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Evaluation of the effects of the metals Cd, Cr, Pb and their mixture on the filtration and oxygen consumption rates in Catarina scallop, *Argopecten ventricosus* juveniles

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Abstract

In this work, we evaluated the effect of sublethal concentrations (LC_{25}, LC_{10} and LC_{5}) of cadmium, chromium, lead, and their mixture on the filtration rate and oxygen consumption rate of Catarina scallop, *Argopecten ventricosus* (Sowerby, 1842), juveniles, in order to evaluate the use of these biomarkers as a reliable tool in environmental monitoring studies, because these metals have been found at high levels in water and sediments in the Mexican Pacific systems. An inverse dose-response relationship was observed when metal concentration and exposure time increased, the filtration rate and oxygen consumption rate reduced. The physiological responses evaluated in this study were sufficiently sensitive to detect alterations in the organisms at 0.014 mg l^{-1} Cd, 0.311 mg l^{-1} Cr, 0.125 mg l^{-1} Pb and 0.05 mg l^{-1} Cd + Cr + Pb at 24 and 72 hrs. Cd showed the most drastic effect. The Catarina scallop juveniles were more sensitive to Cd, Cr and Pb as compared to other bivalves. The biomarkers evaluated are a reliable tool to carry out environmental monitoring studies.

Key words

Argopecten ventricosus, Filtration rate, Heavy metal effects, Oxygen consumption rates, Scallop

Introduction

In last few decades, development of industrial, agricultural, port and tourism activities and increase in human settlements in coastal zones have generated problems in some local area like Ensenada de La Paz (Méndez *et al*., 1998; Shumilin *et al*., 2001) because they are receiving all types of wastes, which alter the natural balance of ecosystems and contribute to the degradation of the environment (Villanueva and Botello, 1998; Walker *et al*., 2001).

The toxic substances entering the coastal systems can be analyzed using different techniques. The most widely used evaluates the degree of deterioration of the aquatic systems and assess the response of the organisms living there.

In last 20 years, scientists have developed various techniques to evaluate the response of the organisms at cellular or molecular level that live in polluted areas or in specimens that are exposed to some type of contaminant in laboratory tests. These indicators termed as biomarkers identify, quantify and understand the importance of exposure to chemicals in the environment that could cause deleterious effects on species. (McCarthy and Munkittrick, 1996; Broeg *et al*., 2005). The use of biomarkers as signals of deleterious effects on the organisms is based on the fact that the first interaction between a xenobiotic and the organism occurs at molecular-cellular level. Such type of interactions helps to establish a cause-effect relationship (Bayne *et al*., 1981; Handy and Depledge, 1999) determining the mechanism of action of the pollutants and in many cases, predicting the damage caused at other levels. At the organism level, alterations produced by xenobiotic can cause deleterious effects in population, for example change in birth rate, feeding rate and growth rate, and can alter the size and recruitment of organisms, affecting the life cycle of the population. These facts may have future implications at the ecosystem level (Walker *et al*., 2001, Neuberger *et al*., 2007).

Physiological responses such as rate of oxygen consumption, feeding rate and growth rate are considered parameters suitable for environmental monitoring programs because they provide valuable information about the organism´s condition in the systems (Handy and Depledge, 1999; Sze and Lee, 2000).

Catarina scallop (*Argopecten ventricosus*) is an important fishing resource in the State of Baja California Sur, since 95% of the national production is fished in this entity. In last 30 years, production of these mollusks has seen a reduction up to 86% (CONAPESCA, 2012). The cause for this decrease is solely attributed to overexploitation, since there are no environmental studies to determine other probable causes that could have originated the destruction of the banks. *Argopecten ventricosus* is distributed from Isla de Cedros, Baja California and the Gulf of California to Northern Peru (Keen, 1971). This species generally lives in shallow waters of coastal lagoons, inlets or protected bays, on muddy or sandy bottoms. Occasionally they are associated with sea grasses and they feed on particles trapped by filtration in the sediment-water interphase. Both conditions make these organisms ideal to be considered as possible "sentinels" or bioindicators in environmental studies (Sobrino-Figueroa *et al*., 2007).

The objective of present study was to evaluate the filtration rate and rate of oxygen consumption in Catarina scallop juveniles exposed to single and combined treatment of Cd, Cr, Pb, in order to evaluate the use of these biomarkers as a reliable tool for environmental monitoring studies. These metals have been found in elevated levels in water and sediments of Mexican Pacific systems (Villanueva and Botello, 1998; Méndez *et al*., 1998; Shumilin *et al*., 2001).

Materials and Methods

Argopecten ventricosus juveniles commonly known as Catarina scallop (1±0.2 cm high and 3-3.5 months old) were obtained from experimental mollusk cultures at the Universidad Autónoma de Baja California Sur.

Static bioassays were carried out for 72 hr. The scallops were exposed to sublethal concentrations (LC_5, LC_{10} and LC_{25}) of Cd, Cr, Pb and their mixture in 1:1 ratio, (0.04, 0.09, 0.2 mg l^{-1} Cd; 0.34, 0.72, 1.7 mg l^{-1} Cr; 0.09, 0.176, 0.41 mg l^{-1} Pb; 0.03, 0.073 0.15 mg l^{-1} Cd + Cr + Pb respectively). The experiment was carried out in an aquaria (25 l) containing 10 scallops per quintuplicate plus a control group without toxic metals (5 aquariums and 50 organisms per treatment) (APHA, 1994; FAO, 1982; 1987).

The test solutions of $CdCl_2$, (Baker 99% purity), $K_2Cr_2O_7$ (Merk 99.5% purity) and $Pb(NO_3)_2$ (Baker 99% purity) were prepared according to the recommendations of APHA (1994) and FAO (1982, 1987) and concentration of metals were estimated by flame atomic absorption spectrophotometry (Perkin Elmer model 3100) (ASTM, 1994). The conditions during the experiments were: temperature 20 °C, salinity 36±1 psu, dissolved oxygen > 4 mg l^{-1}. The marine water used in these assays was filtered and irradiated with UV light. The organisms were fed with the microalgae *Chaetoceros* sp.

After 24 and 72 hrs of exposure, rate of clearance and respiration rate of treated and untreated scallops were measured. The clearance rate (filtration rate) was estimated indirectly by removal of particles (microalgae *Chaetoceros* sp.) from suspension with an inoculum of 498,079±500 cells ml^{-1}, which had been previously determined to be the most adequate, since there was no formation of pseudofeces (Griffiths and King, 1979).

Each assay was carried out in quintuplicate; two organisms from each treatment (metals and mixture) were taken randomly and placed in 3,000 ml containers with constant bubbling. After applying the microalgal inoculum, each hour (for 3 hr), samples were taken (10 ml, in duplicate) from each container, and fixed in 5% formalin. The cell count was done with an optical microscope (American Optical) using a hemocytometer.

The filtration rate was calculated using Griffiths and King (1979) equation.

$$CR = \frac{V \times (\ln [C_i] - \ln [C_f])}{T}$$

Where CR = Clearance rate (l hr^{-1}). V = Chamber volume (l). C_i = Initial cell concentration (cells l^{-1}). C_f = Final cell concentration (cells l^{-1}). T = Time (hr).

The filtration rate was obtained by normalizing clearance with dry weight of the scallops and was expressed as l h^{-1} g^{-1} d.wt. The oxygen consumption rate was estimated following the method of Baber and Blake (1985) using hermetically sealed chambers (static systems) with a volume of one liter and placing one organism inside, for triplicate of each treatment. The initial and final oxygen levels were measured using an oximeter with a minimum reading of 0.1 ppm, (YSI mod 54). Readings were taken every hour for 3 hr. Oxygen consumption was calculated by the formula given by Cech (1990).

$$O_2 \text{ consumption} = \frac{(O_{2\,fi} - O_{2\,ini})\ V}{\text{Time}}$$

Where, $O_{2\,fi}$ = Oxygen concentration at the final observation time (mg l^{-1}); $O_{2\,ini}$= Oxygen concentration at the initial observation time (mg l^{-1}); V= Chamber volume (l). Time between measurements (hr).

The rate of oxygen consumption was obtained by normalizing oxygen consumption with the dry weight of the scallops and expressed as mg $O_2\,h^{-1}\,g^{-1}$ d.wt. The EC_{50} value was calculated from filtration rate and oxygen consumption rate. EC_{50} (effective concentration 50) is the metal concentration that causes 50% reduction in the filtration and oxygen consumption rates compared to control (FAO 1982, 1987; APHA, 1994).

For bioassays of combined metal treatment, the metal interactions were calculated according to Sprague and Ramsay (1965) equation :

$$T.U. = \frac{(A)\ metal}{A_{CL50}} + \frac{(B)\ metal}{B_{CL50}}$$

where, (A) metal = Concentration of metal A in the mixture (mg l^{-1}). A_{CL50} = CL_{50} value at 96 hr (mg l^{-1}) for metal A. (B) metal = Concentration of metal B in the mixture (mg l^{-1}). B_{CL50} = CL_{50} value at 96 hr (mg l^{-1}) for metal B. T.U. (toxic units) < 1 is considered as synergistic effect; T.U. > 1 antagonic effect; T.U. = 1 additive effect. Magnification factor (M.F.) was determined according to Marking (1977).

$$M.F. = \frac{1}{T.U. - 1}$$

Statistical analyses : The data obtained from bioassays were subjected to an exploratory analysis through Kolmogorov-Smirnov test to determine normality. Data were then analyzed using ANOVA test and a multiple comparison with the Tukey test was also applied to determine the statistical significance between the mean values of control and various metal treatments. A significance level of 0.05 was used for the analysis (Sokol and Rohlf, 2000).

Results and Discussion

Results obtained from the filtration rate assays showed an inverse dose response relationship. The rate of filtration is reduced with increase in metal concentration and exposure time.

After 24 hr of treatment with sublethal concentrations (LC_5, LC_{10} and LC_{25}) of Cd, filtration rate values reduced by 12, 31 and 25%, respectively, as compared to the values obtained from control group (Fig. 1). Cr and Pb treatment also showed a decrease in filtration rate of 14 to 48% in Cr treated scallops and 4 to 21% in Pb treated scallops. The values control and in the tests using metals were significantly different ($p < 0.05$), except for LC_5 of Pb (0.09 mg l^{-1}) where the differences was not statistically significant. Chromium caused severe effects on the filtration rate at 24 hr of exposure (Fig. 1).

Bioassays carried out on juveniles exposed to combined metal treatment for 24 hr showed significant difference between treatments and control ($p < 0.05$). LC_5, LC_{10} and LC_{25} value of combined metal treatment showed a reduction of 9 to 15% in filtration rate (Fig. 1).

At 72 hr of exposure, the filtration rate was reduced by 66% to 87% in Cd, 48% to 73% in Cr and 46% to 71% in Pb treatment, respectively (Fig. 2). Combined metal treatment also showed a reduction of 28% to 64% in filtration rate, without showing any synergistic effects (Table 2, Fig. 2). The EC_{50} value of filtration rate during the tests carried out with metals and their mixture at 72 hr of exposure varied between 0.014 and 0.125 mg l^{-1}. Among all metals, Cd showed drastic effect on the physiological parameter (Table 1). The responses observed in the evaluation of the filtration rate at 24 and 72 hr of treatment were significantly different when compared to control and between different metals in all cases ($p \leq 0.05$), with the exception of control values obtained at 24 and 72 hr of exposure.

The rate of oxygen consumption in Catarina scallops reduced with increase in Cd and Cr concentration and time of exposure except for Pb, where an increase in the rate of oxygen consumption was observed (Fig. 3). Cd treatment showed a reduction in O_2 consumption rate between 8% and 24% (Fig. 3). While Cr treatment showed reduction between 25% and 39% as compared to their respective control (Fig. 3). Conversely, the rate

Table 1 : Ec_{50} and confidence intervals ($p<0.05$) values obtained from the evaluation of filtration rate and oxygen consumption rate in tests with *Argopecten ventricosus* juveniles exposed to metals for 72 hr

Metals	EC_{50} (mg l^{-1})	
	Filtration rate (72 hr)	Oxygen consumption rate (72 hr)
Cd	0.014 (0.002 – 0.028)	0.078 (0.064 – 0.093)
Cr	0.311 (0.1 – 0.47)	0.49 (0.38 – 0.63)
Pb	0.125 (0.065 – 0.174)	0.38 (0.28 – 0.52)
Cd + Cr + Pb	0.05 (0.04 – 0.06)	0.13 (0.11 – 0.16)

Table 2 : Toxicity units, type of interaction, and magnification degree in *Argopecten ventricosus* juveniles exposed to metal mixtures (72 hr) in test to evaluate the filtration rate and the rate of oxygen consumption

Metal mixtures	EC_{50} (mg l^{-1})	Toxicity Units	Type of interaction of the mixture	Magnification
Cd + Cr + Pb Filtration rate	0.05	1.57	Antagonism	0
Cd + Cr +Pb oxygen consumption rates	0.13	0.75	Synergistic	1.32 X

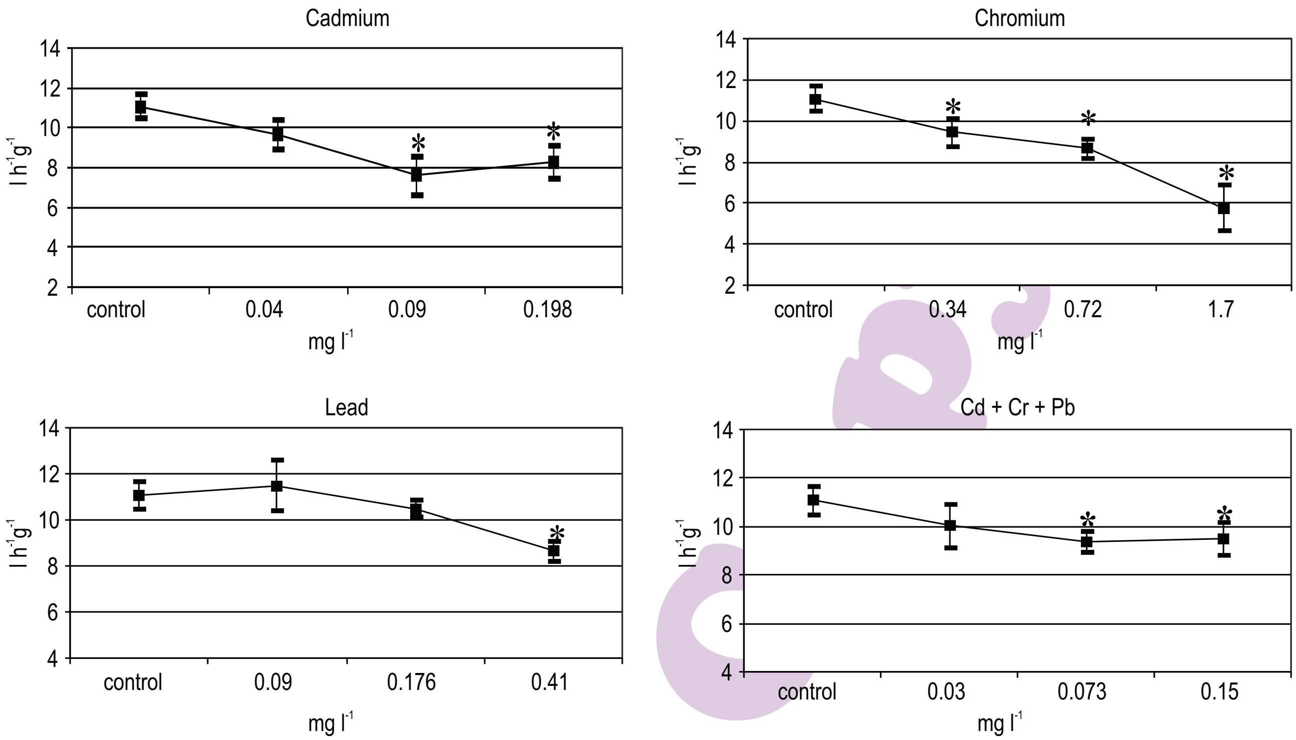

Fig. 1 : Filtration rate (l h^{-1} g^{-1}) of *Argopecten ventricosus* juvenile exposed to toxic metals, Cd, Cr, Pb and their mixture 1:1 for 24 hr. Values are mean of replicate $\pm$ SD; * Significant differences compared with the control ($p < 0.05$)

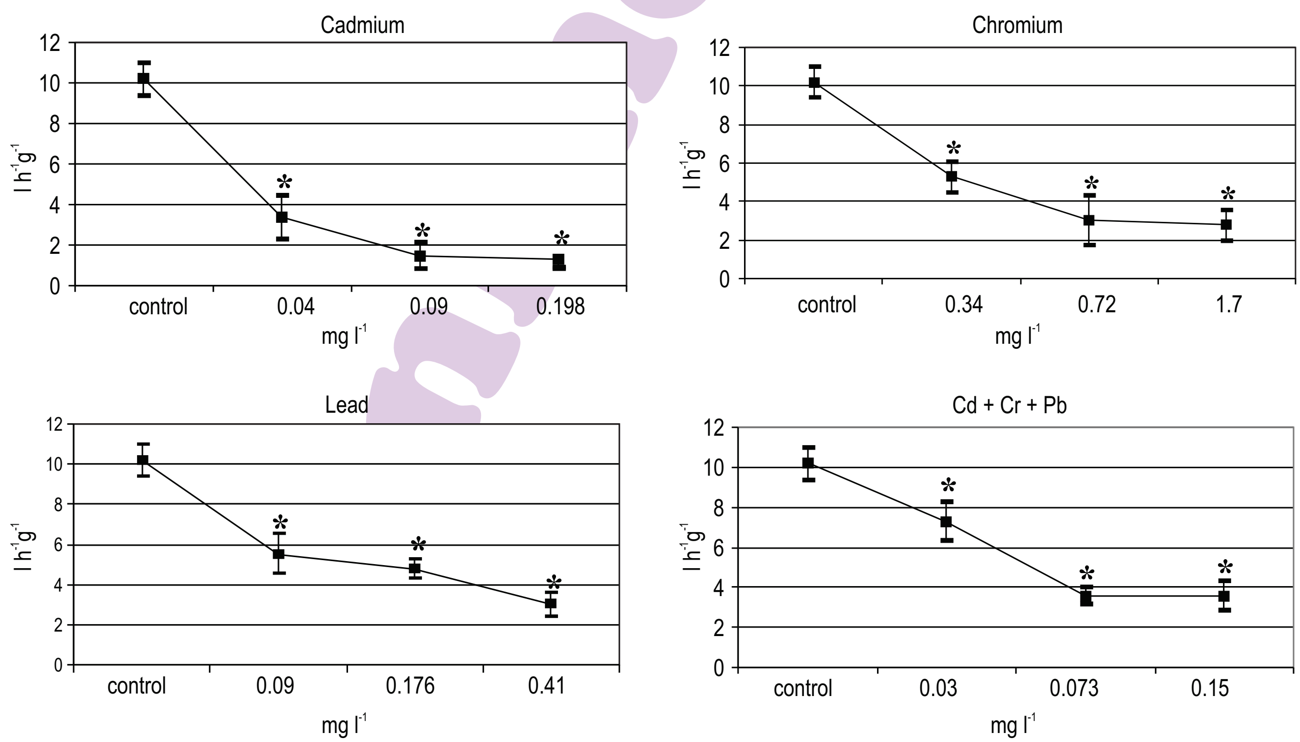

Fig. 2 : Filtration rate (l h^{-1} g^{-1}) of *Argopecten ventricosus* juvenile exposed to toxic metals, Cd, Cr, Pb and their mixture 1:1 for 72 hr. Values are mean of replicate $\pm$ SD; * Significant differences compared with the control ($p < 0.05$)

of oxygen consumption in juveniles treated with Pb showed significant increase ($p < 0.05$) from 130% to 150% as compared to control, after 24 hr of exposure. Combined metal treatment decreased O_2 consumption rate between 8% and 15% (Fig. 3). The rate of oxygen consumption in juveniles exposed to metals for 72 hr was significantly reduced ($p < 0.05$) as compared to their respective control. Cd, Cr, and Pb treatment showed a reduction in the rate of oxygen consumption by 28% to 77%, 40% to 73% and 25% to 48%, respectively (Fig. 4). Combined metal treatment showed a decrease in O_2 consumption rate of 17% to 56% (Fig. 4). There was a synergistic effect due to combined metal interaction at 72 hr of exposure (Table 2).

Significant differences between values the rate of oxygen consumption registered at 24 and 72 hr of metal exposure compared to the control ($p < 0.05$) were observed in all cases, with exception of values obtained for LC_5 and LC_{10} in Pb treatment at 24 hr of exposure.

Moreover, in *A. ventricosus* juveniles the rate of oxygen consumption showed a positive correlation with the clearing rate at 72 hr of exposure ($r^2 > 0.82$; $p < 0.05$). EC_{50} values rate of oxygen consumption calculated at 72 hr of exposure varied between 0.078 and 0.49 mg l^{-1}. Cadmium metal showed the most deleterious effect (Table 1). Bayne *et al*., (1981) and Walker *et al*., (2001), identified a direct relationship between environmental stress and physiological response of the organisms, establishing a cause and effect relationship in a polluted environment which suggests that metabolic processes, such as respiration, filtration rate, and growth are good indicators of the state of the organisms. Furthermore, Martin and Sevareio (1982), Handy and Depledge (1999), and Bodin *et al.,* (2004) observed that there is an inverse relationship between the level of pollutants and the values of physiological functions such as respiration rate and filtration rate.

The filtration rate is intimately linked to the feeding habit of the organisms, thus alterations in this activity directly affects the increase in size and weight of the species. The bioassays carried out on Catarina scallop juveniles showed that the rate of filtration was reduced in function of the increase in metal concentration and time of exposure. Lin *et al*. (1993), Abraham *et al*. (1986), Patel and Anthony (1991), Sze and Lee (2000) and Neuberger *et al*. (2007) observed a decrease in the filtration rate in Japanese oyster, *Villorita cyprinoides*, *Anadara granosa*, *Perna viridis* and bivalve *Donax trunculus*, respectively, exposed to metals.

Kirby and Smith (1972), reported the filtration rate of 10 ± 0.23 l h^{-1} g^{-1} in Atlantic scallop, *A. irradians* under normal conditions. The values recorded in the present study were lower as compared to the values obtained earlier by previously cited researchers.

Watling (1981) determined the EC_{50} of the filtration rate for four species of marine bivalves: *Perna perna*, *Choromytilus*

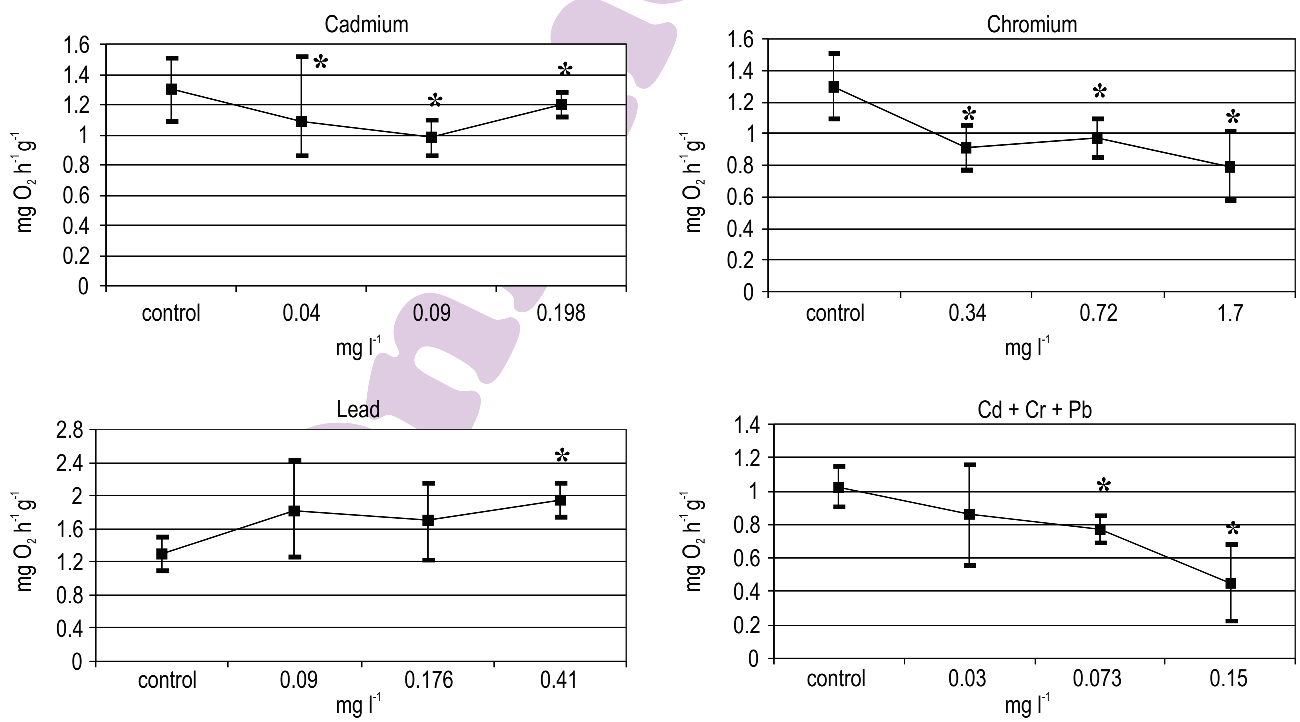

Fig. 3 : Oxygen consumption rate (mg O_2 h^{-1} g^{-1}) in *Argopecten ventricosus* juveniles exposed to toxic metals, Cd, Cr, Pb and their mixture (1:1) for 24 hr. Values are mean of replicate ± SD; * Significant differences compared with the control ($p < 0.05$).

meridionalis, *Crassostrea margaritacea* and *Crassostrea gigas*. The EC_{50} values for Cd varied from 610 to 28000 mg l^{-1} and those for Pb varied from 4170 to 4400 mg l^{-1}, being much larger than the values obtained in the present study (0.014 mg l^{-1} Cd and 0.125 mg l^{-1} Pb), indicating that *A. ventricosus* juveniles are more sensitive to Cd and Pb.

Moreover, the rate of oxygen consumption is considered to be a response that is closely related to changes in the environment and the physiological state of the organism. This rate is a good indicator when the organisms are exposed to any toxic substance, whether experimentally or accidentally. In the present study, juvenile Catarina scallops exposed to Cd, Cr and Pb, showed decrease in the rate of respiration with increase in time of exposure to these metals. Similar findings have earlier been reported by Cheung and Cheung (1995) in *Perna viridis* exposed to cadmium concentrations between 0.15 and 1 mg l^{-1}. Patel and Anthony (1991), Hon-Cheng and Tzong-Shean (1994) and Sze and Lee (2000) observed that sublethal concentrations of silver, cadmium, lead, mercury, copper and zinc acted as respiratory depressors in bivalve mollusks.

Furthermore, the tests carried out on *A. ventricosus* juveniles showed that the rate of oxygen consumption had a positive correlation with the clearing rate at 72 hr of exposure. This fact probably indicates a reduction in feeding, likely causing a lethargic effect (slower metabolism) and thus, a lower demand for oxygen. Similar result was earlier reported in *Perna viridis by* Sze and Lee (2000).

The variation in the values obtained for filtration rate and oxygen consumption in bioassays carried out on Catarina scallop juveniles may be related to the defense mechanisms of these organisms in response to metal exposure. These responses included closing of the valves, mucous secretion and changes in the movement of cilia in the gills (Viarengo et al., 1996; Sze and Lee, 2000; Sobrino-Figueroa and Cáceres, 2009)

Gills are the primary site of attack for toxic substances. Lesions in the gill structure due to metal exposure have been previously reported (Sunilla, 1988; Sze and Lee, 2000). The optical microscope inspection at the end of bioassay showed lesion in the gills consistent with a reduction in their size and loss of apical cilia of the gill cells. Since these structures helps to trap food particles for feeding the scallops, it is logical to suggest that damage to these structures will reduce the feeding capabilities in these organisms.

The physiological parameters evaluated in this study were sufficiently sensitive to detect alterations in the response of the organisms exposed to metals. The concentration of metals used in this study was lower than those prescribed in NOM-001-SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales) which establishes concentrations of 0.1 to 0.2 mg l^{-1} of Cd, 0.5 to 1.0 mg l^{-1} of Cr and 0.2 to 0.4 mg l^{-1} of Pb in residual water discharge to national waters and assets.

It can be concluded that Catarina scallop juveniles were more sensitive to sublethal concentrations of Cd, Cr and Pb as

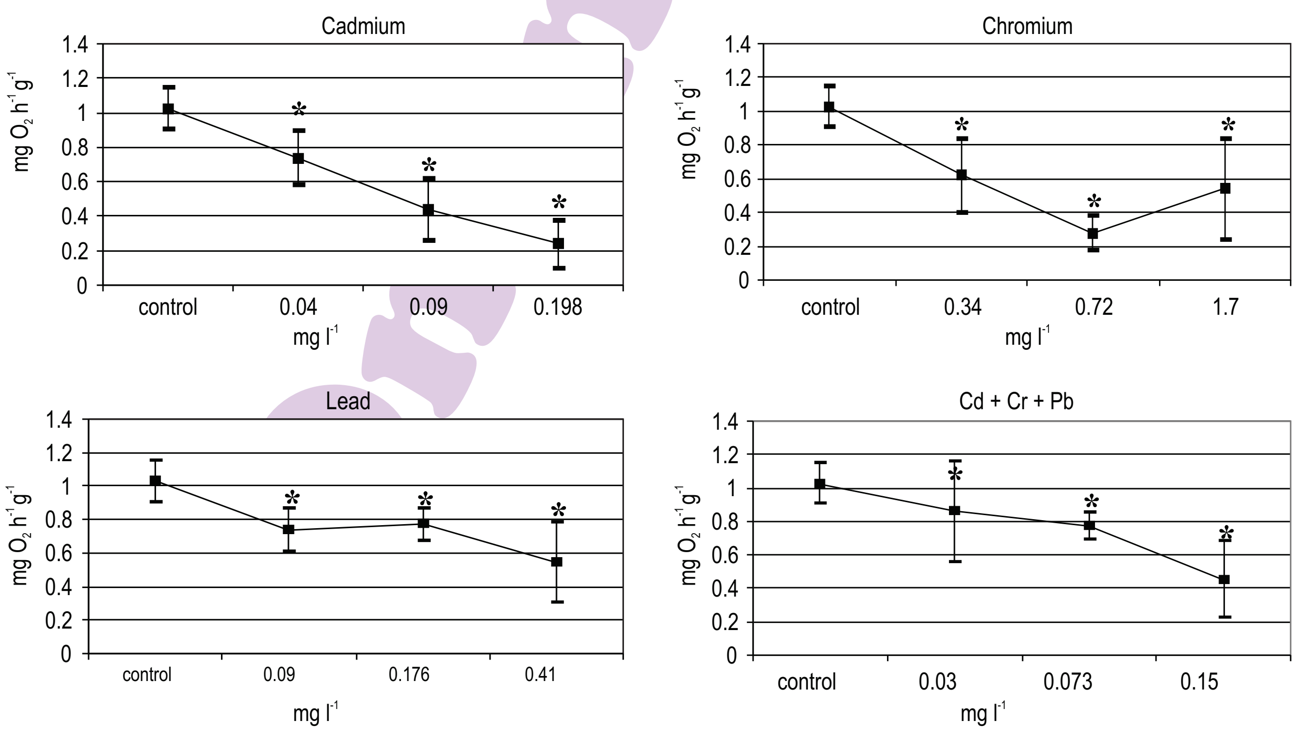

Fig. 4 : Oxygen consumption rate (mg O_2 h^{-1} g^{-1}) in *Argopecten ventricosus* juveniles exposed to toxic metals, Cd, Cr, Pb and their mixture (1:1) for 72 hr. Values are mean of replicate ± SD; * Significant differences compared with the control ($p < 0.05$).*

compared to other bivalves, and the physiological responses studied are a good tool to evaluate the response of these organisms to metal exposure for environmental monitoring studies.

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