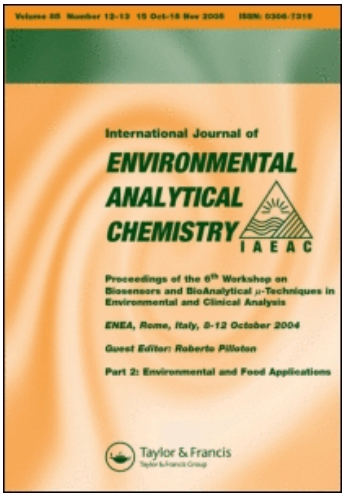


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# The Use of Transplanted *Venerupis Decussata* to Evaluate the Pollution of Heavy Metals and Tributyltin in Marinas

Jose L. Gomez-ariza[a]; Inmaculada Giraldez[a]; Daniel Sanchez-rodas[a]; Teresa Acuñta[a]; Emilio Morales[a]
[a] Departamento de Química y Ciencia de los Materiales, Escuela Politécnica Superior, Universidad de Huelva, La Rabida, Huelva, Spain

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# THE USE OF TRANSPLANTED *VENERUPIS DECUSSATA* TO EVALUATE THE POLLUTION OF HEAVY METALS AND TRIBUTYLTIN IN MARINAS

JOSE L. GOMEZ-ARIZA*, INMACULADA GIRALDEZ, DANIEL SANCHEZ-RODAS, TERESA ACUÑA and EMILIO MORALES

*Departamento de Química y Ciencia de los Materiales, Escuela Politécnica Superior, Universidad de Huelva, 21819 La Rabida, Huelva, Spain*

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Live specimens of the clam *Venerupis decussata* were suspended in seawater of the Mazagon Marina, located in a heavy metal polluted area at the mouth of Huelva Estuary (SW Spain). Clams were preserved in plastic cages and subsamples were recovered every 5 days over a period of 40 days. Water from the marina was sampled every two days during the time course of the experiment. Clams and water were analyzed for metals and organotins. Results showed the accumulation of Mn, Cu, Fe, V, Zn and tributyltin in the bivalves reaching an equilibrium with the surrounding water. Bioconcentration factors ranged from $10^2$ (for V) to $4\times10^3$ (for Cu). Clams also accumulated Al and Pb but a steady state was not reached. A first-order kinetic model was applied to the data and results indicated that rates of accumulation differed in relation to clam size class. Clam mortality increased during the experiment and was total after 42 days which was attributed to the high concentration of Cu in seawater.

*Keywords:* Metals; organotin; *Venerupis decussata*; bioaccumulation

## INTRODUCTION

Estuarine areas are commonly affected by anthropogenic metal pollution as far as they may receive the inputs from mining and industrial activities located along the rivers. Heavy metals are known to be potentially toxic to both aquatic biota and man, requiring exhaustive studies on their presence in the environment. On the other hand, inputs of organotin compounds, mainly tributyltin (TBT), causing harmful effects on non-target organisms. may occur particularly in harbours and marinas by the release from vessel antifouling paints.

* Corresponding author. Fax: +34–959–350311. E-mail: ariza@uhu.es

The evaluation of metal pollution in aquatic habitats is usually performed by the analysis of water, sediment and biological samples, although the latter have more environmental relevance. In fact, metals are accumulated by aquatic organisms providing a time-integrated measure of the pollutant bioavailability by the analysis of their tissues. Bivalves have been the most employed organism as biomonitor accumulating trace metals in direct proportion to their environmental levels [1–4], with storage occurring in metal binding proteins or in granules. However, biomonitors are not always present in the area of study. In such cases, the use of transplantation experiments may be valuable as far as a rapid accumulation observed in control bivalves indicates the presence of pollutants in available forms [5]. In addition, the use of bio-accumulation kinetic models [4] are useful for predicting both the quantitative pollutant content in exposed bivalves and the bioconcentration factors (BCFs) when organisms are not exposed for a sufficiently long period to reach the steady-state. However, most of the bioaccumulation studies are based on data obtained under laboratory conditions which failed to simulate field conditions involving many environmental factors which are known to influence biological uptake.

In this paper, the use of transplanted clams, *Venerupis decussata*, was studied as a biomonitor for the evaluation of the TBT and metal pollution in the Mazagon Marina located at the Huelva Estuary (SW Spain). This estuary is one of the most polluted aquatic environments in Europe as a result of the effects derived from both acid mine drainage and industrial processing plants [6]. A second objective of this investigation was to use a first-order kinetic model to estimate the metal concentration in clams and predict the BCFs.

*V. decussata* is a filter feeder clam of commercial interest, widely distributed in the European waters, living in shallow areas (estuaries, river mouths, bays and coastal lagoons) burrowed in sandy and muddy-sandy substrata at a depth of 8–9 cm and pumping water through the siphons at sediment level. They reach maturity at a length of about 15 mm (one year old). At the age of 4 years, its length is about 40 mm. They spawn twice in a year, in June and September [7,8]. This clam is able to accumulate high metal and TBT concentrations without any apparent detrimental effect, reflecting gradients of pollutant in the surrounding seawater [9,10].

## EXPERIMENTAL

### Accumulation experiments

On January 20, 1998, approximately 800 individual clams, *V. decussata*, were collected from a natural population (selected as a control area) of the Huelva coast far from metal sources (mining and industrial processes) and organotins (boat activities), divided into two class of different shell lengths measured to the

nearest millimetre (25 ± 1 and 36 ± 1 mm, respectively) and transplanted to Mazagon Marina, in which more than 100 pleasure vessels and several fishing boats are usually moored. This marina receives the inputs of heavy metals from mining activities and industrial processing plants located upstream in the Tinto and Odiel Rivers and Huelva Estuary. Clams were placed into 16 plastic cages (34 × 24 × 24 cm) with 1-cm square openings and suspended in seawater at a depth of 1 m and about 2 m above the sediment. 20 clams of each size class were resampled every 5 days after transplantation during 40 days. Each analytical sample consisted of 5 individuals, the biological variation being represented by 4 analytical samples. Algae were cleaned off the cages and the dead clams were counted before placing the cages into the water. Additional samples were retrieved from the control area and served as reference. Water samples were collected from the same points of clams at a depth of approximately 1 m below the surface every two days using 2.5 L polycarbonate bottles being the cap carefully removed under water surface and analyzed for heavy metals, organotins and physico-chemical parameters.



## Reagents, standards and apparatus

All acids and reagents were of analytical or suprapur grade (Merck, Darmstadt, Germany) and solvents were of pesticide grade (Romil, Loughborough, UK). A multi-standard metal solution was obtained from Spex Chemical (Metuchen, NJ, USA) and working solution were prepared in 5% of suprapur grade $HNO_3$ (Merck). Stock and working solution for organotins were prepared in hexane. Water used in all the experiments was distilled and then treated in a Milli-Q system (Millipore, Bedford, MA, USA), All items of laboratory ware were either cleaned with saturated $Na_2Cr_2O_7$ in $H_2SO_4$ during 24 h for organotin analysis or using 50% of $HNO_3$ during 3 days for metal analysis. Then, they were rinsed with distilled water and soaked with Milli-Q water until analysis.

## Analytical procedures

### *Clams*

No gut-cleansing of the collected specimens was performed. Prior to analysis, clam shells were opened, excess water in the mantle cavity was allowed to drain and all soft tissue was removed from the shell with a disposable plastic knife. A portion of 5 g of homogenized wet tissue was treated with $HNO_3$:$HClO_4$ acid mixture (3:1) for 24 h at room temperature and then heated for 5 h at 95°C in Teflon vessels covered with Teflon taps. The digested solution was reduced to

dryness by evaporation and then heated with 2 ml of $HNO_3$ for 15 min. This $HNO_3$ treatment was performed twice and finally the volume was raised to 25 ml with distilled water. Metal analysis except for As was carried out using a ICP-MS (Hewlett Packard, Palo Alto, CA, USA) after spiking the solution with Sc, Rh and Tb as internal standards. Total arsenic in clams was determined using a flow injection system and a non dispersive atomic fluorescence detector (PSA Excalibur: PS Analytical UK) (FIA-HG-AFD). Organotin determination was carried out using a method previously reported in the literature [11], based on an acid digestion (HBr) of a 5 g portion of homogenized wet tissues, organic solvent extraction (dichloromethane), penthylation, Florisil clean up and determination by GC-FPD (Varian, San Fernando, CA, USA). All the samples were analyzed by duplicate and a precision higher than 10% was achieved. Metal and organotin (as cation) levels in clams are expressed in dry weight basis. Water moisture was evaluated drying a portion of sample at 110°C during 24 h until constant weight.

### *Water*

Immediately after sampling the water was filtered using 0.45 μm Whatmann membrane filters (Clifton, NJ, USA). The filtered water was analyzed for several parameters: (i) it was diluted 1:20 with 1 % HNO for metal ICP-MS analysis: (ii) diluted 1:5 with water for arsenic speciation using HPLC-HG-AFD system [12]; and (iii) a 2 L aliquot was analyzed for organotins [13]. Filters were also analyzed for organotins.

Detection limits (DLs) of the different procedures are shown in Table I. The accuracy of the analytical procedures was checked with certified reference materials (CRMs): Tort I (lobster hepatopancreas, from National Research Council, Canada) with certified values for As, Cd, Cr, Co, Co, Fe, Mn, Ni, Pb, V and Zn and CRM-477 (mussel tissue, from Standards, Measurements and Testing Programme) with organotin certified values. Replicate analysis of these CRMs showed good accuracy, with all results comparable with certified values (Table II). Duplicate analysis of water and clam samples also showed good precision with coefficients of variation better than 10%.

Water quality parameters in both control area and Mazagon Marina were evaluated during the time course of the experiment following standardized methods of analysis [14].

## Kinetic model and BCFs

A two-compartment model (water-clam) was used to describe the bioaccumulation of the pollutants by the transplanted clams as the net result of uptake, elimi-

nation and growth processes [15,16]. This model conducts to the following equation when both a negligible growth of the clams and a constant pollutant concentration in the water occur during the experiment:

$$C_A = C_o + k/k' \times C_w(1 - e^{-k't}) \quad (1)$$

where $C_A$ and $C_o$ are the pollutant concentrations in clams (mg $kg^{-1}$) at the exposure time t and t=0 (day), respectively; $C_w$ is the pollutant concentration in water $\mu g\ L^{-1}$); k is the pollutant uptake constant ($10^{-3}$ $day^{-1}$); and k' is the elimination rate constant ($day^{-1}$). Experimental pollutant concentrations in tissues may be fitted to the kinetic model and rate constants may be calculated allowing the prediction the pollutant concentrations at different exposure times.

TABLE I Analytical detection limits (DL, evaluated as 3s of blank for 10 replicate blank analysis) for the determination of metals, organoarsenics and organotins in water $\mu g\ L^{-1}$) and clam samples (mg $kg^{-1}$, dry weight basis)

| *Pollutant* | *Water* | *Clam* | *Pollutant* | *Water* | *Clam* |
|---|---|---|---|---|---|
| Ag | 4 | 0.01 | Ni | 2.4 | 0.02 |
| Al | 12 | 0.05 | Pb | 1.1 | 0.01 |
| As(V) | 0.6 | 0.01 | V | 0.9 | 0.002 |
| Cd | 1.5 | 0.007 | Zn | 15 | 1 |
| Co | 0.7 | 0.001 | TBT | 0.009 | $1.5 \times 10^{-4}$ |
| Cr | 9 | 0.02 | DBT | 0.006 | $1.5 \times 10^{-4}$ |
| Fe | 130 | 1.2 | MBT | 0.004 | $1.5 \times 10^{-4}$ |
| Mn | 2.6 | 0.01 | DMA | 0.5 | --- |
| As(III) | 0.3 | --- | MMA | 0.4 | --- |

TBT (tributyltin); DBT (dibutyltin), MBT (monobutyltin); DMA (dimethyl arsenic), MMA (monomethyl arsenic)

BCFs may be determined in two ways. One method involved the calculation of the concentration ratio of a chemical in an organism to that in water when the steady-state is actually observed. The second method relies on the accuracy of the kinetic model to measure uptake and elimination rate constants. As the exposure time (t), approaches infinity, from equation (1), the BCF will be obtained from the uptake and elimination rate constants:

$$BCF = k/k' + C_o/C_w \quad (2)$$

## Statistical analysis

The data were analyzed statistically for differences using Student's t-test with significance levels of $p<0.05$. Data for the accumulation studies were subjected to quasi-Newton nonlinear regression analysis (CSS: STATISTICA™).

TABLE II Certified and found values (mg $kg^{-1}$, dry weight basis) obtained from the analysis of the certified reference materials Tort-1 and CRM-477

| *Analyte* | *CRM 477* | | *Tort 1* | |
|---|---|---|---|---|
| | *Certified value (mg $kg^{-1}$)* | *Found value (mg $kg^{-1}$)* | *Certified value (mg $kg^{-1}$)* | *Found value (mg $kg^{-1}$)* |
| TBT | 2.20 ± 0.19 | 2.27 ± 0.06 | --- | --- |
| DBT | 1.54 ± 0.12 | 1.47 ± 0.09 | --- | --- |
| MBT | 1.50 ± 0.28 | 1.51 ± 0.09 | --- | --- |
| As | --- | --- | 25 ± 2 | 24.6 ± 0.7 |
| Cd | --- | --- | 26 ± 2 | 25 ± 1 |
| Co | --- | --- | 0.42 ± 0.05 | 0.44 ± 0.07 |
| Cr | --- | --- | 2.4 ± 0.6 | 2.42 ± 0.08 |
| Fe | --- | --- | 190 ± 10 | 184 ± 8 |
| Mn | --- | --- | 23 ± 1 | 22.6 ± 0.7 |
| Ni | --- | --- | 2.3 ± 0.3 | 2.2 ± 0.2 |
| Pb | --- | --- | 10.4 ± 0.2 | 10.5 ± 0.3 |
| V | --- | --- | 1.4 ± 0.3 | 1.6 ± 0.2 |
| Zn | --- | --- | 180 ± 10 | 183 ± 9 |



## RESULTS

### Pollutant levels in transplanted and reference clams and water

The overall mean concentration of each metal in waters collected in the control area and the Marina are given in Table III, and they did not significantly changed during the course of the experiments. Between both areas, significant differences in the concentration of metals were observed for Al, As, Cu, Fe, Mn, Pb, Zn and V (t-test, $p<0.01$) and the ability of *V. decussata* to accumulate these metals could be studied. Otherwise, arsenic (as As(V)) was only found in samples collected in Mazagon Marina and Ag and TBT concentrations were below DLs in both control and marina areas. Metal levels in control clams did not change during the experiment but an increase over time was observed in both large and small transplanted clams (Figure 1). Accumulation was fast during the first 30 days after transplantation and subsequently more gradual. A steady state in which pollutant

concentrations exhibited only minor variations occurred for all pollutants except for Pb and Al. At the end of the accumulation period, pollutant concentrations were about between two (Al, V and Zn) and ten (Cu and TBT) times higher than the initial ones.

TABLE III Average concentration of metals ($\mu g\ L^{-1}$) in water collected from Mazagon Marina and Control Area during the clam transplantation experiment

| *Metal* | *Control Area* | *Mazagon Marina* | *Metal* | *Control Area* | *Mazagon Marina* |
|---|---|---|---|---|---|
| Al | 21 ± 5 | 63 ± 9 | Ni | 5.1 ± 0.3 | 5.1 ± 0.6 |
| As | < DL[a] | 13 ± 2 | Fe | 130 ± 11 | 780 ± 54 |
| Cd | 0.7 ± 0.2 | 0.7 ± 0.2 | Mn | 58 ± 5 | 100 ± 13 |
| Co | 5.7 ± 0.6 | 6.1 ± 0.8 | Pb | < DL | 3.4 ± 0.5 |
| Cr | 13 ± 2 | 14 ± 2 | Zn | 39 ± 6 | 150 ± 17 |
| Cu | 11 ± 2 | 44 ± 4 | V | 4.9 ± 0.3 | 8.8 ± 0.7 |

a. below detection limit



Initial concentration of Ag, As, Cd, Co, Cr and Ni in clams were 0.17 ± 0.06, 23 ± 5, 2.3 ± 0.4, 1.8 ± 0.2, 1.0 ± 0.1 and 2.7 ± 0.1 mg $kg^{-1}$, respectively (average values) and did not increase during the experiment. Moreover, no significant differences between control and transplanted clams were observed for these metals (t-test, $p>0.11$). This result is surprising for As because of the difference between both control area and Mazagon Marina arsenic water concentrations. Moreover, As(V) was the species found in water and it has been reported as readily bioaccumulated by bivalves [17]. The scarce As(V) bioaccumulation suggests that this species is not bioavailable to *V. decussata* or that the clam may regulate their total arsenic content.

Concentrations of Al, Cu, Pb, Zn, V, Fe, Mn and TBT in *V. decussata* decreased as the size of the specimens increased. However, As concentration increased with the body size and concentrations of Ag, Cd, Co, Cr and Ni were relatively independent of the clam size. Water quality parameters remained relatively constant throughout the experimental period, except for the days 12 and 14, when a decrease of salinity and an increase of alkalinity and suspended particulate matter were observed, coinciding with a strong storm (Table V). Mortality in transplanted clams increased during the time course of the experiment from 0% after 10 days to 50% after 30 days. Mortality was total after 42 days.

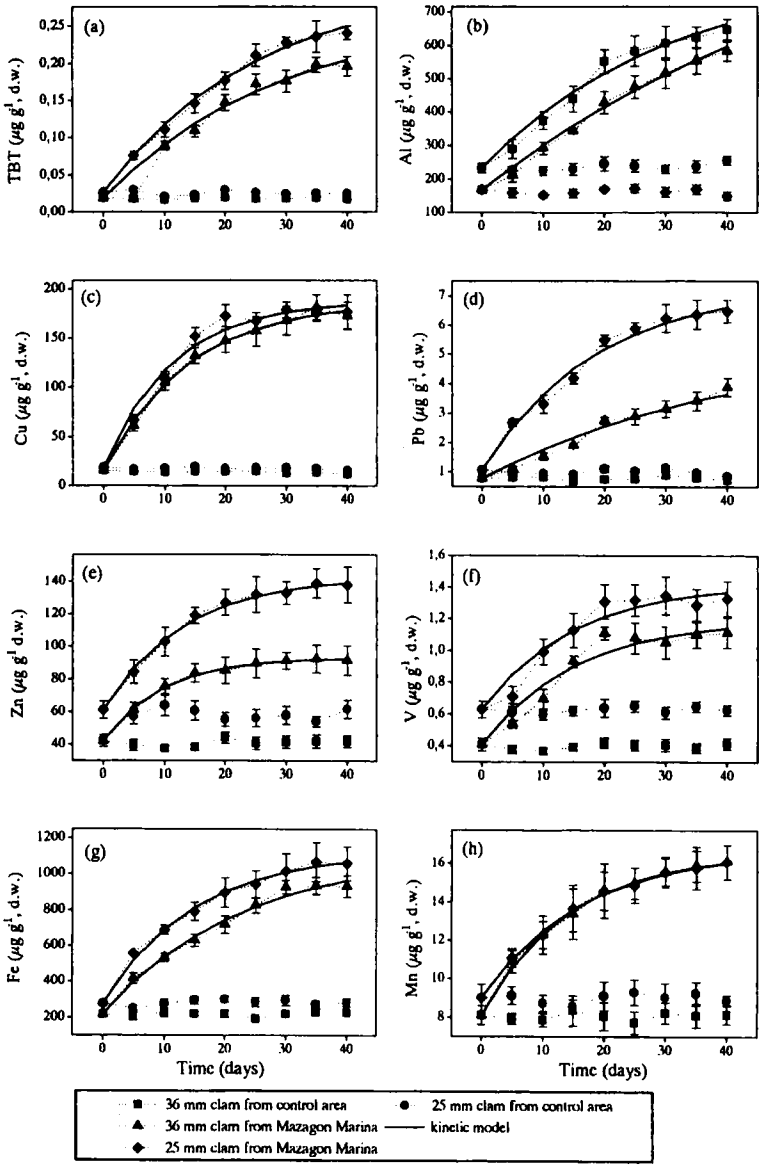


FIGURE 1 Concentrations of metal and tributyltin (TBT) in the soft tissues of clam (*Venerupis decussata*) during the transplantation experiment: (a) TBT; (b) Al; (c) Cu; (d) Pb; (e) Zn; (f) V; (g) Fe; (h) Mn. The error bars represent the standard deviation obtained from the analysis of four groups of five clams

TABLE IV Water quality parameters in Control Area and Mazagon Marina during the clam transplantation experiment

| *Parameter* | *Control Area* | | *Mazagon Marina* | |
|---|---|---|---|---|
| | *day (1–10 and 16–42)* | *day (12–14)* | *day (1–10 and 16–42)* | *day (12–14)* |
| pH | 7.75 ± 0.07 | 7.45 ± 0.05 | 7.76 ± 0.07 | 7.9 ± 0.2 |
| T (°C) | 14.6 ± 0.2 | 14.2 ± 0.2 | 14.4 ± 0.2 | 14.95 ± 0.05 |
| Alkalinity (mg $L^{-1}$) | 100 ± 5 | 92 ± 11 | 96 ± 4 | 60 ± 20 |
| Chlorophyll (μg $L^{-1}$) | 1.9 ± 0.5 | 2.4 ± 0.2 | 2.0 ± 0.3 | 2.2 ± 0.3 |
| Oxygen (mg $L^{-1}$) | 7.6 ± 0.1 | 7.2 ± 0.1 | 7.5 ± 0.3 | 7.6 ± 0.2 |
| Salinity (‰) | 34 ± 1 | 30 ± 1 | 34 ± 2 | 22 ± 4 |
| Suspended matter (mg $L^{-1}$) | 2.3 ± 0.4 | 2.6 ± 0.7 | 2.9 ± 0.4 | 7.6 ± 2 |

TABLE V Kinetic coefficients for accumulation experiments using small (25 mm length) *V. decussata* transplanted to Mazagon Marina for 40 days. k is the uptake rate constant ($day^{-1}$), k' is the depuration rate constant ($day^{-1}$), r is the correlation coefficient, BCFc is the bioconcentration factor calculated as 1000 × k/k' and BCFe is ratio of pollutant content in clam to pollutant concentration in the surrounding water at steady state

| *Pollutant* | *k ($day^{-1}$)* | *k' ($day^{-1}$)* | *r* | *BCFc* | *BCFe* | *BCFe*[a] |
|---|---|---|---|---|---|---|
| Al | 0.30 | 0.032 | 0.98 | $1.3 \cdot 10^4$ | --- | $1.1 \cdot 10^4$ |
| Cu | 0.02 | 0.087 | 0.99 | $6.5 \cdot 10^2$ | $4.0 \cdot 10^3$ | $1.6 \cdot 10^3$ |
| Fe | 0.071 | 0.067 | 0.99 | $1.4 \cdot 10^3$ | $1.3 \cdot 10^3$ | $2.2 \cdot 10^3$ |
| Mn | 0.0046 | 0.061 | 0.99 | $1.6 \cdot 10^2$ | $1.5 \cdot 10^2$ | $1.5 \cdot 10^2$ |
| TBT | --- | 0.039 | 0.99 | --- | --- | --- |
| Pb | 0.096 | 0.052 | 0.99 | $2.2 \cdot 10^3$ | --- | --- |
| V | 0.0058 | 0.065 | 0.97 | $1.6 \cdot 10^2$ | $1.5 \cdot 10^2$ | $1.3 \cdot 10^2$ |
| Zn | 0.048 | 0.075 | 0.99 | $1.6 \cdot 10^2$ | $8.8 \cdot 10^2$ | $1.5 \cdot 10^3$ |

a. for reference clams

## Kinetic model and BCFs

Experimental pollutant concentrations in tissues were fitted to a first-order kinetic model (Figure 1) in order to estimate the rate constants for uptake and elimination processes and predict the BCFs (Tables V and VI). The predicted pollutant concentrations were not significantly different from the observed values and the correlation coefficients of the regression analysis were higher than

0.97, indicating that the model is applicable to the bioconcentration of several pollutants in *V. decussata*.

TABLE VI Kinetic coefficients for accumulation experiments using large (36 mm length) *V. decussata* transplanted to Mazagon Marina for 40 days. k is the uptake rate constant ($day^{-1}$), k' is the depuration rate constant ($day^{-1}$), r is the correlation coefficient, BCFc is the bioconcentration factor calculated as 1000 × k/k' and BCFe is ratio of pollutant content in clam to pollutant concentration in the surrounding water at steady state

| *Pollutant* | *k* ($day^{-1}$) | *k'* ($day^{-1}$) | *r* | *BCFc* | *BCFe* | *BCFe*[a] |
|---|---|---|---|---|---|---|
| Al | 0.22 | 0.015 | 0.99 | $1.7 \cdot 10^4$ | --- | $7.8 \cdot 10^3$ |
| Cu | 0.017 | 0.071 | 0.99 | $5.7 \cdot 10^2$ | $3.9 \cdot 10^3$ | $1.2 \cdot 10^3$ |
| Fe | 0.050 | 0.044 | 0.99 | $1.4 \cdot 10^3$ | $1.2 \cdot 10^3$ | $9.6 \cdot 10^2$ |
| Mn | 0.0046 | 0.061 | 0.99 | $1.6 \cdot 10^2$ | $1.6 \cdot 10^2$ | $1.4 \cdot 10^2$ |
| TBT | --- | 0.039 | 0.99 | --- | --- | --- |
| Pb | 0.096 | 0.052 | 0.99 | $2.2 \cdot 10^3$ | --- | --- |
| V | 0.0057 | 0.065 | 0.97 | $1.3 \cdot 10^2$ | $1.2 \cdot 10^2$ | 81 |
| Zn | 0.041 | 0.10 | 0.99 | $6.6 \cdot 10^2$ | $5.9 \cdot 10^2$ | $1.1 \cdot 10^2$ |

a. for reference clams

A steady state was assumed to occur in both clams cultured in natural areas (control area) and transplanted clams when the pollutant content in tissues remained unchangeable with the time. In both cases, experimental BCFs were calculated and ranged from $10^2$ (for Cr) and $10^4$ (for Al). Higher BCFs were found for non essential elements (Pb, Cd, As) and for metals present at higher concentrations in water (Cu and Fe). BCFs were also calculated from equation (2) and are shown in Tables V and VI. This approach is specially useful when the steady state has not been reached, such as it happened in our study for Pb and Al. Comparative values for experimental and calculated BCFs were obtained for all metals except for Cu where values obtained from the kinetic model were lower than the experimental ones.

## DISCUSSION

Clams exposure during the experiment was limited to dissolved and suspended pollutants present in the water column. However, natural populations of clams are also affected by pollutants from sediment in which are buried. Therefore, the

results from our experiments have some limitations regarding the assessment of harmful actions of pollutants in bivalves.

The high concentrations of As, Cu, Pb, Zn, Fe and Mn in water collected from Mazagon Marina compared to those in the control area may be explained by the influence of both numerous chemical plants in Huelva Estuary and the inputs from Tinto and Odiel Rivers whose waters contain large amounts of metal of pyritic origin from mining activities. Consequently, a significant metal accumulation was observed in tissues of *V. decussata* transplanted to this area. Comparison between the present metal concentrations and those reported in 1989 for the same biological species shows a decrease of As, Cd, and Cr and an increase of Pb and Cu [18]. These changes may be related, on one hand, to the significant decrease of both mining activities and rainfall over the recent years, and, on the other, to the recent touristic development of the zone with additional inputs of Cu, used as antifouling paints in recreational yards, and the starting of a sulphuric plant that introduces extra amounts of Pb.

The comparison of Cu and Zn contents in *V. decussata* from the control area (13 and 42 mg $kg^{-1}$, respectively) with those found in this clam collected from the Portuguese coast [9,19] (Ria Formosa lagoon) next to the study area, resulted in comparable background levels for Cu (10.2 mg $kg^{-1}$) but lower for Zn (115 mg $kg^{-1}$). In urn, the comparison of metal concentrations in two different clams, *V. decussata* (this study) and *Chamelea gallina* [20] both collected from the Huelva coast, reflected the occurrence of Cu, Zn, Mn, Pb and Fe as the main pollutants in the Estuary.

The rate of pollutant accumulation was higher during the initial period of the experiment declining afterwards, this fact being previously checked in *V. decussata* and other bivalves [19,21–23], indicating the presence of pollutants regulation mechanisms. *V. decussata* transplanted to Mazagon Marina took between 20 and 30 days to reach a steady state for Cu, Fe, Mn, TBT, V and Zn. In previous works this clam also reached the equilibrium for Cd in 21–30 days [21,22]. The knowledge of the magnitude of this parameter is important in order to select organisms as biomonitors, although may vary with the level of the pollutant [10] and, moreover, they are site-specific [15]. With this caveat in mind we may compare the time to the steady state of *V. decussata* with that of other bivalves. In general, similar values have been reported for *Circenita callipyga* [23], *Crassostrea gigas* [24] and *Mytilus galloprovincialis* [25], but higher values for *Mytilus edulis* [26], *Crassostrea redalei* and *Crassostrea belcheri* [15], which indicated that these bivalves have to be exposed a longer time before reflecting the actual concentration of pollutants in the surrounding water.

BCFs show the ability of an organism to amplify the signal corresponding to pollutant water concentrations and, therefore, this parameter is important in order

to select a suitable bioindicator. Comparisons of BCF data reported in the literature are sometimes difficult because of the high variability of the results. Those discrepancies may be explained by differences in the type of study (field or laboratory studies), in the test conditions, in the biological species, in the feeding strategy and in the exposure concentrations of pollutants. Bebianno *et al.* [21,32] obtained BCFs for Cd of 400 and 850 for *V. decussata* exposed to concentrations of 400 and 100 $\mu g\ L^{-1}$, respectively, under laboratory conditions. These values are 3.5 and 1.6 times lower, respectively, than those obtained in the present experiment under lower Cd concentration. This inverse relationship between BCFs and exposure concentration was also observed for other metals in our experiments and has also been reported elsewhere [10]. Published data on the metal accumulation by *V. decussata* [9,19,21] together with the BCFs presented in this study suggest that this clam present a similar or lower power of bioaccumulation than that of mussels and oysters. BCFs in *V. decussata* were lower than those found in *M. edulis* [27–29], *M. Galloprovincialis* [22], *C. gigas* [24], *C. Virginica* [28,29], *C. iredali* and *C. Belcheri* [15], reflecting the lower accumulation capacity of this bivalve compared to several species of mussels and oysters. However, comparable BCFs found for *V. decussata* have also been reported for *Dreissena polymorpha*[30,31] and *C. Callipyga* [23].

For our data, the utility of transplanted *V. decussata* in biomonitoring programmes is evident. The rapid metal accumulation observed in this clams transferred to Mazagon Marina indicated the presence of chemical forms which were readily available for the bivalves. Moreover, this technique may sometimes be useful for the estimation of available pollutants in water when they are present below the analytical DL. In this respect, TBT concentration in water from Mazagon Marina was below DL, but may be estimated if one assumes that BCF obtained in laboratory experiments [10] is also valid in field experiments. Using a BCF of 35900[9] and equation 2, a TBT concentration about 7.5 $ng\ L^{-1}$ was estimated for Mazagon Marina, which may cause sublethal effects in non-target organisms such as calcification inhibition in *C. gigas* [32] or imposex in the dogwhelk *Nucella lapillus* [33].

The size dependence of element concentrations found in this study makes possible the classification of metals in three groups: (i) metals showing increasing concentration with size, implying that the element is accumulated faster than the clam grow, (ii) metals exhibiting concentrations independent of the size, which suggests that excretion equals uptake for these elements; and (iii) metals showing a decreasing concentration with increasing size, due to physiological effects causing differences in metabolic, feeding or filtration rates by age. Given that both metal concentration and kinetic rate constants in *V. decussata* are size-dependent, a limited size range should be chosen for analysis during bio-

monitoring based on the use of natural or transplanted clams. This has also been observed in other species of bivalves such as *C. Gallina* [20], *M. Edulis* [26,34,35], *M. Galloprovincialis* [36], *Saccostrea cucullata* [37], *Isognomon sognomon* [37], *Macoma balthica* [38] and *C. virginica* [39].

The high mortality found in transplanted clams during the experiment may be hardly attributed to the changes in water quality physico-chemical parameters observed between days 2 and 16 because of the resistance of *V. decussata* to low values of salinity for short periods of time [7]. Therefore, mortality may be attributed to the high copper exposure concentration, due to the known high toxicity of this element for bivalves, such as *M. edulis* [40] and *D. Polymorpha* [41]. Stephenson and Taylor [42] exposed *V. decussata* to concentrations of 10 and 100 $\mu g\ L^{-1}$ of Cu and mortality was total after 50 days for the higher concentration (which is only about twice than that found in Mazagon Marina). In addition, copper exposed *V. decussata* were greatly affected by a concentration of 10 $\mu g\ L^{-1}$, which induced a rapid decline of scope for growth during the first 48 h of exposure [19].



## CONCLUSION

*V. decussata* accumulated metals from the surrounding water and the use of this biomonitor in both wild natural populations and transplanting experiments may eliminate the tedious and expensive analysis of water. However, more studies, including laboratory experiments on accumulation and depuration, are needed to establish the real value of this organism to represent the pollutant presence in the environment, obtaining relationships between metal content in their tissues and metal concentration in water, as well as the time necessary to attain metal tissue concentrations in near-equilibrium with seawater.

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