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Cadmium Accumulation and Kinetics in *Solea senegalensis* Tissues under Dietary and Water Exposure and the Link to Human Health

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Abstract: Bioaccumulation of cadmium was assessed in different tissues of the benthic fish *Solea senegalensis*. Juvenile Senegalese soles were simultaneously exposed to cadmium-contaminated diet (*Hediste diversicolor*) and water during 14 days and allowed to depurate for another 14 days. Cadmium content was measured in muscle, gills, liver and intestine, with recorded values increasing in these tissues in this same order. Muscle showed a considerably lower cadmium accumulation after 14 days of uptake. Cadmium kinetics in juvenile Senegalese soles revealed that the highest uptake flux of this metal occurred in the intestine. Cadmium depuration from the liver was not detected, which suggests the existence of a storage compartment for this metal in *Solea senegalensis* during uptake and depuration. Comparisons between maximum acceptable values for cadmium in the muscle, the Target Hazard Quotient and the Estimated Weekly Intake, indicated that acceptable limits were not exceeded, and the muscle of juvenile Senegalese soles could be considered safe for human consumption.

Keywords: benthic fish; uptake flux; *Hediste diversicolor*; metal; toxicokinetics; environmental contaminant

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1. Introduction

Estuarine ecosystems are dynamic environments located on the transition zone between marine and freshwater habitats and they are widely considered of high ecological and economic value [1,2]. A considerable number of marine species have been used in several studies as biological indicators of habitat quality [2–4]. Some of those species are of high socio-economic value and commonly use estuarine ecosystems as nursery grounds [4,5]. Such interface position makes these ecosystems highly susceptible to drastic fluctuations of environmental conditions, either due to natural processes or anthropogenic activities [1]. The accumulation of metals in estuaries has long been considered as a significant threat to their biodiversity [6]. Even though metals occur naturally in the environment, the increase of anthropogenic activities close to coastal areas has enhanced the input of metals to estuarine and marine ecosystems [7]. While certain metals, such as copper and zinc, are essential to biological processes, others like cadmium are considered non-essential and therefore, even when present at low concentrations, can be a potential threat to aquatic organisms [8,9].

The toxic effects and patterns of cadmium accumulation have already been assessed at low trophic levels using several phytoplanktonic [10–13] and zooplanktonic organisms [14–18]. Nonetheless, constraints (for example, ethical) often discourage the use of higher trophic levels, such as fish, in this type of studies, which limits our understanding on the negative impacts caused by contaminants. Bioaccumulation is commonly used as a general term to describe the level of concentration of a given chemical in an organism in relation to

external exposure concentration (for example, dietary, dermal) [19]. When the lower levels of an aquatic trophic web are exposed to metals, such as cadmium, bioaccumulation is likely to occur [20–22]. Depending on metal-organism interactions (for example, ways of uptake, storage, detoxification and elimination), metals can be transferred bottom-up along trophic webs to higher trophic levels, such as fish, and eventually reach humans [23]. Organisms accumulate biologically available forms of metals, thus enabling the continuous monitoring of pollutants in the environment [24]. Accordingly, Munger et al. [25] have highlighted free metal ion activity to be a more accurate way to predict bioaccumulation in target organisms.

In addition to the limited number of studies assessing cadmium accumulation in marine vertebrates, available works generally only consider a single route of exposure to this metal, either diet or water [7,26–29]. However, considering that in natural environments marine organisms are often simultaneously exposed to multiple contaminated compartments, the assessment of multiple routes of exposure is an important step towards a more realistic understanding of cadmium accumulation patterns, not only in the organism itself, but also within and between different levels of marine/estuarine trophic webs [30,31]. Single-route approaches may not only lead to underestimations of total metal accumulation, but also foster a biased perception of metal distribution in internal tissues, both being major obstacles to an accurate understanding of the effects upon ecosystems and human health.

Fish bioaccumulation experiments can be particularly important for benthic species, living in close contact with metal-contaminated sediments and feeding upon sediment-dwelling macroinvertebrates highly prone to the dermal uptake of metals [28]. Furthermore, as some of these benthic fish are often relevant components of human diets, they can easily be an important pathway for metal exposure in humans. For predictive purposes, the use of integrated approaches coupling fish bioaccumulation and human consumption indices (for example, Target Hazard Quotient (THQ)) can allow an improved insight into both environmental and human health [32,33].

The present study aimed to assess and understand: (i) the bioaccumulation patterns of cadmium in juvenile Senegalese sole, *Solea senegalensis*, a species present in the coastal waters of the Mediterranean and eastern Atlantic [34], which is widely used for human consumption, targeted by commercial fisheries and cultured in earthen ponds and intensive fish farms using recirculated aquaculture systems (RAS) [35,36]; and (ii) the possible risks and implications to human health of consuming muscle of Senegalese sole exposed to cadmium. To achieve the first objective, we simultaneously exposed juveniles of Senegalese soles to waterborne and dietary cadmium (using as prey an estuarine benthic organism, the ragworm, *Hediste diversicolor*) for an uptake period of 14 days, followed by another 14 days of depuration. Fish were then sampled at different time points during uptake and depuration for cadmium content measurements in different tissues (muscle, gills, liver and intestine). Concerning the second objective, a non-carcinogenic Target Hazard Quotient (THQ) established by the US Environmental Protection Agency [37] was used, as well as the Estimated Weekly Intake (EWI) to evaluate the potential risks of cadmium to human health via consumption of Senegalese sole.

2. Materials and Methods

2.1. Sole Stock

A total of 32 juvenile *Solea senegalensis* (with an average (±STDV) wet weight of 17.77 ± 2.04 g and total length of 11.88 ± 0.68 cm) were provided by Safistela—a commercial Senegalese sole hatchery from group Sea8 located in Póvoa de Varzim, Portugal. Juvenile Senegalese soles were transferred under constant temperature and in the dark, followed by an acclimation period to laboratory conditions. Senegalese soles were maintained during acclimation (14 days) and experimentation (28 days) in a recirculated modular system (RMS), as described below and represented in Figure 1.

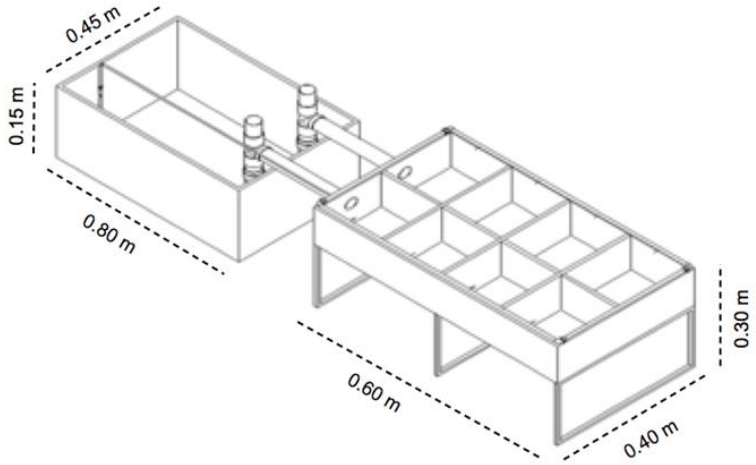


Figure 1. Recirculated Modular System (RMS). Each module is divided into 8 compartments. Each compartment is supplied in parallel with filtered artificial seawater (ASW) through one individual inlet pipe, derived from the main inlet pipe manifold system.

2.2. Polychaete Stock

Ragworms *Hediste diversicolor* were sampled by hand during low tide from a reference non-contaminated site area at Praia da Barra, Aveiro, Portugal (40°38′13.3″N 8°44′29.4″W) [38]. Organisms were kept in artificial seawater (ASW), previously prepared by mixing Tropic Marin Pro Reef Salt (Tropic Marin® Pro Reef, Wartenberg, Germany) with water purified by reverse osmosis (Aqua-win RO-6080, Kaohsiung, Taiwan) at a salinity 35 and a temperature of 18 °C. During acclimation (4 days), *H. diversicolor* were kept in clean ASW and fed *ad libitum* with TetraMin® fish flakes. This procedure aimed at purging their digestive tract from natural dietary items before providing these polychaetes as diet to Senegalese soles [39]. After this period, polychaetes were contaminated with cadmium for the uptake phase or supplied directly as non-contaminated diet during the depuration period.

2.3. Test Chemical

Anhydrous cadmium chloride (CAS No. 10108-64-2, Sigma-Aldrich, Darmstadt, Germany) was used to prepare a Cd^{2+} stock solution (1 g Cd L^{-1}) using a Millipore® Academic Milli-Q system. The tested concentration of 6.88 µg of Cd L^{-1} at salinity 35 was achieved through dilution without changing the final water salinity in previously prepared ASW. This non-lethal concentration was chosen as an environmentally relevant concentration [29,40,41] and used for the waterborne exposure of both Senegalese soles and *H. diversicolor* (used as contaminated diet).

2.4. Basic Set-Up of the Recirculated Modular System (RMS)

Recirculated modular systems were assembled using materials and equipment readily available in local or online stores, thus facilitating its replication across other laboratories (see Figure 1). A total of 4 RMS were used and each system module was composed of one high-density polyethylene food-grade (HDPE) tank (0.80 m long, 0.45 m wide, and 0.15 m high) with a maximum functional volume of approximately 36 L, connected to an HDPE filtration tank (0.60 m long, 0.40 m wide, and 0.30 m high), operating with the same water volume capacity (36 L). The tank was divided into 8 compartments (0.20 m long, and 0.225 m wide) built with perforated plates of HDPE. The RMS operated with ASW, with filtered water in the filtration tank being pumped through a polyvinyl chloride (PVC) inlet pipe system using a submerged water pump (Eheim Compact 1000, Deizisau, Germany) with a regulated total flow of approximately 800 L h^{-1}. Each compartment, stocked with one *S. senegalensis*, was supplied in parallel with filtered ASW through one individual inlet pipe, derived from the main inlet pipe manifold system. Mechanical filtration was

guaranteed by a FSI–XOI 50 µm filter bag (Tropical Marine Center, Hertfordshire, UK) connected to the outlet pipes, from the main tank to the filtration tank. Biological filtration was ensured by submerged bioballs (approximately 10 L). Experiments were performed in a temperature-controlled room. No sediment was provided to avoid the potential loss of metals through adsorption. Temperature was maintained at 20 ± 1 °C and salinity at 35 ± 1. Water chemistry remained within optimal reference levels for *S. senegalensis* aquaculture during the experiment (NH^3/NH^{4+} < 1 mg L^{-1}, NO^{2-} = 0 mg L^{-1}, NO^{3-} < 10 mg L^{-1}, $PO_4{}^{3-}$ = 0 mg L^{-1}, Ca^{2+} = 420–480 mg L^{-1}, pH = 7.9, and D.O. > 6.6 mg L^{-1} (>80% saturation)).

2.5. Experimental Design—Dietary and Water Exposure

An acclimation period of 14 days was used prior to testing for Cd bioaccumulation in Senegalese soles. After acclimation, a two-phase experiment was conducted, consisting of an uptake phase where 32 *S. senegalensis* fish were exposed to cadmium-contaminated ASW and fed with cadmium-contaminated polychaetes, followed by a depuration phase where fish were transferred to clean ASW and provided with non-contaminated polychaetes. The uptake and depuration phases each lasted a total of 14 days (for a total experimental period of 28 days). Juvenile Senegalese soles were fed daily with 1% of their wet body weight, in order to maintain a constant body lipid content [42]. Polychaetes provided during the uptake phase were previously exposed for 48 h to the same cadmium concentration as the Senegalese soles used in the present experiment. Non-contaminated polychaetes were kept in clean ASW. The internal cadmium concentration measured in *H. diversicolor* used as the contaminated diet to juvenile soles during the uptake phase was 0.2 ± 0.02 µg g^{-1} of organism, while cadmium in non-contaminated *H. diversicolor* being provided as a clean diet during the depuration phase was below the detection limit and therefore considered as non-significant (0.008 ± 0.004 µg g^{-1}). The bottom of the RMS was siphoned daily throughout the experiment to remove any uneaten food and fecal pellets. The required volume of new ASW contaminated with cadmium (uptake phase) or not contaminated (depuration phase) was added to compensate for losses (~7 L) during routine daily cleaning procedures, as well as ensuring a stable Cd concentration in the exposure tanks. The uptake phase was comprised of six sampling times (at days 0, 1, 3, 7, 10, and 14) whereas the depuration phase included four sampling times (at days 17, 21, 24, and 28). At each sampling point, three replicates were randomly sampled, each one with one juvenile Senegalese sole. All fish were anesthetized by placing them in ice and euthanized by decapitation prior to dissection. Two major tissue fractions were sampled: edible (muscle) and non-edible (intestine, gills and liver). All sampled tissues were rinsed with ultrapure water, flash frozen with liquid nitrogen and stored at −80 °C until toxicokinetics analysis.

2.6. Chemical Analysis

Chemical analysis of the water was performed using inductively coupled plasma-mass spectrometry (ICP–MS) for cadmium. Samples from the stock solution (n = 3) and from the concentration tested (n = 3) were acidified, using HNO_3 (67–70%, J.T. Baker Ultrex II, for metal trace analysis) until pH < 3, after spiking to assess contamination accuracy.

All tissues were rinsed with ultrapure water to remove excess of medium, freeze-dried for 48 h to a constant weight, weighed and then digested using a mixture of acids, HNO_3 and $HClO_4$ (v/v, J.T. Baker Ultrex II Ultra Pure), at a ratio of 7:1 using 4 heating cycles [43]. The residues were taken up with 1 mL of 0.1 M HNO_3 (J.T. Baker Ultrex II Ultra Pure) and cadmium content was measured using Graphite Furnace Atomic Absorption Spectrophotometry (Perkin-Elmer PinAAcle 900Z). Values presented here are calculated and corrected per dry weight of the organisms. For every digestion cycle, three replicates of blanks and three replicates of certified reference material (CRM) (DOLT-5, Dogfish liver CRM for trace metals and other constituents) were used to estimate the accuracy of the method (cadmium concentration, 14.5 ± 0.6 mg kg^{-1}). The detection limit was 0.186 µg L^{-1} (n = 20). Recovery of cadmium from the certified reference material was 108%.

2.7. Toxicokinetic Models

The uptake and depuration kinetics of cadmium in the tissues of Senegalese soles exposed to both contaminated water and diet were described using a first-order one-compartment model. The model assumed that the background concentration in each of the Senegalese soles tissues is a fixed value C_o at time zero and does not take part in depuration. Equation (1) represents the uptake phase, while Equation (2) represents the depuration phase.

For the uptake phase the model used reads:

$$Q(t) = C_o + \frac{u_f}{k_2} \times (1 - e^{(-k_2 \times t)}) \quad (1)$$

For the depuration phase the model used reads:

$$Q(t) = C_o + \frac{u_f}{k_2} \times (e^{(-k_2 \times (t - t_0))} - e^{(-k_2 \times t)}) \quad (2)$$

where,

Q(t) is the Cd concentration in the tissue in μg g^{-1} of dried weight of each tissue at sampling time t,

C_o is the initial Cd (background) concentration in the Senegalese sole's tissue in μg g^{-1} of dried weight of each tissue at time 0,

u_f is the total uptake flux (water and diet uptake flux) into the Senegalese sole's tissue in μg of Cd^{2+} g^{-1} day^{-1} [44],

k_2 is the depuration rate parameter per day,

t_0 is the time at which organisms were transferred to freshly prepared uncontaminated water and diet in days, and

t is the sampling time in days.

Both equations used for describing cadmium uptake and depuration patterns were fitted simultaneously, as recommended by Guideline 305 [42].

2.8. Statistical Analysis

The kinetic parameters used by the model were calculated using non-linear regression analysis by simultaneously fitting the uptake and depuration equations to data using the least Sum of Squares (SS) coupled with the Levenberg–Marquardt algorithm in the SPSS Statistical Package version 20 (IBM Corp. Armonk, NY, USA).

The time (expressed in days) that each tissue required to eliminate half the amount of cadmium (DT_{50}), was calculated as:

$$DT_{50} = \frac{\ln(2)}{k_2} \quad (3)$$

The concentration of Cd at steady state (C_{org}^{ss}) in μg g^{-1} in the organism was derived from the model Equation (1) by substituting $t = \infty$.

2.9. Health Risk Assessment

The estimation of THQ for potential non-carcinogenic effects of cadmium in human health followed the methodology provided by US EPA Region III Risk-Based Concentration Table [37], with THQ being calculated as follows:

$$THQ = \frac{EF \times ED \times FIR \times C_m}{RfD \times BW \times AT} \quad (4)$$

where,

EF is the exposure frequency in days year^{-1} (365 days year^{-1}),

ED is the exposure duration in years,

FIR is the food ingestion rate in kg day^{-1} person^{-1} (0.0334 for the world [45] and 0.1559 for Portugal [46]),

C_m is the metal concentration in fish at steady state in mg kg^{-1},

RfD is the reference dose in mg kg^{-1} day^{-1} (0.001 for cadmium [37]),

BW is the body weight in kg, and

AT is the average exposure time for Cd (365 days year^{-1} × ED).

In the current study we used the same exposure duration values and body weight as Vieira et al. [47] according to US EPA [48] for nine different age groups (Table S1).

The Estimated Weekly Intake (EWI) of cadmium was calculated using the following equation:

$$EWI = \frac{Cm \times WIR}{BW} \quad (5)$$

where,

C_m is the metal concentration in fish at steady state in mg kg^{-1},

WIR is the weekly food ingestion rate in kg week^{-1} person^{-1} (0.2338 for the world [45] and 1.0913 for Portugal [46]), and

BW is the body weight in kg.

3. Results

3.1. Chemical Analysis

Results from the chemical analysis for both the stock and test solution showed a variation of 2 and 6%, respectively from the nominal ones, thus confirming the accuracy of the spiking technique. The Visual MINTEQ equilibrium model estimated the percentage of free ionic cadmium to be 4.5%, free ion concentration at 0.31 µg L^{-1} and free ionic activity at 0.09 µg L^{-1}.

No fish mortality was recorded during the 14 days of acclimation, neither during the 28 days of the bioassay.

3.2. Toxicokinetics of Cadmium in Senegalese Soles

After 14 days of cadmium uptake none of the tissues' internal concentration reached a steady state phase and Senegalese soles exposed to cadmium through water and diet presented differences in the accumulation patterns of each tissue. An increase in cadmium internal concentration was recorded for all tissues over time in the following order: muscle (0.003 µg g^{-1}, not possible to calculate STDV/SE, n = 1, Figure 2) < gills (0.34 µg g^{-1}, STDV = 0.06, SE = 0.03, Figure 2) < liver (0.76 µg g^{-1}, STDV = 0.17, SE = 0.1, Figure 2) < intestine (2.1 µg g^{-1}, STDV = 2.2, SE = 2.1, Figure 2). The kinetic parameters of all tissues for cadmium ions activity calculated using a first-order one-compartment model are summarized in Table 1. Fish intestine showed the highest cadmium uptake flux, followed by the liver, the gills and lastly the muscle. When comparing depuration rates, the fastest one was observed in the intestine and muscle followed by the gills and lastly the liver, where no depuration was recorded. The toxicokinetics model estimated the steady state concentration for each tissue in the following order: muscle (0.02 µg g-1) < gills (1.60 µg g^{-1}) < intestine (2.86 µg g^{-1}). No steady state concentration was estimated for the liver as the internal concentration recorded kept increasing indefinitely due to the absence of depuration.

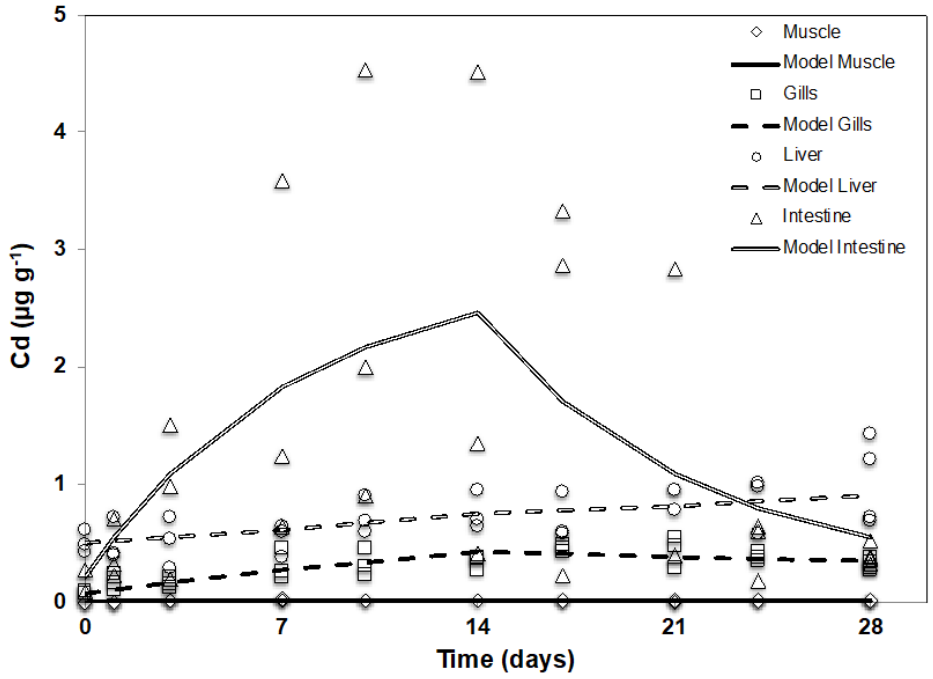


Figure 2. Uptake and elimination kinetics of cadmium in the muscle, gills, liver and intestine of juvenile *Solea senegalensis* exposed to cadmium-contaminated artificial seawater (ASW) and diet (polychaete *Hediste diversicolor*). Symbols represent measured cadmium concentration in the different organs, and lines represent the predicted uptake and depuration using toxicokinetic models.

Table 1. Uptake and depuration kinetics parameters of cadmium in the muscle, gills, liver and intestine of juvenile *Solea senegalensis* exposed to both cadmium-contaminated artificial seawater (ASW) and diet (polychaetes *Hediste diversicolor*). C_o is the initial (background) concentration in the sole's tissues; u_f is the total uptake flux (water and diet uptake flux) into the soles' tissues; k_2 is the depuration rate; DT_{50} is the time each tissue required to eliminate half the amount of cadmium; and C_{org}^{ss} is the concentration of cadmium at steady state (SE in brackets for C_o values; 95% CI in brackets for u_f and k_2 values).

Fish Tissue	C_o (µg Cd g^{-1})	u_f (µg Cd^{2+} g^{-1} day^{-1})	k_2 (day^{-1})	DT_{50} (Days)	C_{org}^{ss} (µg Cd g^{-1})
Muscle	0.00 (-)	0.002 (0.001–0.003)	0.11 (0.03–0.20)	6.1	0.02
Gills	0.08 (0.01)	0.03 (0.02–0.04)	0.02 (0–0.04)	38.5	1.60
Liver	0.50 (0.05)	0.01 (0.002–0.02)	−0.05 (-)	-	-
Intestine	0.21 (0.06)	0.36 (0.13–0.59)	0.14 (0.03–0.24)	5.1	2.86

3.3. Health Risk Assessment

The concentrations used to estimate the THQ values in the present study were lower than the maximum acceptable limit set by the EU Commission Regulation (EC) No 1881/2006 [49], which is set at 0.05 mg kg^{-1} wet weight. The THQ values determined for cadmium were lower than the ones for juvenile *S. senegalensis* in the present study (Table 2). When considering world consumption, EWI values ranged from as low as 0.009 to 0.334, while those for the Portuguese population peaked at 1.559 (Table 2).

Table 2. Risk assessment for non-carcinogenic effects in humans by cadmium measured in the muscle of juvenile *Solea senegalensis* using Estimated Weekly Intakes (EWI) (μg kg^{-1} body weight) and Target Hazard Quotients (THQ) for different age groups. Provisional Tolerable Weekly Intake (PTWI) of cadmium is set at 2.5 μg kg^{-1} body weight by the European Food Safety Agency (EFSA) (2012).

Age Group	EWI (1)	THQ (1)	EWI (2)	THQ (2)
Children 1–3 years	0.050	0.007	0.234	0.033
Children 4–6 years	0.033	0.005	0.156	0.022
Children 7–10 years	0.022	0.003	0.102	0.015
Adolescents 11–14 years	0.014	0.002	0.064	0.009
Adolescents 15–19 years	0.010	0.001	0.049	0.007
Adults 20–24 years	0.010	0.001	0.045	0.006
Adults 25–54 years	0.009	0.001	0.043	0.006
Adults 54–64 years	0.009	0.001	0.043	0.006
Seniors > 65 years	0.010	0.001	0.045	0.006
Age Group	**EWI (3)**	**THQ (3)**	**EWI (4)**	**THQ (4)**
Children 1–3 years	0.334	0.048	1.559	0.223
Children 4–6 years	0.223	0.032	1.039	0.148
Children 7–10 years	0.146	0.021	0.682	0.097
Adolescents 11–14 years	0.092	0.013	0.428	0.061
Adolescents 15–19 years	0.070	0.010	0.326	0.047
Adults 20–24 years	0.065	0.009	0.303	0.043
Adults 25–54 years	0.061	0.009	0.283	0.040
Adults 54–64 years	0.061	0.009	0.283	0.040
Seniors > 65 years	0.065	0.009	0.303	0.043

(1) THQ/EWI values according to world fish consumption using cadmium concentration in muscle after 14 days. (2) THQ/EWI values according to Portugal's fish consumption using cadmium concentration in muscle after 14 days. (3) THQ/EWI values according to world's fish consumption using cadmium concentration in muscle at steady state. (4) THQ/EWI values according to Portugal's fish consumption using cadmium concentration in muscle at steady state.

4. Discussion

4.1. Toxicokinetics

Metal accumulation in marine fish has received increasing attention in recent years [6,7,40,50,51]. However, few studies have considered bioaccumulation load due to simultaneous waterborne and dietary exposure [30,31]. When it comes to marine fish, and to the authors' best knowledge, no study to date ever addressed the effects of a simultaneous exposure to both cadmium-contaminated water and diet. International guidelines, such as OECD Guideline 305 [42], only refer to bioaccumulation of metals in fish either through water or diet, which makes the estimation of bioaccumulation and pathway contribution in case of a two route exposure a challenging task. Coupling bioaccumulation experiments with human health indices of risk can offer a promising solution to enhance predictive ecotoxicology. However, increasing the realism in exposure scenarios is essential for improving accuracy of estimates, which requires accounting for multiple exposure routes.

The exposure of Senegalese soles to cadmium during 14 days was not enough for any of the tissues being studied to reach a steady state concentration, nor did the 14 days of depuration seemed to be enough for cadmium to be fully eliminated. These findings are in agreement with previous studies that showed the increased capacity that marine fish have to bioaccumulate and store cadmium when exposed either via water or diet, as well as their limited ability to eliminate it from their body [6,52–54]. The bioaccumulation patterns and kinetic parameters recorded for cadmium exhibited considerable differences between the different tissues surveyed in the present study. Such variability can be attributed to the fact of cadmium displaying a specific affinity to different tissues [52,55]. Cadmium accumulation in juvenile *S. senegalensis* tissues via a two-way exposure followed a similar

pattern of distribution to other marine/estuarine fish via a one-way exposure (waterborne or dietary) [26,29–31,56], overall demonstrating higher concentrations of the metal to be present in the intestine followed by the liver, the gills and lastly the muscle. The intestine has been considered a relevant target for cadmium, for either waterborne or dietary routes of exposure, presenting therefore a significant burden of accumulated cadmium [55]. Previous studies have shown that apart from the intestine, dietary cadmium tends to be mostly accumulated in the kidney and liver [26,27,55], whereas waterborne cadmium is more prone to be accumulated in the gills [30]. Independently of routes of exposure, cadmium tends to accumulate mainly in the liver and kidney, as well as in the intestine and gills [26].

The edible fraction of Senegalese soles (muscle) showed the slowest uptake rates, as well as the lowest accumulated cadmium concentration, among all sampled tissues. Muscle is one of the tissues, where the least amount of cadmium is usually accumulated [57], most likely due to the comparatively lower blood circulation that occurs in this specific tissue. Vieira et al. [47] reported values of cadmium in the edible fraction of three commercially available fish (*Sardina pilchardus*, *Scober japonicus* and *Trachurus trachurus*) caught in Portuguese waters and purchased from local markets, ranging from 0.0056 to 0.0084 mg kg^{-1}, wet weight. Cao et al. [29] reported cadmium accumulation in muscle tissues to be the lowest when compared to gills, liver and kidney and ranging from 0.25 to 0.66 mg kg^{-1} upon Japanese flounders, *Paralichthys olivaceus*, exposure to waterborne cadmium concentrations ranging from 2 to 8 mg g^{-1} for 28 days. Cadmium accumulation in the muscle did not seem to significantly change with time after exposure of *P. olivaceous* to low dosed cadmium (up to 50 μg L^{-1}). Results suggest that muscle may not be an appropriate bioindicator tissue for environmental cadmium concentrations, as cadmium burden is not always proportional to cadmium contamination in the environment. However, it consists of an edible part of the fish to humans and therefore, its use in food safety and human health risk assessment studies is considered imperative.

Gills play a key role in fish physiology, as they are responsible for ion, acid-base regulation and gas exchange, as well as for the excretion and elimination of nitrogenous waste products [58–61]. It is known that Cd^{2+} tends to bind to the gills at specific sites (biotic ligand) inhibiting Ca^{2+} uptake and metabolism and ultimately disrupting the integrity of the epithelium [59]. Gills are in direct contact with waterborne cadmium and possibly this metal is transferred and redistributed through the circulatory system to the rest of the fish body and vice versa. Slow depuration of cadmium from the gills could indicate such a pattern, as assimilated cadmium, either waterborne or dietary, can enter the body circulation by the intestine through blood and reach the gills, causing damage [26]. Elevated values of cadmium in the branchial epithelium of fish exposed to either waterborne or dietary cadmium due to the induction of metallotheionein-like proteins (MTLPs) synthesis as a detoxification mechanism have been previously reported, and those proteins are usually found stored in the chloride cells of the gill [26,40,58,62].

High cadmium concentration in the liver, compared to the rest of the tissues, can be attributed to the fact that this particular organ significantly contributes to the detoxification and storage of cadmium in fish [26,27,29]. Along with the kidney, liver is known to be related with the production and storage of MTLPs, as well as the existence of gluthathione (GSH) in the liver cytosol, responsible for binding to metals as a detoxification mechanism through metal sequestration [52,56,63,64]. Jebali et al. [63] support the theory that MTLPs are tissue specific and showed that the highest content recorded was in the kidney, followed by the liver and the gills. No depuration was observed in the present study from this tissue. It is likely that during depuration MTLPs and GSH are detoxifying cadmium by binding it and subsequently storing it in the liver, thus rendering it inert [27]. The inability of this particular organ to eliminate cadmium can ultimately promote hepatotoxicity; this is likely to occur due to the depletion of MTs and GSH promoted by the "spill-over" phenomenon [56], as cadmium is considered a hepatotoxicant [64]. The liver has been

previously proposed as a suitable bioindicator of water contamination as it bioaccumulates cadmium and reflects the concentrations found in the surrounding environment [55].

The highest cadmium concentration after waterborne and dietary exposure was found in the intestine, approximately a 3-fold increase compared to the liver and a 6-fold increase when compared to the gills. Such an outcome was somehow expected due to the direct contact/passage of the diet through the intestine, after initial digestion in the stomach, and the high assimilation efficiency of nutrients and metals displayed by this particular organ [65]. The exposure of the Atlantic cod, *Gadus morhua*, to cadmium indicated a 3-fold difference in the concentration measured in the intestinal mucosa when compared to the gills, suggesting that the intestine might be the primary uptake site for several waterborne metals, including cadmium [58]. Marine fish have a higher drinking rate (approx. 0.5% of their body weight per hour) when compared to their freshwater counterparts, due to the hyperosmotic environment they inhabit. This environmental driver may be responsible for the higher proportion of total cadmium that accumulates in the intestine as a result from waterborne exposure [27,29,57]. Kuroshima [66] observed that preferential cadmium accumulation in the killifish *Fundulus heteroclitus* switched from the gills to the intestine with increasing salinities, thus suggesting a higher metal uptake rate through the ingestion of seawater to maintain osmotic balance. However, dietary uptake also plays a significant role in the cadmium load present in the fish intestine. Dang and Wang [40] showed significant differences in cadmium bioaccumulation in the digestive tract of marine grunts, *Terapon jarbua*, after 14 days of exposure to increasing dietary cadmium compared to waterborne contamination. However, the same authors also reported that in long-term exposures (4 weeks) cadmium bioaccumulation in the intestine was mainly due to waterborne cadmium rather than dietary. Such a result could be due to the distinct routes of cadmium uptake and/or depuration by the intestine, as it may depend on the form cadmium is stored in the prey (e.g., TAM-Trophically Available Metals) and/or the digestive and assimilative efficiency of the fish. Kalman et al. [56] suggested that the intestinal walls have the capacity to store cadmium and may later be involved in the elimination and/or redistribution of the metal to the rest of the tissues, which may explain the high depuration rate determined in the intestine. Dang and Wang [40] showed that MTLPs production in the intestine induced by either waterborne or dietary cadmium after 14 days of exposure, was considerably higher when compared to the gills or the liver.

The biological half-life of cadmium in the tissues surveyed in the present study, with the exception of the liver, varied from 6 to 39 days, while other studies have reported estimates ranging from 24 to 200 days [30,31,52]. These differences can be attributed to phylogenetic issues (different species) [43,67], differences in the age and size of monitored specimens (e.g., juvenile vs. adults) [68], routes for cadmium exposure (e.g., waterborne vs. dietary) [43], as well as different abiotic conditions (for example, temperature, pH, salinity, zinc concentration, presence of organic matter, amongst others) [67].

4.2. Health Risk Assessment

The concentration of cadmium measured in the muscle of juvenile *S. senegalensis* after exposure to an environmentally relevant scenario was lower than the maximum acceptable limit set by the EU Commission Regulation (EC) No 1881/2006 [49] at 0.05 mg kg^{-1} wet weight. The analysis of non-carcinogenic risks showed THQ values < 1 for all age groups exposed in the concentrations found in the current study. Such results indicated that an exposed population could be considered safe if consuming fish with the amount of cadmium measured in the current study. Vieira et al. [47] suggested a more intensive selection and compilation of information and data, concerning real ingestion rates. Children's intake rate is often higher comparable to their body weight than that of an adult and exposure frequency for each species consumed and age groups can be refined, so these estimates can be improved.

The European Food Safety Agency (EFSA) [69] has recommended a Provisional Tolerable Weekly Intake (PTWI) of cadmium of 2.5 µg kg^{-1} body weight. All EWIs estimated

here were within the PTWI levels proposed by EFSA [69]. The Portuguese population is estimated to be ingesting weekly higher levels of cadmium compared to the mean world population. Such a result would be expected due to a higher consumption of seafood compared to the mean world value [70]. Values of ca. half the proposed maximum limits were estimated for the Portuguese population in the youngest age groups (children 1–3 years and children 4–6 years) when compared to older ones. Decreasing EWI with increasing age would result from the dilution that the metal undergoes due to increasing body weight, when compared to younger age groups. For both populations to exceed the PTWI of cadmium, the weekly food ingestion rate would have to be higher than 12 kg week^{-1} person^{-1} for the youngest age group, and higher than 64 kg week^{-1} person^{-1} for the oldest age group. As such, the scenarios at which PTWI of cadmium could be exceeded are rather unrealistic to occur.

Comparisons between the maximum acceptable values for cadmium in the muscle, THQ and EWI show that cadmium measured in the muscle of juvenile Senegalese soles in the present study is within acceptable limits and can be considered safe for human consumption.

5. Conclusions

The bioaccumulation profile of cadmium in *S. senegalensis* differs with the tissue being analyzed. Upon cadmium exposure from contaminated diet and water, the highest concentrations for this metal were found in the intestine and the liver, while the muscle showed a very low concentration. The liver showed no cadmium depuration while depuration was observed for other tissues, which was probably due to its detoxifying and storage role in fish. Gills showed a high binding capacity of cadmium also during the depuration rate, suggesting an internalization of cadmium in cells and a probable redistribution through the plasma. Even though Senegalese soles from cadmium-contaminated environments could be safe for human consumption due to low cadmium levels in the muscle, as THQ values reported are below one, they may comprise a potential risk to higher predators, such as bigger fish, birds or mammals. This study has provided indications that bioaccumulation through a two-way exposure scenario is an important parameter to take into consideration when assessing contamination by non-essential metals like cadmium. Further studies using higher exposure concentrations, field populations and assessing subcellular compartmentalization to prey and individual tissues are advised to better understand uptake routes and storage compartments of cadmium.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4441/13/4/522/s1, Table S1: Exposure Duration (ED, in years) and Body Weight (BW, in kg) values according to Vieira et al. (2011) and US EPA (2008) for nine different age groups.

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