Research Article

Copper, zinc, cadmium and lead inputs and outputs in the maternity section of a commercial shrimp hatchery

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ABSTRACT. The aim of this work was to determine the amounts of copper, zinc, cadmium and lead which enter the tanks of the maternity section of a Mexican commercial hatchery, and those that are discharged to the environment. The most important inputs of Cu were chemicals for water treatment and disease prevention, feeds and the metal dissolved in influent water. Suspended solids and feeds were the two most important inputs of Zn, and feeds and chemicals were the main sources of Pb and Cd. Most metal concentrations may be considered safe for shrimp culture, but the concentrations of Cd of three formulated diets were close or above safe levels. The annual 2013 output of this hatchery was approximately 1.3×10^9 post-larvae, and the estimated metals discharged to the environment by the maternity section were 351 g of Cu, 1,190 g of Zn and 1.35 and 0.02 g of Pb and Cd, respectively. The amounts of Pb and Cd are equivalent to those of the fertilizers spread on between 0.5 and 1.5 ha of the agricultural land of Sinaloa State.

Keywords: shrimp hatcheries, shrimp feeds, metal inputs, metal discharges, environmental impact.

Ingresos y descargas de cobre, zinc, cadmio y plomo en la sección de maternidad de un laboratorio de producción comercial de postlarvas de camarón

RESUMEN. El objetivo de este estudio fue determinar las cantidades de cobre, zinc, cadmio y plomo que entran a los estanques de la sección de maternidad en un laboratorio comercial de producción de postlarvas en México, así como aquéllas descargadas al ambiente. Los principales ingresos de Cu fueron los productos químicos usados en el tratamiento y prevención de enfermedades, alimentos y metal disuelto en el agua que ingresa. Los sólidos suspendidos y los alimentos fueron los dos principales ingresos de Zn, mientras que los alimentos y los productos químicos de los tratamientos fueron los principales ingresos de Pb y Cd. La mayoría de las concentraciones de metales pueden ser consideradas como seguras para la producción de postlarvas de camarón, pero las concentraciones de Cd de tres dietas formuladas estuvieron cercanas y por encima de los niveles de seguridad. La producción anual de este laboratorio en el 2013 fue aproximadamente $1,3 \times 10^9$ post-larvas, y la descarga estimada de metales al ambiente por la sección de maternidad fue de 351 g de Cu, 1,190 g de Zn y 1,35 y 0,02 de Pb y Cd, respectivamente, que es comparable a la cantidad de Cd y Pb usados para fertilizar entre 1,5 y 0,5 hectárea de la superficie agrícola del Estado de Sinaloa.

Palabras clave: producción de postlarvas, alimento, ingresos de metales, descargas de metales, impacto ambiental.

INTRODUCTION

Mexican commercial shrimp culture relies on hatchery-produced post-larvae (PLs) and the Sinaloa State, with more than 22 hatcheries, is the main supplier of this commodity (Industria Acuícola, 2013). For their operation, these hatcheries obtain water from natural sources, such as wellpoints, artesian wells or nearby water bodies, which may be significant metal sources. Additionally, metals are contained in formulated shrimp feeds (Lacerda *et al.*, 2009; Soares *et al.*, 2011), and any other substance which enters the culture system is a possible additional source. Apart from their effects on survival and growth, metals will be eventually discharged into the aquatic environment, and might become an environmental threat.

In this study we measured the inputs and outputs of Cd, Cu, Pb and Zn in two of the >40 holding tanks of the maternity section of a commercial hatchery (outdoors area of the hatchery where PL (post-larvae) are held for hardening from stage PL_6 to the date of harvest and shipping), in order to identify the most important sources of potentially toxic substances supplied to post larvae during daily routines, and to determine if the operation of these laboratories might be a significant source of impact for the environmental quality of Mexican coastal systems.

MATERIALS AND METHODS

The field phase of this study lasted 13 days and was carried out in spring 2013 in the maternity section of the hatchery Aquapacific SA de CV (Agua Verde, El Rosario Municipality, Sinaloa, Mexico). The day before stocking (March 25, 2013), the two 45 m^3 tanks used for this work (Tanks 36 and 40) were filled to the 35 m^3 mark. Water samples (5 L) were obtained and filtered through 47 mm Whatman GF-C filters, acid washed and of known weight. Residues of sea salt were removed with 3-5 mL of distilled water and filters were kept frozen at -20°C. The particle-free water was acidified to pH < 7 adding the appropriate amount of nitric acid (trace metal grade) and preserved in separate containers until analysis.

The day after filling, 2 m^3 of microalgae culture (*Chaetoceros* sp., approximately 10^6 cells mL^{-1}) were added to each tank, the PL (post-larvae) were stocked into the tanks, which were filled to full capacity. The daily maintenance routine started on that day, with the exception of the 30% daily water changes that began on April 1st, at post-larval stage PL_{11}.

Three samples of 100 post-larvae and of the microalgae culture (50 mL concentrated on Whatman GF-C filters) were stored at -20°C until processing and, starting on that day, triplicate samples of all items (feed, probiotics, water and preventive treatments) entering each tank were obtained and preserved in labeled containers, annotating at the same time the amount used for each tank.

Samples (1 g, in triplicate) were obtained of the multivitamin product, of the seven types of formulated feeds, and of the probiotics and sugar which were applied daily excepting the days of preventive (anti-biotic or antimycotic) treatment. Four different products were used for treatment during this work, each provided once on different days (type and manufacturer of feeds and treatments not mentioned due to our agreement to protect the hatchery's trade secrets).

Starting on the date of continuous daily water replacements (mean salinity 36 ±1, flow rate: 12 L min^{-1}, equivalent to 17,280 L day^{-1} and to a daily renewal of 38.4%), water samples (5 L) were obtained at 8 h intervals from the inflow and outflow. The three daily samples of inflow and outflow were pooled to obtain one composite sample of the inflow and outflow of each tank. These were filtered through GF-C filters. Particle-free water and filters were preserved as the samples obtained on the day of tank filling.

Tank 36 was harvested after 10 days of culture (April 4), two days earlier than tank 40. In both cases, samples of organisms and water were obtained at the time of harvest, after which we proceeded to scrape the walls to obtain samples (1 g in triplicate) of the adhering biofilm. The same amount of sediment was collected in triplicate from the bottom of each tank.

The percentages of each metal retained by post-larvae biomass (%MS) were calculated as

$$\%MS = 100\,(FM-IM)/TI$$

where FM and IM = final and initial metal content (mg) of post-larvae and TI = total inputs-IM

The concentrations of dissolved metals in the water samples were determined with a GFAAS, VARIAN SpectrAA220 atomic absorption spectrometer, injecting directly the sample into the detection system with flame atomic absorption spectroscopy (FAAS). Graphite furnace analysis (GFAAS) was used for samples with readings below the limit of detection for FAAS (Páez-Osuna *et al.*, 2015). Pb and Cd were not detected with GFAAS even after concentration of 1 L sample on NH_4OH-pretreated Chelex 100 columns and elution with 40 mL of HNO_3 (2 M). Metals in the particulate samples were determined after acid digestion of freeze dried samples of known dry weight according to Ruelas-Inzunza & Páez-Osuna (2007). As for water, FAAS or GFAAS were used depending on concentrations. All glassware and containers were acid-

washed as in Moody & Lindstrom (1977). Blanks were used for accuracy (ranges: Cu = <0.013; Zn = <0.312; Pb = 0.310-0.420; Cd = 0.014-0.33 mg L^{-1}, respectively) and for detection limits, which were 0.012 µg g^{-1} for Cd (organic solids) and 0.034 µg L^{-1} for water samples; 0.03 µg L^{-1} for Pb, 0.09 µg g^{-1} for Cu and 0.009 µg g^{-1} for Zn (GFAAS). Analysis of certified reference material DOLT-4 (National Research Council of Canada) gave recovery values of 91.5% for Cu, 96.2% for Cd, 103.4% for Zn and 105.6% for Pb (n = 3 in all cases).

Results are given in µg g^{-1} (dry weight) with the exception of water samples (µg L^{-1}).

RESULTS

Inputs

Tank 36 was harvested two days earlier than tank 40. For this reason, with the notable exception of post-larvae (3.2 and 3.0x10^6 PL, equivalent to 13.95 and 13.08 kg, wet weight, for tanks 36 and 40, respectively) all inputs to tank 36 were lower than in 40. Therefore, in view of the lower stocking density, the final biomass harvested in tank 40 was lower than that obtained in tank 36 (Table 1).

The concentrations of dissolved Pb and Cd of the water used for tank filling and daily water renewals were consistently below the respective limits of detection. The mean value determined in the case of dissolved Cu was two orders of magnitude or higher than that of dissolved Zn, while Zn was the most abundant metal determined in the suspended solids of incoming and effluent water. Concentrations of both metals were highly variable, with coefficients of variation >50% (Table 2).

Reflecting their role as essential metals, the contents of Cu and Zn of the post-larvae were more than two orders of magnitude higher than those of Cd and Pb (Table 2). Apart from the initial addition of microalgae, both tanks received daily four types of formulated feed from stocking to PL$_{11}$. One of these was supplied also after this stage, while the remaining three were discontinued and substituted by three different feeds. With the exception of feed F4 (decapsulated, freeze dried *Artemia* cysts) all feeds were rich sources of the essential Cu and Zn. Feeds F2, F4 and F6 had Pb contents close or higher than 0.2 µg g^{-1} while F1, F3, F5 and F7 had high amounts (≥0.4 µg g^{-1}) of the non-essential Cd (Table 2).

Each type and brand of antibacterial or antifungal treatments was used only once in each tank, presumably to minimize the possibility of generating resistant strains; other treatments (EDTA, vitamins, probiotics)

Table 1. Total inputs and outputs to tanks 36 and 40 (water in L, solids in g). SS: suspended solids; PL: post-larvae

	Tank 36	Tank 40	Tank 36	Tank 40
	Inputs		Outputs	
Water	111,240	145,800	111,240	145,800
SS	530	544	6,525	10,390
PL	14,930	14,630	32,240	27,060
Biofilm	-	-	7.21	8.80
Sediment	-	-	462	168
Feed 1	6,380	7,720	-	-
Feed 2	5,350	7,400	-	-
Feed 3	3,180	3,530	-	-
Feed 4	2,170	2,240	-	-
Feed 5	4,910	10,370	-	-
Feed 6	2,970	6,450	-	-
Feed 7	2,500	5,060	-	-
Microalgae	438.2	438.2	-	-
Treatment 1	180	180	-	-
Treatment 2	200	200	-	-
Treatment 3	100	100	-	-
Treatment 4	525	525	-	-
Vitamin mix	1,000	1,200	-	-
Probiotics	140	180		
Sugar	1,060	1,320		
EDTA	1,750	2,150		

were suspended on that date. Treatment T1 stands out for its high Cu and Cd contents. Cd was high also in treatment T2, and Zn was not detected in any of these products (Table 2).

Outputs

As in the influent, concentrations of dissolved Pb and Cd were below the respective limits of detection in effluent water; Zn values remained unchanged while those of Cu increased to more than twice the value of the influent. In the case of post-larvae, final concentrations of the four metals were not different from those determined at the time of stocking (Table 2).

Among solid outputs, biofilm and post-larvae had the highest concentration of Cu, while suspended solids were the most important output in the case of Zn, Pb and Cd. However, values determined in the suspended solids of the effluents were significantly lower than those of the influent and the most notable decreases were those detected for Zn and Pb, which were equivalent to 13.3 and 16.1% of the respective values of influent water (Table 2).

Tank 36

In terms of mass, the most important input of Cu was due to preventive treatments (27.7%), followed by post-larvae, feeds and dissolved Cu of influent water (26.4,

Table 2. Mean concentrations (± standard deviation) in µg g^{-1} (water in µg L^{-1}) of Cu, Zn, Pb and Cd in the influent and effluent water, in suspended solids (SS), feeds and chemical and biological treatments and post-larvae (PL) cultured in tanks 36 and 40. Feeds F1 to F7 and treatments T1 to T4: brand protected by trade secret; n: number of triplicate samples.

	Cu	Zn	Pb	Cd
		Inputs		
Water (n = 24)	6.62 ± 4.04	0.04 ± 0.03	<LD	<LD*
SS (n =24)	28.7 ± 15.0	17,800 ± 12,800	28.6 ± 15.1	3.37 ± 1.85
PL (n = 3)	58.0 ± 4.8	78.4 ± 5.3	0.12 ± 0.09	0.08 ± 0.03
Feeds				
F1 (n = 2)	28.90 ± 0.48	163.0 ± 4.3	0.140 ± 0.039	0.394 ± 0.026
F2 (n = 2)	9.89 ± 0.53	131.0 ± 6.8	0.230 ± 0.049	0.125 ± 0.006
F3 (n = 2)	35.80 ± 0.25	169.0 ± 1.9	0.066 ± 0.007	0.446 ± 0.102
F4 (n = 2)	3.69 ± 0.30	85.7 ± 3.5	0.199 ± 0.023	<LD
F5 (n = 2)	29.90 ± 0.08	159.0 ± 0.7	0.166 ± 0.011	0.52 ± 0.11
F6 (n = 2)	55.3 ± 1.7	295.0 ± 9.2	0.347 ± 0.055	0.136 ± 0.004
F7 (n = 2)	33.50 ± 0.26	163.0 ± 4.9	0.046 ± 0.005	0.510 ± 0.051
Microalgae	7.98 ± 2.37	74 ± 27	3.770 ± 1.620	1.11 ± 0.48
Treatments				
T1 (n = 1)	9.670 ± 1.880	<LD	0.092 ± 0.013	0.149 ± 0.062
T2 (n = 1)	0.103 ± 0.034	<LD	0.088 ± 0.045	0.131 ± 0.078
T3 (n = 1)	0.27 ± 0.15	<LD	0.041 ± 0.004	0.010 ± 0.001
T4 (n = 1)	0.091 ± 0.008	<LD	0.084 ± 0.015	0.083 ± 0.056
Vitamin mix (n = 3)	884 ± 120	<LD	0.104 ± 0.007	0.006 ± 0.001
Probiotic (n = 3)	0.162 ± 0.066	<LD	0.041 ± 0.009	0.003 ± 0.002
Sugar (n = 3)	0.200 ± 0.063	<LD	0.081 ± 0.034	<LD
EDTA (n = 3)	0.112 ± 0.031	<LD	0.243 ± 0.062	<LD
		Outputs		
Water (n = 24)	15.7 ± 5.20	0.04 ± 0.03	<LD	<LD
SS (n = 24)	14.2 ± 15.3	2,370 ± 950	4.59 ± 5.18	1.06 ± 0.18
Biofilm (n = 3)	62 ± 10	142 ± 10	0.35 ± 0.05	0.23 ± 0.01
Sediment (n = 3)	21.7 ± 4.8	132 ± 26	0.47 ± 0.14	0.13 ± 0.05
PL (n = 3)	58.0 ± 4.8	78.4 ± 5.3	0.12 ± 0 .09	0.08 ± 0.03

LD: detection limits: Pb = 0.03 µg L^{-1}; Cd = 0.012 µg g^{-1} and *0.034 µg L^{-1}; Zn = 0.009 µg g^{-1}

23.0 and 22.4%, respectively), while 61% of the total Zn input was due to suspended solids and 31.1% to formulated feeds, most of the balance being due to PL.

The inputs of Pb to tank 36 were mainly due to suspended solids (42.9%), followed in equal parts (≈25%) by preventive treatments (mainly EDTA for water treatment) and feeds, while the most important source of Cd were feeds (76.4%), followed by suspended solids and post-larvae (Table 3).

The major outputs of Cu were post-larvae and the dissolved Cu of effluent water (50.7 and 47.3%, respectively), while suspended solids and post larvae made up almost 100% of the outputs of Zn (78.3 and 21.1%, respectively). Suspended solids contained most of the outputs of the non essential Pb and Cd, with percentages higher than 80% for both metals (Table 3).

Tank 40

The PL of tank 40 remained in this section two days longer than those of tank 36. For this reason, feeds contained 32.4% of the total Cu inputs of this tank. Other important sources of Cu were preventive treatments (25.8%) and suspended solids, which represented 22.4% of the total inputs of this metal. As in the case of tank 36, suspended solids were the highest input of Zn (57%), and feeds (36.9%) and preventive treatments made up the remainder 43%.

Suspended solids were also the main contributors (41.3%) to the total inputs of Pb to tank 40, followed by feeds and in a slightly lower proportion by preventive treatments, while the most important sources of Cd were feeds with 81.7%, followed by suspended solids and post-larvae, in almost equal parts.

Table 3. Total Cu, Zn, Cd and Pb inputs and outputs (mg) in tank 36 of the post-larvae maternity section. SS: suspended solids, PL: post-larvae, ND: not detected.

	Cu	Zn	Pb	Cd
Inputs				
Water	736	4.07	ND	ND
SS	15.2	8940	11.01	1.29
Feeds	757	4.551	6.40	9.35
PL	866	1.171	1.81	1.13
Treatments	909	ND	6.45	0.46
Total	3.284	14.665	25.67	12.23
Outputs				
Water	1.746	4.65	ND	ND
SS	64	9.387	20.12	8.48
Biofilm	0.45	1.02	0.003	0.002
Sediment	10.03	60.98	0.22	0.06
PL	1.870	2.528	3.90	2.43
Total	3.691	11.981	24.24	10.97

Table 4. Total Cu, Zn, Cd and Pb inputs and outputs (mg) in tank 40 of the post-larvae maternity section. SS: suspended solids, PL: post-larvae, ND: not detected.

	Cu	Zn	Pb	Cd
Inputs				
Water	965	5.34	ND	ND
SS	17.5	10.803	13.59	1.61
Feeds	1.404	6.997	9.32	12.66
PL	848	1.147	1.77	1.10
Treatments	1.116	ND	8.23	0.12
Total	4.327	18.952	32.91	15.49
Outputs				
Water	2.289	6.09	ND	ND
SS	264	19.280	35.71	10.88
Biofilm	0.54	1.25	ND	ND
Sediment	3.65	22.2	0.08	0.02
PL	1.569	2.121	3.27	2.04
Total	4.127	21.431	39.06	12.94

The most important Cu outputs were the dissolved fraction and the post-larvae (55.5 and 38%, respectively) while, in the case of Zn, suspended solids and post-larvae (90 and 9.9%, respectively) were practically the only outputs. As in tank 36, data show that the internal concentrations of the non-essential Cd and Pb of post larvae remained unchanged, increasing their total load of these metals by amounts equivalent to the increase of their dry mass. For this reason, suspended solids were the main output of Pb and Cd, with percentages of 91.4 and 84.1%, respectively (Table 4).

DISCUSSION

Copper

The mean copper concentration determined in the water used for tank filling and water exchanges was more than twice the concentration of 3.1 µg L^{-1}, that EPA (2013) suggests as acceptable for long term exposure of marine or brackish water organisms (CCC: criterion continuous concentration) and the difference was even higher in the case of the mean value recorded in effluent water (15.7 µg L^{-1}). However, according to Boyd (2009), the maximum tolerable concentration for the culture of aquatic organism in hard or marine water is 70 µg L^{-1}, which is five and ten times higher than the values detected in influent and effluent water, respectively.

With the exception of feeds F2 and F4, the mean Cu concentration determined in foods was from three to almost five times higher than the 10-11 µg g^{-1} recommended by Piedad-Pascual (1989) and Ikem & Egilla (2008) for shrimp and fish feeds, although this did not seem to affect post-larvae survival since, accor-

ding to the estimates of the biologist in charge, survival in both tanks was >80% and final biomass was 200% or higher than the initial value.

In the case of tank 36, post-larvae retained close to 41% of all Cu inputs in their biomass and most of the rest was discharged as dissolved Cu, while the percentage retained in tank 40 was only 21%. The remaining was discharged with the effluent water, most as dissolved and the remaining as particle-associated Cu.

Zinc

The Zn content of most feeds was close or within the range of values (100-150 µg g^{-1}) indicated by Piedad-Pascual (1989) and Davis & Kurmaly (1993) as adequate for fish and shrimp culture, with the exception of F6, which had twice the highest of these values. Water concentration was well below the CCC value (81 µg L^{-1}) indicated by EPA (2013) as acceptable for aquatic communities, and post-larvae of tanks 36 and 40 retained 10.1 and 5.5% of all Zn inputs, respectively, equivalent to approximately 30 and 14% of the amount supplied as shrimp feeds. Most of the excess was discharged as suspended solids.

Lead

As a non essential, potentially toxic metal, Pb should not be present in animal feeds or in the substances introduced into the culture system. However, its presence as contaminant is unavoidable and tolerated in concentrations even higher than those found in this study. For instance, Pb was detected in feeds used in U.S. federal hatcheries, where the mean Pb content was 0.78 µg g^{-1} (Maule *et al.*, 2007) and values between

0.35 and 0.37 µg g^{-1} were determined by Anhwange *et al.* (2012) in feeds for cultured fish, and even higher values (3.58 µg g^{-1}) were found in shrimp feeds (Shamshad *et al.*, 2009). All these values, which are higher than the upper limit of the range found in this study, are below the tolerable limit (5 µg g^{-1}) established by European Union legislation on undesirable substances in formulated animal feeds (EU, 2013), and are far lower than the 30 µg g^{-1} allowed by the American Association of Feed Control Officials (Hanks, 2000).

For this reason, although it is highly unlikely that these amounts might represent a threat for postlarval shrimp health, between 25 and 28% of the total Pb which entered the shrimp tanks came from shrimp feeds. This might raise some concern, especially considering that similar or slightly lower (88%, for tank 40) input was due to preventive treatments, which are supposed to preserve shrimp health.

One additional consideration is that only between 9 and 5% of the Pb inputs were retained within the shrimp biomass of tanks 36 and 40, respectively. The remaining was discharged as particulate suspended matter with the effluent water, thus becoming a potential environmental threat.

Cadmium

Microalgae had the highest Cd content of all feeds, but in view of the small amount supplied their contribution to total Cd of larval feeds was only 5.2 and 3.8% for tanks 36 and 40, respectively. Three out of the seven types of formulated feeds (F3, F5 and F7) used in this hatchery had mean Cd concentrations close or higher than the maximum admissible level (0.5 µg g^{-1}, dw) established by European legislation (EU, 2013) and mentioned by Hanks (2000) for the U.S. food industry. These and suspended solids to a lower extent (close to 10%), were practically the only Cd inputs, between 11.7 and 6.5% of which were incorporated in post-larval biomass of tanks 36 and 40, respectively, while the remaining was discharged with the effluents as particulate Cd.

In this case therefore, unlike Pb, the Cd content of formulated feeds might become a double threat, to the health of shrimp post-larvae as well as to the environment, which receives the remaining 89-92% of the Cd input.

Discharges to the environment

The two tanks used for this study yielded approximately 9.5x10^6 post-larvae ready for the market. To achieve this production, this laboratory discharged 4.38 g of Cu, 2.63 g of which added to the original concentration, 28.8 g of Zn (with an enrichment of 8.92 g), 0.06 g of Pb, which represents a 100% increase in comparison to the influent, while 95% of the 0.02 g of Cd discharged to the environment came from shrimp feeds and treatments.

By the end of 2012, the total production of this hatchery was 1.267x10^9 post-larvae, equivalent to 25.4% of the total produced in Sinaloa and 13.14% of the national production (4.99x10^9 and 9.64x10^9 post-larvae, respectively: Industria Acuícola, 2013). Under the assumption that inputs and outputs remained unchanged, the estimated total amount discharged during 2012 by that particular production area of this hatchery would have been in the order of 584 g of Cu, 3,840 g of Zn, and 7.5 and 2.5 g of Pb y Cd, respectively. Once corrected for the original concen-trations, these amounts would mean an estimated total addition to the environment of 350.8 g of Cu, 1,190 g of Zn and 4.2 and 2.2 g of Pb and Cd, respectively.

According to national statistics, close to 50% of the 1.3x10^6 ha of agricultural land of Sinaloa State is dedicated to intensive agriculture, including irrigation and fertilization (INEGI, 2007), and its estimated consumption of phosphatic fertilizers in 2010 was 0.12x10^6 Mg, equivalent to 10.2% of the apparent national consumption (1.16x10^6 Mg: Claridades Agropecuarias, 2013). Therefore, the average appli-cation rate would be 0.2 Mg (200 kg) ha^{-1}yr^{-1}.

Phosphatic fertilizers contain several metals in amounts that depend on their origin as well as on production techniques. Cu contents range from 22 to 130 mg kg^{-1}, with a mean value of 58 ± 45 mg kg^{-1}; Zn varies from 13.3 to 515 mg kg^{-1} (mean 245 ± 174 mg kg^{-1}). The contents of Cd are 0.1 to 60 mg kg^{-1} (mean 21.0 ± 24.6 mg kg^{-1}) and those of Pb range from 3 to 44.5 mg kg^{-1}, with a mean content of 14.5 ± 17.1 mg kg^{-1} (Pezzarossa *et al.*, 1990; Chandrajith & Dissanayake, 2009; Ahialey *et al.*, 2014). The amounts discharged by this hatchery would seem to represent a tolerable impact, since they are lower than those contained in the fertilizers used in 25 to 30 ha in the case of the essential Cu and Zn, and in between 0.5 and 1.5 ha for Cd and Pb, respectively.

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