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Levels of arsenic, mercury and selenium in *Clarias gariepinus* from Sagua la Grande River, Cuba

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Abstract – This study first reports concentrations of arsenic (As) and selenium (Se) in fish tissue of *Clarias gariepinus* from Sagua la Grande River in the Villa Clara Province, Cuba. We also confirm the mercury (Hg) levels in these fish obtained previously. Individuals were captured in three areas near Sagua la Grande City, where this fish is a common source of food for the city inhabitants. Concentrations range of As, Hg and Se (in wet weight) were 0.01–0.11 $\mu g.g^{-1}$; 0.03–0.24 $\mu g.g^{-1}$ and 0.75–3.87 $\mu g.g^{-1}$, respectively. As and Se levels were positively correlated ($n = 19$, $\rho = 0.673$, $P < 0.05$). High levels of Se were found in fish tissue and in 31.6% ($n = 6$) of individuals captured exceeded the threshold value for Se toxicity, which means that Se is likely to produce adverse consequences on the fish themselves or on the wildlife organisms that eat them. As and Se concentrations were positively correlated with fish weight and length ($P < 0.05$). Fish from irrigation canal have higher Se concentrations than fish captured in the other two stations. However, no significant differences were found between Hg and As concentrations in fish at the three sampling stations. Finally, as the Se concentrations in *C. gariepinus* were abnormally high, we suggest the need for studies about Se sources in the zone, the effect of Se in fish and the intake associated with fish consumption.

Key words: Arsenic / mercury / selenium / *Clarias gariepinus* / Sagua la Grande River

Introduction

Arsenic (As), mercury (Hg) and selenium (Se) are chemical elements of high environmental and toxicological interest. In aquatic environments these elements have complex biochemical cycles that include the formation of organic species. Furthermore, Se and Hg tend to increase all along the trophic chain (Donohue and Abernathy, 1999; Clarkson and Mago, 2006; Luoma and Presse, 2009).

Arsenic, especially in its inorganic forms, is considered a toxic element, which can cause cancer in humans (Kapaj *et al.*, 2006). Fish are important sources of As in human diet (Baeyens *et al.*, 2009; Sirot *et al.*, 2009). Although in these products, As is mainly present as organic species that are much less toxic, some authors refer that a certain fraction can be found in the toxic inorganic form, so it is always of concern (Moreau *et al.*, 2007; Sirot *et al.*, 2009; Williams *et al.*, 2009).

Hg in the aquatic environment tends to become organic species and bioaccumulate through food chains, so that fish consumption is the most dangerous source of exposure for humans to the pollutant (Boudou and Ribeyre, 1997; UNEP Chemicals, 2002). Hg species (mainly methylmercury) can cross the cerebral membranes leading to neurological disorders (Environment Canada, 2002). Their effects can be especially severe on foetuses, because this toxic compound can pass from the mother to her offspring (Sanfeliu *et al.*, 2003).

Se is an essential element for human health and it is well known for its antioxidant activity (Dumont *et al.*, 2006). However, above the relatively low normal physiological requirements, it can be toxic (Letavayová *et al.*, 2006). In aquatic systems, Se is accumulated through food chains, resulting in the exposure of animals, such as fish and birds, from higher trophic levels causing them problems in their reproductive systems which adversely affect populations (Lemmy, 1993, 1996; Luoma and Presse, 2009).

Some researchers have reported an antagonistic effect of Se to Hg species in different animals. Moreover, it has long been observed that Se protects animals from the toxicity of both inorganic Hg and MeHg. However,

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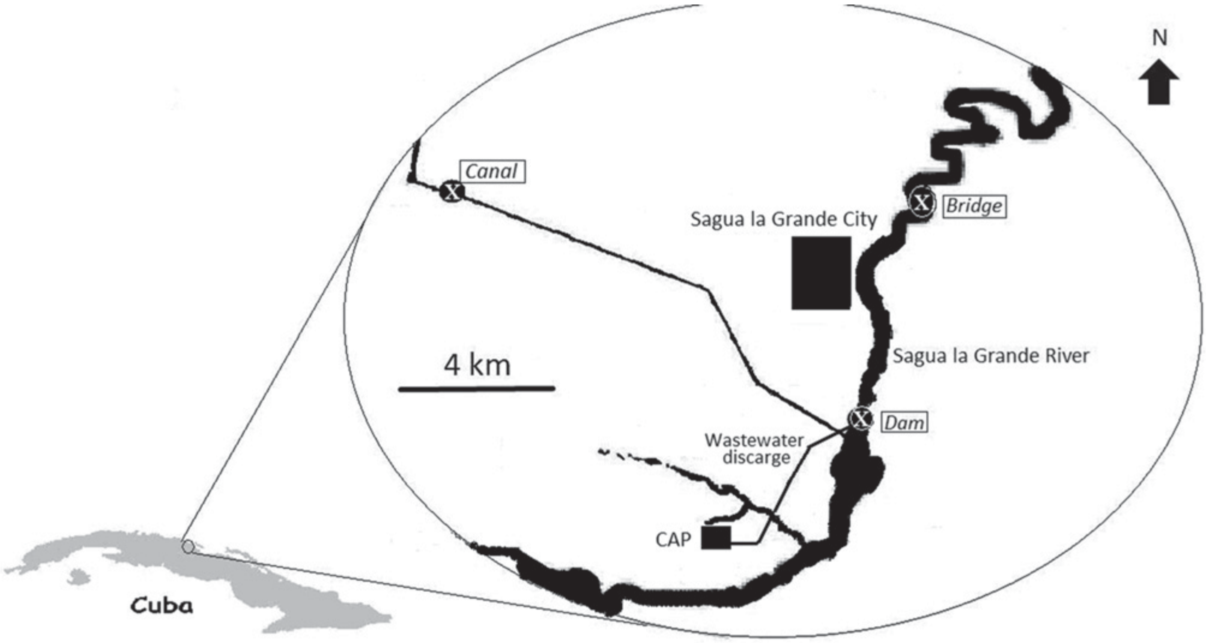

Fig. 1. Sample stations within Sagua la Grande river watershed. Sampling stations are indicated with names inside squares. CAP, Chlor-alkali plant.

additive or even synergistic effects of Hg and Se have also been reported (Khan and Wang, 2009). Mechanisms of interaction of selenium with heavy metals are not yet completely elucidated (Yang *et al.*, 2008).

In the northern province of Villa Clara, Cuba, there are several factors that make interesting to study the above mentioned elements. A mercury-cell chlor-alkali plant (CAP) has caused widespread contamination with Hg in the Sagua la Grande River for several years. Gonzalez (1991) found that the area was polluted with mercury a few years after the beginning of plant operation. Díaz-Asencio *et al.* (2009) also reported a significant enrichment of mercury in the estuary sediment in recent years, reaching a maximum level of 2.68 $\mu g.g^{-1}$ around 1990. Moreover, Olivares-Rieumont *et al.* (2012) found levels of mercury ranging from 0.190 to 0.690 $\mu g.g^{-1}$ dry weigh (dw) in oyster tissues (*Crassostrea rizophorae*) collected at the Sagua la Grande River estuary and offshore mangrove keys 19 km downstream of CAP.

De la Rosa *et al.* (2009a) reported that people living near the Sagua la Grande River are moderate fish consumers, with a weekly intake of freshwater fish of 2.7 times a week. For consumers, *Clarias gariepinus* was second in preference (32%) among favorite fish species. This versatile omnivore specie was introduced in Cuba for fish farming and has rapidly spread in most Cuban rivers, reducing native fish populations and standing at the top of the food webs. (De la Rosa-Medero and Campbell, 2008; De la Rosa *et al.*, 2009b). De la Rosa *et al.* (2009a) found Hg levels in *C. gariepinus* tissue higher than those found in other fish species in the Sagua la Grande River. Although Hg levels do not exceed the 0.5 $\mu g.g^{-1}$ wet weight (ww), established by the Cuban authorities as maximum recommended limit (NC-493, 2006), the concentrations found in fish represent an important risk especially for vulnerable population groups (De la Rosa *et al.*, 2009a, 2009b).

Due to *C. gariepinus* being introduced recently into Cuban watersheds, there is little information about pollutant levels in fish tissue. Therefore, the primary objective of this study is to corroborate the Hg levels obtained by De la Rosa *et al.* (2009b) in *C. gariepinus* tissue from the Sagua la Grande River and determine levels of other potential toxic elements such as As and Se; the latter in its dual role of essential at a normal range and toxic at high concentrations. We were particularly interested in determining the levels of these three elements in fish tissue and their relationships, as well as assess the dependence of element concentrations with fish size and capture sites.

Materials and methods

Sampling

Individuals of *C. gariepinus* were collected from three sampling stations in June 2007: two located on the Sagua la Grande River and the other in an irrigation canal (Fig. 1). Sampling stations are consistent with those used in 2006 by De la Rosa *et al.* (2009b). The "Dam" station was located south of the city and downstream from the CAP effluent discharge point. The "Bridge" station was set up north of the city and downstream from it, whereas the "Canal" station is approximately 10 km west of the city.

Fish selected for this study were in the same length ranges of those evaluated by De la Rosa *et al.* (2009b) (36–74 cm), in order to make comparisons with previous findings. Fish of this size are usually consumed by the

Table 1. Operational parameters of the ICP-MS system.

Plasma conditions	
Forward power	1250 W
Plasma gas flow rate	15 $L.min^{-1}$
Auxiliary gas flow rate	0.73 $L.min^{-1}$
Nebulizer gas flow rate	0.83 $L.min^{-1}$
Nebulizer	Meinhard
Spray chamber	Impact Bead Quartz Spray
Measurement parameters	
Acquisition mode	Continuous
Isotope monitored	^{202}Hg, ^{75}As and ^{82}Se
Dwell time per point	200 ms
Replicates	3

population of the area. A total of 19 fish were collected and distributed as follows: "Dam" (six fish), "Bridge" (seven fish) and "Canal" (six fish) and were caught using polyethylene nets and the traditional techniques of fish-hooks.

Immediately after capture, fish were measured and weighed, and dorsal fillets were extracted and frozen at $-20\,^{\circ}C$. Tissue samples were then thawed, homogenized and lyophilized in the laboratory. Samples were kept at $4\,^{\circ}C$ prior to laboratory analysis.

Analysis of As, Hg and Se

Fish samples were analyzed at the Analytical Chemistry Department from the Universidad Complutense de Madrid, which has a long experience with metrology in chemistry related-activities through both its participation and/or co-ordination in numerous projects of reference material certification (BCR).

Fish tissue samples (about 500 mg) were accurately weighed, and placed in a Teflon digestion vessel, after adding 2.5 mL of HNO_3 (65%) and 1 mL H_2O_2 (30%) and digested in a microwave oven at 43% power output. The pressure was kept at 20 psi during 15 min, increased to 40 psi for 30 min and kept 1 h at 85 psi. The final volume was adjusted to 25 mL with ultrapure water for analysis. A quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Thermo X-Series (Thermo Electron, Windsford, Cheshire, UK) equipped with a Meinhard nebulizer, a Fussel Torch, and an Impact Bead Quartz Spray Chamber cooled by a Peltier system was used for determination of As, Hg and Se. The mass calibration of the ICP-MS instrument was tuned daily with a solution containing 1 $\mu g.L^{-1}$ of Li, Be, Co, Ni, In, Ba, Ce, Pb, Bi and U in 5% (v/v) HNO_3. The operation conditions and data acquisition parameters are summarized in Table 1.

The validation of analytical methodology was performed, detection and quantification limits were: 0.11 and 3.52 $ng.g^{-1}$ ww for As, 0.10 and 0.37 $ng.g^{-1}$ ww for Hg and 4.07 and 13.7 $ng.g^{-1}$ ww for Se, respectively. For analytical accuracy assessment, the procedure was separately applied to three certified reference materials (CRMs): Tuna fish tissue CRM-627 certified for As (4.8 ± 0.3 $\mu g.g^{-1}$), Tuna fish tissue CRM-463 certified for Hg (2.85 ± 0.16 $\mu g.g^{-1}$), and Antarctic Krill Murst-ISS A22, certified for Se (7.37 ± 0.91 $\mu g.g^{-1}$). The measured concentrations were: 4.3 ± 04 $\mu g.g^{-1}$ for As in CRM-627, 2.80 ± 0.02 $\mu g.g^{-1}$ for Hg in CRM-463 and 7.42 ± 0.52 $\mu g.g^{-1}$ for Se in Antarctic Krill Murst-ISS A22, no significant differences were detected between the CRMs certified values and the experimental ones.

Statistical analysis

Data were evaluated to check normality using the Shapiro–Wilk test. As data showed significant evidence for non-normal distribution, non-parametric tests for statistical analysis were used. The Spearman's rank correlation (ρ) was used to assess possible relationships between body fish size and concentrations of trace elements levels, and also inter-elements effects. The Kruskal–Wallis *H*-test was used to compare the body fish size (length and weight) and elements concentrations between sampling stations, followed by a Mann–Whitney *W*-test as *post-hoc* test. A multiple regression model was used to assess element concentrations contributing significantly to the variance in fish length. The level for significance was chosen as p value less than 0.05.

Results and discussion

Total concentrations of As, Hg and Se in *C. gariepinus*

As, Hg and Se concentrations for each fish fillet samples are presented in Figure 2. Arsenic concentrations varied between 0.01 and 0.11 $\mu g.g^{-1}$ ww (median 0.03 $\mu g.g^{-1}$ ww, for $n = 19$). These concentrations are lower in all cases than the recommended maximum concentration limit by the Cuban standard of 1 $\mu g.g^{-1}$ ww of As for fish consumption (NC-493, 2006) and in the same order of magnitude reported by other authors in freshwater fish. Hinck *et al.* (2009) reported average concentration of 0.11 $\mu g.g^{-1}$ ww for As in fish collected at 111 sites from some important river basins from the USA, with a maximum of 1.95 $\mu g.g^{-1}$ ww for whole body. During a survey on fish fillets from Oak Ridge Reservation in Tennessee, USA, Burger and Campbell (2004) detected the presence of As in a concentration range between 0.085 and 0.140 $\mu g.g^{-1}$.

Hg concentrations ranged between 0.03 and 0.24 $\mu g.g^{-1}$ ww (median 0.16 $\mu g.g^{-1}$ ww, $n = 19$) (Table 2), similar values were found by De la Rosa *et al.* (2009b) in fillets of *C. gariepinus* captured in this river in 2006 (0.07 to 0.38 $\mu g.g^{-1}$ ww, median 0.18 $\mu g.g^{-1}$ ww, $n = 27$). None of the samples exceeded the maximum limits recommended by the Cuban authorities for fish consumption of 0.5 $\mu g.g^{-1}$ ww of Hg (NC-493, 2006). Only two samples (10%) had levels higher than 0.2 $\mu g.g^{-1}$ ww, limit recommended by the World Health Organization for

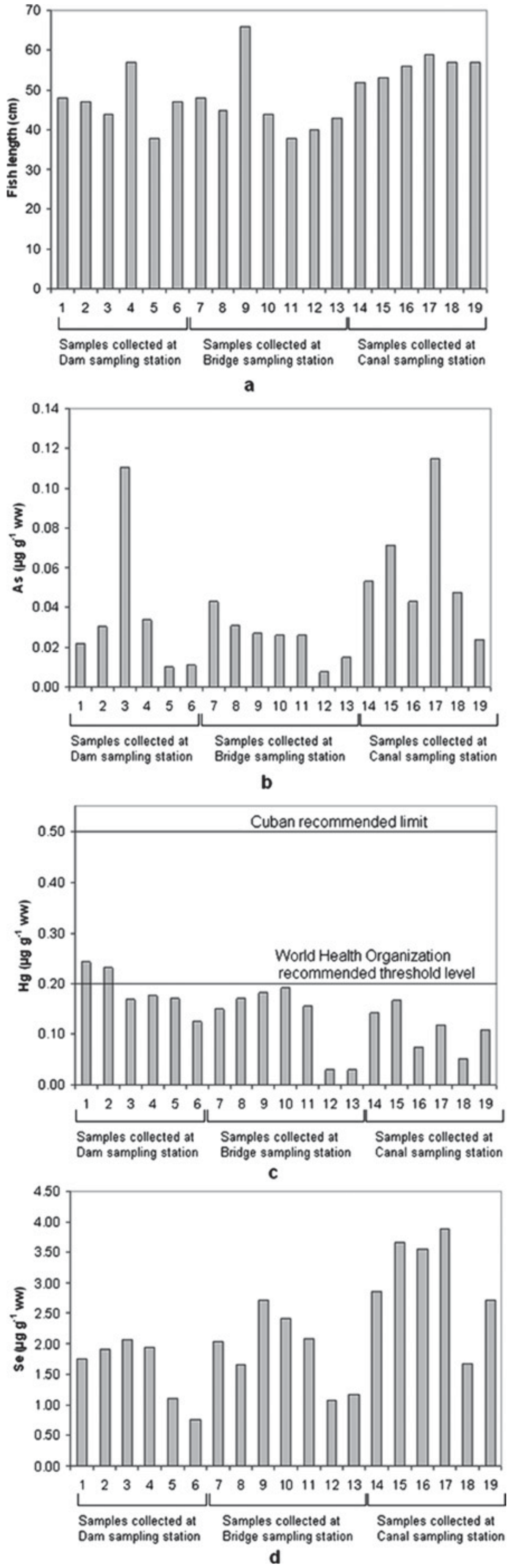

Fig. 2. Fish length (a) and As (b), Hg (b) and Se (c) concentrations (µg.g^{-1} ww) in fish fillet of *C. gariepinus*.

fish consumed by vulnerable people (World Health Organization, 1990).

Se concentrations in fillets of *C. gariepinus* were between 0.75 and 3.87 µg.g^{-1} ww (median 2.03 µg.g^{-1} ww, $n = 19$) or between 2.3 and 15.5 µg.g^{-1} dry weight (dw). These levels are higher than the average values reported for other freshwater fish. For instance, in the Savannah River, USA, were found average levels of Se in fillet between 0.21 and 0.64 µg.g^{-1} ww for various species (Burger *et al.*, 2001). Hinck *et al.* (2009) studied piscivorous and benthivorous fish at 111 sites from US river basins and reported a mean of 0.59 µg.g^{-1} ww of Se in whole fish with a maximum value of 4.66 µg.g^{-1} ww. Lemly (1993) proposed an Se reference concentration of 4 µg.g^{-1} dw in whole body as threshold values for selenium toxicity involving reproductive failures and teratogenic deformities in young fish, although adults can survive and appear healthy. For muscle, this value is 8 µg.g^{-1} dw (Lemly, 1993) or 2.6 µg.g^{-1} ww according to Burger and Campbell (2004) when a moisture value of 67% is used for the conversion. This threshold value for selenium toxicity is exceeded in 31.6% ($n = 6$) of *C. gariepinus* individuals captured in the Sagua la Grande River, which means that Se is likely to produce adverse consequences on the fish themselves or on the wildlife organisms that eat them. Five of the samples that exceed the threshold value were collected in the "Canal" sampling station.

There are no previous reported background levels of Se in fish fillets in Cuba. Moreover, levels of Se are not regulated for fishing products in the Cuban Food Sanitary Standard (NC-493, 2006). However, on the basis of existing information about industrial sources in the area, there is no evidence of any anthropogenic sources that could increase Se concentrations in the region. For that reason, we hypothesized that high Se levels in fish may be related to existing levels of this element in the aquatic environment, although there are no reports of Se concentrations in soils and sediments in the area either. More information is needed about Se levels in the aquatic environment of the Sagua la Grande River to reach a definitive conclusion about transfer mechanisms toward the fish; however, our results suggest bioaccumulation of selenium by *C. gariepinus*. High concentration of Se found in these fish is an interesting finding. Although the number of samples taken in this study is not large, so it may be considered as preliminary, it provides the basis for further studies in order to verify the presence of this element in the area.

Correlations between element concentrations and fish size

As shown in Table 3, the only statistically significant correlation inter-elements was found between As and Se concentrations ($\rho = 0.673$, $P < 0.05$). Although it has been reported that As and Se are metalloids with similar chemical properties, biological interactions between these two elements depend on their chemical forms (Zeng *et al.*,

Table 2. Summary data and As, Hg and Se levels in *C. gariepinus* in the three sampling stations.

		Sampling station		
		"Dam"	"Bridge"	"Canal"
Number of individuals		6	7	6
Total weight (g)	Median	1000	540	1668
	Range	425–1900	390–2420	1380–1840
Total length (cm)	Median	47	44	57
	Range	38–57	38–66	52–59
As (μg.g^{-1} ww)	Median	0.03	0.03	0.05
	Range	0.01–0.11	0.01–0.04	0.02–0.11
Hg (μg.g^{-1} ww)	Median	0.17	0.16	0.11
	Range	0.13–0.24	0.03–0.19	0.05–0.17
Se (μg.g^{-1} ww)	Median	1.84	2.04	3.20
	Range	0.75–2.07	1.08–2.72	1.67–3.87

Table 3. Spearman's rank correlations between element concentrations and fish size. Given are correlation coefficients.

	As	Hg	Se	Total length	Total weight
As	–	NS	0.673*	0.527*	0.480*
Hg		–	NS	NS	NS
Se			–	0.566*	0.497*
Total length				–	0.969*
Total weight					–

*Statistical significance $P < 0.05$.
NS, not significant.

2005). Significant correlations between As and Se in individuals from other fish species as *Morone chrysops* were also reported (Burger and Campbell, 2004).

Se and Hg concentrations were not statistically correlated($P > 0.05$; Table 3). Although some authors have found significant correlations between these two elements in the fillets of different species the statistical relationship may not exist. For example, Lima *et al.* (2005) in Piriá River and in Grande Lake assessed levels of Hg and Se in ten different fish species, but they only found statistically significant relationships between Hg and Se for *H. malavaricus* and *Leporinus* sp. species. Others like Burger *et al.* (2001) found in Savannah River only significant correlations for three fish species of the eleven examined. Although several authors have reported an antagonistic effect of Se on Hg metabolism, mechanisms of this antagonism are unclear (Yang *et al.*, 2008). Yang *et al.* (2010) found that when Se concentrations in fish fillets were below certain values, usually Hg levels were high. This possibly suggests that threshold concentrations of Se in fish must be reached before a clear protective role of Se against Hg assimilation become noticeable. A similar trend is observed between these two elements in *C. gariepinus*; however, more data are needed to reach conclusive results.

Significant and positive correlations were observed between As and Se concentrations in fillets and body fish length and weight (Spearman correlations with $P < 0.05$) (Table 3). It is reported that the primary sources of Se in fish is related to diet (Luoma and Presse, 2009). Fish can accumulate Se found in their environment and this process can be relatively fast (Lemly, 1999). *C. gariepinus* are voracious omnivorous, which increases their weight in a short time, making them ideal for growing in farms. Schram *et al.* (2008) conducted experiments with this fish to achieve enrichment of Se in fish tissue providing a diet rich in Se, showing that it is possible to increase the levels of Se in fish fillet in a relatively short time. In Cuban water bodies, this fish quickly occupies the top of the food chain and feeds from various sources such as detritus, filamentous algae, zooplankton, macrophytes, aquatic and terrestrial insects, nematodes, mollusks, crustaceans, birds, and fish (De la Rosa-Medero and Campbell, 2008). Similar behavior was reported by Desta *et al.* (2007) for these fish from Lake Awassa, Ethiopia. *C. gariepinus* capture their prey by gulping them with a rapid opening of the mouth and then retaining them either on the gillrakers or fine recurved teeth arranged on dentary, premaxillary, vomerine and pharyngeal bands. This fish exhibits a variety of feeding strategies including sucking the surface for terrestrial insects and plant fragments washed into the water by heavy rains and pack-hunting of small cichlids. They can suck the prey along with water and suspended particles. Ingestion of particles (biotic and abiotic) is an input way of selenium into living organisms (Luoma and Presse, 2009). Moreau *et al.* (2007) have found that Se tends to be magnified through the consumption of algae and invertebrates, so the levels of selenium in *C. gariepinus* apparently reflects levels found in all these sources in the aquatic system of the Sagua la Grande River.

No significant correlation ($P > 0.05$) was found between Hg and fish size (either length or weight) (Table 3). A similar result was obtained by De la Rosa *et al.* (2009b) who suggested that this behavior could be explained by the dietary habits of *C. gariepinus* in Sagua la Grande River. Owing to its omnivorous nature, it can feed on fish but also on small terrestrial insects, mollusks and fruits (Toledo-Perez *et al.*, 2007; Zheng *et al.*, 1988) depending on the availability of food. This ability to change food source might affect this correlation, as the body mass increases not only due to fish consumption, which is the most recognized source of accumulation of Hg in the aquatic food chain.

Relationships between locations and element concentrations in *C. gariepinus*

As and Hg concentrations were similar in the fillets of the fish from the three sampling stations ($H = 5.239$, $P > 0.05$ and $H = 5.820$, $P > 0.05$, respectively). Although no significant differences between the medians were found for mercury among the three stations, at "Dam" there is a tendency for higher values. Similar results were found in an earlier work (De la Rosa *et al.* 2009b). However, it appears that the high mobility of these fish, likely reflects homogeneity in exposure to Hg throughout the region.

It were observed significant differences among Se concentrations in the different sites ($H = 7.423$, $P < 0.05$). Se concentrations in fish captured in the sampling station "Canal" were significantly higher than levels found in fish from the other two stations ("Canal" and "Dam": $W = 32.0$, $P < 0.05$; "Canal" and "Bridge": $W = 37.0$, $P < 0.05$). On the other hand, there were significant size differences among the fish as a function of location ($H = 6.102$, $P < 0.05$ and $H = 6.075$, $P < 0.05$ for weight and length, respectively), with fish collected from "Canal" being the largest.

"Canal" station is located approximately 10 km away from Sagua la Grande City, at a site with difficult access, while the other two stations are closer to the city, so water pollution and overfishing could affect fish size in these latter stations. This behavior (regarding fish size) was also observed in fish collected in a previous year (De la Rosa *et al.*, 2009b).

A total of 39.0% of the variability in log length of the fish ($P < 0.10$) was explained by a multiple regression model, which included As, Hg and Se concentrations as independent variables. Only Se concentration entered significantly in the model (coefficient value $\beta = 0.05$, $P < 0.05$). This result suggests that differences between Se concentrations in the sites may be related to differences in the size of fish rather than to location. As it was mentioned above, there is no evidence of any Se pollution sources in the studied area.

Conclusions

Results confirm previous studies relate to Hg concentration in *C. gariepinus* from the Sagua la Grande River, Cuba, and provide new information about low As levels in the fish. High levels of Se were found in *C. gariepinus* fillets. In 31.6% of individuals Se concentrations exceeded threshold values for Se toxicity, which means that Se is likely to produce adverse consequences on the fish themselves or on the wildlife organisms that eat them. Significant differences were found for Se in fish captured in the sampling stations, probably influenced by differences in fish size at these stations. Owing to the high levels of Se found in fish, it is necessary to assess the likely sources of Se in the region and to study the effect of Se in fish and the intake associated with fish consumption.

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