Cadmium bioaccumulation in aquatic oligochaetes using a biodynamic model: a review of values of physiological parameters and model validation using laboratory and field bioaccumulation data

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1 Introduction

Oligochaete worms have colonized a wide range of aquatic and semi-aquatic environments, from freshwater to brackish and marine habitats. Different species occupy a variety of microhabitats found in sediments, as well as in aquatic vegetation and decomposing organic matter. Most species are detritivorous, selectively feeding on bacteria, algae and mineral particles rich in organic matter (Coler et al. 1968; Harper et al. 1981a, b; Wavre and Brinkhurst 1971), although a few oligochaetes are predatory. Tubificines and lumbriculids are common oligochaete taxa that can dominate freshwater benthic macroinvertebrate communities (Brinkhurst and Jamieson 1971; Verdonschot 2006), and may account for 50-80% of the biomass in the majority of lakes, rivers and reservoirs (Poddubnaya 1980).

Aquatic oligochaetes have been widely used in sediment ecotoxicity and bioaccumulation studies (see review by Rodriguez and Reynoldson 2011), and their usefulness in Environmental Risk Assessment has been highlighted by Chapman (2001) and Egeler and Römbke (2007). Among those most frequently used are the cosmopolitan species *Lumbriculus variegatus* (Lumbriculidae), *Limnodrilus hoffmeisteri* and *Tubifex tubifex* (Tubificinae), although other species have also been utilized, notably *Branchiura sowerbyi* (Rhyacodrilinae) in tropical regions (Lobo and Espindola 2014). These sediment-dwelling species have been included in standardized protocols for laboratory and field ecotoxicology research: USEPA (2000), ASTM (2005) and OECD (2007, 2008). Their entire life cycle occurs in the sediment, therefore the uptake of pollutants can occur via two different routes: through the integument (porewater) and through digestive epithelia (ingested particles and porewater). In the field, *L. variegatus*, *T. tubifex* and *L. hoffmeisteri* are adapted to a wide range of environmental conditions and can tolerate high levels of pollution (e.g. see Table 3.3, in Rodriguez and Reynoldson 2011).

Sediment-dwelling organisms meet their nutritional requirements from the organic fraction of sediment and the microorganisms associated with this material. Nonetheless, sediment is a poor food source thus massive volumes must be processed in order to obtain sufficient nutrients (Lopez and Levinton 1987). Some oligochaete species are known as upward conveyors, since they ingest huge quantities of underlying sediment and egest feces at the sediment-water interface. These worms build galleries in the sediment, and play an important role in the bioturbation of lakes and rivers through their burrowing activity (Matisoff et al. 1999; Mermillod-Blondin et al. 2001, 2005; Nogaro et al. 2009). This has implications with regard to the physical and chemical properties of the sediment (e.g. sediment "pelletization", changes in redox potential, detritus processing, and nutrient recycling). Bioturbation effects also include the transport of pollutants from the sediment and their release back into the water column (Ciutat et al. 2005; Hunting et al. 2012; Karickhoff and Morris 1985).

Physiological parameters related to digestive processes, such as feeding rates and habits, assimilation efficiencies, and selective feeding on certain sediment particle sizes, have been used for modeling the bioaccumulation of xenobiotics (Leppänen 1995). Most works quantifying egestion/defecation rates and absorption efficiencies in aquatic oligochaetes date back to the 1970´s to 90´s; however, in the assessment of metal bioaccumulation, physiological parameters have rarely been mentioned. In 1999, Martinez-Madrid

et al. included data on egestion rates, growth rates and cocoon biomass in a *T. tubifex* chronic bioassay, at 13 sites with varying degrees of pollution. More recently, different toxicokinetic models have been used to assess metal assimilation efficiencies in *T. tubifex*, both in spiked-sediment (Gillis et al. 2004; Steen Redeker et al. 2004) and water-only exposures in the laboratory (Steen Redeker and Blust 2004), though only for Cd and Zn.

In the last decade, in an attempt to arrive at a more comprehensive understanding of the relative importance of physiological parameters in the study of trace metal bioaccumulation in invertebrates, the use of a biodynamic model (Luoma and Rainbow 2005) has gained importance. The biodynamic concept provides a framework for explaining how and why trace element bioaccumulation differs between metals, species, and environments. This concept includes the application of bioenergetic-based kinetic bioaccumulation models (Reinfelder et al. 1998; Newman and Unger 2003). It is based on the idea that infaunal organisms can accumulate metals from both water and food, thus bioaccumulation is expected to occur when the rate of metal uptake summed across all sources (solution and diet) exceeds the combined rates of elimination via the gut, excretion or other mechanisms (e.g. epidermal lysosomes, elimination of chloragosomes, autotomy; reviewed by Rodriguez and Reynoldson 2011). However, several difficulties arise in the application of the biodynamic model in sediment-ingesting freshwater oligochaetes due to the complexity of estimating some of the physiological parameters. In sediment-dwelling aquatic oligochaetes, the uptake of contaminants occurs via both epidermal and digestive processes, the latter usually prevailing over the epidermal route (Méndez-Fernández et al. 2014). The biodynamic model has succeeded in explaining metal bioaccumulation in the upward conveyor polychaete *Arenicola marina* at a range of sediment exposure concentrations (Casado-Martínez et al. 2009a, b, 2010 a, b). More recently, the biodynamic model has been applied in the oligochaete *L. variegatus* exposed to copper ions and copper oxide nanoparticles (Ramskov et al. 2015).

In the present paper, we reviewed published data on some of the physiological parameters related to digestive processes in three aquatic oligochaete species widely used in standardized bioassays (*L. variegatus*, *L. hoffmeisteri* and *T. tubifex*), as a first step in examining the potential of using the food ingestion rates and assimilation efficiencies reported in the bibliography in a biodynamic model. By using these physiological parameters, we aimed (1) to build a biodynamic model to predict Cd bioaccumulation in detritivorous aquatic oligochaete species, (2) to evaluate the predictions of our model with independently measured tissue residue data in *T. tubifex* exposed to Cd spiked-sediment bioassays in the laboratory and (3) to validate the biodynamic model by comparing predicted *vs*. field Cd bioaccumulation data, in order to identify the limits of its application.

2 Physiological parameters: a review

The following sections describe the relevant information on digestive physiological parameters (i.e. ingestion rates and assimilation efficiencies) reported in the scientific, peer-reviewed literature for aquatic oligochaete species, in particular *T. tubifex*, *L. hoffmeisteri* and *L. variegatus*. In this review, we selected

those publications that also included data on worm biomass and sediment characteristics (i.e., organic content and/or silt-clay percentage). All data originally given in wet weight were expressed on a dry weight basis, by assuming 90% water content (Gillis et al., 2002; Maestre et al., 2009; Méndez-Fernández et al., 2013). Data on metal concentration originally expressed in micromoles of metal were converted to micrograms. In the following sections the term "tubificids" refers to the oligochaete species of the subfamily Tubificinae.

2.1 Ingestion rates

Aquatic oligochaetes are considered to be continuous feeders; therefore it is assumed that egestion/defecation rates are good approximations of ingestion/feeding rates (Cammen 1980; Martinez-Madrid et al. 1999), the former being easier to measure. Differences in ingestion rates between populations can be due to alterations in feeding behavior, which depend on water and sediment characteristics, such as: 1) sediment particle size, i.e., ingestion rates can be influenced by the selection of a particular sediment fraction and the grain-size frequency distribution of the sediment, 2) sediment total organic content (TOC) or variation in the composition of the associated microbial communities, 3) water temperature and dissolved oxygen concentration, and 4) the presence of toxic chemicals in the sediment that can induce avoidance behavior, with worms either starving or lying on the sediment surface in order to minimize exposure to high levels of pollutants via digestive epithelia or through the body wall, via porewater.

The first three of the aforementioned characteristics have been studied by a number of authors. It is well documented that *T. tubifex* feeds selectively on fine-grained particles (< 63 μm) (Kosiorek 1974; Rodriguez et al. 2001; Tevesz et al. 1980), whereas *L. hoffmeisteri* appears to be more eclectic with respect to the sediment particle size ingested, although it can adopt a strict limivorous regime (Juget 1979). In the field, *T. tubifex* prefers fine sediments (Juget 1979) with some organic content (Verdonschot 1981), while *L. hoffmeisteri* can be associated with either sandy (Juget 1979) or muddy sediments (Birtwell and Arthur 1980; Giere and Pfannkuche 1982; Mildward et al. 2001). *L. variegatus* prefers sandy sediments (Chekanovskaya 1962), although preferential feeding on particles < 100 μm has also been reported in this species (Lawrence et al. 2000). Ingestion rates and particle selection by deposit feeders are also thought to be related to the organic matter associated with the particles (Cammen 1980); however, taking TOC as a typical surrogate measurement of the nutritional quality of sediments has not been found to be a good predictor of nutrient availability for worms (e. g. *L. variegatus*: Ankley et al. 1994). Data on the quality and density of the microorganism communities present in the sediment is probably more relevant than TOC for interpreting differences in ingestion rates, but this information is rarely reported in the literature. Wavre and Brinkhurst (1971) studied the diet of three aquatic oligochaetes that ingested the heterotrophic aerobic bacteria present in sediments. The authors indicated that bacteria were the primary food source for the worms and that different species were specialized deposit feeders, selecting detritus with a particular microflora composition. Later, in 1972, Brinkhurst et al. showed the importance of the sediment microflora in the feeding biology of single *vs.* complex cultures of tubificine species.

A critical issue that modifies ingestion rates in oligochaetes is autotomy, a mechanism related to asexual reproduction by architomy in *L. variegatus*, and to the elimination of metals in *T. tubifex*. Autotomy impedes sediment ingestion in *L. variegatus,* for a period of 6-7 days following division of the worm (Leppänen and Kukonnen 1998), as well as the elimination of chemicals in the feces, until the new mouth or anus is formed. During this period, the uptake of chemicals is thought to be restricted to the integumentary pathway.

Under experimental conditions water temperature is usually kept constant, but there is some variability in reports describing the relationship between temperature and digestive parameters. Appleby and Brinkhurst (1970) reported that, in both *T. tubifex* and *L. hoffmeisteri,* defecation rates increased with temperature, and the worms gained weight, with a peak occurring at 18°C. However, linear increments in feeding rates with increasing temperature have been reported within a range of 7-22°C (McCall and Fisher 1980), and 4-20°C (White et al. 1987; Leppänen and Kukonnen 1998). Regarding dissolved oxygen levels, whilst normoxic conditions are usually maintained in laboratory experiments (e.g. > 2.5 mg l^{-1}; ASTM 2005), temporal hypoxic or anaerobic conditions can occur in the field. The percentage survival of *L. hoffmeisteri* at dissolved oxygen concentrations of 0.1-0.2 mg/l was 24% to 30% at 15°C and 25°C, respectively (Aston 1973); whereas Reynoldson (1987) observed that *T. tubifex* maintained under anoxic conditions showed an average survival of 60% for up to sixteen weeks at 9.7-12°C. *T. tubifex* and *L. hoffmeisteri* are known for their capacity for anaerobic metabolism (Gnaiger and Staudigl 1987; Schöttler 1978), and defecation rates seem to be independent of dissolved oxygen down to 0.5 mg/l, only decreasing to zero at < 0.3 mg/l (Volpers and Neumann 2005).

The fourth characteristic that we identified as having an effect on ingestion rates was sediment avoidance, an ecologically relevant response in sediment-dwelling organisms, which has been proposed by different authors as an escape response in the presence of polluted sediment (Amiard-Triquet 2009; Weis 2014). Reductions in the burrowing activity of worms due to the presence of pollutants have been quantified (White and Keilty 1988; Keilty et al. 1988; Meller et al. 1998; Bettinetti and Provini 2002; Rodriguez et al. 2006). In bioassays, this behavior is associated with the presence of worms at the sediment surface over long periods, with scarce movements, and a concomitant reduction in both burrowing activity and the production of fecal pellets (Martinez-Madrid et al. 1999; Méndez-Fernández et al. 2013, 2014). Body biomass reduction in worms exposed to highly polluted sediments has been found to be similar to that shown in worms under starving conditions (worms exposed to calcinated sediments) and has been interpreted as the consequence of sediment avoidance behavior (Martinez-Madrid et al. 1999).

We considered the above miscellaneous factors when searching for relevant data on feeding and defecation rates. Thus, the database used for the biodynamic model includes primarily laboratory data run at 18-22ºC under normoxic conditions, which was the case in most of the laboratory studies. However, data reported under different temperature, food, or oxygen concentration regimes was also considered, for comparative purposes. Data for egestion rates were also differentiated, depending on whether the worms were exposed to unpolluted (bioassay controls, reference sites, and non-toxic conditions) or polluted sediments. Another

important issue when considering the variety of data was worm biomass, since larger individuals have higher egestion rates. To tackle this problem, egestion rates were standardized to 1 mg (dw) worm biomass, according to the formula $Yst = (Wst/We)^b * Ye$, where *Yst* and *Ye* represent the standard and experimentally recorded egestion rates, respectively; *Wst* is the standard worm weight, 1 mg dw; *We* is the weight of the experimental worms; and *b* is the allometric coefficient that scales the physiological rates to body weight, set at 0.771 by Cammen (1980) for deposit feeders, implying that ingestion rates are controlled by their metabolic requirements. *Yst* values were used as weight-specific ingestion rates in the model (see below).

2.2 Assimilation efficiencies

In aquatic oligochaetes, urinary excretion is usually disregarded (Brinkhurst et al. 1972) and assimilation efficiency (AE) is calculated as the fraction of absorbed products that is incorporated into body tissues (Penry 1998). However, measuring the parameters required for the estimation of assimilation efficiencies from the sediment matrix is problematic, hence assimilation efficiencies in ecotoxicity studies with benthic invertebrates are difficult to calculate, and some AE data reported are, in fact, absorption efficiencies (Penry 1998).

Wang and Fisher (1999) carried out a revision of the most commonly available techniques for measuring assimilation efficiencies. They looked at two main approaches: radiotracer and mass balance. In freshwater oligochaetes a dual-tracer technique has been applied for the determination of assimilation efficiencies of organic pollutants (Klump et al. 1987; Kukkonen and Landrum 1995; Lu et al. 2004). Another approach, consisting of the use of gamma-emitting radioisotopes, has been extensively used in several aquatic invertebrates, with successful application of the biodynamic model, e.g., in the marine polychaetes *Arenicola marina* (Casado-Martínez et al. 2009a, 2010a) and *Nereis diversicolor* (Rainbow et al. 2009), and in the oligochaete *L. variegatus* (Ramskov et al. 2015).

The mass balance method requires the quantification of total ingestion and egestion. This approach has been used by Brinkhurst and Austin (1979), who calculated AE values for *T. tubifex* and *L. hoffmeisteri* ranging from 2.7% to 5.7%, with an overall mean value of 4.1%. However, this method requires adequate measurement of both total ingested sediment and the fraction digested, which is not possible in most cases. Alternatively, the ratio method proposed by Conover (1966) quantifies the ratios between ash-free dry weight in both food and feces to obtain AE, applying the equation $AE = [(F' - E')/(1- E') * F'] * 100$, where F' is the ash-free to dry weight ratio of the ingested sediment, and E' is the same ratio calculated for feces. The ratio method relies on the assumption that only the organic component of food is significantly affected by the digestive process (Conover 1966). In the present study, data on sediment and fecal organic content originally reported as LOI% (Loss on Ignition) were converted to total organic carbon (TOC%) using the conversion factor from Nelson and Sommers (1996), which is based on the assumption that organic matter contains 58% organic carbon.

2.3 Biokinetic parameters in Cd bioaccumulation by aquatic oligochaetes

233 At steady state the uptake of a contaminant is balanced by elimination and growth, to give a value known

234 as constant concentration in the consumer (C_{ss}) (Reinfelder et al. 1998). The concentration of a trace

235 element in a consumer due to food ingestion ($C_{ss,f}$) is given by the function: $C_{ss,f} = (AE*IR*C_F)/(k_e+g)$,

236 where AE is the assimilation efficiency (%), IR is the weight-specific ingestion rate, C_F is the chemical

237 concentration in the food, k_e is the loss rate constant after uptake from the food (d^{-1}), and g is the growth

238 rate (d^{-1}). Based on the available literature on oligochaetes, one of the main entry routes for Cd is through

239 the ingestion of sediments (Hare et al. 2001; Warren et al. 1998). More recently, in the paper published by

240 Camusso et al. (2012), the importance of the dietary uptake of Cd (sediment ingestion) in the oligochaete

241 *Lumbriculus variegatus* was demonstrated. Additionally, Méndez-Fernández et al. (2014) showed that

242 ingested sediment in *Tubifex tubifex* was a good predictor of toxicity that was explained by Cd

243 bioaccumulation in the worms.

244

245 Regarding the loss rate constant in *T. tubifex*, Gillis et al. (2004) described Cd depuration as a two-

246 compartment model, with the first compartment representing the total body concentration of metal that is

247 easily mobilized, and the second compartment representing the total body concentration of metal that is

248 more tightly bound to worm tissues. These compartments have measurable loss rate constants: k_g, the

249 depuration rate from the gut, and k_e, the depuration rate from body tissues ($k_e = 0.81$ d^{-1}, $k_g = 56.1$ d^{-1},

250 transformed from Gillis et al. 2004). Metal dilution due to organism growth has been ignored in the model

251 since the growth rate constant (adult worms= -0.03 to 0.03 d^{-1}) in our data was much lower than the loss

252 rate constants (see Wang and Fisher 1999).

253

254 To test the accuracy of the biodynamic model in *T. tubifex* bioassays, we used published data on metal

255 tissue residues following long-term Cd exposure (28 days) from Gillis et al. (2002) and Méndez-Fernández

256 et al. (2013), in Cd spiked-sediment experiments. The worms in Gillis et al. (2002) came from a population

257 strain of *T. tubifex* from the Canadian Great Lakes, while the worms in Méndez-Fernández et al. (2013)

258 came from a population strain from a mountain stream in northern Spain. The sediment Cd concentration

259 ranged from 75.3 - 508.0 µg g^{-1} dw in Gillis et al. (2002) and 0.5 - 161.9 µg g^{-1} dw in Méndez-Fernández

260 et al. (2013). In the spiking procedures, the mixture and equilibration periods differed between the two

261 studies. In the former, 90-min of mixing was followed by a 3-week equilibration period, and the latter

262 comprised 4-h of mixing followed by a 1-week equilibration period. Sediment digestion procedures were

263 also different, 5% HCl digested in Gillis et al. (2002), and following EPA3052 (65% HNO_3 + HF) in

264 Méndez-Fernández et al. (2013).

265

266 Additionally, we wanted to explore the accuracy of the biodynamic model for aquatic oligochaetes exposed

267 to field sediments in laboratory bioassays and for field-collected worms. For the application of

268 physiological parameters in the model, we tested different IR values obtained from reported values in the

269 literature, related to non-toxic *vs.* toxic effects. For those cases where toxicity was not reported, when

270 sediment metal concentrations were higher than the Potentially Effective Concentration (PEC, MacDonald

271 et al. 2000) the sediments were considered as toxic, and if lower than the PEC as non-toxic. When exposed

272 to toxic sediments, worms can demonstrate sediment avoidance behavior, resulting in a lower IR value that

we have included in the model. Ramskov et al. (2015) found that in *L. variegatus* the AE value remained approximately constant across an increasing Cu exposure, suggesting a relation to a decreasing IR as a behavioral response. Therefore, in our approach for validation of the biodynamic model, only IR values, not AEs, were chosen depending on exposure to polluted (or toxic) *vs.* unpolluted (or non-toxic) sediments. The AE values used in the model were selected depending on the taxa reported, as defined in the bibliographic source, that is, an AE median value of the three oligochaete species was used for taxa reported as "oligochaetes"; the median of *L. hoffmeisteri* and *T. tubifex* for taxa reported as tubificid worms; and for *T. tubifex,* the median value calculated for this species (related to the < 63 µm sediment fraction).

3 Results

3.1 Physiological parameters

The laboratory-based defecation rates measured in several aquatic oligochaete species were used as an approximation for ingestion rates (IR) (data for unpolluted and polluted conditions are reported in Supplementary Material Tables S1 and S2, respectively). A summary of the descriptive statistics of the reviewed IR values is shown in Table 1 for *T. tubifex*, *L. hoffmeisteri* and *L. variegatus*, at a temperature range of 18-23°C and under normoxic conditions. In Table 2, IR values are reported for various temperatures and under both normoxic and hypoxic conditions.

Table 1 Ingestion rates (mg mg^{-1} d^{-1}) in *T. tubifex, L. hoffmeisteri* and *L. variegatus* measured under unpolluted *vs.* polluted conditions, and at 18-23°C. Abbreviations: SE: Standard Error of the mean. Data sources can be found in Tables S1 and S2.

	T. tubifex		*L. hoffmeisteri*		*L. variegatus*	
Ingestion rates	Unpolluted	Polluted	Unpolluted	Polluted	Unpolluted	Polluted
Mean	9.4	4.5	29.5	1.3	15.4	3.7
SE	1.6	1.6	4.1	0.4	3.9	1.4
Median	7.8	2.4	24.5	1.7	11.5	2.1
Minimum	2.6	1.8	3.5	0.5	0.6	0.5
Maximum	26.7	10.4	80.0	1.7	38.8	8.2
n	16	5	23	3	11	5

Table 2 Ingestion rates (mg mg^{-1} d^{-1}) in *T. tubifex and L. hoffmeisteri* reported in the literature, at different temperatures and under normoxic or hypoxic conditions. Abbreviations: SE: Standard Error of the mean. Source data from Volpers and Neumann (2005).

	T. tubifex		*L. hoffmeisteri*	
Ingestion rates	12-14ºC Normoxia	10.5ºC Hypoxia	12-14ºC Normoxia	10.5ºC Hypoxia
Mean	14.5	39.3	3.5	18.4
SE	1.9	4.5	0.5	4.6
Median	15.9	40.6	3.5	17.5
Minimum	8.1	25.3	1.7	4.7
Maximum	19.3	51.3	5.0	36.1
n	6	6	6	6

298 Median IR values (mg mg^{-1} d^{-1}) measured in unpolluted conditions and at a temperature range of 18-23°C

299 were: 7.8 for *T. tubifex*, 24.5 for *L. hoffmeisteri* and 11.5 for *L. variegatus*. In polluted conditions the values

300 were lower: 2.4 (*T. tubifex)*, 1.7 (*L. hoffmeisteri)* and 2.1 (*L. variegatus)*. In the absence of pollutants,

301 comparisons between the three species showed significant differences (Dunn´s test, p < 0.05), indicating

302 the importance of using individual values calculated for each species when possible. Conversely, in polluted

303 conditions, these differences were not significant (Dunn´s test, p > 0.05), and IR was reduced to similar,

304 low levels in all three species. In all cases, the differences in IR measured in unpolluted *vs.* polluted batches

305 were significant (Kruskal-Wallis test, p < 0.05). The maximum IR values recorded in unpolluted conditions

306 were similar for *T. tubifex* (26.7 mg mg^{-1} d^{-1}) and *L. variegatus* (38.8 mg mg^{-1} d^{-1}), and were much higher

307 for *L. hoffmeisteri* (80 mg mg^{-1} d^{-1}). In the presence of pollutants, the maximum IR values for all three

308 species were less than 10.5 mg mg^{-1} d^{-1}, with minimum values being well below 2 mg mg^{-1} d^{-1}.

309

310 **Table 3** Assimilation efficiencies (AE, %) for three oligochaete species, calculated using the Conover or
311 dual-tracer method (see section 2.2 for details on the methods). Abbreviations: SE: Standard Error of the
312 mean. Data sources can be found in Tables S3 and S4.

Assimilation Efficiencies	Conover method		Dual-tracer method	
	T. tubifex	*L. hoffmeisteri*	*L. hoffmeisteri*	*L. variegatus*
Mean	9.3	10.2	9.7	15.4
SE	1.4	1.0	2.1	2.0
Median	8.7	9.5	9.0	13.0
Minimum	3.4	6.4	2.7	10.9
Maximum	19.6	14.6	16.1	25.6
n	12	9	6	8

313

314 Ingestion rates at temperatures below 15°C, which more closely mimic water temperatures in the field in

315 temperate regions, were only available from one study (Appleby and Brinkhurst 1970), which estimated IR

316 using the inverted method to obtain the feces (see Table S1). At 12-14°C, median IR values in *T. tubifex*

317 were 15.9 mg mg^{-1} d^{-1}, but this value was not significantly higher than the result obtained at 18-23°C

318 (Kruskal-Wallis test, p > 0.05). Contrastingly, IR values were found to be significantly lower at 12-14°C

319 than at 18-23°C in *L. hoffmeisteri* (3.5 mg mg^{-1} d^{-1} *vs.* 24.5 mg mg^{-1} d^{-1}) (Kruskal-Wallis test, p < 0.05).

320 Ingestion rates measured in worms under hypoxic conditions (Volpers and Neuman 2005), showed higher

321 median values at 10.5°C than at 12-14°C for both *T. tubifex* (40.6 mg mg^{-1} d^{-1}) and *L. hoffmeisteri* (17.5 mg

322 mg^{-1} d^{-1}).

323

324 Assimilation efficiency (AE) values, calculated using the Conover and dual-tracer methods, are reported in

325 Supplementary Material Tables S3 and S4. Median AE values using the Conover method (Table 3) were

326 8.7% for *T. tubifex* and 9.5% for *L. hoffmeisteri*. Using the dual-tracer method, median values were 15.9%

327 for *L. hoffmeisteri* and 13.0% for *L. variegatus* (Table 3)*,* while no data were found for *T. tubifex.*

328 Comparisons between species using the same method were not significant, nor were they for *L. hoffmeisteri*

329 using different methods (U Mann-Whitney test, p >0.05), although the higher variability found in *L.*

330 *variegatus* AE values may have caused misleading results in statistical analyses. Assimilation efficiencies

for each species, independently of the method, ranged from 3.4 - 19.6% for *T. tubifex*, 2.7 – 16.1% for *L. hoffmeisteri* and 10.9 - 25.6% for *L. variegatus*.

3.2 Application of the biodynamic model to laboratory Cd spiked-sediment bioassays with *Tubifex tubifex*

The physiological parameters used to generate the biodynamic model were obtained from the present literature review, and uptake and elimination rates from kinetic experiments by Gillis et al. (2004) with *T. tubifex* (see Table 4). Cadmium tissue residues predicted by the biodynamic model (Table 5) were obtained using several combinations within the range of AE and IR values reported in Table 4, depending on the exposure conditions reported in each case.

Table 4 Bioaccumulation parameters used to generate biodynamic model predictions of accumulated Cd concentrations in *T. tubifex*, Tubificids, or Oligochaetes. Abbreviations: IR, Ingestion rate (g g^{-1} d^{-1}); AE%: Assimilation efficiency; Kg (d^{-1}), the depuration rate from the fast compartment; Ke (d^{-1}), the depuration rate from the slow compartment, in a two-compartment model.

Species	Parameter	Median; Min-Max	Reference
T. tubifex	IR[1]	Unpolluted: 7.8; 2.6-26.7 Polluted: 2.4; 1.8-10.4	Present review
	AE[2]	9.1; 3.6-19.6	Present review
Tubificids	IR[1]	Unpolluted: 10.6; 1.7-80 Polluted: 1.90; 0.5-10.4	Present review
	AE	9.6; 2.7-36.4	Present review
Oligochaetes	IR[1]	Unpolluted: 11.1; 0.6-80 Polluted: 2.00; 0.5-10.4	Present review
	AE	11.3; 2.7-36.4	Present review
T. tubifex	k$_g$	56.110	Gillis et al., 2004
	k$_e$	0.812	Gillis et al., 2004

[1] At normoxic conditions and at 12-23ºC
[2] Only for sediment fraction < 63 μm, see Table S3

Measured tissue residues in *T. tubifex* using data from Cd-spiked experiments by Gillis et al. (2002), were in good agreement with our predictions when the physiological parameters used were the maximum values reported in the literature for both AE (19.6%) and IR (26.7 g g^{-1} d^{-1}) (Fig. 1), though when median values were used tissue concentrations were underpredicted by a factor of 2.7-10 (see values in Table 5). However, we found that when median AE (9.1%) and IR (7.8 g g^{-1} d^{-1}) values were applied to the biodynamic model the predicted Cd tissue concentrations were very close to the actual concentrations measured in laboratory bioassays by Méndez-Fernández et al. (2013) for sediment exposures < 80.8 μg Cd g^{-1} dw, although the results were underpredicted at the highest sediment exposure (Fig. 1, Table 5).

Table 5 Predicted tissue Cd concentrations (μg g^{-1} dw) from the biodynamic model using the range and median values of the parameters as presented in Table 4, and measured tissue Cd concentration in 28-day chronic sediment bioassays with *T. tubifex*. Sediment Cd concentrations for each of the exposures are also indicated. Superscripts: 1, Gillis et al. 2002; 2, Méndez-Fernández et al. 2013.

| Sediment Cd Concentration[1] | Tissue Cd concentration[1] | | | Sediment Cd Concentration[2] | Tissue Cd concentration[2] | | |
| | Predicted | | Measured | | Predicted | | Measured |
	Median	Range			Median	Range	
75.3	93.9	12.4-692	254	0.46	0.58	0.1-4.25	0.2
152	189	25.0-1395	1068	13.9	17.3	2.3-128	7.8
208	259	34.2-1912	2635	26.9	33.6	4.4-248	19.2
301	375	49.5-2769	3415	51.1	63.8	8.4-470	28.1
412	514	67.8-3792	3617	80.8	101	13.3-743	248
508	634	83.5-4671	4553	161	202	26.6-1488	6789

In the Cd chronic bioassay with *T. tubifex* published by Méndez-Fernández et al. (2013), all worms suffered autotomy when exposed to 161.9 µg Cd g^{-1} dw sediment. The worms were observed at the sediment surface, movements were scarce, and neither fecal pellets nor galleries were present, inferring the existence of sediment avoidance behavior. Therefore, we explored the results of the prediction in the biodynamic model in the presence of a possible reduction in IR by *T. tubifex*. The inclusion of a sediment avoidance factor, through a reduction in the theoretical IR in the contaminated sediment (median value: 2.4 g g^{-1} d^{-1}), with the same AE, resulted in a predicted tissue Cd concentration of 59.4 µg Cd g^{-1} dw, a value much lower than the tissue concentration measured at the highest sediment exposure (6789.3 µg Cd g^{-1} dw).

3.3 Application of the biodynamic model to field-collected aquatic oligochaetes or those exposed to field sediments in laboratory bioassays

Data on sediment Cd concentration and Cd tissue residues reported in papers from several sources have been used to apply the biodynamic model to aquatic oligochaetes, both in laboratory bioassays (Gillis et al. 2002; Méndez-Fernández et al. 2013, 2014, 2015) and field-collected worms (Hernández and Egea 1987; Krantzberg 1994; Protano et al. 2014; Say and Giani 1987; Singh et al. 2007). Reviewed sediment Cd concentrations ranged from 0.03 to 508 µg g^{-1} dw, while measured tissue Cd residues ranged from 0.01 to 6789 µg g^{-1} dw. A significant linear regression was obtained between sediment Cd concentration and measured Cd tissue concentration in aquatic oligochaetes using log-transformed data (n= 77, F= 147.01, p< 0.001), with a high coefficient of determination (R^2= 0.67, Fig.2).

We used the information on environmental conditions (i.e. temperature, dissolved oxygen, polluted or toxic sediments) reported in the original papers to refine the physiological parameters utilized in this review as much as possible (Table 4). The values used were: a median IR (g g^{-1} d^{-1}, dw) of 7.8 for *T. tubifex*, 10.6 for tubificids (*T. tubifex* and *L. hoffmeisteri* database), and 11.1 for undetermined oligochaetes (*T. tubifex*, *L. hoffmeisteri* and *L. variegatus* database) exposed to unpolluted or non-toxic sediments, at temperatures ranging from 12-23°C. When worms were exposed to polluted or toxic sediment a median IR value of 2.4 was used for *T. tubifex* and 1.90 for tubificids. An AE median value of 9.1 % was used for *T. tubifex*, derived from a data series where sediment TOC in the fraction <63 µm was given (see Table S3), while median AEs of 9.6% and 11.3% were used for tubificids and oligochaetes, respectively.

396   The biodynamic model accurately predicted Cd bioaccumulation across a range of measured tissue

397   concentrations of 0.1-100 µg Cd g^{-1} dw. The results showed that 81.3% of predicted concentrations were

398   within a factor of five of the 1:1 line of the predicted-measured data (Fig. 3). Predicted data were also highly

399   correlated with measured data (Pearson's r=0.77, p=0.000, n=49, log-transformed data) within a tissue

400   range of 0.1-100 µg Cd g^{-1} dw. However, when considering the entire range of tissue concentrations, the

401   accuracy of predicted-measured values decreased to 67.5% (n=77). Most of the discrepancies between

402   predicted and measured data were associated with the lower range of Cd tissue concentrations, < 0.01 µg

403   g^{-1} dw (Fig. 3), with the model overpredicting the results by up to two orders of magnitude for some field

404   sites. At Cd tissue concentrations of > 100 µg g^{-1} dw, field data reported from Krantzberg (1994) were

405   underpredicted by a factor of 64-213 (see Fig.3), although if maximum values of AE and IR are applied, as

406   in Gillis et al (2002) (see section 3.3), the predicted values are more accurate (only underestimated by a

407   factor of 2.8-9.2). The application of high AE and IR values is supported by the fact that in both of these

408   studies the sediment and the oligochaete population came from the North American Great Lakes (Canada).

409

410

411   **4 Discussion**

412   The range of Assimilation Efficiencies (AE) of 2.7 - 36.4% calculated for three freshwater oligochaetes in

413   the present review, is somewhat in accordance with the range of values (< 2.5 to 19%) reported for

414   geophagous earthworms, based on ingested soil organic matter (Curry and Schmidt 2007), and is even

415   closer to the Cd AE reported for deposit-feeder polychaetes, using radiolabelling techniques, e.g. 3.3-43.6%

416   in *Arenicola marina* (Casado-Martinez et al. 2009a). More recently, Ramskov et al. (2015) obtained similar

417   Cu AE values of 30% and 24-41% in *L. variegatus*, after exposure to Cu ions and Cu oxide nanoparticles,

418   respectively. Steen-Redeker et al. (2004) measured Cd AE in *T. tubifex* using radiolabelling techniques,

419   and reported a very low value of 0.09% that the authors attributed to competition with Zn, but this result

420   could also be due to mucus production by the epidermal layer, which acts as a barrier to metals in aquatic

421   oligochaetes (Bouchè et al. 2000; Méndez-Fernández et al. 2014).

422

423   The biodynamic model accurately predicted Cd bioaccumulation for *T. tubifex* in laboratory bioassays using

424   Cd-spiked sediments. The best predictions were obtained when using different AE and IR values for each

425   experiment (Fig.1). Interestingly, for worms exposed to muddy sediments in Great Lakes (Canada), as in

426   Gillis et al. (2002 and 2004: 0.35% OC, 94.4% silt-clay) and Krantzberg (1994), prediction of Cd tissue

427   residues was improved when the maximum values of AE and IR reported in the literature were used in the

428   model, suggesting that the worms may belong to the same source population. This implies that the

429   application of the biodynamic model requires careful and critical selection of physiological parameter

430   values in studies where these figures have not been directly measured. Laboratory measurement of the

431   elimination rate constants for different oligochaete groups may therefore be of interest for improving

432   predictions in relation to field data.

433

434   Despite differences in the Cd exposure concentration range utilized in Cd-spiked experiments, similar

435   maximum values for Cd bioaccumulation have been reported, namely 4553.3 µg Cd g^{-1} dw (Gillis et al.

436   2002) and 6789.3 μg Cd g^{-1} dw (Méndez-Fernández et al. 2013). These values related to an almost complete
437   inhibition of reproduction in the former study, and to a complete lack of reproduction plus generalized
438   autotomy in the latter. Interlaboratory differences may be due to both population genetics and culture
439   sediment characteristics (e.g. particle size distribution, organic content and natural chemical concentrations,
440   e.g. Reynoldson et al., 1996), and also to differences in detoxification mechanisms (e.g. autotomy and
441   mucous barriers to metal uptake). The use of maximum IR and AE values in the biodynamic model applied
442   to the laboratory data in Méndez-Fernández et al. (2013) improved prediction of the maximum exposure
443   (1488.45 μg Cd g^{-1} dw), but the predicted value was still roughly five times lower than the measured tissue
444   concentration. This possibility has been discarded because a decrease in growth rate was recorded during
445   these experiments, suggesting a stressful situation at the highest concentration. Thus, we interpret that the
446   higher Cd tissue concentration measured could be a consequence of incomplete (or lack of) gut purgation
447   in autotomised worms, or related to difficulties in separating the Cd in tissue from the Cd associated with
448   the mucus barrier. This would result in a higher whole body Cd concentration, but not all of this Cd is
449   metabolically active (Rainbow 2002). The use of lower ingestion rates when there is sediment avoidance,
450   as reported by Méndez-Fernández et al. (2013), did not improve predictions of Cd tissue concentration.
451   This suggests that sediment avoidance prevented Cd bioaccumulation via dietary uptake, but not through
452   the integument, via porewater (which was reported to contain 352.3 μg Cd l^{-1} at the beginning of the
453   experiment). The inclusion of the integumentary uptake route would probably result in better predictions,
454   especially in high pollution scenarios, where there is an excess of metal in the porewater (Méndez-
455   Fernández et al., 2014).
456
457   The Sediment Quality Guidelines for Cd proposed by MacDonald et al. (2000) and the Threshold value for
458   aquatic life, 1.5 μg Cd g^{-1} dw, proposed by the Oregon Department of Environmental Quality (DEQ 2007),
459   were used to discuss the environmental risk associated with the predictions of the biodynamic model
460   (Fig.2). Cadmium concentration in freshwater sediments worldwide usually lies within the range of 0.1-
461   3000 μg Cd g^{-1} dw (Moore and Ramamoorthy 1984), although background concentrations in European
462   stream sediments are normally less than 43.1 μg Cd g^{-1} dw (median value of 0.48 μg Cd g^{-1} dw, n= 848;
463   Salminen et al. 2005). In the present review, sediment concentrations ranged from 0.03 to 508 μg Cd g^{-1}
464   dw and exceeded the Cd threshold levels proposed by MacDonald et al. (2000) at 61% of the sites reviewed.
465   These locations largely corresponded to sites where tissue concentration exceeded the threshold value of
466   1.5 μg Cd g^{-1} dw for aquatic life (Fig.2). Cadmium sorption and precipitation/dissolution processes are
467   governed by a complex set of environmental variables, such as temperature, oxygen, pH, sediment particle
468   size, and other sediment characteristics (Delmott et al. 2007). These factors could be responsible for the
469   data dispersion in measured Cd tissue residues related to Cd sediment concentration. On the other hand,
470   differences in Cd bioaccumulation can also be due to physiological or genetic variation, as reported for
471   Canadian and Spanish populations of *T. tubifex* in an intercalibration exercise (Reynoldson et al. 1996) and
472   in other studies (Maestre et al. 2009).
473
474   Dispersion of the data in the tissue predicted *vs*. measured data representation (Fig. 3), which occurs mainly
475   outside the range 0.1-100 μg Cd g^{-1} dw, could be explained by the variation in analytical techniques used

476 for tissue measurements; by differences in Cd bioavailability (as explained above); or by the presence of
477 metal mixtures. For instance, the presence of Zn reduces Cd uptake in aquatic organisms due to antagonistic
478 effects (Back 1990; Norwood et al. 2007; Steen Redeker et al. 2004). Conversely, some metals may enhance
479 the bioaccumulation of others (Borgmann et al. 2008; De Jonge et al. 2013). Most data overpredicted by
480 the biodynamic model were associated with tissue concentrations below 0.1 µg Cd g^{-1} dw, obtained in
481 bioassays using sediments from areas of the Nalón River Basin (Spain), where the lithology is rich in
482 different metals (mainly As and Hg, but not Cd) (Méndez-Fernández et al. 2015). Nevertheless, predictions
483 were below the threshold value of 1.5 µg Cd g^{-1} dw in all these cases, and do not imply a change in the risk
484 assessment due to Cd bioaccumulation. However, future development of European, national or regional
485 thresholds for metals in both sediment and the tissues of target macroinvertebrate species could alter the
486 risk assessment.

488 At the other extreme (Fig. 3), high Cd tissue concentrations in field-collected oligochaetes usually resulted
489 in underpredicted bioaccumulation values. In the case of field data from Krantzberg (1994), the existence
490 of summer anoxia in the sediments when the oligochaete worms (Canadian population) were sampled may
491 have caused the deviation. Studies on ingestion rates and assimilation efficiencies under anoxia are
492 contradictory. Volpers and Neumann (2005) suggested that worms stop feeding below 0.3 mg O$_2$ l^{-1},
493 however some tubificid species can grow and feed actively despite severe hypoxia (down to 0.005 mg O$_2$
494 l^{-1}) and sulfide atmospheres (Fend et al. 2016). Worm behavior in the field under temporal hypoxia or
495 anoxia in the water column is unknown, which makes model predictions difficult. For instance, Fisher and
496 Beeton (1975) demonstrated that oligochaetes burrowed deeper into the sediment during hypoxic conditions
497 and returned to the surface when conditions improved.

499 Prediction of field bioaccumulation is further complicated by the existence of benthic communities where
500 there are mixed, interacting species. The distribution and abundance of aquatic oligochaete species is
501 determined by the quantity and quality of food available (Moore 1979), and mixed populations of
502 oligochaetes can discriminate between different species of bacteria (Coler et al. 1968). Therefore, the
503 quality of organic matter and the associated microflora in the sediment appears to be more important than
504 the quantity of organic matter, in terms of digestive processes and metal bioaccumulation. Food quality has
505 been shown to have a great effect on metal assimilation in marine bivalves (Wang and Fisher 1996), and
506 the microbial communities in the alimentary tract of deposit feeders are relevant for digestive processes,
507 since intracellular digestion by microbes is an excellent mechanism for achieving differential retention of
508 food components (Lopez and Levinton 1987). Mutualistic interactions between closely related oligochaete
509 species, related to fecal microflora, have been demonstrated in the field (Brinkhurst 1974; Milbrink 1993),
510 and these could also have an effect on assimilation efficiencies.

5 Conclusions

514 The biodynamic model accurately predicts Cd tissue concentrations in oligochaete worms over a range of
515 measured concentrations, from 0.1 to 100 µg Cd g^{-1} dw, but different ingestion rate and assimilation

efficiency values need to be applied under different exposure conditions to improve predictions. The determination of more reliable physiological parameters (including uptake and elimination rates) for use in biodynamic models requires further experimentation, in order to assess the influence of temperature, dissolved oxygen, and probably sediment particle-size distribution. The presence of metal mixtures and other contaminants in field sediments complicates predictions, due to changes in worm behavior (e.g. sediment avoidance) when sediments are toxic and/or to antagonistic or synergic effects between metals. In the current global climate change, an increase in water temperature will play a direct role in the physiology of aquatic organisms, and in the interaction of metals with metabolic processes (Sokolova and Lannig 2008). Consequently, predictions of metal bioaccumulation are likely to be affected. The biodynamic model could be of great help in facilitating prospective risk assessments in these changing scenarios.

6 Summary

This study reviews certain physiological digestive parameters in the literature that could be used to predict tissue residues in aquatic oligochaetes using the biodynamic model. Predictions were evaluated with independently measured Cd bioaccumulation data in sediment bioassays and field oligochaetes. The parameter review focused on three species commonly used in ecotoxicity testing and bioaccumulation studies: *Tubifex tubifex (Tt), Limnodrilus hoffmeisteri (Lh)* and *Lumbriculus variegatus (Lv)*. Median Ingestion rates (g g^{-1} d^{-1}, dw) at unpolluted conditions were 7.8 (*Tt*), 24.5 (*Lh*) and 11.5 (*Lv*), while results were lower (1.7-2.4) at polluted conditions. Assimilation efficiencies ranged from 3.4 - 19.6% (*Tt*), 2.7 – 16.1% (*Lh*), and 10.9 - 25.6% (*Lv*). The biodynamic model accurately predicted Cd tissue concentration in *T. tubifex* exposed to spiked sediments in laboratory bioassays. Comparisons of predicted *vs*. measured Cd tissue concentration in bioassays or field aquatic oligochaetes suggest that the biodynamic model can predict Cd tissue concentration within a factor of five in 81.3% of cases, across a range of measured tissue concentrations from 0.1 to 100 µg Cd g^{-1} dw. Predictions can be refined by using physiological parameter values that have been measured under varying environmental conditions (e.g. temperature, dissolved oxygen). The model can underestimate tissue concentration by up to one order of magnitude when worms are exposed to highly contaminated sediments. Contrarily, predictions overestimate tissue concentration by up to two orders of magnitude when the measured Cd < 0.1 µg g^{-1} dw, although in most cases these predictions do not fail bioaccumulation-based risk assessments, using a tissue threshold value of 1.5 µg Cd g^{-1} dw.

555   this work was possible thanks to all the "oligochaetologists" who have advanced the understanding of
556   oligochaete biology.

557

558   **Conflict of interest**
559   The authors declare that they have no conflict of interest.

560

561

562   **References**

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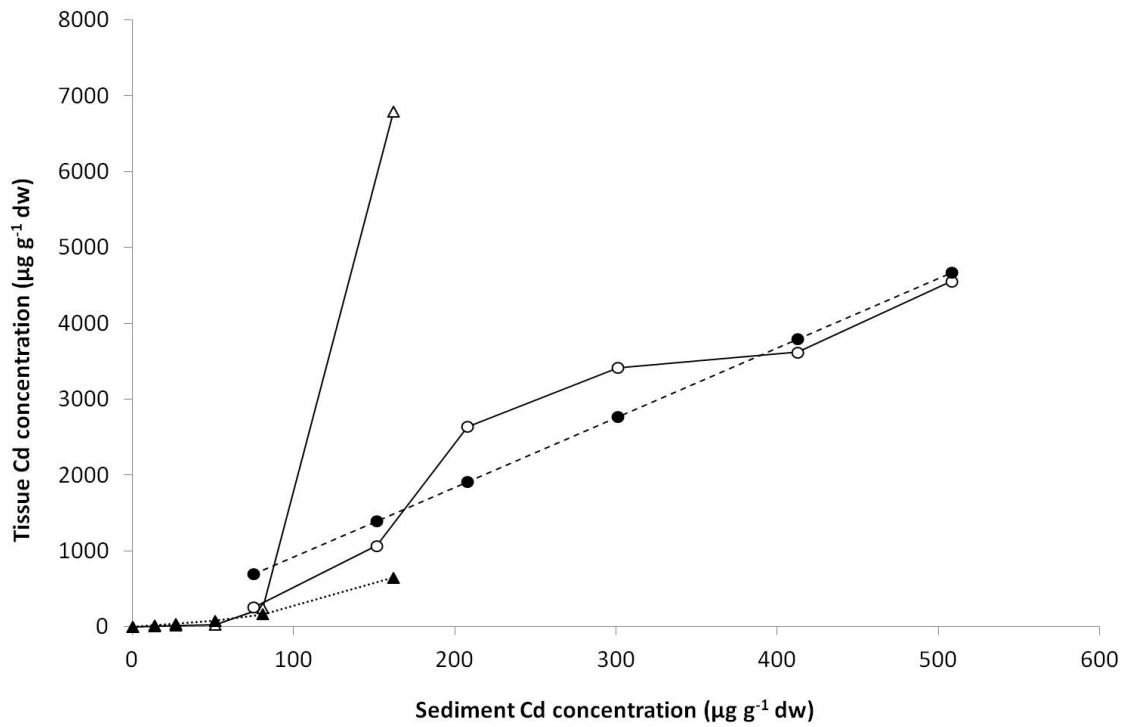
Fig.1 Tissue Cd concentration measured in *T. tubifex* 28-day sediment exposure and best predicted values from the model plotted against Cd sediment concentration. Symbols: Méndez-Fernández et al. (2013), measured (△) and predicted (▲); From Gillis et al. (2002), measured (○) and predicted (●)
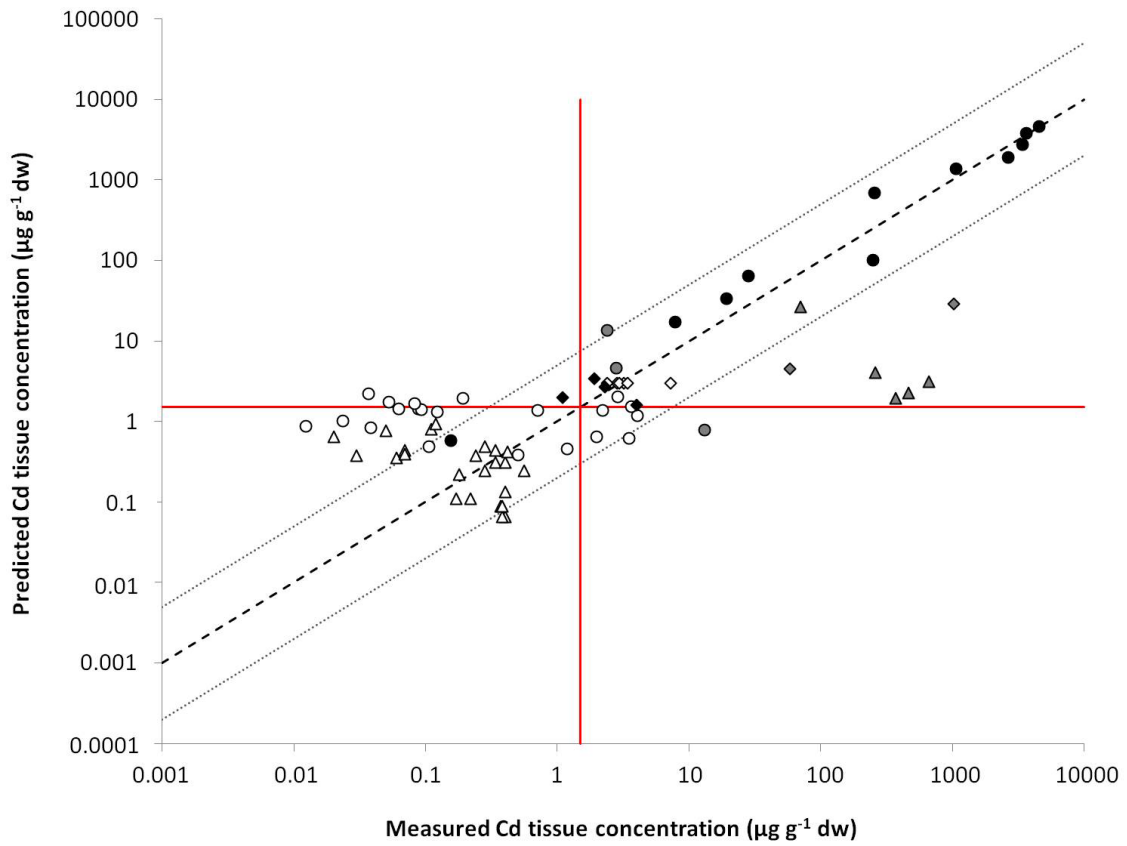
$$y = 1.13x + 0.01$$
$$R^2 = 0.67$$

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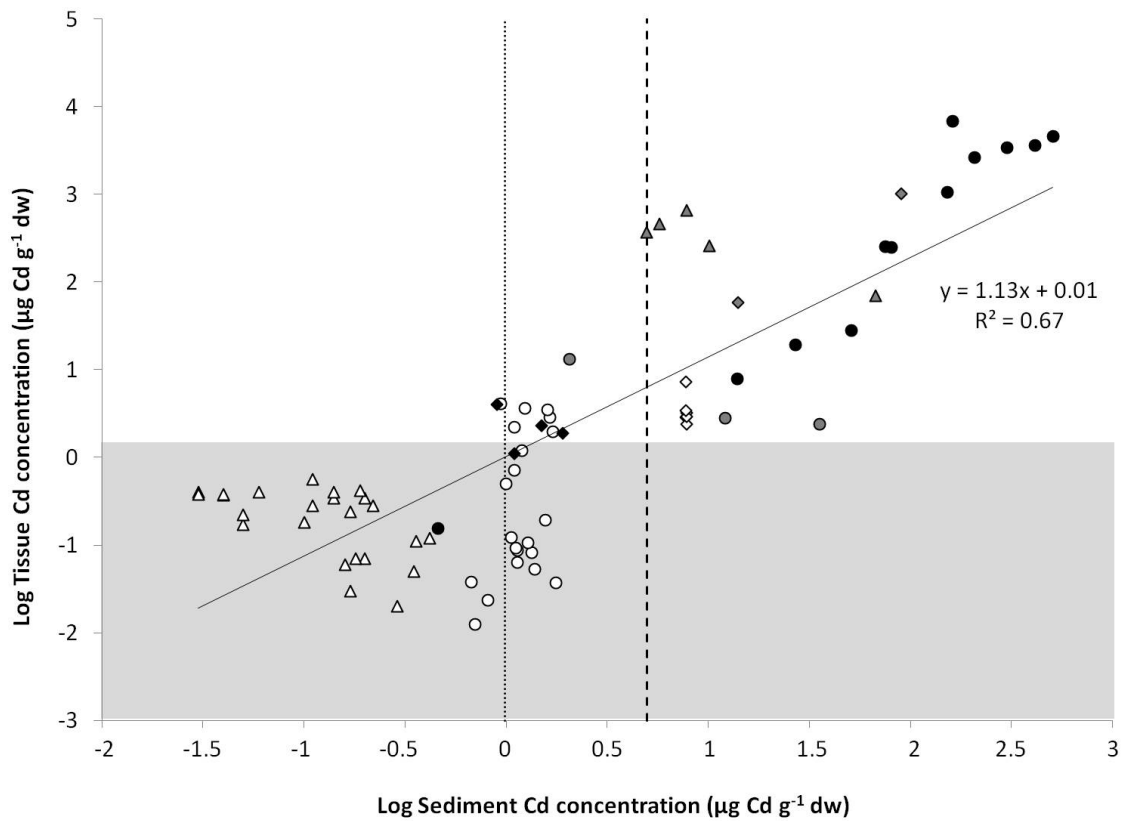
Fig.2 Measured Cd concentration in freshwater oligochaetes after exposure to field-collected sediments or collected directly from the field. The solid line represents the linear regression between sediment concentration and bioaccumulated Cd. The Sediment Quality Guidelines for Cd proposed by MacDonald et al. (2000) are indicated as the Threshold Effect Concentration value (0.99 µg Cd g[-1] dw, dotted line) and Probable Effect Concentration value (4.98 µg Cd g[-1] dw, dashed line); the grey area groups sites where tissue Cd concentration is below the Threshold value for aquatic life proposed by the Oregon Department of Environmental Quality (2007). Symbols: *T. tubifex*: Spiked-sediments from this review (●), Méndez-Fernández et al. 2014 (○), Méndez-Fernández et al. 2015 (◐); Tubifex sp.: Singh et al. 2007 (◇); Tubificids: Hernández and Egea 1987 (◆), Say and Giani 1981 (◆); Oligochaetes: Protano et al. 2014 (△), Krantzberg 1994 (▲)

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837　**Fig.3** Relation between predicted and measured Cd bioaccumulation in freshwater oligochaetes exposed to

838　field-collected sediments in bioassays or collected directly from the field. The dashed black line represents

839　a perfect model fit of 1:1 for predicted *vs.* measured Cd accumulation; the dotted grey lines represent error

840　within a factor of five; and the solid red lines indicate the Threshold value for aquatic life proposed by the

841　Oregon Department of Environmental Quality (2007), 1.5 μg Cd g^{-1} dw. Symbols are as in Fig.2

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