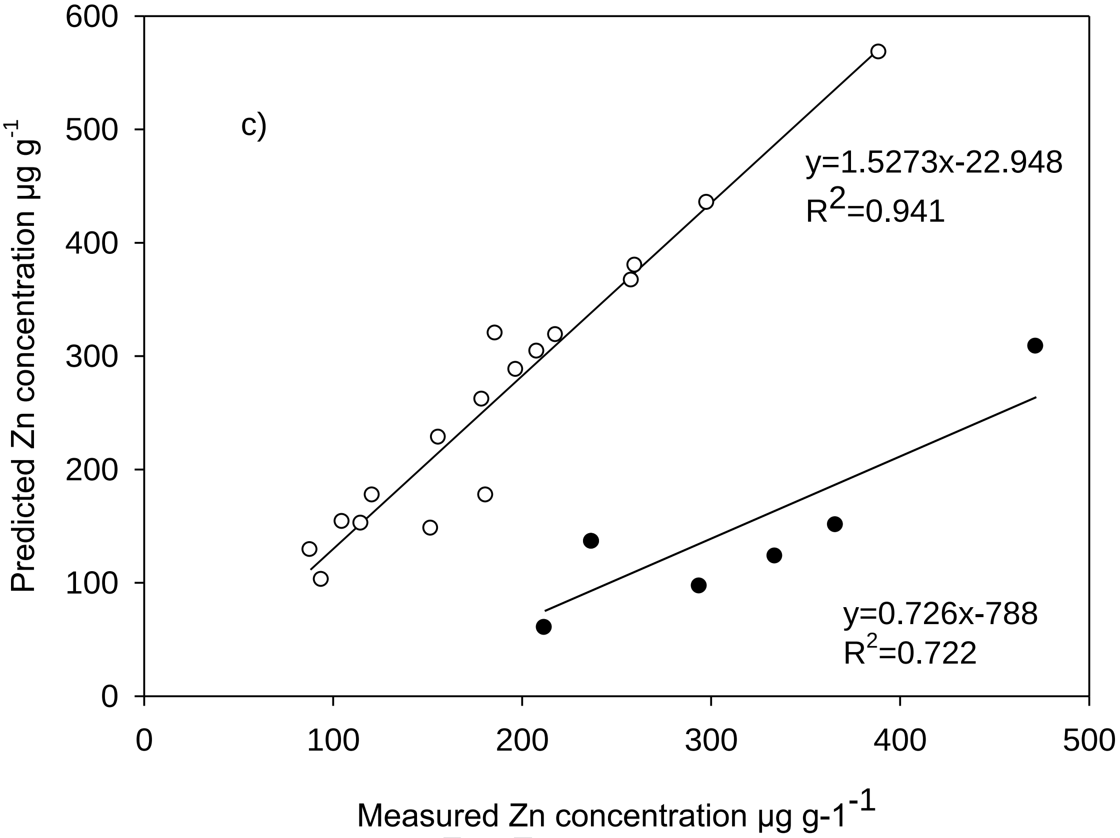


Predicted Cd conc (µg g-1)
2.5
2.0
1.5
1.0
0.5
0.0
0.00
0.01
0.02
0.03
0.04
0.05
Growth rate constant per day
BW
RSP
PZ
Predicted Ag conc (µg g-1)
0.6
0.5
0.4
0.3
0.2
0.1
Predicted Zn conc (µg g-1)
350
300
250
200
150
100
50
0

Figure 3.



a)
y = 1.7633x+0.2465
R2 = 0.688
Predicted Cd concentration µg g-1
Measured Cd concentration µg g-1
0
1
2
3
4
5
6
7
0.0
0.5
1.0
1.5
2.0
2.5
3.0
b)
y = 0.6622x+0.2929
R2 = 0.825
Predicted Ag concentration µg g-1
Measured Ag concentration µg g-1
0
2
4
6
8
10


c)
y=1.5273x-22.948
R2=0.941
y=0.726x-788
R2=0.722
Predicted Zn concentration µg g-1
Measured Zn concentration µg g-1-1
600
500
400
300
200
100
0
0
100
200
300
400
500