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BIOACCUMULATION OF METALS AND EVALUATION OF GOLDEN MUSSELS ENCRUSTED ON DIFFERENT SCREENS OF NET CAGES*

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ABSTRACT

This study aimed to evaluate the characterization and bioaccumulation of metals of the golden mussel encrusted in the screens of net cages installed in the reservoir of Itaipu Binacional. The experimental design was completely randomized, composed of two types of screens (PVC and Bezinal) distributed on four sides (Sides I, II, III, and IV) of the net cage. The total mass, the average mass, and the dimensions (width, height, and length) of the mollusks were evaluated. The mussels adhered to the Bezinal screen presented a higher average mass, length, height, and width than those colonized on the PVC screen ($p < 0.05$). However, the PVC screen provided a greater ($p < 0.05$) total mass of encrusted mussels. Regardless of the screen used, the average mass and length of mussels had higher values on Side I ($p < 0.05$) than to the Sides II and IV, but not different from Side III. The mussels evaluated showed high levels of metals, and those encrusted in the Bezinal screen presented higher levels of Al, Zn and Cr than the individuals on the PVC screen ($p < 0.05$). The use of a Bezinal screen is more efficient than a PVC screen, as an antifouling material. Mussels with shorter lengths had a higher incidence on the PVC screen. The mussels encrusted in the Bezinal screen bioaccumulate higher content of Al, Zn, and Cr. The type of screen used in the net cages influences the mass and size of adhered mussels, as well as can interfere with the metal accumulation in the golden mussel.

Keywords: aquaculture area; *Limnoperna fortunei*; heavy metal; antifouling screens.

BIOACUMULAÇÃO DE METAIS E AVALIAÇÃO DO MEXILHÃO-DOURADO INCRUSTADO EM DIFERENTES TELAS DE TANQUES-REDE

RESUMO

Objetivou-se avaliar a caracterização e a bioacumulação de metais do mexilhão-dourado incrustado nas telas de tanques-rede instalados no reservatório da Itaipu Binacional. O delineamento experimental foi inteiramente casualizado, composto por dois tipos de telas (PVC e Bezinal) distribuídas em quatro lados (Lados I, II, III e IV) do tanque-rede. Foram avaliadas a massa total, a massa média e as dimensões (largura, altura e comprimento) dos moluscos. Os mexilhões aderidos na tela Bezinal apresentaram maior massa média, comprimento, altura e largura do que aqueles colonizados na tela PVC ($p < 0,05$). No entanto, a tela PVC proporcionou uma maior ($p < 0,05$) massa total de mexilhões incrustados. Independente da tela utilizada, a massa média e o comprimento de mexilhões apresentaram maiores valores no Lado I ($p < 0,05$) em relação aos Lados II e IV, mas não diferindo do Lado III. Os mexilhões avaliados apresentaram níveis elevados de metais, sendo que os incrustados à tela Bezinal revelaram teores mais elevados de Al, Zn e Cr do que os indivíduos na tela PVC ($p < 0,05$). O uso da tela Bezinal é mais eficiente em relação à tela PVC como material anti-incrustante. Os mexilhões com menores comprimentos tiveram uma maior incidência na tela PVC. Os mexilhões incrustados à tela Bezinal bioacumulam maior teor de Al, Zn e Cr. O tipo de tela utilizado nos tanques-rede influencia na massa e tamanho dos mexilhões aderidos, bem como pode interferir na acumulação de metais no mexilhão-dourado.

Palavras-chave: área aquícola; *Limnoperna fortunei*; metal pesado; telas anti-incrustantes.

INTRODUCTION

The fish cultivation in net cages in hydroelectric power plant reservoirs is an intensive system that has contributed to increasing national aquaculture production. However, one of the problems found in this fish production system in artificial reservoirs is the inlay of golden mussel *Limnoperna fortunei* (Dunker, 1857) on the screens of the net cages. Materials such as wire, galvanized wire coated with PVC, and steel are generally used to make these screens (Oliveira et al., 2014; Godoy et al., 2018).

The *L. fortunei* is a bivalve of heteromyarian external morphology, shows fine fibers (byssus), which adhere to various solid structures that develop agglomerates, reaching about 4 cm in length (Pestana et al., 2010; Canzi et al., 2014). The species has a high degree of dominance because of its invasive potential, due to its characteristics of high prolificacy, easy dispersion, colonization, and stabilization in different environments, causing environmental and economic losses. Currently, *L. fortunei* can be considered the freshwater mollusk that causes enormous economic and environmental damage in the South American continent (Ávila-Simas et al., 2019).

The golden mussel a highly invasive species in Brazil and has caused productive, economical, and biological impacts in reservoirs (Furlan-Murari et al., 2019). Cage fouling increases production costs as they reduce the durability of structures and require frequent cleaning of the screens. Furthermore, the fouling blocks water exchange from net cage and biologically analyzing can be a source of contamination or pollution for aquaculture in reservoirs (Costa et al., 2018; Freire and Marafon, 2018; Vianna et al., 2019). Rezende Ayroza et al. (2019), when investigating the cage fish farm as a vector for golden mussel invasion, concluded that fish breeding in cages contributes to the establishment of the *L. fortunei* in reservoirs by elevating larval density and the nutritional status of the mollusk. The colonization of this freshwater bivalve mollusk in the reservoir varies during the year in response to seasonality.

The first appearance of the golden mussel in the Itaipu reservoir was found inside the Itaipu Binacional hydroelectric power plant, in April 2001. Two to three individuals per m^{-2} were seen, with a length ranging from 0.6 to 3.5 cm, showing that the mollusk has been in the environment for over a year (Canzi et al., 2014).

The entry of exotic species into ecosystems, especially aquatic ones, can lead to biodiversity losses, mainly due to losses of native species and imbalances in the natural ecosystem (Tokumon et al., 2018). Due to the problems that are invading mollusks causing in aquatic ecosystems and industrial and productive activities, the number of studies covering the characterization and survey of these invasive species has increased (Freire and Marafon, 2018).

World population growth has led to an increase in pollution of water resources, with chemical contamination being more and more frequent. Bivalve mollusks are abundant in aquatic environments, have a high tolerance to pollutants, and can remove substances such as heavy metals, pesticides and toxins present in water. However, mussels bioaccumulate these substances in their tissues, and other aquatic organisms through the food chain. Currently, mollusks have been used in research because they are an environmental bioindicator, as they can reflect the levels of contaminants (Marengoni et al., 2013). Thus Amaral et al. (2019) concluded that *L. fortunei* a possible bioindicator of the quality of aquatic environments contaminated by heavy metals.

Considering the negative impacts of golden mussel fouling on fish cultivation in net cages and the different types of screens, there is a need to evaluate the relationship between screens for cages and the characteristics of the size and mass of mollusks and the accumulation of metals. In this study, we tested the hypothesis that the golden mussel incrustation, its performance, and accumulation of metals may be favored by the type of screens used in the cage fish farm. Thus, the study aimed to evaluate the characterization and bioaccumulation of metals in the golden mussel *L. fortunei* encrusted in two types of screens for net cages installed in an aquaculture area to produce of tilapia, in the reservoir of the Itaipu Binacional hydroelectric plant, in Entre Rios do Oeste, Paraná, Brazil.

MATERIAL AND METHODS

The study was carried out at the Itaipu Binacional hydroelectric power plant reservoir, in an area of the São Francisco Verdadeiro Aquaculture Park (24°41’10.23”S, 54°14’28.00”W), in Entre Rios do Oeste, belonging to the Paraná III River watershed, in the western Paraná, from September 2017 to June 2018. According to data from the Paraná Meteorological System (SIMEPAR), the months had an average temperature of 22.4°C and accumulated precipitation of 2168.2 mm.

The water quality of the reservoir was monitored monthly in three locations along the longitudinal axis of tilapia farming, in the upstream portion (24°41’10.00”S, 54°14’01.00”W), at the reference point (24°41’10.23”S, 54°14’28.00”W), and downstream (24°40’52.30”S, 54°14’36.12”W) of the net cages. The physical and chemical variables (temperature, dissolved oxygen, pH, conductivity, total solids, and turbidity) were evaluated using a portable multiparametric probe, Horiba®, model U-54G, and the Secchi disk measured the water transparency.

The average values (minimum - maximum) of temperature (24.54 - 28.9°C), transparency (0.95 - 1.5 m), dissolved oxygen (6.05 - 8.8 mg L^{-1}), pH (7.01 - 8.88), conductivity (51 - 66 µS cm^{-1}), total solids (0.03 - 0.04 g L^{-1}), and turbidity (11.8 - 27.54 NTU) of the water showed no differences between the upstream, downstream, and reference point. The mean values of the water quality variables remained within the standards recommended for class II freshwater bodies of CONAMA Resolution No. 357/2005 (Brasil, 2005) and remained in the normal conditions recommended for the Nile tilapia cultivation (Mallasen et al., 2012).

The evaluation of the encrusted mussels was carried out in an adapted experimental net cage structure, used to support the screens. Two types of 19 mm mesh screens (NBR 10,118) were used, being in galvanized wire coated with PVC (Belgo Plastic®) and wire coated with a Zn-Al alloy (95% zinc and 5% aluminum), called Bezinal (Belgo Bezinal®). The experimental units were constituted of 20 x 20 cm collectors, representing the different screens of net cages. Forty-eight collectors were used, randomly distributed on the four sides of the net cage (Figure 1) and, after

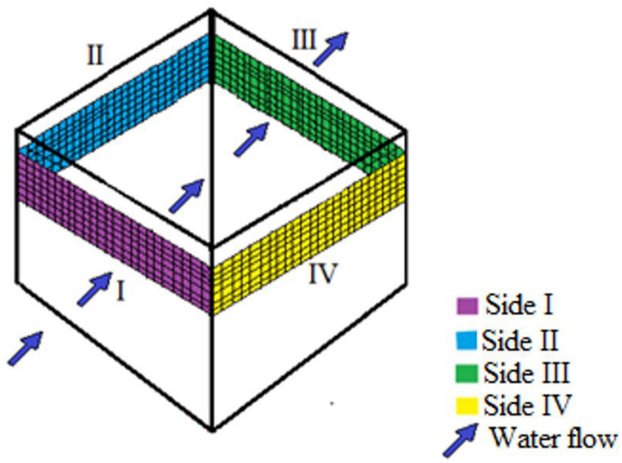

Figure 1. Illustration simulating the sides of the net cage installed in a tilapia cultivation area in the Itaipu Binacional reservoir, in Entre Rios do Oeste.

nine months of submersion, the encrusted mussels were scraped and removed. Then, the organisms were packed in previously identified packages and were preserved in 70% alcohol and transported to the laboratories.

The mass and dimensions measurements of the mussels encrusted on the screens were verified in 24 experimental units on the four sides (Sides I, II, III, and IV) of the net cage. Sides I and III represent the front and back, respectively, and Sides II and IV represent the left and right sides, respectively, concerning the water flow. The individuals were counted, and, with the aid of a precision digital scale (Bel Engineering UMark 250A), biomass was measured to determine the mean individual mass (MIM) and the total mass of mussels (TMM) per unit area (0.04 m^2). To assess the dimensions, height, width, and maximum length were measured using a 0.01 mm digital caliper (Digmess). The length was considered as the distance from the anterior extremity, located just below and in front of the umbos to the posterior extremity of the shell. The method of Sturges was performed in 10 classes with 2 mm intervals (> 10, 10-12, 12-14, ... 26-28 mm) of the average lengths of golden mussel encrusted to verify the distribution incidence of the number of individuals into different length classes, concerning the two types of screens used.

The bioaccumulation of cadmium (Cd), lead (Pb), aluminum (Al), zinc (Zn), and chromium (Cr) in the mussels was carried out with the extraction of 24 collectors from the four sides of the net cages. The organisms were stored in a freezer (-20°C). The samples were sent to the laboratories of Unioeste, in Marechal Cândido Rondon, where they were dried in an air-forced circulation oven at 55 ± 5°C for 72 hours. Then, they were ground and submitted to the nitro perchloric digestion. At the Unioeste Environmental and Instrumental Chemistry Laboratory, metals were determined by atomic absorption spectrometry, flame modality, with GBC analytical equipment, model 932 AA (Welz and Sperling, 1999; AOAC, 2005).

Statistical analyzes were performed using the statistical program SAS Institute Inc (2014). The response variables were compared between the following factors: type of screens (PVC and Bezinal) and the sides of the net tank (I, II, III and IV). The values obtained for the variables of mean individual mass, total mass, length, height, and width of the mussels were subjected to analysis of variance (ANOVA) at 5% probability and, in case of significant difference, the Tukey test (p <0.05) was applied. The Chi-square test (p <0.05) was used to compare the differences in the incidence of individuals in the mussel length classes between the two types of screens (PVC and Bezinal). The average metal concentrations in the mussels were subjected to analysis of variance, and Student's t-test was applied.

RESULTS

The ANOVA results showed that there was a significant difference in the individual mass, total mass, length, height, and width of the golden mussel between the two types of screens (PVC and Bezinal) and in the individual mass and the length between the four sides of the net cages (p <0.05). However, there was no evidence of a significant interaction between screens and side. The mussels encrusted on the Bezinal mesh had a higher mean mass, length, height, and width than those encrusted on the PVC coated mesh (p <0.05). However, the PVC screen provided a higher (p <0.05) total mass (TMM) of mussels encrusted concerning the Bezinal screen (Table 1).

Regardless of the screen used, the mean individual mass (MMI) and the length of the mussels (LG) had higher values on Side I (p <0.05) than on Sides II and IV, but not differing from Side III. There was no effect of the side on the total mass (TMM), the height (HT), and the width (WD) of mussels encrusted on the net cage (Table 1).

The mollusks had a minimum length of 3.4 and a maximum of 29 mm. There was a low incidence of individuals with a length of up to 10 mm and between 26 and 28 mm, with no statistical differences being observed by the Chi-square test in these length orderings (Figure 2).

Individuals with lengths between 10 to 20 mm had a higher incidence on the PVC screen. However, the ordering of the size of the golden mussel in the lengths of 20 to 22 mm did not show any significant difference, while in the lengths between 22 to 26 mm there was a greater frequency of mussels on the Bezinal screen by the Chi-square test (Figure 2).

Regarding the determination of trace metals, there was no significant difference of average concentrations of Cd or Pb in the mussels encrusted on the Bezinal and PVC screens. However, mussels encrusted in the bezinal mesh showed higher levels of Al, Zn and Cr (p <0.05) than the individuals on the PVC screen (Table 2).

Table 1. Mean individual mass (MIM), total mass (TMM), length (LG), height (HT), and width (WD) of golden mussel encrusted in two types of screens and sides of net cage installed in tilapia cultivation area, in the Itaipu Binacional reservoir, in Entre Rios do Oeste.

Screen	MIM (g)	TMM (g)	LG (mm)	HT (mm)	WD (mm)
Bezinal	0.46[a]	195.91[b]	18.20[a]	8.39[a]	7.09[a]
PVC	0.33[b]	731.64[a]	16.62[b]	7.57[b]	6.26[b]
Side					
I	0.46[a]	468.36[a]	18.09[a]	8.17[a]	6.64[a]
II	0.35[b]	447.77[a]	16.86[b]	7.78[a]	6.68[a]
III	0.41[ab]	548.71[a]	17.75[ab]	8.16[a]	6.76[a]
IV	0.37[b]	390.26[a]	16.96[b]	7.80[a]	6.60[a]
Probability					
Screen	<0.0010	<0.0010	<0.0010	<0.0010	0.0061
Side	0.0111	0.1438	0.0103	0.0784	0.9788
Screen x Side	0.6962	0.1778	0.1733	0.2251	0.4464
CV (%)	13.23	24.03	3.69	4.07	9.73

Different letters in the same column differ from each other by the Tukey test at 5% probability.

Table 2. Concentrations (mg kg^{-1}) of trace metals in the golden mussel collected in Bezinal and PVC screens of net cages installed in a tilapia cultivation area in the Itaipu Binacional reservoir, in Entre Rios do Oeste.

Screen	Concentrations (mg kg^{-1})				
	Cd	Pb	Al	Zn	Cr
Bezinal	5.15±0.69	161.33±9.63	1318.48±191.71	80.62±30.58	5.48±1.85
PVC	4.82±0.57	155.47±11.13	1141.74±154.61	31.92±7.84	2.60±1.26
Probability	0.2179	0.1937	0.0235	<0.001	<0.003
CV (%)	12.78	6.59	14.13	4.07	39.75

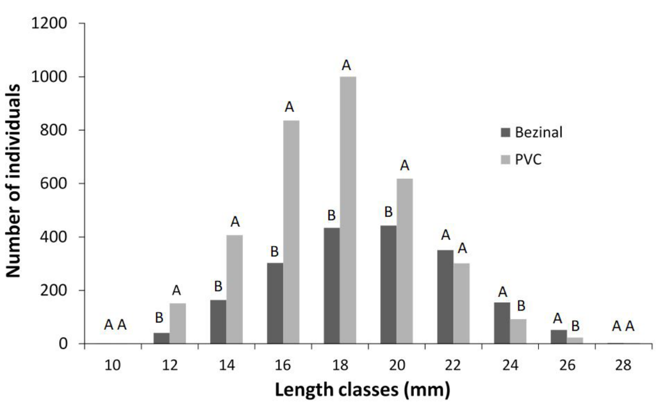

Figure 2. Ordering of average lengths of golden mussel encrusted in Bezinal and PVC screens of net cages installed in a tilapia cultivation area in the Itaipu Binacional reservoir, in Entre Rios do Oeste. Different letters within classes differ by the Chi-square test (p <0.05).

DISCUSSION

The coating composition of the screens was possibly responsible for the differences in masses (TMM and MIM) and dimensions (LG, HT, and WD) of mussels. The higher encrustation of PVC screens may have provided the lowest values of mass and dimensions of individuals, due to the greater demand for space and food.

The water flow can explain the higher value of mass and length of the mussels on Side I (p <0.01) than Sides II and IV and the absence of difference concerning Side III. Sides I and III represent the front and rear parts of the net cages concerning the flow of the water. The greater circulation of water may have favored the dispersion of post-larvae and increased the availability of food for mussels, contributing favorably to the growth of these invaders.

Costa et al. (2012) evaluating the encrustation and body parameters of *L. fortunei* in empty net cages and populated with silver catfish *Rhamdia quelen*, observed a greater amount and average size of mussels in net cages containing fish. The authors believe that the event is linked to the greater supply of nutrients available, with a possible relationship between the development of mollusks in the months of high temperatures and an increase in the stock of fish.

The cultivation of fish in net cages is a system of a high density of storage, which needs a great renewal of water, being dependent on efficient screens. The screens with a high degree of clogging, due to the adherence of *L. fortunei*, obstruct the opening of the mesh, preventing the water renewal process from being carried out efficiently, promoting the reduction of oxygen and preventing the dispersion of debris (Costa et al., 2012; Oliveira et al., 2014). Also, they can cause stress to farmed fish, increasing the risk of developing diseases and negative zootechnical results and, consequently, compromising the socio-economic issues of production. The need for frequent cleaning of the encrusted screens raises operating costs, besides reducing the useful life of the material. Thus, the bioinvasion of mussels negatively impacts the cost of tilapia production in net cages, mainly in small fish farms (Costa et al., 2018), as in the aquaculture area evaluated in Entre Rios do Oeste.

The presence of zinc in the composition (95%) of the Bezinal screen may have contributed to the differences in the masses and dimensions of the adhered mussels. According to Pereira et al. (2010), the metallic materials have a smooth surface, low porosity, and are easily oxidized, releasing compounds that are toxic to the mussel.

Faria et al. (2006) evaluating anti-fouling materials for mussels, found, in a laboratory study, that PVC presented a high mean disconnection force; however, materials that undergo oxidation such as zinc did not show any mussel adhesion. The authors also observed a high percentage of dead organisms adhered to the zinc surface concerning PVC. In the same study, in the field evaluation, they found that the substrate containing zinc obtained less mussel incrustations than PVC. The authors explain that polymers, such as PVC, may have greater encrustation compared to metallic materials, due to the formation of a biofilm that can help organisms to adhere more easily. They also justify that zinc oxide, resulting from the oxidation of zinc, is a biocide for the golden mussel and may have increased the mortality of the adhered individuals.

Pereira et al. (2010) selected and tested materials and coatings with anti-fouling properties for the control of *L. fortunei*. The ceramic material coated with base paint with commercial zinc oxide showed the least encrustations concerning paints with the presence of copper and others with anti-fouling properties, proving the good efficiency of zinc in controlling the fouling of golden mussel.

Vianna et al. (2019) evaluating the clogging by golden mussel in different materials for making screens of net cages, found the lowest levels of clogging in Bezinal screens concerning PVC-coated screens. However, the authors highlight the lower cost/year of the PVC screen compared to Bezinal. The high encrustation of mussels in net cages increases production costs, as they reduce the durability of the structures and demand frequent cleaning of the screens (Costa et al., 2018).

In the evaluated screens, low frequencies of mussels with a length of less than 10 mm and greater than 26 mm were found (Figure 2), with a maximum length of 29 mm. Santos et al. (2008), evaluating the individual growth of *L. fortunei* over a year, found the predominance of mussels with a length between 5 to 7 mm and observed the presence of individuals up to 39 mm length.

The golden mussel is considered adult, capable of reproduction, with 5 mm length, being able to measure 20 mm in the first year of life, 30 mm in the second, and 35 mm in the third (Boltovskoy and Cataldo, 1999; Santos et al., 2008). In this context, the presence of mussels with high lengths in the present study may have been favored by the increased supply of nutrients present in the water, due to the intensive cultivation of fish in the aquaculture area, and by the period of the experiment, which was predominantly in the spring, summer, and autumn. Boltovskoy and Cataldo (1999) found that mussels show accelerated growth during summer and reduced growth in winter, while Santos et al. (2008) observed a greater growth of the golden mussel during the spring and summer. Furthermore, Rezende Ayroza et al. (2019) confirmed that fish farming in net cages favors colonization and nutritional status of *L. fortunei* and its performance in the reservoir varies during the year in response to seasonality.

The predominance of mollusks in lengths from 12 to 20 mm was verified on the PVC screen. However, from the 24 mm length, individuals appeared more frequently on the Bezinal screen. The fact that Zn is toxic to mollusks may have contributed to higher mortality of younger individuals and with a shorter length in the Bezinal mesh.

The mussels present in the Bezinal screen showed higher levels of Zn ($p < 0.01$) and Cr ($p < 0.03$), possibly due to the oxidation of the screen and the absorption of metals by the mussels (Table 2). This fact reinforces the hypothesis that the Zn present on the screen may be bioaccumulated, causing toxicity to the mussels.

The concentration of Al in the evaluated mollusks is above that recommended by the international food legislation, which is 0.51 mg kg^{-1} (ATSDR, 2008). The Zn content determined in mussels is in a range close to the maximum value estimated by ANVISA (Brasil, 1998) in food from fisheries, which is 50 mg kg^{-1}, and within the range stipulated by FAO (1992) (30 to 100 mg kg^{-1}). The concentrations of Cd, Pb, and Cr found show values well above that specified by ANVISA (Brasil, 1998) for bivalve mollusks, which is 2.0 and 1.5 mg kg^{-1}, respectively for Cd and Pb, and in foods from fisheries which have a limit of 0.1 mg kg^{-1} for Cr. The high levels of these metals can lead to the intoxication of several species present in this habitat. Some species of fish such as silver catfish *Rhamdia quelen* and pacu *Piaractus mesopotamicus* can ingest the golden mussel (Godoy et al., 2018), and may indirectly affect humans, through the translocation of metals through the food chain (Marengoni et al., 2013). Ávila-Simas et al. (2019) found that approximately 40% of the fish species evaluated in the upper Uruguay River ingested the golden mussel and suggests that *L. fortunei* is predated by different species according to the hydrodynamics of the environment.

In the metabolism of living organisms, essential metals (Cr and Zn), fundamental for the organism, and non-essential metals (Pb, Cd, and Al) can be toxic when present in high concentrations. The ingestion of non-essential metals, such as cadmium, can cause a series of consequences to humans, such as diseases and their accumulation in the kidneys and liver (Fernandes and Mainier, 2014).

Marengoni et al. (2013), in a study evaluating the bioaccumulation of heavy metals and nutrients in the golden mussel, in another aquaculture area of the reservoir of the Itaipu Binacional hydroelectric power plant, observed concentrations of 3.33, 35.25, 22.91, and 4.08 mg kg^{-1} for Cd, Pb, Zn, and Cr, in mussels. The values of the metal concentrations found by the authors were lower concerning the present study, except for the Cr concentration (2.60 mg kg^{-1}) observed on the PVC screen (Table 2).

Assessing the concentrations of heavy metals in mussels *Perna perna*, in Ilha de Santana (SE), Carvalho et al. (2001) verified the concentrations of 0.11; 0.54; 395; 24.8 and 0.38 mg kg^{-1}, respectively, for Cd, Pb, Al, Zn, and Cr, and it was found that the values were much lower concerning the concentrations observed in the continental environment, in the aquaculture area of tilapia cultivation, in the reservoir of Itaipu Binacional (Table 2).

The degradation of water resources can lead to a series of negative consequences, such as the reduction of aquaculture productivity and compromise the food security of organisms grown in aquaculture. Pollution can originate from domestic sewage, garbage, agricultural and livestock waste, which can cause changes in water quality, eutrophication, and bioaccumulation of heavy metals, among others. The golden mussel can be a bioindicator that allows evaluating pollutions in the aquatic environment and the influence of the intensive system of fish in net cages in the environment since this cultivation system generates a supply of nutrients and minerals that can have negative effects on the existing fauna (Marengoni et al., 2013; Rezende Ayroza et al., 2019).

CONCLUSIONS

The use of the Bezinal screen, regardless of the sides of the evaluated net cage, is more efficient concerning PVC screen as an anti-fouling material because it favors a smaller total mass of golden mussel encrusted. However, the coated wire of the Zn-Al alloy (Bezinal) does not prevent the clogging of the mesh caused by the mollusks in the evaluated conditions. Mussels with shorter lengths tend to have a higher incidence in the PVC screen. Mollusks encrusted on the Bezinal screen bioaccumulate a higher content of Al, Zn, and Cr than those adhered to PVC screen. High levels of Cd, Pb, Al, Zn, and Cr were determined in the encrusted mussels regardless of the type of mesh evaluated.

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