American Journal of Environmental Sciences 1 (3): 239-248, 2005
ISSN 15553-345X

A Model for Bioaccumulation of Metals in *Crassostrea virginica* from Apalachicola Bay, Florida

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Abstract: Kinetics of bioaccumulation of the trace metals (Cd and Zn) by *C. virginica* were investigated in this study. A computer program, Oyster Bioaccumulation Model (OBM), was developed to simulate the accumulation of the metals from both dissolved (water column) and particulate (food) phases by *C. virginica*. The model is based on two main attributes: (1) the oyster biological characteristics such as gill morphometry, feeding growth rate, respiration rate and lipid composition and (2) the physicochemical properties of metals, which include aqueous diffusivity and partition coefficients. For some data from Chesapeake bay, simulation results compared well with field results. Predicted Bioaccumulation Factor (BAF) parameters (1.5×10^4 for Cd and 6.6×10^4 for Zn) for Apalachicola Bay show that elemental concentrations in *C. virginica* are up to 4 orders of magnitude greater that that of the water column. Furthermore, the model is shown to be flexible enough to be utilized in other estuaries.

Key words: Bioaccumulation, heavy metals, dissolve, particulate, *Crassostrea virginica*, filter-feeder, gill

INTRODUCTION

Heavy metal bioaccumulation in filter-feeders such as the oyster, *Crassostrea virginica* is the net result of uptake and elimination of the metals in ambient water. As a food source for humans and the aquatic food chain, oysters are known[1-4] to have high enough metal content to: (1) impact the health of the entire ecosystem through trophic transfer and (2) are a potential health risk to humans because of consumption. For these reasons, it was recognized by investigators such as Liao et al.[5] and Lim and Dim[3] that bioaccumulation assessment of aquatic organisms is an important component of the evaluation of the health of the ecosystem.

Over the past decade a considerable amount of interest has been generated regarding the kinetics of metal bioaccumulation in bivalves[3,6-8]. Most previous kinetic models using the oyster *C. virginica* employed data collected under controlled laboratory conditions. However, laboratory experiments cannot duplicate field conditions because of the many complex environmental factors. Additionally, previous bioaccumulation studies did not consider the very important physiological properties of oysters such as oxygen consumption, filtration and growth rates and the fluid mechanics of the filtered water and food processing on the gills of bivalves.

In this study a bioenergetic-based kinetic model was developed not only on the basis of mass balance[7] but also with consideration of physiological properties such as respiration, growth and gill activities that are known to be critical for metal uptake from both dissolved and particulate phases[9,10]. The model presented here is adopted from the Food and Gill Exchange of Toxic Substances (FGETS) model developed by Barber and Suarez[11]. Originally applied for fish, a modified version of the simulation program of FGETS was written in FORTRAN to simulate the soft tissue concentrations of Cd and Zn in oyster. The prospect of applying FGETS to oysters was founded on the recognition by Hamelink et al.[12] that gills of vertebrates and invertebrates share a number of common features such as short diffusion distance between water and circulatory fluid and large surface area to facilitate gas exchange.

Our model considered both biological attributes of the oysters and the physico-chemical properties of the metals that determine diffusive exchange across gill membranes and the digestive tract. Some physiological as well as physical parameters needed for the model formulation were compiled from the literature[5,7,13]. These parameters include metal assimilation efficiency from ingested food, the metal uptake rate constant from the dissolved phase and the metal efflux rate constant. Biological parameters such as growth rate are also incorporated into the kinetic model to calibrate for growth dilution of trace metals in tissue. Additionally, we considered field condition parameters such as actual dissolved metal concentrations in the water column, temperature and the metal concentrations in suspended particles.

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Study area: Apalachicola Bay is a coastal estuary with barrier islands and significant freshwater input. The bay with its sounds acts as a transitory buffer zone between the freshwater of the Apalachicola River and the saline water of the Gulf of Mexico. The Apalachicola River is formed by the confluence of the Chattahoochee and Flint Rivers. Based on patterns of water movements in the bay, six sites were selected along two transects (Fig. 1). The sites were selected to follow the flow of fresh and saline water into the bay. A differential GPS instrument was utilized to accurately locate the sites and facilitate repetitive sampling.

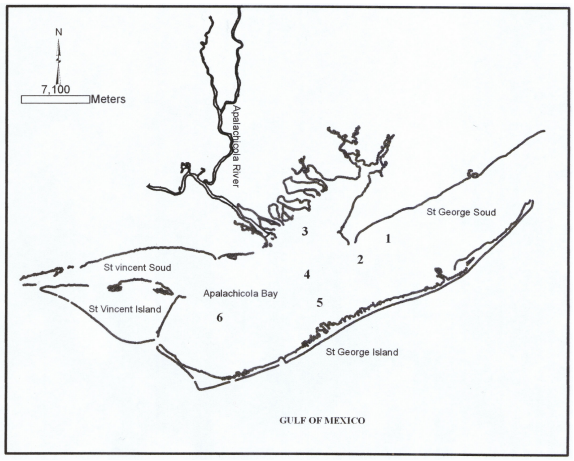

Fig. 1: Map of Apalachicola bay showing the 6 sampling sites. The sites were selected along two transects

MATERIAL AND METHODS

Oysters: *Crassostrea virginica* samples were collected at the six selected sites in the Apalachicola Bay in years 2002-2003 (Fig. 1). Sample collection and preservation techniques were adopted from the method described in the Guidance Document for Metals in Shellfish[14] and the NS&T mussel watch project[15]. Market-size oysters with similar shell length of about 8 to 9 cm were selected and their soft tissue analyzed for metal content. Wet and dry weights were also recorded for the soft tissue of individual oysters.

For the purpose of this study, the gill area of the oysters was determined by careful dissections of the gill of 30 individual animals. The gills were placed in watch glasses containing deionized water at room temperature for about 4 hr and measurements of length and width were conducted using a magnifying glass.

Water and particulate matter analysis: Surface water was collected at a depth of 1 meter using a vertical water-sampling instrument from Aquatic Instruments Inc. The samples were homogenized and vacuum filtered using 0.45 μm pre-washed and pre-weighed Whatman membranes in the laboratory. The membrane filters were acid digested and analyzed for particulate metals while the filtrated water samples were analyzed for dissolved metals. Water chemistry parameters such as temperature and salinity were measured on site. The particulate matter is considered here to be the source of ingested metal by the oyster.

The atomic absorption spectrometry (AAS) technique was utilized for the elemental analysis of the oyster tissue, particulate matter and the filtered water. Samples were first acid digested following certified EPA methods (3020A and 3050B) and the oyster tissue was analyzed by Flame AAS while both the filtered-water and particulate matter were analyzed using Graphite AAS. The quality control of the analysis consisted analyzing NIST standard reference materials 1566b oyster tissue, 3108 for Cd and 3168 for Zn.

MODEL DEVELOPMENT

The development of a model often begins with the construction of a conceptual diagram[16]. With the knowledge that models are simplified version of the real world, in this study, we consider a single compartment organism as suggested by Reinfelder et al.[7] and the food-chain approach[11]. The kinetic model of heavy metal bioaccumulation is based on the simple conceptual model in which the concentration of a metal is primarily controlled by the balance between uptake, elimination and growth (Fig. 2).

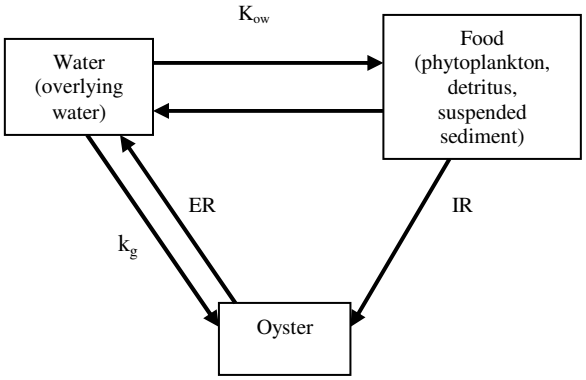

Fig. 2: Conceptual model of pathways of metal uptake and elimination in *Crassostrea virginica*

Model formulation: The model developed in this study is called the Oyster Bioaccumulation Model (OBM) for heavy metals. In fact, the model is a modified version of the Food and Gill Exchange of Toxic Substances (FGETS) model developed by Barber et al.[11] for fish. In this model, *C. virginica* is considered to take up heavy metals from the water column via the gills and through the consumption of food in the digestive tract. With the consideration of mass balance, elimination of metal occurs via egestion of fecal matter and metabolic transformation, which result in net growth after some energy loss via respiration. The OBM is based upon the following assumptions:

* Oyster larvae are considered to have no metal in the tissue at year zero.
* There is a gill transport system. On the gill surface, water circulates through interfilament (Fig. 3) openings while particles are conveyed by ciliary action toward the labial palps[17]. Therefore, it is assumed that dissolved metal and oxygen diffusion into the system occurs through gill membrane only. Thus the gill total area controls the dissolved metal uptake and the oxygen exchange.
* It is assumed that the gill filaments act as parallel ducts (Fig. 3) in which interfilament water has laminar flow (Fig. 4). The same assumption was used by Jorgensen[18,19] who demonstrated that the laminar flow on bivalve gills is due to a very low Reynolds's number.
* It is also assumed that metal exchange across the oyster gill occurs by simple diffusion.

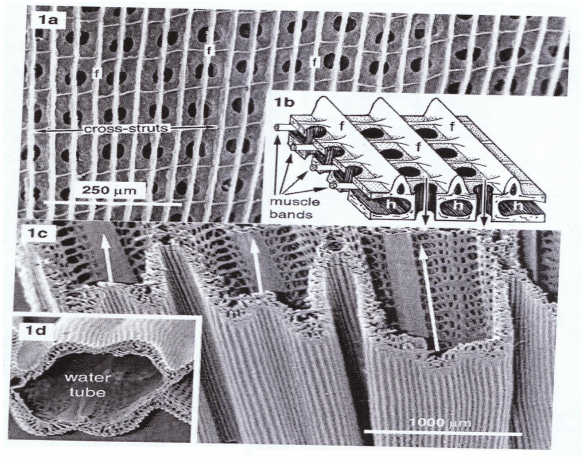

Fig. 3: Eulamellibrach (*C. virginica*) gill showing a valve with parallel filaments (f) (adapted from Medler and Silverman[41]

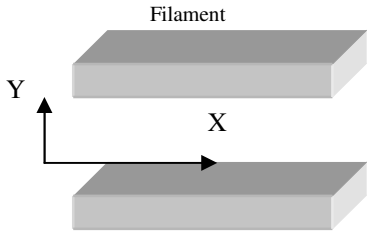

Fig. 4: Schematic representation of interfilament water flow in laminar motion

Based on the model assumptions, bioaccumulation of metals in *C. virginica* can be modeled using the following mass balance equation[11]:

$$\frac{dB_f}{dt} = G + F \quad (1)$$

where B_f (µg) is the time dependent total metal body burden in micrograms, t is time in days, G and F represent the net metal uptake through the gill from the dissolved phase and through the digestive tract from food (particulate matter), respectively. The subscript *f* indicate the metal under consideration. These metal are either zinc or cadmium.

Growth rate is incorporated into the kinetic model to calibrate for growth dilution, which according to Reinfelder et al.[7] is a very important parameter along with the metal efflux rate to model bioenergetics flux in *C. virginica*. The rate of change of oyster body mass is given by Barber and Suarez[11]

$$\frac{dW}{dt} = I_r - E_r - R - S_d - E_x \quad (2)$$

where W (g) denotes the time dependent mass of *C. virginica* (growth), *t* is the time in days, I_r is the feeding rate, E_r is the egestion rate, *R* is the routine respiration rate, E_x is excretion and S_d is the specific dynamical action or respiration other than routine respiration.

The time dependent total body concentration was simulated as C_f as shown below

$$C_f = \frac{B_f}{W} \quad (3)$$

The model is therefore composed of terms involving oyster mass, bioenergetics and physiological metal-specific parameters and physico-chemical characteristics of the metals. The bioenergetic behavior data required to estimate the parameters were generally available for fish[11,20,21]. Because no general equations for shellfish mollusks are available, it is assumed that equations used to estimate bioenergetics for fish were also suitable for that of shellfish. Previous work by Liao et al.[5] used a similar assumption to parametize some bioenergetic terms for Zn bioaccumulation in the mollusk *Haliotis diversicolor*.

Modeling uptake from water: In equation (1), G represents the net metal uptake from the dissolved phase in the water. With the interfilament water having laminar flow and at steady state, it is assumed that metal exchange across the oyster gill occurs by simple diffusion (Fig. 4). Therefore, Fick's first law of diffusion is applied to model this process, which can be formulated as[11]

$$G = S_g k_g (C_w - \frac{C_a}{K_{aw}}) \quad (4)$$

where S_g is the oyster's total gill area (cm^2), k_g is the metal mass conductance (cm s^{-1}) across the gill, K_{aw} is the partition coefficient of the contaminant between the blood of the oyster and water. C_w and C_a are metal concentrations (µg mL^{-1}) in the interfilament water and the oyster's blood, respectively.

In fluid mechanics, the chemical mass conductance, k_g, can be defined as a function of the chemical diffusion rate and the distance between filaments. With the assumptions that adjacent gill filaments are considered as parallel plates (Fig. 4) and the exhalent gill water is at steady-state with laminar

flow, metal transfer to filaments can be modeled using the differential equation below

$$\frac{d^2 f(x)}{dx^2} + \lambda^2 (-\frac{1}{2\mu D}\frac{dp}{dy})(h^2 - x^2) f(x) = 0 \quad (5)$$

Where λ is the eigenvalue of the equation, μ is the viscosity of the fluid between the filaments, P is the fluid pressure along the filament, y is the position along the filament, h is one-half of the distance between filaments and D is the diffusion constant of the metal in the fluid.

The above differential equation is known as the classical Graetz problem used in fluid mechanics to determine mass transfer in fluids with laminar flow between parallel permeable plates[22-24]. It was demonstrated that, with the appropriate boundary conditions such as symmetry about the center line, solutions to equation (5) is reduced to the determination of λ_n and f_n which are the nth eigenvalue and corresponding eigenfunction, respectively. The eigenvalues can be determined and using the first eigenvalue, the mass conductance can be expressed as follows[11]:

$$k_g = \frac{D}{h}\left(\frac{1 - 0.99\exp(-\frac{2}{3}N_{G_z})}{\frac{1}{2}N_{G_z}}\right) \quad (6)$$

where N_{G_z} is the Graetz number.

Modeling the uptake from food: Heavy metal bioaccumulation in *C. virginica* includes contributions from food. The net metal uptake from food can be represented by the following mass balance equation:

$$F = C_p I_r - C_e E_r \quad (7)$$

where C_p is the metal concentration in the ingested food and C_e is the metal concentration in the feces. Assuming that a constant fraction of the ingested metal is eliminated in the feces, E_r can be expressed by

$$E_r = (1 - \alpha_f) I_r \quad (8)$$

where α_f is the oyster's assimilation efficiency for food.

In the OBM, as in FGETS it is assumed that

$C_e = K_e C_a$ and $C_a = \frac{C_f}{K_f}$. Here K_e is the distribution coefficient between feces and water and K_f is the distribution coefficient between water and the soft tissue. It follows from equations (7), (8) and the expression just given for C_e and C_a that

$$F = \alpha_c C_f I_r \quad (9),$$

where α_c is the assimilation efficiency for the metal. The assimilation efficiency of the heavy metal from food is the fraction of the ingested metal that remains in the tissue of the oyster after it has emptied its gut of undigested material[13,25].

Combining equations 7, 8 and 9, the assimilation efficiency for the metal α_c can be expressed as

$$\alpha_c = 1 - (1 - \alpha_f)\frac{C_e}{C_p}$$

$$\text{or } \alpha_c = 1 - (1 - \alpha_f)\frac{K_e C_f}{C_p K_f} \quad (10)$$

This equation shows that the assimilation efficiency for metal decreases as the total body burden of the metal increases. Elsewhere, Barber et al.[11] have demonstrated that the assimilation efficiency α_c decreases exponentially with time.

Also in OBM as in FGETS:

$$K_f = P_a + P_l K_l + P_o K_{oc} \quad (11)$$

where P_a, P_l and P_o are the fraction of the animal that is aqueous, lipid and non-lipid organic matter, respectively and K_{oc} is the organic carbon partition coefficient.

Concentration of the toxin in tissue is related to the fat fraction. The partition of the chemical compound between the lipid and aqueous phases is parametized as K_l. The partition coefficient K_l is a function of K_{ow} and is given by the following allometric expression

$$K_l = a K_{ow}^{0.785} \quad (12)$$

where the constant a has the theoretical value of 1.27[11].

We used the functional forms R, S_d and E_x given in Barber and Suarez[11]. These expressions follow $R = \frac{12}{32}\cdot RQ \cdot O_r$, $S_d = \Phi F$, and $E_x = \frac{17}{14} NC(R + S_d)$. Where RQ is the number of liter of carbon dioxide respired per liter of oxygen consumed. The value used for RQ was 1.0. The oxygen consumption rate O_r was given by an allometric expression. The value of the parameter Φ is 0.17. The quantity NC is the mass ratio of nitrogen to carbon in the oyster soft tissue. The value used for NC was 0.25. The expression for R, S_d and E_x are based on the assumptions that ammonia, carbon dioxide and water are the only waste products of oyster metabolism and that body mass loss due to respiration is determined by the loss of carbon dioxide.

Modeling the bioenergetic flux: Feeding processes in the bivalve provides the input energy necessary for anabolism (growth) and catabolism (respiration and excretion). Many early workers demonstrated that *C. virginica* efficiently feeds on natural assemblages of phytoplankton, nanozooplankton, detritus, bacteria and even dissolved organic matter[16,26]. Langdon and Newell estimated that suspended cellulosic detritus and bacteria combined would contribute 6% to the metabolic carbon and 27% to the metabolic nitrogen requirements while detrital organic matter contribute up to 60% of the carbon requirements of *C. virginica*.

According to Dame[16] the biomass growth rate in *C. virginica* is expressed as:

Growth rate = Feeding rate - respiration – excretion (13)

Equation (13) is another representation of the equation 2.

Newell and Langdon[17] and then Haure et al.[27] have indicated that feeding or the bioenergetic flux and oxygen consumption in *C. virginica* is controlled by environmental factors such as the seston concentration and the ambient temperature and some endogenous factors such as filtration rate. The relationships between feeding rate or oxygen consumption as a function of temperature and body mass can be described using non-linear statistical models previously defined by Bougrier et al.[28] for the pacific oyster

$$Y = [a + (b * c^{T})] * W^{d} \quad (14)$$

where Y is the physiological function to be determined (feeding rate (g h^{-1}) or oxygen consumption rate (mg O_2 h^{-1}), T (°C) is the temperature, W (g) the tissue mass and a,b,c and d are constants.

In the OBM, equation 14 was replaced by a similar temperature-dependent power function[11],

$$Y = \exp(f_0(T - T_{opt}))f_1 W^{f_2} \quad (15)$$

with T_{opt} the preferred temperature and f_0, f_1 and f_2 are allometric parameters. Note that when T equals T_{opt}, the exponential factor of equation 15 is reduced to unity, consequently in that case equation 15 can be written as:

$$Y = f_1 W^{f_2} \quad (16)$$

Equation (16) is the traditional allometric expression used to parametized diverse physiological functions. Here, *Y* denotes feeding and oxygen consumption rates, but the same allometric function is used to parametize the total gill area and the density of filaments (filament per mm) on the gill.

Allometric expressions of the type $Y = Y_o W^{b}$ are often used to parametize variables. According to West and Brown[29], the expression is "life's universal scaling law", which explains with a simple power law, the behavior of complex biological processes such as metabolic, growth and respiration rates. These authors as well as Newell and Langdon[17] have indicated that the weight exponent b is perhaps the most important constant in the expression as its value is almost invariable and approximates a simple multiple of ¼. In the OBM, this value was assumed to be theoretically 0.785. Since the weight exponent b is less than unity, any physiological parameter determined using the allometric expression will be higher for small bivalves than for large individuals[17].

Predicting the bioaccumulation factor (BAF): The bioaccumulation factor is a constant that relates elemental concentrations in the oyster tissue to those in the in the environmental water. It is therefore, an important parameter[3], which can be predicted using a computer model. Once the whole-body concentration of a metal at steady state is determined using the model simulation, the dimensionless BAF coefficient can be predicted with the following equation:

$$BAF = \frac{[wholebody]_{steadyState}}{C_w + C_p} \quad (17)$$

where C_w and C_p are metal concentrations in the dissolved and particulate phases, respectively.

RESULTS

Model implementation: Some dependent and independent parameters that are critical for the implementation of the model are derived from field and laboratory measurements of samples collected from the study site. It is not possible to estimate all of the physiological parameters without adequate laboratory experiments. However, it is possible to estimate these parameters from the literature. The same approach was used by Liao and co-workers who based their Zn bioaccumulation study on a preliminary database adapted from Thomann[30] and Barber et al.[11]. Table 1 show the resulting parameters used in the model implementation.

In Table 1, H_{lamel} is one-half of the distance between two neighboring filament, X_{lamel} is the length of the filament and V_{lamel} is the flow speed of the interfilament water.

Table 1: Parameters used for model implementation

	Cd	Zn	Source
C_w (µg L^{-1})	$0.23_w/0.2_s$	$11.2_w/7.0_s$	This study
C_p (µg L^{-1})	$6.0_w/6.0_s$	$13.0_w/11.0_s$	This study
k_{ow}	$5*10^5$	$7*10^5$	Ambrose 1999
H_{lamel} (cm)	0.05	0.05	Newell et al., 1996
X_{lamel} (cm)	1.50	1.50	This study
V_{lamel} (cm s^{-1})	0.60	0.60	Jones et al., 1992
AE (%)	60.0	73.0	Reinfelder et al., 1997
T (°K)	298	298	This study
S (ppt)	25.0	25.0	This study
pH	7.8	7.8	This study

C_w = dissolved metal C_p = particulate metal

AE=metal assimilation efficiency, S=salinity, T=temperature (°K)

Metal concentrations in *C. virginica*

Quality control: Elemental concentrations of Cd and Zn in actual oysters were determined in the laboratory using the atomic absorption spectrometric (AAS) technique. The accuracy of the analytical technique was verified with the analysis of the standard reference materials oyster tissue (SRM-1566b) from the National Institute of Standards and Technology (NIST). The quality assurance results illustrated in Table 2 show good precision with respect to the SRM's certified values.

Cd and Zn in oysters: The concentrations of Cd and Zn in oyster tissue are presented in Table 3. The results indicate that in the oysters collected from Apalachicola Bay, average concentrations (in µg g^{-1} dry-weight) of

Table 2: Quality control performance with SRM-1566b (oyster tissue) values are Mean± 95 % CL (µg g^{-1} dry-weight)

	SRM1566b		
Metals	Measured	Certified	%recovery
Cd	2.26 ±0.43	2.48 ± 0.08	91
Zn	1437 ± 18	1424 ± 46	101

3.39 ± 0.15 for Cd and 737 ± 134 for Zn were determined in the winter. In the summer the average concentrations (µg g^{-1} dry-weight) in for Cd and Zn are 2.56 ± 0.16 and 431 ± 58, respectively (Table 4). Similar results were reported by Fisher et al.[31] at some unpolluted sites in Tampa Bay, Florida. Furthermore, in the Carolinas O'Connor et al.[32] have reported comparable values for the Status and Trends program.

Table 3: Seasonal variation of Cd and Zn concentrations in *Crassostrea virginica.v*Values are Mean± 95 % CL (µg g^{-1} dry-weight)

Sites	Cd		Zn	
	Winter	Summer	Winter	Summer
ST-1	3.78±0.81	2.65±0.07	527±27	263±24
ST-2	3.81±0.26	1.86±0.14	837±38	593±38
ST-3	3.18±0.25	2.89±0.25	1296±45	619±28
ST-4	3.42±0.56	2.33±0.12	806±13	395±25
ST-5	3.24±0.52	2.85±0.21	614±18	374±26
ST-6	2.88±0.16	2.78±0.10	343±33	343±12
Mean	3.39±1.18	2.56±0.39	737±76	431±65

Table 4: Summary of data set used for validation simulation. The data were retrieved from the EPA Chesapeake Bay Program[35] files VA106TOT and VATISSUE. Ranges of C_w and C_p are given in (µg L^{-1}) and elemental concentration in C. virginica is in ((µg g^{-1} dry-weight). Reported simulation results are the steady state concentrations

	Cd	Zn
C_w	0.2 (DML-0.2)	1.0-12.0
C_p	9.0 (1.0-9.0)	40.0 (10-40)
T (°K)	297	283
S (ppt)	7-40	
Location	Chesapeake Bay, VA	
Concentration in oyster		
Simulation	3.0	2000
Reported values	0.9-4.2	700-4000

Simulation: Here, the OBM was used to simulate total metal (Cd and Zn) body burden B_f and growth, W. The simulation time was long enough for the body burden to reach a plateau, which suggests a steady state concentration.

The time dependent total body concentration was generated as C_{fi} (i indicating the metal under study Zn or Cd) as indicated earlier $C_{fi} = \frac{B_f}{W}$.

The OBM computer program is written in FORTRAN. The output results were further analyzed with the commonly used GNUPLOT software. All simulation graphs were generated using the GNUPLOT.

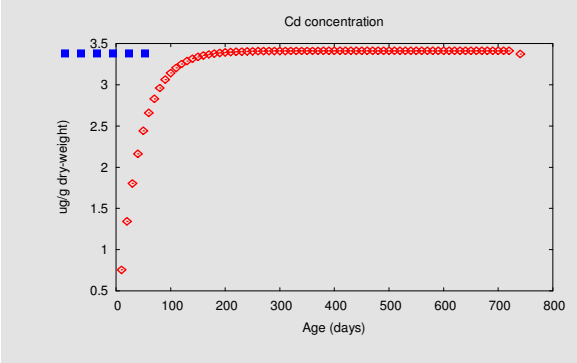

Fig. 5a: Simulated concentration of Cd in the whole soft tissue of *Crassostrea virginica* (winter). The steady state concentration is represented by the dashed line

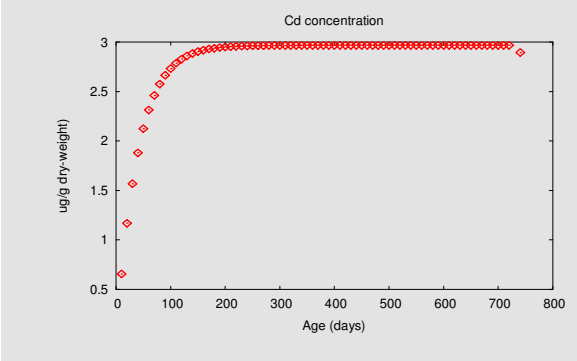

Fig. 5b: Simulated concentration of Cd in the whole soft tissue of *Crassostrea virginica* (summer)

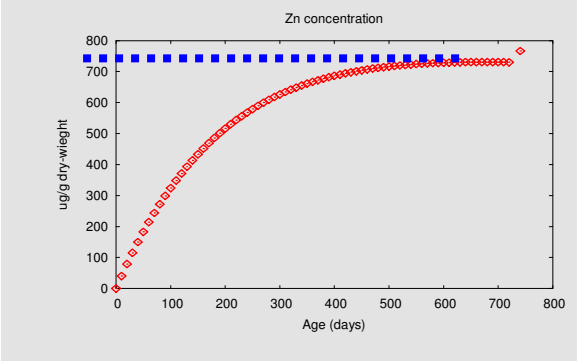

Fig. 6a: Simulated bioconcentration of Zn in the whole soft tissue of *Crassostrea virginica* (in winter). The steady state concentration is represented by the dashed line

Cadmium simulation: Long-term Cd whole-body concentration in *C. virginica* was simulated using data in Table 4. The output result is graphically represented in Fig. 4a and 4b as the variation of elemental concentration as a function of oyster age (days). It can be observed from Fig. 4a and 4b that the graph

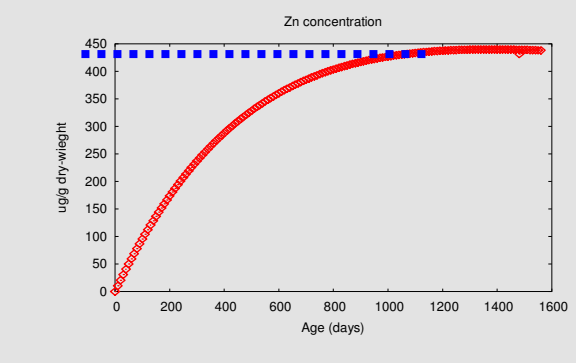

Fig. 6b: Simulated bioconcentration of Zn in the whole soft tissue of *Crassostrea virginica* (in summer). The steady state concentration is represented by the dashed line

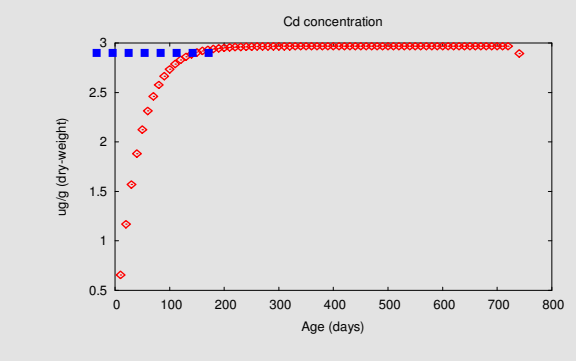

Fig. 7: Simulated bioconcentration of Cd in the whole soft tissue of *C. virginica* in Chesapeake Bay. The steady state concentration is represented by the dashed line

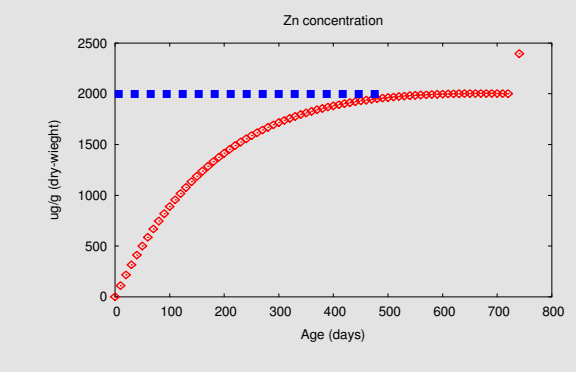

Fig. 8: Simulated bioconcentration of Zn in the whole soft tissue of *C. virginica* in Chesapeake Bay. The steady state concentration is represented by the dashed line

representing elemental concentration has a very steep slope between 0 and 100 days this is indicative of rapid metal uptake in juvenile oyster. The slope; however, slowly decreases between 100 and 300 days (about 10 months) where the net bioaccumulation reaches steady state[5,30]. The curves are similar to the whole-body concentration curves described by Thomann[30] in his study of an equilibrium model of microcontaminants in aquatic food chains. In the Cd simulation, the steady state concentration (dashed line) was reached when the oyster whole-body concentration is about (in μg g^{-1} dry-weight) 3.5 during the winter and 3.0 for the summer.

Zinc simulation: The computer simulation of Zn bioaccumulation in the oyster whole-body tissue is represented by Fig. 6a for winter and 6b for summer. The curves, once again, preserve the general characteristics of a steady state model with a first phase of rapid metal bioaccumulation rate (steep slope) followed by a plateau phase, which corresponds to steady state. Analogous results were reported by Cutshall[33] and Liao et al.[5] who conducted laboratory studies of Zn bioaccumulation in oysters. Figure 4a indicates that juvenile oysters of 0 to 400 days old (about 14 months) can rapidly take up Zn in their tissue to levels that approximate 450 μg g^{-1} dry-weight. The graph also shows that in the oyster of 400 to 1000 days old (about 2.5 years), the uptake rate is slower, however; the whole-body metal concentration can reach an equilibrium level of about 730 μg g^{-1} dry-weight in the winter. In the summer however, the results (Fig. 6b) show that the steady state level is significantly lower (450 μg g^{-1} dry-weight) than the winter's simulation value. This may be the consequence of high particulate concentrations in the winter. At the steady state level, the net accumulation is balanced by growth dilution and elimination.

Bioaccumulation factor determination: The Bioaccumulation factor (BAF) was defined earlier using the following expression

$$BAF = \frac{[wholebody]_{steadyState}}{C_w + C_p}.$$

Using the winter simulation results of Cd and Zn concentrations in the oyster at steady state and the respective metal concentrations of both dissolved and particulate phases

$$BAF_{Cd} = \frac{3.4\,ppm}{(0.23+6.0)*10^{-3}\,ppm} = 5.5*10^{2},$$

and

$$BAF_{Zn} = \frac{730\,ppm}{(11.2+13.0)*10^{-3}\,ppm} = 3.0*10^{4}.$$

The results of the BAF suggest that elemental concentrations in *C. virginica* are about two to four orders of magnitude higher than metal concentrations in the surrounding water. It is well documented elsewhere that the American oyster *Crassostrea virginica* is capable of bioaccumulating heavy metals in their soft tissue to levels that are greater than that in the water in which they live. According to Roesijadi[34],

concentrations of metals in the tissue of bivalves are about four orders of magnitude higher than those in surrounding water. Therefore, the BAF results reported here are in agreement with data reported in the literature.

Model validation: The applicability of the OBM to other estuarine environments was assessed with data from Chesapeake Bay, Maryland. The data used for this validation simulation is summarized in Table 4. All other parameters such as biological attributes are left the same as for previous calculations because identical species of oysters are found in the Chesapeake Bay. The data set used was obtained from documents prepared by the Environmental Protection Agency (EPA) Chesapeake Bay Program[35]. The choice of Chesapeake Bay is based not only on data availability but also on some physical similarities such as the ranges of salinity and temperature.

Validation results are illustrated in Fig. 7 for Cd and Fig. 8 for Zn. Simulation results of Cd and Zn bioconcentrations in *C. virginica* show steady state concentrations (μg g^{-1} dry-weight) of about 3.2 and 2000, respectively. Although the computer simulation did not generate the maximum value reported for Cd and Zn in Chesapeake Bay, these values are clearly within the respective concentration ranges reported by Chesapeake Bay Program (Table 4). It can be seen that the Cd results is close to the reported average value of 3.6 μg g^{-1} dry-weight and the Zn result is within the reported range. These comparisons suggest that the OBM model is flexible enough to be applied in other estuarine environments.

DISCUSSION

The computer simulation results for both Cd and Zn at steady state are comparable to the average concentrations found in the actual oyster tissue (Table 3). The Cd and Zn results are in agreement with a field study conducted by Fisher et al.[31], which reported values (in μg g^{-1} dry-weight) of 2.22 for Cd and 1260 for Zn in Tampa Bay, Florida, a site that is about 250 miles south of Apalachicola Bay.

The computer simulation results of both metals are in agreement with field results obtained from oyster tissue analysis. This is an indication that the OBM model is a good representation of metal uptake in *C. virginica*.

As previously mentioned Fig. 4 and 5 showed curves that have typically three phases. The first phase, with a large positive slope is characterized by a very rapid increase in the bioaccumulation of the metals in juvenile oysters. This perhaps is due to the higher growth rate generally observed in any younger organisms, which are developing with higher metabolic rates. Therefore, this observation in juvenile oysters (4 to 7 months) is simply due to a normal higher rate of food uptake and metabolism. For example, Newell and Langdon[17] demonstrated that the clearance rate is much greater in juvenile than in adult *C. virginica*. The second phase on the curve is characterized by a slow uptake rate. In this phase, net metal bioaccumulation can still be observed but at a slower rate than the previous one. The third phase is represented by the horizontal portion of the curves. It corresponds to metal uptake at steady state in adult oysters (over 15 months old). It is noticed by comparing Fig. 4 and 5 that the steady state level was reached at different times for the two metals. However, the physiological mechanisms that govern the steady state are still the same. At this stage, although physiological mechanisms of consumption and assimilation are still taking place in the oyster, metal bioaccumulation reaches a steady state[30]. In this computer simulation study, the steady state concentrations (μg g^{-1} dry-weight) were reached at 3.5 for Cd and 730 for Zn in the winter season. A slight drop in the dissolved and particulate metal concentrations in the summer resulted in a decrease in the steady state metal concentrations (μg g^{-1} dry-weight) to 3.0 and 450 for Cd and Zn, respectively.

The steady state concentrations are expected to fluctuate according to the prevailing environmental conditions. In fact, it can be seen in Fig. 6 and 7 that the validation simulations run with the outside data set, yielded different steady state concentrations. Additionally, several laboratory studies[3,5,33] conducted with diverse chemical and physical conditions resulted in a variety of outcomes in terms of the level of the steady state concentrations. Based on this observation, it is concluded that the steady state concentration is site specific. Therefore, the results obtained from the use of the OBM are specific steady state concentrations for Apalachicola Bay.

The results of this study suggest that that the concentration of Zn is approximately 3 orders of magnitude higher than that of Cd (Table 3). There are three reasons for this. First, in contrast to Cd, Zn is well documented as an essential trace metal in the physiology of bivalves[36,37]. This suggests that physiologically, Zn should have higher assimilation efficiency (AE) as compared to that of Cd. A previous study by Ke et al.[13] reported an AE value for Zn that is about twice that of Cd. Differences in efflux rate could also be the cause of the difference between levels of the concentrations of Cd and Zn in oysters. Wang et al.[38] reported efflux rates of 0.01 and 0.03 d^{-1} in oyster and Reinfelder et al.[37] have concluded that small differences in efflux rates may have substantial effects on trace metal bioaccumulation with non-essential elements (Cd) being excreted at a faster rate. With the Zn efflux rate being slightly smaller than that of Cd, the bioaccumulation rate of Zn is consequently greater. The second argument that explains the difference is based on the presence in bivalve tissues of low-molecule sulphur-rich metal binding proteins such as

Metallothionein (MT)[31,34,39,40]. Metallothionein is known to selectively complex certain heavy metals such as Cu and Zn[40] making these elements the most abundant in oyster tissue. Additionally, Roesijadi[34] has described MT in *C. virginica* is related to the metabolism of Cu and Zn. Since MT is capable of binding high concentrations of metal, it was suggested that the role of MT was also to protect the animals from metal toxicity[31]. However, it was shown that these essential metals could be displaced at the sulphur binding sites by toxic metals and the rate of such displacement by Cd is higher than other metals making Cd the third most abundant metal in oyster tissue[36,34,39,40]. Finally, the selective retention of Zn to a different degree is due to the chemistry of the element. One of the most important chemical characteristics of a metal is the octanol/water partition coefficient $K_{ow.}$ As it was mentioned earlier in the model development, the partition coefficient is an important parameter which determines the bioavailability of metal in ingested food or water.

Several studies on bioaccumulation of heavy metals in bivalves[3,9,13] were focused on the laboratory determination of the bioconcentration factor (BAF). Based on this study it is suggest that the BAF should be determined under field conditions because in non-controlled environments, slight changes can cause significant shifts. However, the BAF can be modeled and predicted using steady state concentrations. Since the prediction of the steady state concentrations are site specific, the resulting BAFs are also site specific.

CONCLUSION

A bioaccumulation model describing the long-term uptake and retention of Cd and Zn in the oyster (OBM) was developed based on both dissolved metal and metal in food pathways. For the model implementation, the input parameters were derived from this study; however, some bioenergetic and physiological parameters were compiled from the literature.

The model predictions are in agreement with sample field results. Therefore, it can be concluded that the OBM can effectively be applied to predict long-term bioaccumulation of Cd and Zn in *C. virginica*. Furthermore, the model allows the prediction of the site specific BAF, which is an important parameter in the determination of both total body burden of heavy metals along with the water chemistry in Apalachicola Bay. Therefore, the OBM model can be used as an efficient biomonitoring tool for long-term assessment of heavy metals in the Apalachicola Bay ecosystem.

The OBM model was be effectively validated with data from Chesapeake Bay. *Crassostrea virginica* is ubiquitous and abundant in costal waters. These facts and the flexibility of the OBM model make it ideal for use in other estuarine environments.

ACKNOWLEDGEMENTS

We are grateful for the financial support provided by the National Oceanic and Atmospheric Administration's Environmental Cooperate Science Center Cooperative, the United States Department of Education's Title III Grant to Florida A&M University. In addition, we thank the staff at the Apalachicola National Estuarine Research Reserve for technical assistance.

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