Effects of Lead in white shrimp (*Litopenaeus schmitti*) metabolism regarding salinity[#]

Efeitos do Chumbo no metabolismo do camarão branco (*Litopenaeus schmitti*) em relação à salinidade

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Abstract

Penaeid shrimps are important resources for worldwide fisheries and aquaculture. In Brazil, *Litopenaeus schmitti* is an important commercially exploited species, and the ideal animal for studying the impairment caused by the effects of heavy metals often detected in coastal areas. The main purpose of the present study was to detect the acute toxicity of lead (Pb) in *L. schmitti* and to investigate its effect on oxygen consumption and ammonia excretion for different salinities. This has not been studied in this species before. Lead was significantly more toxic at salinity 8 than at 20 and 33. The oxygen consumption and ammonia excretion were estimated through experiments performed on each of the fifteen possible combinations of three salinities (33, 20 and 8), at the temperature of 21 °C. Cadmium showed a significant improvement in oxygen consumption at salinity 8, and results show that the oxygen consumption increases with respect to the lead concentration. At the highest lead concentration employed (2.12 10^{-2} mg/L), the salinity 8 and the temperature at 21 °C, oxygen consumption increases 131% in relation to the control. In addition, after separate exposure to lead, elevation in ammonia excretion was obtained, which was 88.2% higher than the control. The results show that lead is more toxic to *L. schmitti* at lower salinities.

Keywords: *Litopenaeus schmitti*. Lead. Oxygen Consumption. Ammonia. Salinity.

Resumo

Os camarões penaeus são importantes recursos da indústria de pesca mundiais e da aquacultura. No Brasil, *Litopenaeus schmitti* é uma espécie importante comercialmente explorada e o animal ideal para estudar o prejuízo causado pelos efeitos de metais pesados frequentemente descobertos em áreas costeiras. O objetivo principal do presente estudo foi descobrir a toxicidade aguda do chumbo (Pb) em *L. schmitti* e investigar o seu efeito sobre o consumo de oxigênio e a excreção de amônia em diferentes salinidades. Não se estudou esse efeito nessa espécie antes. O chumbo foi significativamente mais tóxico na salinidade 8 do que 20 e 33. O consumo de oxigênio e a excreção de amônia previram-se por meio de experimentos executados em cada uma de quinze combinações possíveis de três salinidades (33, 20 e 8), na temperatura de 21 °C. O cádmio mostrou uma melhora significativa no consumo de oxigênio na salinidade 8, e os resultados mostram que o consumo de oxigênio aumenta com respeito à concentração de chumbo. No momento da concentração de chumbo mais alta empregada (2.12 10^{-2} mg/L), a salinidade 8 e a temperatura em 21 °C, o consumo de oxigênio aumentou 131% em relação ao controle. Além disso, depois da exposição separada ao chumbo, verificou-se que o chumbo é mais tóxico para *L. schmitti* na salinidade mais baixa.

Palavras-chave: *Litopenaeus schmitti*. Chumbo. Consumo de Oxigênio. Amônia. Salinidade.

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INTRODUCTION

Erroneously referred to as "heavy metals" or "trace metals", these elements form an integral part of the environment and living matter naturally present in small concentrations[1]. Some of them stand out because they are essential to living organisms, though in minimum quantity; other trace elements, such as lead, do not have any known biological functions and its effects upon biota elements are usually deleterious[2].

Lead occurs as an environmental contaminant in a wide range of chemical and physical forms which strongly influence its behavior and effects on the ecosystem being assimilated by organisms in the form of ions as much as in the form of complex. The presence of this metal in marine environment occurs as a result of processes such as erosion and soil leaching and they still appear ubiquitous from the precipitation of lead dust from the atmosphere due to its extensive use in paint industries, drums, pipe and additives in petroleum products and their environmental concentration varies from location to location[3,4].

In its inorganic form lead is present in different oxidation states (0, I, II e IV), from which Pb^{2+} is the most stable form among the ionic species, which leads to be thought of as the form in which this element is bioaccumulated by aquatic organisms[2,3]. Similarly, lead organic compounds known as neutral organometallic compounds, such as tetraethyl lead and tetramethyl lead, tend to be bioaccumulated by organisms because they are lipophilic (property that facilitates its movement through biological membranes)[2].

Zagatto and Bertoletti[2] state that the toxicity of this metal ion is mainly due to its ability to infer in the metabolism (blocking, shifting the essential ion or modifying the active conformation) and also to its low mobility (they become gradually insoluble as the molecular mass increases) which causes them to build up, deeply modifying the metabolism of the organism. Jackson, et al[3], reported that lead seems to be metabolized through the calcium channels and thus accumulates in skeletal tissues.

In recent years, several studies such as by Barbieri, et al[5,6,7,8,9], have been conducted regarding the bioaccumulation of metals to determine toxicity in many species of organisms. In the case of shrimp, in addition to being a benthic species with non-pelagic larval stage, this organism is also considered great for toxicity studies[10] especially for high availability throughout the year, relatively low cost without counting on ease of handling, and also a great sensitivity to contaminants which carries great reliability and accuracy of the assessment of response to the pollutant even when working in short periods of exposure[3]. They are considered to be good water quality indices, with the ability to reflect the environmental deterioration before it reaches the population or community.

Bioenergetic changes are among the most sensitive indicators of pollution stress[7]. Exposure to metals in the aquatic environment produces many physiological changes in crustaceans, including changes in metabolic activity. An organism's respiratory rate (amount of energy released from the oxidation of the substrate feed) is a sensitive and useful measure of their daily energy consumption[6]. Changes in oxygen consumption as a result of exposure to sub-lethal pollutants can affect the crustaceans' ability to feed, escape from predators, or even reproduce. Therefore, based on the values of the oxygen amount consumed by an animal for a certain period of time, it is possible to evaluate the energy spent during the same period to keep its vital processes and thus infer on assimilation when it is exposed to a contaminant[8].

Another way to evaluate the physiological behavior is the measure of ammonia excretion. Most of the aquatic invertebrates excrete ammonia as an end product of protein metabolism. Due to possessing high solubility and small molecular weight, the ammonia diffusion is very fast and can be lost not only necessarily by the kidneys, but also across the body surface in contact with the water[11]. This excretion is a percentage of the organism's energy losses and also represents part of the contribution of aquatic invertebrates in nutrient recycling in aquatic environments[9].

Temperature and salinity are considered to be the main abiotic factors that regulate the bioavailability of a contaminant in the water. On shrimp, due to being poikilotherm, the metabolic rate varies directly along with the change of the ambient temperature. On the other hand, salinity changes influence on metabolic rate as a result of a series of amendments on physico-chemical (desorption and solubility) and physiological (osmoregulation, membrane permeability) processes and it may also exist a complex interaction between these two variables, with one acting as a modulation factor upon the effect of another[12,13].

Therefore, through observation of the lesser adverse effects it is possible to establish the tolerable exposure level, in other words, before observing drastic demonstrations of the toxicity's effect it is possible to evaluate the exposure conditions through behavioral changes by using biotic factors (physiological condition) and controlling the abiotic factors (temperature, salinity and metal concentration).

This work's hypothesis is that with the increase in salinity, a sequacious increase of lead toxicity would happen, revealed by the change of the oxygen consumption and ammonia excretion.

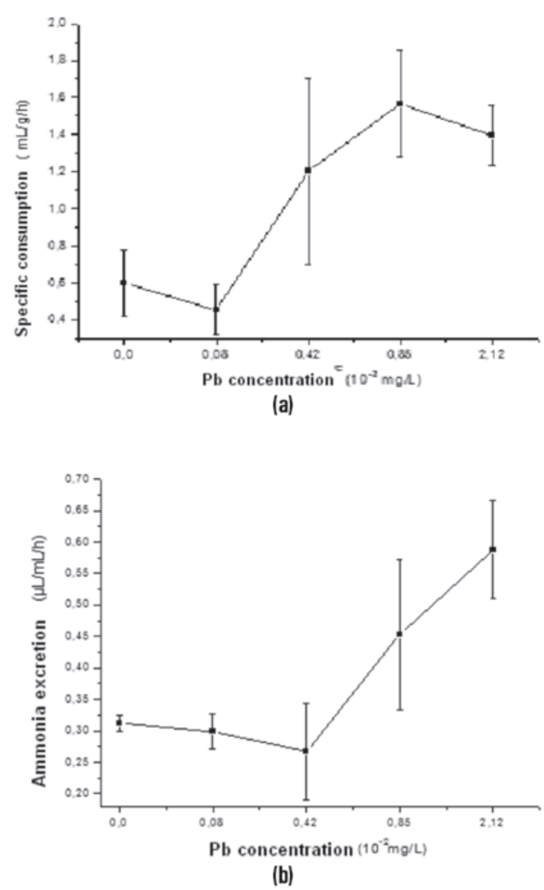
METHOD

On this experiment 60 white shrimp (*Litopenaeus schmitti*) with mass approximately 8.25 g and 1.25 g collected in the Iguape-Cananeia Lagoon complex were used. The shrimp specimens were acclimatized in the salinity of 30‰ to 20 ºC with constant aeration, cleaning and daily renewal of the water for a period of 5 days prior the experiments. For the purpose of measuring routine metabolism three replicas were used in each concentration at salinities 8‰, 20‰ and 30‰ with varying temperature between 21 ºC and 22 ºC. These salinity values are within the range to which white shrimp is subject during their life cycle. Four different concentrations were tested, in addition to a control (0.08, 0.42, 0.85 and 2.12 10^{-2} mg of Pb/L). The choice of the Lead Chloride concentration used in this study was based on data obtained in previous ascertainment of lethal concentration at 50% (LC50). Before starting the experiment, the animals were kept individually in respirometric tubular chambers with continuous water circulation for 60 minutes in order to minimize the stress caused by handling. At the end of the acclimation period Lead Chloride ($PbCl_2$) was separately introduced in an amount determined in respirometers with a precision pipette in order to achieve the desired final concentration. In order to finding out the exact concentration of dissolved lead in experiments, an analysis was done through chromatography with atomic absorption. The difference between the concentrations determined at the beginning and end of the confinement (after 30 minutes) were used to calculate the oxygen consumption (mL/g/h) and ammonia excretion (µl/mL/h) taking into account the respirometer volume and the animal's wet weight. The dissolved oxygen was determined through the Winkler method and the ammoniacal nitrogen based upon the phenolhypochlorite method[14]. Average oxygen specific consumption and ammonia excretion by shrimp were evaluated through variance analysis.

RESULTS

For shrimp acclimatized at salinity 8‰ oxygen specific consumption increased with the increment of lead concentration. The average oxygen specific consumption of shrimp exposed to 0.08 and 2.12 x 10^{-2} mg Pb/L was 1.57 and 1.40 mL of oxygen/g/h, respectively (Figure 1a).

Figure 1. Variation of Specific Consumption (a) in milliliters of oxygen per gram per hour and variation of ammonia excretion (b) in micro liters of ammonia per milliliters per hour for white shrimp acclimatized at salinity 8‰ with temperature varying between 21 °C and 22 °C depending on the exposure to different concentrations of Lead Chloride ($PbCl_2$) during 30 minutes (n = 3)



These values represent an increment of 159% and 131% in relation to control (Table 1). Using the Tukey statistical test ($p < 0.05$), it was found that the average oxygen specific consumption at the concentration of 2.12 10^{-2} mg/L of Lead, on the used salinity is significantly different in relation to the respective control.

Table 1. Oxygen Specific Consumption (OSC) in milliliters of oxygen per gram per hour for white shrimp acclimatized to different salinities in temperature ranging between 21 and 22 °C, depending on the exposure to different concentrations of Lead Chloride ($PbCl_2$) during 30 minutes. Standard Deviation (SD) and percentage relative to the control are also shown. Each value represents an average of 3 determinations

	Lead Concentration (10^{-2} mg/L)														
	0.0			0.08			0.42			0,85			2.12		
Salinity	**OSC**	**SD**	**%**	**OSC**	**SD**	**%**	**OSC**	**SD**	**%**	**OSC**	**SD**	**%**	**OSC**	**SD**	**%**
8‰	0.603	0.176	100	0.457	0.134	24.2*	1.20	0.503	99.0	1.56	0.287	159	1.39	0.162	131
20‰	2.59	0.531	100	2.75	0.915	6.20	2.10	0.460	18.9*	1.35	0.348	47.9*	1.10	0.557	57.5*
30‰	1.74	0.592	100	1.48	0.386	14.9*	1.85	0.381	6.30	0.827	0.186	52.5*	0.757	0.531	56.5*

* Represents the decrease in metabolic rate relative to the control.

Ammonia excretion of shrimp acclimatized to salinity 8‰ varied depending on the increase of lead concentration, more evident in higher concentration values (Figure 1b). At the highest lead concentration (2.12 10^{-2} mg/L) used, the average excretion reached 0.588 µL/mL/h, showing increase of ammonia excretion of 88.2% in comparison with the average value of animals of the control group (Table 2). By using the Tukey statistical test ($p < 0.05$), it was found that the average ammonia excretion at the highest concentration used is significantly different in relation with to the control.

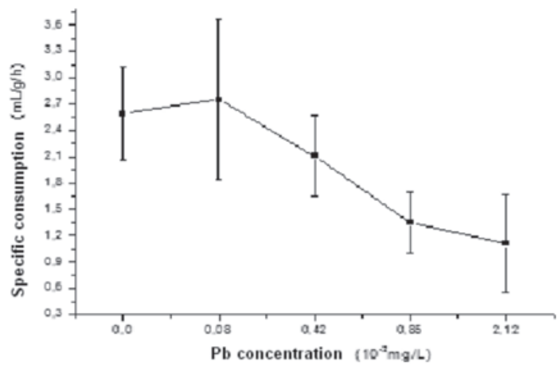
Table 2. Specific excretion (SE) in microliters of ammonia per milliliters per hour for the white shrimp acclimatized to different salinities in temperature varying between 21 and 22 °C, depending on the exposure to different concentrations of Lead Chloride ($PbCl_2$) during 30 minutes. Standard deviation and percentage relative to the control are also shown. Each value represents an average of 3 determinations

	Lead Concentration (10^{-2} mg/L)														
	0.0			0.08			0.42			0,85			2.12		
Salinity	**SE**	**SD**	**%**	**SE**	**SD**	**%**	**SE**	**SD**	**%**	**SE**	**SD**	**%**	**SE**	**SD**	**%**
8‰	0.312	0.013	100	0.299	0.027	4.30*	0.267	0.077	14.5*	0.453	0.119	45.0	0.588	0.078	88.2
20‰	0.246	0.114	100	0.245	0.035	0.30*	0.148	0.023	39.7*	0.094	0.036	61.7*	0.084	0.033	66.0*
30‰	0.152	0.100	100	0.049	0.022	67.8*	0.189	0.006	24.3	0.081	0.007	47.0*	0.100	0.072	34.4*

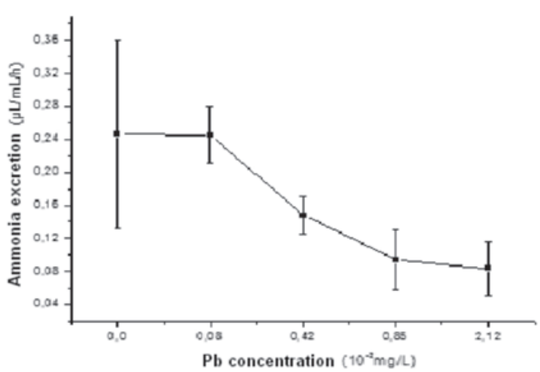
* Represents the decrease in metabolic rate relative to the control.

On shrimp acclimatized to salinity 20‰, unlike what happened to salinity 8‰, the oxygen specific consumption declined as there was an increase of lead concentration. The results presented at Figure 2a clearly show the inhibitory effects on oxygen consumption caused by increased lead concentration. During 30 minutes of experiment the levels of oxygen consumption of the shrimp exposed to 0.08 and 2.12 10^{-2} mg Pb/L were 47.9% and 57.5% lower than control, respectively (Table 1). By using the Tukey statistical test ($p < 0.05$), it was noted that the average oxygen specific consumption at the lead concentration of 2.12 10^{-2} mg/L, at the used salinity, it is significantly different in relation with to the control.

Figure 2. Variation of Specific Consumption (a) in milliliters of oxygen per gram per hour and variation of the ammonia excretion (b) in micro liters of ammonia per milliliters per hour for the white shrimp acclimatized to salinity of 20‰ with temperature varying between 21 and 22 °C depending on the exposure to different Lead Chloride concentrations ($PbCl_2$) during 30 minutes (n = 3)



(a)

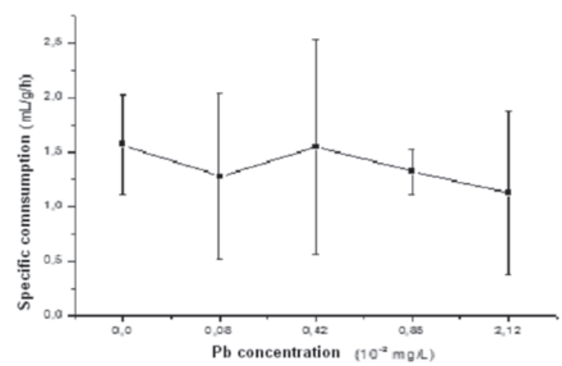


(b)

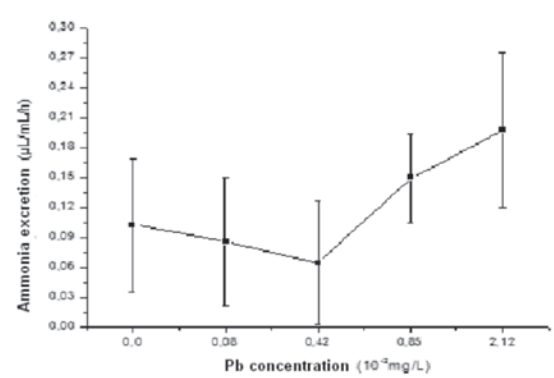
The ammonia excretion of shrimp acclimatized to salinity 20‰ accompanied the decrease of oxygen consumption observed on the same salinity, therefore it decreased due to increased lead concentration, more evident in higher concentration values (Figure 2b). At the highest lead concentration (2.12 10^{-2} mg/L) used, the average excretion reached 0.084 µL/mL/h showing decrease of ammonia excretion of 66.0% in relation with to the control (Table 2). By using the Tukey statistical test ($p < 0.05$), it was noted that the average ammonia excretion at the highest concentration used is significantly different in relation with to the control.

On shrimp acclimatized to salinity 30‰ the oxygen specific consumption generally decreased with the increment of lead concentration, the consumption at to salinity 30‰ lower than consumption at salinity 20‰. We must take into account that the increased salinity promotes decreased water solubility of oxygen, which can contribute to a more evident decrease (Figure 3a). The average oxygen specific consumption of shrimp exposed to 0.08 and 2.12 10^{-2} mg Pb/L was 0.827 and 0.757 mL of oxygen/g/h, respectively.

Figure 3. Variation of specific Consumption (a) in milliliters of oxygen per gram per hour and variation of the ammonia excretion (b) in micro liters of ammonia per milliliters per hour for the white shrimp acclimatized to salinity of 30‰ with temperature varying between 21 °C and 22 °C depending on the exposure to different concentrations of Lead Chloride ($PbCl_2$) during 30 minutes (n = 3)



(a)



(b)

These values represent a decrease of 52.5% and 56.5% compared to the control (Table 1). By using the Tukey statistical test ($p < 0.05$), it was noted that there are no significant differences between the average do the oxygen specific consumption in relation with to the control. On the ammonia excretion of shrimp acclimatized to salinity 30‰, in higher concentrations an increase of excretion was observed (Figure 3b), these values not being significantly different in relation with to the control.

DISCUSSION

Absorption and retention of lead in the respiratory tract are related to several factors, and among others, one can mention the salinity, hygroscopicity, concentration, larval stage, respiratory rate and exposure duration[8,9]. Studies on the effect of other toxic against crustaceans' respiration show that both decrease as the increase in oxygen consumption rate is directly related to the factors mentioned above. The Airways are an excellent entrance of toxic substances to aquatic organisms, especially for organisms that, throughout their life cycle, migrate from one environment to the other causing regular changes in oxygen consumption to maintenance of homeostasis[15,16].

The increment of respiratory rate can be caused both by the increase of lead concentration (Figure 1a) as by the increase in salinity due to the osmoregulation process (Figures 1a and 2a – controls). It should be noted that this was a laboratory experiment using sea water with only two parameters involved, namely lead concentration and salinity. In nature, all the development stages of this animal are exposed to many other potentially dangerous contaminants and different abiotic conditions, in other words, these metabolic wastes could increase even more, causing changes in survival rate, influencing more steeply in growth, reproduction, ability to escape, etc.

It is known that the increase of salinity, in addition to decreasing the solubility of the metal, also promotes decreased water solubility of oxygen, and the response observed regarding crustaceans is the decrease in respiratory rate. The present experiments show the decrease in respiratory rate as we incited the increase of salinity, regardless of the shrimp being exposed to the presence of the metal (Figures 1a, 2a and 3a – controls). The crustaceans, in spite of having an excellent ability to regulate their oxygen consumption patterns[6] decreased their rate at 57.5% when exposed to the highest lead concentration (Figure 2a).

Studies on the effects of mercury toxicity on the crab *Eriocheir sinensis*, showed that there was an increase in toxic effects at low salinities on this species[17]. The authors report that mercury interacts with the osmoregulation mechanism preventing the animal's ability of osmoregulation. For the gastropod *Thiara tuberculata* exposed to heavy metals (mercury and copper), the increase of salinity led toxicity to decrease, reflected by the decrease of oxygen consumption[18], the same found on this study for *L. schmitti,* regarding lead. Hall e Anderson[19] made a review analyzing the influence of salinity on toxicity of many classes of chemical substances, finding a toxicity decrease as salinity increased. Studies of CL50 done with *Cyprinodon variegatus*, in 96 hours of exposition to Cadmium (Cd^{+2}) at Chesapeake Bay on threes salinities, (15‰, 20‰ and 25‰), had shown that the higher the salinity, the lower is the Cadmium's toxic effect on fish[20], the same occurring on this study with shrimp. In our work with lead, significant toxicity changes with the salinity decrease were found.

The decrease in oxygen consumption may be related to cytological damage because the branchiae are probably the first target when it comes to contamination by metals, which includes thickening of bronchial epithelium and profound changes in the patterns of haemolymph in the branchiae, with concomitant increase in haemolymph vacuolation and reduced spaces, causing stagnation and perfusion[6].

The relation between effect's intensity, concentration and exposure time, depends on the life stage and health conditions of the organism at risk. It is known that due to the larvae being particularly sensitive, their exposure to potentially toxic substances must be avoided since the effect of contamination by metals may very well represent lethal consequences for the development stages of estuarine biota. Jackson, et al[3], studied the effect of lead on the larval development of crustacean *Collianassa kraussi* at different salinities and showed that larval mortality was always greater in salinities less than 20 and 24 than at higher salinities of 30 and 35 (units). In the case of white shrimp, it is possible to say that he is "naturally" protected since its life cycle begins in highly saline environments. In the present study, at higher salinities the toxic effect of lead was practically null, even for the highest concentration (2.12 10^{-2} mg of Pb/L). In other words, there were not any significant differences between the average of the control animals and those who were exposed to the highest concentration of lead.

It is important to remember that, individually or in combination, the metal ions pose a great risk to the environment and are considered to be one of the most important factors of pollution of coastal waters. Since lead is naturally on high concentrations at region studied (21, 22) this fact is definitely a reason of concern so it is needs to study its combined effect with other metals in future investigations. Jackson, et al[3], reported the synergistic effect between lead and zinc in *Collianassa kraussi* showing that 50% of the mortality was inflicted upon eggs and larvae in lower concentrations when compared to lead and zinc when separated.

Eysink, et al[21], evaluated the levels of contamination by heavy metals and organochlorine pesticides of the water and the sediment of river Ribeira and the Iguape-Cananeia Estuarine Lagoon Complex and noted that lead exceeded in up to 550 and 100 times the recomended limit for water and sediment, respectively. Even in the absence of evidence with respect to biomagnification along the trophic chain[3,21,22] it is known that a great variety of organisms aquatic might assimilate e accumulate high lead concentrations, with the residence time related to the absorption stage, in the case of shrimp for example, as of the post larval (average size between 6 and 7 mm of length), they start to show bentonic habits, in other words, during a great deal of time they remain buried in the sediment running the food hunt[23].

Ammonia is one of the final catabolism products, mainly of amino acids. These, in addition to being used in skeleton components and body structure, might even be more important than ions in maintenance of the osmotic pressure on shrimp[8,9]. The increase in ammonia excretion at low salinities reflects in increased catabolism of amino acids (Figure 1b) even when shrimp were exposed to the highest lead concentrations.

The influence of salinity on the bioaccumulation of metals is more complex and specific to each element. In general it has been recorded that there is greater bioaccumulation for metals when salinity decreases, however, the opposite can also occur[2,24]. In salinity 20‰ there was inhibition of ammonia excretion on shrimp exposed to sub lethal lead concentrations (Figure 2b). Although there are not any confirmed evidences, we can assume that decrease in ammonia excretion in the presence of lead can be attributed to a reduction of metabolic rate (Figure 2a) or even to interaction between lead and ammonia production. According to Zagatto and Bertoletti[2] the lead assimilation has the tendency to rise with the salinity's increase, but the differences found on this study might be related to the lead concentrations used, the shrimp species, and other abiotic factors such as salinity and temperature. However, many studies still need to be conducted to determine the relationship between exposure to heavy metals and ammonia excretion.

CONCLUSION

Variations in the shrimp metabolism according to the lead concentrations at the three salinities employed followed as tendency to metabolic rate. In the experiment conducted, at salinity 8‰, toxicity increased expressed with the increased oxygen consumption, in other words, speeding the contaminant's assimilation. There was a significant direct relationship with salinity: as salinity decreased, toxicity of the lead employed in this study has increased.

The hypothesis that with increasing salinity, would therefore happen an increased toxicity of metal[2,13] revealed by oxygen consumption and ammonia excretion, was not proved, on the contrary, with the salinity increased, there was a decrease of toxicity.

The database available in the literature, together with those obtained in this study show that the quantification of the effects of pollutants on their metabolic rates might be a very safe way to monitor environmental quality before profound deleterious effects occur. The methodology employed in this study provides reliable data for monitoring environmental quality in addition to providing grants to study the shrimp's ecophysiology and ecotoxicology.

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