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Arsenic Speciation in Farmed Hungarian Freshwater Fish

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Arsenic speciation analysis was carried out on freshwater farmed fish collected from an area with elevated groundwater arsenic concentrations in Hungary as well as from outside of the area (control samples). The arsenic species were determined by high-performance liquid chromatography−inductively coupled plasma mass spectrometry on methanol extracts of the muscle tissue from the fish. Catfish (*Claries gariepinus*) were raised in geothermal water where the average total arsenic concentrations were 167 (contaminated sites) and 15.1 ng As mL^{-1} (control); they were all fed an artificial diet containing 2880 μg As kg^{-1} total arsenic, mostly present as arsenobetaine. In the catfish, the accumulated total arsenic (2510−4720 μg As kg^{-1}) was found mostly in the form of arsenobetaine suggesting that uptake of arsenic was dominated by their diet. Carp (*Cyprinus carpio*) were cultured in surface lakes with no significant arsenic pollution and had total arsenic concentrations ranging from 62 to 363 μg As kg^{-1}. The arsenic species found in the carp extracts differed markedly from those in the catfish in that no arsenobetaine was detected. Most samples of carp from the investigated sites contained low concentrations of As(III) (arsenite), As(V) (arsenate), MA (methylarsonate), and DMA (dimethylarsinate), and no other compounds were detected. The four individuals from the control site, however, all contained appreciable levels of oxo-arsenosugar-glycerol and oxo-arsenosugar-phosphate. Indeed, the oxo-arsenosugar-phosphate dominated the speciation pattern for these carp contributing about 75% of the sum of species. The contrast between these two freshwater aquaculture species regarding total arsenic and arsenic species has relevant toxicological aspects in terms of food safety.

KEYWORDS: Arsenic speciation; HPLC-ICP-MS; freshwater fish; food safety

INTRODUCTION

In southeastern Hungary, elevated arsenic concentrations are observed in the groundwater due to the special geogenic situation. In a survey of this area, arsenic concentrations up to 300 ng mL^{-1}, most of which was present as arsenite, were reported (*1*). Southeastern Hungary is also noted for freshwater fish production, and around 50% of the fish farms in Hungary are located there (*2*). In addition to the commonly grown carp and bream, African catfish are also cultured in this area; the carp and bream are grown in ponds supplied with surface water whereas African catfish, which are native to tropical areas, are cultivated in tanks filled with geothermal water (28 °C).

It is well-known that marine fish can contain high concentrations of arsenic and that this arsenic is present mainly as the nontoxic arsenobetaine (AB; **Figure 1**) (*3*). Freshwater fish, however, contain much lower arsenic concentrations, and whereas arsenic compounds in marine fish have been studied intensively, there are only a few publications dealing with arsenicals in freshwater fish.

The few studies so far on arsenic species in freshwater fish show a rather complicated picture. Shiomi and co-workers investigated rainbow trout (*Salmo gairdneri*) and Japanese smelt (*Hypomesus nipponensis*) and found that AB accounted for 80 and 60% of the extractable arsenic compounds, respectively (*4*). Inorganic arsenic, MA (methylarsonate), DMA (dimethylarsinate), TETRA (tetramethylarsonium ion), and AB/TMAO (the method was unable to distinguish between these two nontoxic arsenic compounds) were reported to be present in muscle and kidney of bream (*5*). Kaise et al. investigated five fish species from an arsenic-contaminated freshwater environment (*6*). Trimethylated arsenic species (because of the employed method, AB could not be determined directly), as well as DMA, dominated in extracts of the fish species. Mozambique mouthbreeder (*Oreochromis mossambicus*), collected from a Blackfoot disease area in Bangladesh, contained AB and DMA as major arsenicals (*7*). Zheng and Hintelmann found AB and DMA as the dominating arsenicals in Northern pike, whereas in yellow perch and pumpkinseed inorganic arsenic was the major

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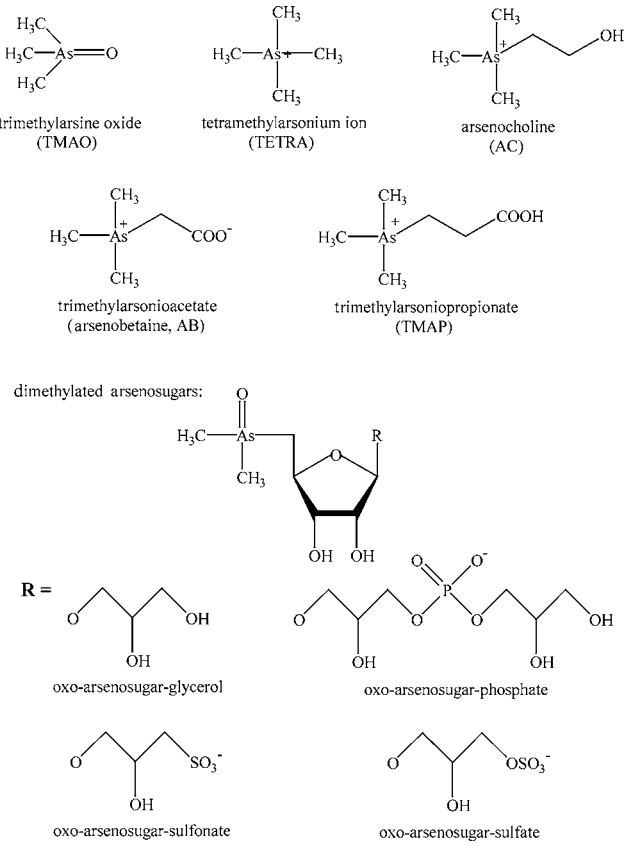

Figure 1. Structures of arsenic compounds relevant to this work (compounds are drawn in their most deprotonated form).

detectable arsenic species (*8*). Slejkovec et al. investigated nine species of freshwater fish belonging to four different families for arsenic species (*9*). They reported AB to be present in almost all samples, in most of them as the major extractable arsenic compound. As(III), DMA, TMAO, and an unknown cationic arsenic compound were also detected in some of the samples.

The possible human health consequences of arsenic in food have resulted in several countries imposing a maximum permissible concentration for this element. Usually this concentration is based on total arsenic, although it is well-recognized that the various arsenic species found in food items vary greatly in their toxicity, from the nontoxic AB to the toxic inorganic arsenic species As(III) and As(V). Future discussions in the European Union on trace elements and food safety are likely to include consideration of the elemental species present, and legislation will be framed accordingly.

Farmed freshwater fish are a popular part of the diet for Hungarian people. These fish, however, are cultured in water with naturally high arsenic concentrations. Knowledge of the arsenic concentrations and species present in the Hungarian farmed fish could help in the evaluation of the food safety of these products and identify possible toxicological risks associated with their consumption. We report an investigation into the arsenic speciation in muscle tissues of two species of freshwater edible fish, obtained from eight fish farms in Hungary.

MATERIALS AND METHODS

Reagents. All solutions were prepared with Milli-Q (18.2 MΩ cm) water. Concentrated nitric acid (Merck; p.a., pro analysis) was further purified in a quartz sub-boiling distillation unit. Methanol (puriss p.a.), formic acid (puriss p.a.), and ammonium dihydrogen phosphate (p.a) were purchased from Fluka (Buchs, Switzerland); and pyridine (p.a.), $Na_2HAsO_4 \cdot 7H_2O$, aqueous ammonia solution (25%, suprapur), sodium borohydride (p.a.), sodium hydroxide (p.a.), and hydrochloric acid (32%, p.a.) were obtained from Merck (Darmstadt, Germany). Standard solutions (1000 μg As cm^{-3}) for the identification and quantification of arsenic compounds were prepared as described elsewhere (*10*). Arsenosugars were isolated from natural sources and purified as described elsewhere (*11*). For total arsenic determinations, Ge (50 ng cm^{-3}) was used as an internal standard.

Instrumentation. Fish and fishfood samples were freeze-dried in a Christ Alpha 1-4 freeze-drying system (Christ, Osterode am Harz, Germany). The dry samples were pulverized in a Retch ZM 1000 mill (Retsch, Haan, Germany) equipped with a titanium rotor and a 0.25 mm sieve. Digestions for total arsenic determinations were performed with a Milestone ultraCLAVE II microwave digestion system (EMLS, Leutkirch, Germany). Total arsenic determinations and arsenic speciation analyses were carried out with an Agilent 7500c inductively coupled plasma mass spectrometer (ICPMS) (Agilent, Waldbronn, Germany) as the detection system. An Agilent 1100 Series high-performance liquid chromatography (HPLC) system (Agilent) consisting of a solvent degassing unit, a binary pump, an autosampler, and a thermostated column compartment was used as the chromatographic system.

Samples. Fishing locations, fish species, number of fish taken, their average length, together with the origin of water samples are summarized in **Table 1**. Fish as well as water samples were collected from eight sampling sites situated in different aquatic ecosystems (**Figure 2**). Six of the eight sampling sites (1, 2, 3, 5, 6, and 7) were situated in the area affected by arsenic groundwater pollution. The fish lake in Látrány (site 8) was selected as a reference point for carp samples (*Cyprinus carpio*), as it is situated 250 km west from the chosen high arsenic area. Samples of African catfish (*Claries gariepinus*) were also taken from a fish farm at Tuka (site 4), which is 80 km north of the chosen area. In Szarvas, three separate sites were sampled as follows: two different groundwater wells for catfish (sites 2 and 3) and one fish lake for carp production (site 7). In general, catfish samples were collected from sampling sites where warm groundwater was used for fish culture, while carp samples were taken from surface fish lakes.

The diets of the two fish species were quite different. Catfish cultured on the fish farms received fishmeal from an automated feed system, whereas carp were reared under natural conditions where they have an omnivorous diet based on plants, insects, and crustaceans (*12*).

Sample Collection. From each sampling point (**Figure 2**), water samples were collected in polyethylene vials. The water samples were acidified to 1% HNO_3 (v/v) and stored at 4 °C until analysis.

African catfish (ca. 50 cm total length) were collected from four fish tanks filled with geothermal water, while the common carp (ca. 40 cm) were purchased from fish farms of Szajol, Biharugra, Szarvas, and Látrány (**Table 1**). Each fish was rinsed with deionized water, and a sample of muscle tissue (ca. 200 g) from just behind the pectoral fin was removed, frozen, and then freeze-dried and pulverized. Fishmeal was freeze-dried and pulverized before analysis.

Procedures. *Sample Digestion.* For total arsenic determinations, portions of freeze-dried muscle tissue (0.5 g), fishmeal (0.5 g), or standard reference material DOLT-2 (0.2 g, dogfish liver, National Research Council Canada, Ottawa, Canada) were weighed into quartz tubes (12 cm^3) and concentrated nitric acid (5 cm^3) was added. The quartz tubes were then closed with Teflon caps and placed in the sample rack. After the autoclave was loaded with argon to a pressure of 40 bar, the samples were heated to 250 °C, and this temperature was maintained for 30 min. The digests were then diluted to 50 cm^3 with Milli-Q water.

In the case of carp samples, total arsenic concentrations of the extracts were also determined. Thus, a portion (2 cm^3) of the filtered extract was transferred to a quartz tube (12 cm^3) and concentrated (65 v/v%) nitric acid (2 cm^3) was added. The decomposition of the extracts was carried out as described above. The digests were diluted to 10 cm^3 with Milli-Q water.

The acidified water samples were measured directly without any further sample preparation. Total arsenic concentrations in the acid digests and in the acidified water samples were determined by ICPMS using an external calibration curve established with As(V) solutions.

Table 1. Size of the Fish, Origin, and Total Arsenic Concentrations of the Collected Fish and Water Samples[a]

	sampling sites: code and named location	collected sample	*N*	AVG[b] length (cm) (RSD%)	total As (μg kg^{-1}) (RSD%)	type of water sample	total As in the water samples (ng mL^{-1}) (RSD%, $n = 3$)
1	Füzesgyarmat		10	51 (6)	4720 (12)	groundwater	162[c]
2	Szarvas water well A	African catfish	10	49 (8)	2980 (18)	groundwater	152 (0.5)
3	Szarvas water well B	(*Clarias gariepinus*)	8	42 (4)	2510 (32)	groundwater	187 (0.5)
4	Tuka		8	46 (4)	2650 (14)	groundwater	15.1 (1.4)
5	Szajol		10	40 (5)	168 (62)	surface water	2.5 (21)
6	Biharugra	common carp	10	44 (5)	62.2 (38)	surface water	0.7 (7.1)
7	Szarvas fish lake	(*C. carpio*)	8	36 (7)	90.3 (44)	surface water	13.2[c]
8	Látrány		6	39 (3)	363 (11)	surface water	3.2[c]
		fishmeal	3		2880 (1.7)		

[a] *N*, number of individuals. [b] AVG, average. [c] Less than three samples were available.

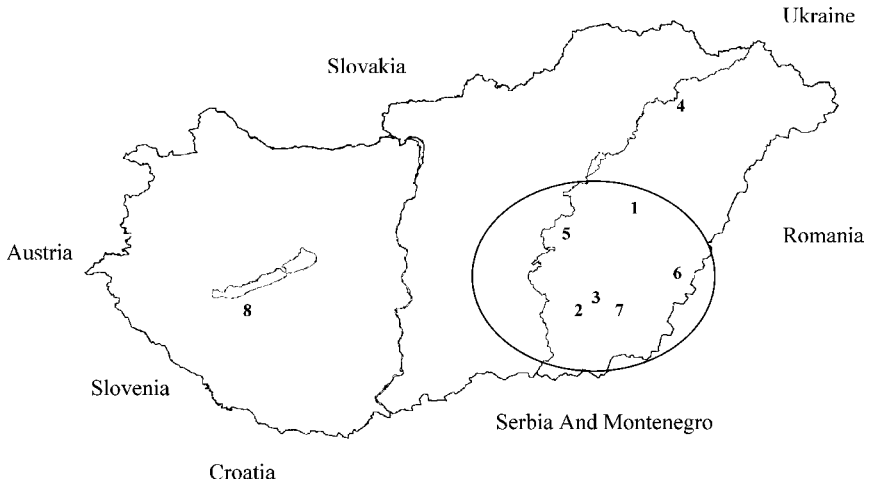

Figure 2. Sampling locations in Hungary. The circle line delineates the arsenic-affected groundwater supply. Key: 1, Füzesgyarmat; 2, 3, and 7, Szarvas; 4, Tuka; 5, Szajol; 6, Biharugra; and 8, Látrány.

The accuracy of total arsenic determinations was checked by analyzing certified reference material DOLT-2 (certified arsenic concentration 16.6 ± 1.1 mg kg^{-1}); the measured total arsenic concentration was 15.5 ± 1.3 mg kg^{-1} ($n = 6$).

Sample Extraction. Portions of the freeze-dried powders (500 mg of catfish, 1000 mg of carp, and 1500 mg of fishmeal) were weighed directly into polypropylene tubes (15 cm^3) and extracted with methanol (10 cm^3) by shaking top over bottom at ambient temperature overnight. After centrifugation (1090*g* for 10 min), portions (2 cm^3) were transferred to polypropylene tubes. The solutions were evaporated to dryness at room temperature in a centrifugal lyophilizer (Maxi Dry Lyo, Heto-Holten, Allerød, Denmark). To the evaporated residues, water was added to a total mass of 2.0 g (catfish) and 1 g (carp, fishmeal). After they were shaken vigorously, the extracts were filtered through 0.22 μm Nylon filters (CAMEO 25NS, Osmonics, Minnetonka) and the filtrates were analyzed for arsenic speciation. In this way, extracts were prepared from four to six individual fish from all sites.

Arsenic Speciation Analysis. Arsenic species were determined by HPLC-ICPMS. The HPLC separations were performed on a Hamilton PRP-X100 (Reno, United States) anion exchange column (25 cm × 4.1 mm i.d., 10 μm particles) with 20 mM aqueous ammonium dihydrogen phosphate of pH 5.6 at 40°C and a flow rate of 1.5 cm^3 min^{-1} and a Zorbax 300-SCX (Agilent) cation exchange column (15 cm × 4.6 mm i.d., 5 μm particles) with 20 mM aqueous pyridine of pH 2.3 at 30 °C and a flow rate of 1.5 cm^3 min^{-1}. The analytical columns were protected by guard columns filled with the same stationary phases. The outlet of the HPLC column was directly connected via PEEK capillary tubing (0.125 mm i.d.) to the nebulizer of the ICPMS system, which served as arsenic selective detector. To improve the detection limits when performing anion exchange chromatography, a Hydride Generation Accessory (Agilent Technology) was installed between the HPLC system and the ICPMS (*13*). The ion intensity at *m*/*z* 75 (^{75}As) was monitored using the "time-resolved" analysis software. Additionally, the ion intensities at *m*/*z* 77 (^{40}Ar^{37}Cl, ^{77}Se) and 82 (^{82}Se) were monitored to detect possible argon chloride (^{40}Ar^{35}Cl) interferences on *m*/*z* 75. Instrumental settings used throughout this work were described in detail elsewhere (*10*). Peak areas were determined using the ICPMS chromatographic software Version C.01.00 (Agilent).

Arsenic species in extracts of the samples were identified by matching retention times with those of standard compounds. The assignments are usually straightforward except for As(III); under our anion exchange chromatographic conditions, As(III) elutes very close to the solvent front and hence is not well-resolved from neutral and cationic arsenicals, in particular the oxo-arsenosugar-glycerol. Although the use of the Hydride Generation Accessory provides some selectivity for As(III), because this arsenical is a strongly hydride-forming species, when cation exchange chromatography revealed the presence of significant quantities of oxo-arsenosugar-glycerol and/or TMAO (both of which produce hydrides under our conditions), the As(III) content could not be quantified. There also remains the possibility of unknown, hydride active arsenicals eluting at the void volume and thus contributing to the signal attributed to As(III). Although we consider this unlikely, the values reported for As(III) should be considered as maximum values.

RESULTS AND DISCUSSION

Total Arsenic Concentrations. The total arsenic concentrations in the water samples collected from the different locations are given in **Table 1**. The highest arsenic concentrations were measured in groundwater samples from Füzesgyarmat and Szarvas (sampling sites 1−3; **Figure 2**) where the average total arsenic concentration found was 167 ng As mL^{-1}, which is 16

Table 2. Arsenic Compounds Determined in the Samples[a]

sample sampling site	fishmeal (ng As/g)	catfish[b] (μg As kg^{-1})				carp[b] (μg As kg^{-1})			
		1	2	3	4	5	6	7	8
AB	1930 ± 10[c]	5010 ± 430	3190 ± 710	2170 ± 610	2900 ± 500	<2.5	<2.5	<2.5	<2.5
TMAP	7.3 ± 0.5[c]	19.4 ± 4.6	17.5 ± 2.5	trace	16.7 ± 1.5	<2.5	<2.5	<2.5	<2.5
TMAO	<1.7	<10	<10	<10	<10	<2.5	<2.5	<2.5	trace
AC	6.1 ± 0.4[c]	<10	<10	<10	<10	<2.5	<2.5	<2.5	<2.5
TETRA	15.3 ± 0.7[c]	10.6± 2.1	trace	trace	<10	<2.5	<2.5	<2.5	<2.5
As(III)	24.0[d]	28.2 ± 3.1	26.8 ± 2.4	24.1 ± 3.0	19.3 ± 3.1	0.9 ± 0.6	0.6 ± 0.1	0.7 ± 0.1	n.d.
DMA	17.9[d]	7.6 ± 1.7	9.6 ± 5.2	6.8 ± 1.3	4.9 ± 1.2	2.1 ± 1.7	1.3 ± 0.5	1.6 ± 0.2	10.7 ± 1.0
MA	5.0[d]	27.0 ± 1.8	28.4 ± 3.5	29.1 ± 3.9	25.7 ± 4.0	1.0 ± 0.5	0.5 ± 0.2	1.1± 0.5	1.1 ± 0.5
As(V)	<1.7	trace	trace	10.1 ± 5.9	trace	trace	trace	trace	trace
oxo-arsenosugar-phosphate	<3.3	<20	<20	<20	<20	<5	<5	<5	74.2 ± 6.3
oxo-arsenosugar-glycerol	<1.7	<10	<10	<10	<10	<2.5	<2.5	<2.5	3.8 ± 0.7[e]
unknown peak									10.5 ± 3.2[e]
summed species/ total As × 100 (%)	70	101	108	97	120	2.3	4.9	4.6	29

[a] ND, not determined. [b] $n = 4$–6 individuals. [c] $n = 3$. [d] $n = 2$. [e] Quantified by TETRA.

times higher than the maximum permissible limit for drinking water (10 ng As mL^{-1}) recommended by the World Health Organization (*14*). The groundwater sample collected in Tuka (sampling site 4) showed only 15.1 ng As mL^{-1}. The generally high arsenic concentrations found in these groundwater samples are in good agreement with the results published by Csanády (*1*). The arsenic concentrations in the surface water samples were generally low (≤3.2 ng As mL^{-1}) with the exception of the surface water collected from the Szarvas fish lake, which contained 13 ng As mL^{-1}. Probably, the relatively high arsenic concentration found in the Szarvas fish lake is because the water originates from the Körös river, which is known for its anthropogenic arsenic pollution (*15*).

Mean total arsenic concentrations in the carp samples ranged from about 60 to 360 μg As kg^{-1}, well within the range reported for other freshwater fish collected from nonpolluted environments (*9*, *16*). However, the total arsenic concentrations determined in catfish ranged from 2510 to 4720 μg As kg^{-1}, much higher than the published data for other freshwater fish even when compared with data from contaminated sites (*6*−*8*, *17*, *18*). Arsenic present at 2880 μg As kg^{-1} as a natural constituent of the fishmeal fed to the catfish is the probable explanation for the observed higher arsenic concentrations in the catfish from our study. The arsenic concentrations in neither carp nor catfish appeared to be correlated to water arsenic concentrations.

Arsenic Speciation. The arsenic speciation analysis of the samples revealed marked differences in the case of two fish species (**Table 2**). In the catfish samples, AB was detected as the major compound constituting >90% of the total arsenic (**Figure 3**). Several other arsenic species were also present but only as trace constituents, namely, TMAP, TETRA, DMA, MA, As(V), and As(III). In contrast, none of the carp samples contained any detectable AB; in most cases, the only arsenic species present were the four simple anions, namely, As(III), As(V), MA, and DMA. The exception is the sampling site 8 (control site), where in all carp samples the dominant presence of oxo-arsenosugar-phosphate was found. We detected also the oxo-arsenosugar-glycerol, a trace of TMAO and an unknown peak eluting between AC and TETRA on the cation exchange column in all individuals of this group (**Figure 4a**,**b**). In general, concentrations of the arsenicals in the carp samples, however, were much lower (5−50-fold) than those found in the catfish.

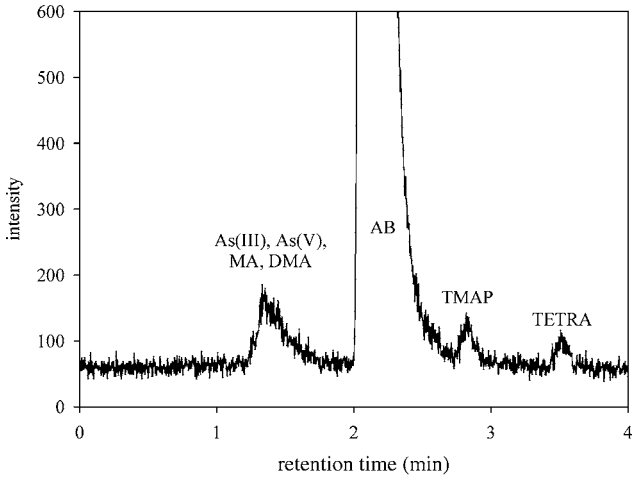

Figure 3. Cation exchange HPLC-ICP-MS chromatogram of a catfish sample collected at Füzesgyarmat (sampling site 1) (column, ZORBAX 300-SCX; column temperature, 30 °C; mobile phase, 20 mM pyridine at pH 2.3; injection volume, 50 mm^3; and flow rate, 1.5 cm^3 min^{-1}).

The large difference between the carp and the catfish results is best explained by their diets rather than by the arsenic pollution of their water media. Arsenic speciation analysis of fishmeal (**Table 2**) fed to the catfish showed a preponderance of AB, in addition to small amounts of several other arsenicals found in catfish. Of interest though is the fact that As(V) was not detected in the fishmeal but was present in all samples of catfish. Possibly, the source of As(V) was the high arsenic water in which the catfish were reared, or it may result from in vivo oxidation of As(III) present in the fishmeal. Also of interest was the absence of AC in all of the catfish samples despite this arsenical being present in the fishmeal. This may simply be an analytical consequence of poorer detection limits obtained for the fish samples. Alternatively (or perhaps in combination with poorer detection limits), this result may reflect lower accumulation for AC as compared to AB and TMAP. For example, when the marine fish *Aldrichetta forsteri* was given AC in its diet, it did not accumulate this arsenical but rather converted the AC to AB (*19*).

The presence of appreciable concentrations of AB in the catfish raises the question as to the origin and role of this compound in fish. The fact that high concentrations of AB occur

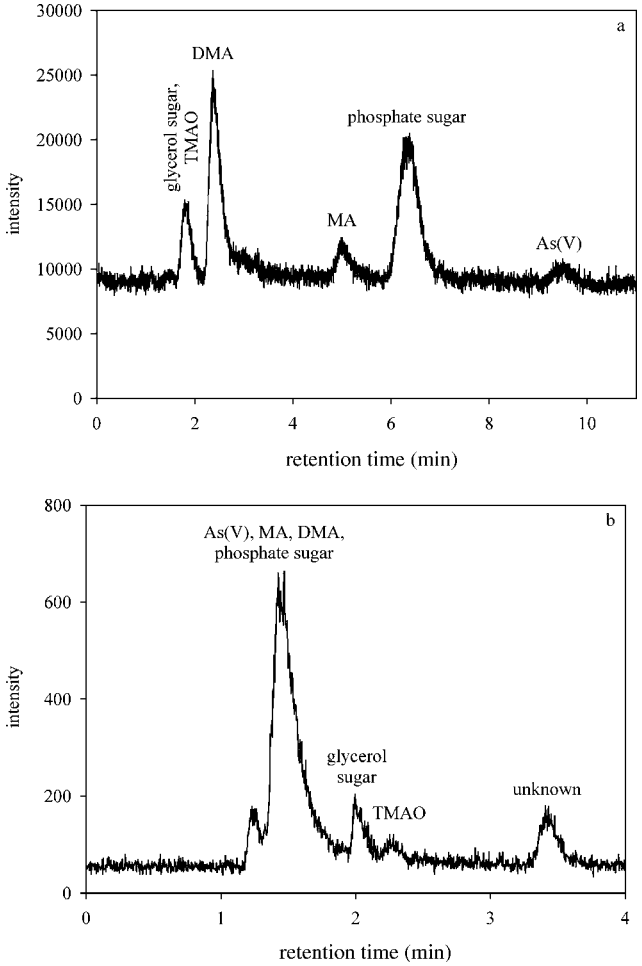

Figure 4. (**a**) Anion exchange HPLC-HG-ICP-MS chromatogram of a control carp sample from Latrany (sampling site 8) (column, Hamilton PRP-X100; column temperature, 40 °C; mobile phase, 20 mM $NH_4H_2PO_4$ at pH 5.6; injection volume, 50 mm^3; and flow rate, 1.5 $cm^3\ min^{-1}$). (**b**) Cation exchange HPLC-ICP-MS chromatogram of a control carp sample from Latrany (sampling site 8) (column, ZORBAX 300-SCX; column temperature, 30 °C; mobile phase, 20 mM pyridine at pH 2.3; injection volume, 50 mm^3; and flow rate, 1.5 $cm^3\ min^{-1}$).

in marine fish, but not in freshwater fish, suggests that salinity of the ambient water may be a factor. Osmoconforming animals, such as molluscs, retain small charged organic molecules when in high salinity waters and release them when salinity is reduced. It appears reasonable that AB, structurally very similar to a major osmolyte glycine betaine, behaves similarly and thus occurs at a higher concentration in osmoconforming animals taken from marine waters. The AB found in osmoregulating fish may be just a consequence of the AB content of their diets. In our study, fish (carp) on a natural diet contained no AB, whereas fish (catfish) given AB in their diet were able to accumulate this arsenical to levels comparable with those found in marine fish. On the basis of these results, it seems that ingested AB can be easily accumulated in fish irrespective of its freshwater or marine origin. This observation is consistent with results from a recent laboratory study, which showed that both freshwater- and saltwater-adopted Atlantic salmon accumulated AB equally well (*20*).

Although most of the carp samples showed a very simple pattern of arsenic species [the four simple anions As(III), As(V), MA, and DMA], the carp sampled from Látrány (site 8) were very different in that they also contained the oxo-arsenosugar-phosphate and oxo-arsenosugar-glycerol. Indeed, these arsenicals occurred in all four individual samples from Látrány at fairly uniform concentrations and constituted >75% of the sum of arsenic species in each case. Arsenosugars can occur in freshwater algae and plants (*21*, *22*), and these are the likely source of the arsenosugars in carp from Látrány.

The work reported here has several points of toxicological interest. It is well-known that AB is a nontoxic form of arsenic whereas the inorganic forms are toxic. Thus, although the catfish cultured in Hungary have moderate concentrations of arsenic (up to ca. 5 mg kg^{-1} dry mass), this is almost entirely present as the harmless AB and hence presents no concerns for human health whatsoever. The carp, grown in lakes in Hungary on natural diets, contain low concentrations of arsenic (generally <0.2 mg kg^{-1}), which, even if totally present as inorganic arsenic, would not constitute a significant health risk to human consumers. For carp from the one site (Látrány, site 8), which contained slightly higher arsenic concentrations (ca. 0.3 mg kg^{-1}), inorganic arsenic was a minor constituent, and most of the detectable arsenic was present as arsenosugars.

This leads to a point of fundamental importance in the toxicological assessment of arsenic species in food, namely, how much of the total arsenic present in the original sample is accounted for by speciation analysis. For the catfish investigated here, 97−120% of the total arsenic was accounted for by extraction followed by HPLC-ICPMS analysis (>90% of which was AB; **Table 2**). Probably the high concentrations of AB in our catfish samples (see below) have influenced the high extraction efficiency observed for this species. We consider these data acceptable and believe that they provide an adequate picture of arsenic speciation in these samples. For the carp, however, only 2−29% of the total arsenic was accounted for by our speciation analysis. Low extraction efficiencies for arsenic have been previously reported for some freshwater fish (*9*). In our work, the efficiency of the extraction was determined by the digestion of the extracts and compared to the total arsenic amount incorporated in the carp samples. Surprisingly, total arsenic analysis of the extracts showed that the extraction yield was actually between 50 and 70% in each investigated carp sample, which means that most of the apparent losses occurred at the HPLC-ICPMS stage. The discrepancies between the total arsenic in the extracts and the sum of arsenic species eluting from the HPLC column remain unexplained and will be the subject of further studies. However, the data generated emphasize the importance of mass balance calculations as an integral part of quality control of the chosen speciation analysis method. We note in closing that despite the apparent low recoveries of arsenic species in carp, the total arsenic concentrations were so low that these fish do not represent a significant toxicological concern. Indeed, the very low arsenic concentrations in these samples may well exacerbate the problems of low recovery encountered in our speciation analyses.

In summary, the work reported here has shown that (i) freshwater fish when exposed to AB in food can accumulate this arsenical to appreciable concentrations and (ii) a combination of total arsenic analysis and arsenic speciation analysis can identify foods of possible toxicological concern.

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