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# Arsenic speciation in fish from Greek coastal areas☆

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ABSTRACT

Arsenic speciation analysis was conducted on fish samples (sardine and anchovy) collected from six areas along the Greek coastline, *i.e.* Artemisium Straits, Thermaikos Gulf, Amvrakikos Gulf, Strymonian Gulf, Thracian Sea, and Elefsina Gulf. Total arsenic levels ranging from 11.8 to 62.6 mg As/kg dry weight were determined. Arsenobetaine, a non-toxic form of arsenic, was found to be the main arsenic species, present at 8.6 to 58.8 mg As/kg dry weight, accounting for 67–95% of the total arsenic. Also detected in all fish samples was dimethylarsinic acid, although at considerably lower concentrations, ranging from 0.072–0.956 mg As/kg dry weight. Monomethylarsonic acid was detected at low levels in all anchovy samples, and only in sardines from one area. Finally, inorganic arsenic in the form of arsenate was detected only in fish at one area, indicating the possible effect of an environmental parameter on its presence at detectable amounts. Statistical analysis revealed the environmental variables, such as salinity, total organic carbon and nitrogen, ammonium, phosphate, total phosphorus, dissolved oxygen and pressure index, are potentially correlated to As species concentrations. Furthermore, based on factor analysis, the biological parameters, such as fish weight, lipids, protein and ash content, that are correlated to As species concentrations of fish were also identified. The interrelationship of arsenobetaine and dimethylarsinic acid concentrations within each fish species was evaluated.



## Introduction

Greeks rely heavily on seafood diets, especially fish and crustaceans, as a result their main exposure to arsenic (As) is *via* the consumption of seafood that contains elevated levels of As in various chemical forms, *i.e.* arsenic species (Cullen and Reimer, 1989; Edmonds and Francesconi, 1987). It is therefore critical from a food safety point of view for As speciation analysis to be conducted in order to determine As species and their quantities that are being ingested by humans and thus enable more accurate risk assessments (Fowler et al., 2015; Francesconi, 2010; Moreda-Piñeiro et al., 2012). Because many of the marine organisms in question originate from Greek coastal areas with considerable industrial activity, it is of interest to establish As species concentration levels and thus be able to identify if and when marine organisms are being exposed to elevated As concentrations in their environment.

Detection of As contamination in seafood and other marine organisms is critical both because of health risk and environmental issues. This is because different As species exhibit

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different physicochemical properties and toxicities (Cullen and Reimer, 1989; Hughes, 2002; Moe et al., 2016), *i.e.* inorganic As species, arsenite iAs(III) and arsenate iAs(V), are more toxic than the organo-As(V) forms, such as arsenobetaine (AsB), a compound that has been established to be non-toxic and the main As component in fish (Neff, 1997; Sharma and Sohn, 2009). However, it should be stressed that detecting As contamination in fish present in the natural environment is particularly difficult, mainly because fish bioaccumulate As naturally. Therefore, even when marginally elevated As concentrations are found in marine biota, it is extremely difficult to associate these levels directly to anthropogenic contamination or even naturally occurring elevated As levels. This is because the source of AsB, the main As species in marine fish, is not completely understood (Popowich et al., 2016; Zhang et al., 2016b), and thus it is believed to originate from their diet (Azizur Rahman et al., 2012; Cullen and Reimer, 1989; Larsen and Francesconi, 2003; Zhang et al., 2012) or as some studies have suggested to form as part of their metabolism (Foster and Maher, 2016; Zhang et al., 2016b). So the dietary status of fish may be affecting the extent of As bioaccumulation and thus As species levels (Maher, 1985; Maher et al., 1999; Zhang et al., 2016c), especially AsB levels (Amlund et al., 2006a; Larsen and Francesconi, 2003; Zhang et al., 2016b). In addition, environmental factors can affect dietary supplies/food availability (Bonanno et al., 2014; Martino and Houde, 2010; Rumolo et al., 2016) and thus indirectly influence As species levels.

So far only a limited number of As speciation studies in fish collected from Greek coastal areas have been conducted, especially for fish species that are of high consumption such as the sardines and anchovies analyzed here. To the best of our knowledge only the study by Schaeffer et al. (2005) has so far reported As speciation for fish originating from Greek coastal areas. Also, limited reports for As speciation in these fish species, from other areas around the world, have been made so far (Kucuksezgin et al., 2014; Moreda-Piñeiro et al., 2012; Muñoz et al., 2000; Özcan et al., 2016; Rattanachongkiat et al., 2004; Schoof and Yager, 2007).

The objective of the present study was to determine As species levels in two fish species, *i.e.* sardine and anchovy, originating from various areas along the extended Greek coastline. These results are compared to those found in a 2005 study involving the same fish species and similar Greek coastal regions (Schaeffer et al., 2005). It is also of interest to investigate the correlation of environmental and fish biological parameters with As species in fish. This is expected to provide improved insight into the factors affecting the bioaccumulation and metabolism of As species in fish by distinguishing them in terms of fish diet and fish metabolism. Detailed statistical analysis was performed in order to help reveal existing correlations between As species and biological and environmental parameters.

## 1. Materials and methods

### 1.1. Sample collection and preparation

Sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*) samples ($n$ = 180) were collected from six different coastal areas in Greece, Artemisium Straits (ART), Thermaikos Gulf (THE), Amvrakikos Gulf (AMV), Strymonian Gulf (STR), Thracian Sea (THR) and Elefsina Gulf (ELE), shown in Fig. 1. All fish samples belonged to a gonad stage level 1. These fish species are consumed in high amounts (approximately 40% of the population is a high consumer of these fish) (Schaeffer et al., 2005). Sampling from all areas took place from September to October 2013.

Immediately after collection, total length and total weight of fish were measured, all edible parts (skin, flesh and bone) were dissected, placed in zip-lock bags and stored at −20°C. All samples were subsequently freeze-dried and stored under dry conditions. For each fish species and area, three composites were prepared by mixing and homogenizing five individuals per composite. Chemical determinations were carried out in triplicate for each composite, thus providing nine replicate analyses for each fish species and area.

### 1.2. Reagents and standards

Aqueous solutions containing individual arsenic compounds were prepared from sodium arsenite (iAs(III)), $NaAsO_2$ (BDH Chemicals Ltd., Poole, England), from sodium arsenate dibasic heptahydrate $Na_2HAsO_4{\cdot}7H_2O$ (iAs(V)), from dimethylarsinic acid (DMA) or cacodylic acid (Fluka) and from monosodium acid methane arsonate sesquihydrate (Chem Service, West Chester, PA) referred to in the remainder of this manuscript as monomethylarsonic acid (MMA). Arsenobetaine (AsB) or 2-(trimethylarsonio)acetate stock solution was obtained from Sigma-Aldrich. These As species stock solutions were diluted with deionized water (Milli-Q 18.2 MΩ cm) to the desired concentrations before use. For the analytical procedures the following chemicals were used: di-ammonium hydrogen phosphate from Sigma-Aldrich, and hydrogen peroxide and nitric acid from Merck. Total As standard of 10,000 μg/mL from CPI International was used for making standards for total As determination. Indium standard of 1000 μg/mL from CPI International was used to prepare the internal standard for total As.

### 1.3. Instrumentation

A Flexar high performance liquid chromatography (HPLC) pump (Perkin Elmer) was used for mobile phase delivery at 1 mL/min for the HPLC separations. The pump outlet was connected to a Rheodyne injection valve fitted with a 20 μL loop, followed by an anion exchange HPLC column. The column effluent was introduced into the inductively coupled plasma mass spectrometer (ICP–MS) *via* a pneumatic nebulizer (Meinhard, Elemental Scientific Glassblowing, Golden, CO). The ICP–MS used was a NexION 300xx ICP–MS (PerkinElmer, Shelton, CT, U.S.) which had been optimized for maximum. In sensitivity, $CeO^+$ levels below 3%, and $Ce^{++}$ levels below 2.5%. Additional m/z ratios were also monitored in order to detect any $ArCl^+$ interferences (m/z 77), as well as Se (m/z 78 or 82) in case corrections were needed.

### 1.4. Total arsenic determination

Concentration of total arsenic was determined in each composite separately using a modification of the US EPA