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Anodic stripping voltammetry with gold electrodes as an alternative method for the routine determination of mercury in fish. Comparison 2 with spectroscopic approaches 3

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Abstract 11

The applicability to the determination of mercury in tuna of square wave anodic stripping 12 voltammetry (SW-ASV) conducted at both solid gold electrode (SGE) and a gold nanoparticle-13 modified glassy carbon electrode (AuNPs-GCE) was demonstrated. Mercury content in two 14 certified materials and in ten samples of canned tuna was measured. The performances of the 15 16 electrodes were compared with one another as well as with two spectroscopic techniques, namely cold vapour atomic absorption spectroscopy (CV-AAS) and a direct mercury analyser (DMA). The 17 results found pointed out that both SW-ASV approaches can be considered both suitable, easy and 18 alternative methods to monitor mercury concentration in tunas, since they allowed to reach accurate 19 quantification at concentration values lower than the maximum admissible level in this matrix 20 ([Hg]=1 mg/kg_{wet weight,ww}). In particular, mercury detection at the AuNPs-GCE showed a LOQ in 21 fish-matrix of 0.1 µg/l, corresponding to 0.06 mg/kg_{ww}, with performance comparable to that of 22 23 DMA.

- 24
- 25

Keywords: mercury, gold nanoparticle-modified glassy carbon electrode, solid gold electrode, 26 27 direct mercury analyser, cold vapour atomic absorption spectroscopy

28

29 **1** Introduction

Heavy metals are considered among the most alarming forms of pollution in the aquatic 30 environment because of their toxicity and accumulation by marine organisms. Fish absorb heavy 31 metals from the surrounding environment depending on a variety of factors such as the 32 characteristics of the species under consideration, metal concentrations in water and exposure 33 period, as well as abiotic factors such as temperature, salinity, pH, and seasonal changes (Ginsberg 34 & Toal, 2009). The distribution of metals varies among fish species, since it depends on age, 35 development status and other physiological factors. Tuna, as a top predator, being able to 36 concentrate high amounts of heavy metals are also used for biomonitoring of environmental 37 38 contamination (Has-Schon, Bogut, & Strelec, 2006).

Several metals, such as iron, copper, zinc, and manganese play an essential role in biological systems; other elements, as mercury, lead, and cadmium are toxic, even in trace amounts, and they have been included in the regulations of the European Union for contaminants in foodstuffs EC (Commission Regulation, 2008). Mercury, in particular, is a known toxicant which is present in the environment as a result of both natural processes and anthropogenic activities. Fish accumulate considerable amount of this metal in their tissues and, in fact, the primary source of mercury contamination in man is through eating fish (Inskip & Piotrowsiki, 1995).

In the last years, the consumption of fish has increased in importance due to the high protein supply, 46 and low saturated fat and omega fatty acids content that are known to contribute to good health. 47 Consequently, the health risk associated with consumption of fish contaminated by heavy metals is 48 an important global concern. The techniques generally adopted for Hg determination are cold 49 vapour atomic absorption spectroscopy (CV-AAS) (Souza-Araujo, Giarrizzo, & Lima, 2015), cold 50 vapour atomic fluorescence spectroscopy (CV-AFS) (Fricke, Götz, Schleyer, & Püttmann, 2015) 51 52 and inductively coupled plasma mass spectrometry (ICP-MS) (Sasmaz, Akgül, Yıldırım, & Sasmaz, 2016). These methods are well established, but they are affected by several drawbacks, such as 53 lengthy analysis times, the use of expensive equipment, the lack of multi elemental analysis, the 54 incapacity for speciation studies (Bagheri, Afkami, Saber-Tehrani & Khoshsafar, 2012) and they 55 cannot be used for field-analysis. Furthermore, several complex steps must be performed, and these 56 57 require specially trained personnel. Thus, the availability of simple, inexpensive and rapid methods suitable for the routine determination of mercury in food samples is highly desirable. 58

Recently, new devices for direct mercury analysis have been developed, which automatically
perform both sample decomposition and Hg detection by AAS, with short analysis times and low
limit of quantitation, LOQ = 0.010 mg/kg wet weight) (Squadrone, Benedetto, Brizio, Prearo, &
Abete, 2015).

2

Also electrochemistry offer quite attractive routine analysis capabilities, because it is sensitive, 63 inexpensive, simple, fast and can be performed with miniaturized, portable instrumentation. Several 64 electrochemical methods have been developed for mercury determination in different matrices, 65 especially in water (Martín-Yerga, Gonzáles-García & Costa-García, 2013). Focusing the attention 66 to fish analysis, most of them are based on the preconcentration of Hg onto the working electrode 67 and subsequent stripping, primarily using anodic stripping voltammetry (ASV). In particular, the 68 U.S. Environmental Protection Agency (USEPA) has recommended the use of stripping 69 70 voltammetry for mercury analysis (EPA, Method 7472, 1996).

Even if voltammetric methods are the most commonly reported, some potentiometric methods have also been developed for Hg determination (Clevenger, Smith, & Winefordner, 1997). Other authors suggest the use of anodic stripping chronopotentiometry–because of the low contribution of capacitive current-to the measured signal in comparison to ASV (Kurmaz & Gultyai, 2010).

75 Some reported ASV methodologies are based on the use of simple bare electrodes as working 76 electrode (WE), especially based on carbon and gold. Interestingly, gold thin-layer electrodes made 77 from compact discs (CD-ROM) were employed successfully for mercury determination in fish after a digestion step (Radulescu & Danet, 2008). Unfortunately, solid electrodes suffer from memory 78 79 effects resulting from the difficult removal of mercury from their surface (Martín-Yerga, Gonzáles-García & Costa-García, 2013). Therefore, reusing the electrode without interferences remains an 80 important challenge to obtain a WE that fulfils the requirements for routine analysis in accredited 81 laboratories. With this purpose a large number of modified WEs have been studied. Screen-printed 82 carbon electrodes (SPCEs) modified with gold films have been employed and have achieved LODs 83 as low as 0.9 µg/L (Meucci, Laschi, Minunni, Pretti, Intorre, Soldani, & Mascini, 2009). Tamer et 84 85 al. used a platinum electrode modified with poly(3-hexylthiophene) (Tamer, Oymak & Ertaş, 2007). Also carbon paste electrodes (CPEs) modified with several species able to complex and 86 preconcentrate Hg(II) have been used. For example, CPEs have been modified with several species 87 able to complex and preconcentrate Hg(II). Afkhami et al. modified a CPE with nitrobenzoyl 88 diphenylmethylenphosphorane (N-BDMP) for the simultaneous determination of Cd²⁺ and Hg²⁺ in 89 90 various samples, fish included (Afkhami, Madrakian, Sabounchei, Rezaei, Samiee, & Pourshahbaz, 2012). Also different silica species functionalized with complexing groups have been employed for 91 92 the modification of GCE and CPEs, For example, fish analysis was performed using silica 93 nanoparticles functionalized with a Schiff base (Afkhami, Madrakian, Ghaedi, Rezaeivala, 2013). 94 The same authors also used a CPE modified with multi-walled carbon nanotubes (MWCNTs) and 95 3-(4-methoxybenzylideneamino)-2-thioxothiazolodin-4-one, as a new synthesized Schiff base, for 96 the simultaneous determination of Pb(II) and Hg(II) by ASV in several samples, including tuna fish

(Afkhami, Bagheri, Khoshsafar, Saber-Tehrani, Tabatabaee & Shirzadmehr, 2012). The unique 97 properties of nanotubes enhance the effect of the complexing agent improving the analytical signal 98 of this electrode in comparison to the previous example. Bagheri et al. developed a pasting binder 99 for CPE based on a triphenylphosphine-modified carbon nanotube composite with a room 100 temperature ionic liquid (RTIL) for sensitive and simultaneous determination of Cd(II), Pb(II) and 101 Hg(II) in fish (Bagheri, Afkhami, Khoshsafar, Rezaei, Shirzadmehr, 2013). Recently Ramenzani et 102 al. (Ramezani, Mashhadizadeh, Jalilian & Mehdi Aghilic, 2015) develop a CPE modified with 103 104 MWCNTs, AuNPs and 1,2-bis[5,2-thiolmethyl-sulphide-1,3,4-oxadiazol-2-yl]-ethane (BTMSOE) 105 as a novel ion-carrier: with this MWCNTs/AuNPs/BTMSOE-CPE they reached very low LOD also 106 in fish matrix.

Table 1 shows a comparison among the analytical performance of electrochemical methods appliedfor mercury determination in fish published in the literature previously described.

Despite this numerous electroanalytical approaches reported in literature, EPA still suggests the use of a GCE modified with a gold film, since the modification required is very simple and permits to work with a renewable surface. However it is not possible to determine whether bare gold electrodes and gold film electrodes exhibit significant differences in analytical performance (Abollino, Giacomino, Malandrino, Piscionieri, & Mentasti, 2008, Martín-Yerga, Gonzáles-García & Costa-García, 2013).

In view to propose a technique suitable for routine analyses, cheap and easy to be applied, in this 115 study we tested the performance of ASV using a solid gold electrode (SGE) (Abollino, Giacomino, 116 Malandrino, Piscionieri, & Mentasti, 2008) and a home-made gold nanoparticle- modified glassy 117 carbon electrode (AuNPs-GCE), and assessed their applicability for mercury determination in fish. 118 The AuNPs-GCE, had previously been developed in our laboratory, and applied to the 119 quantification of Hg in the low ng/L range in aqueous solutions and in solid certified materials 120 having different matrices (Abollino, Giacomino, Ginepro, Malandrino & Zelano, 2012). The main 121 advantages of this type of electrode are i) the very easy procedure required for electrode 122 123 modification, ii) the possibility to work with a renewable surface (as in film electrodes), dissolving 124 the gold nanoparticle layer and depositing a new one when a worsening of the response is noticed, and in addition, iii) the large surface area of the deposited nanoparticles, that gives rise to an 125 126 improvement of the analytical performance (in particular lower detection limit) in comparison to conventional electrodes. 127

Two reference materials, namely *Tuna Fish BCR 463* and *Tuna Fish ISPRA T-22*, and ten commercial samples of canned tunas (CTs) were analysed for mercury by ASV, using SGE and AuNPs-GCE. The results were compared with those obtained with two spectroscopic techniques,

- namely conventional CV-AAS and a direct mercury analyser (DMA), to better assess the
 advantages and drawbacks of the electrochemical approach for Hg determination.
- 133 Finally, for the sake of completeness, we compared mercury concentrations found in the considered
- 134 CTs with the maximum admissible value for this element established by the European Legislation.
- 135

136 **2 Experimental**

137 2.1 Apparatus and Reagents

Digestions of samples were performed in polytetrafluoroethylene (PTFE) bombs, with a Milestone
 MLS-1200 Mega microwave laboratory unit (Milestone, Sorisole, Italy).

140 Voltammetric analyses were performed with a PGSTAT 10 potentiostat (Eco Chemie, Utrecht, The

141 Netherlands) coupled to a 663 VA Metrohm (Herisau, Switzerland) stand equipped with an AuNPs-

142 GCE working electrode prepared from a commercial Metrohm GCE (see below) or a solid gold

electrode (SGE), a glassy carbon counter electrode and an Ag/AgCl/KCl (3M) reference electrode.

144 The analyser was interfaced to a personal computer; the operational conditions were selected and

voltammogramms were visualised and processed with the aid of GPES 4.9.

Morphological characterisation of the electrode surface was performed by scanning electron microscopy, SEM (LEICA Microsystems, Germany) using a Stereo scan 410 SEM Inspect FTM with Field Emission Gun.

The roughness of the obtained AuNPs-layer was investigated by Atomic Force Microscopy (AFM) using a Danish Micro Engineering Scanning Probe Microscope, SPM (AFM/Scanning Tunneling Microscope, STM) Microscope, with a DME Igloo stage with 50 µm DS95-50E SPM head for fluid environment, integrated optical axis on cantilever and total positioning and approach control via CCD PSU camera (DME 2350) and a fully digital hold C26 Dualscope/Rasterscope controller.

- A DMA-80 Direct Analyser (FKV SrL, Torre Boldone, BG, Italy) was employed; the analyses were carried out in IZSPLV laboratory in Torino. The instrument features a circular, stainless steel, interchangeable 40-position autosampler, and can accommodate both nickel and quartz boats depending on the requirements of the application. It requires regular grade oxygen as a carrier and decomposition gas. The instrument is equipped with a Hollow Cathode Lamp ($\lambda_{Hg} = 253,7$ nm) and a Si-photodiode sensor.
- The sample solutions were also analysed by with a model 1100 B AA Spectrometer (Perkin Elmer,
 Waltham, MA, USA) equipped with a MHS-20 Mercury Hydride System accessory. Argon was

adopted as carrier to conduct the vapours to the atomization cell. The instrument is equipped with an Electrodeless Discharge Lamp ($\lambda_{Hg} = 253,7$ nm) and a Photomultiplier detector. NaBH₄ and KMnO₄ solutions were used for the formation of volatile hydrides and to monitor the reaction respectively.

A chemometric processing of the experimental results was performed by ANOVA, with the aid ofan XLStat 7 software package, used as a Microsoft Excel plug-in.

Analytical grade reagents were used. A 1000 mg/l standard solution of mercury was prepared from
 HgCl₂ in 0.012 M HCl. More diluted Hg(II) standard solutions were prepared from the concentrated
 standards.

171 Calibration standards for DME-80 were prepared using a NIST traceable stock solution of 1000 172 mg/l Hg preserved in 5% HNO₃. Working standards of 0.1 and 1 mg/l were prepared and preserved 173 in 3.7% HCl and stored in amber glass vials.

High purity water (HPW) obtained from a Milli-Q apparatus (Millipore, Bedford, USA) was usedthroughout.

176 100 mg/l stock solutions of HAuCl₄· $3H_2O$ (Sigma, > 99.9% trace metals basis) in HPW were 177 prepared and used for the deposition of gold nanoparticles onto the electrode.

For the CV-AAS analysis a 3% sodium borohydride solution in 1% NaOH was used as reducingreagent.

180 2.2 Procedures

181 *2.2.1 Samples and sample pretratments*

182 *Tuna Fish BCR 463* ([Hg] = 2.85 ± 0.16 mg/kg) and *Tuna Fish ISPRA T22* ([Hg] = 4.43 ± 0.34 183 mg/kg) were analysed to value the efficiency and the accuracy of each technique for mercury 184 quantification in this type of matrix. The concentration in *ISPRA T22* is not certified, so we used the 185 value reported in literature by Detcheva and Grobecker as reference concentration (Detcheva & 186 Grobecker, 2006).

187 Ten samples of CT were purchased in local supermarkets. Samples were transported to the 188 laboratory and coded 1-10 for easy identification. Then, each can was opened and the sauce drained. 189 The samples of tuna fish were grinded in a mortar and they were freeze-dried in order to obtain a 190 powdered sample, useful to be analysed in different times and with different techniques. For ASV and CV-AAS analysis, a dissolution of the matrix was required. Aliquots of 0.5 g of *Tuna Fish BCR 463* and *Tuna Fish ISPRA T-22* were transferred into the bombs and digested without any pretreatment with a mixture of 3 ml of HNO₃ and 3 ml of H_2O_2 . Aliquots of 0.25 g of CTs were treated in the same way. The following heating program of the microwave unit was adopted: 250 W for 1 min; 0 W for 1 min; 250 W for 5 min; 400 W for 5 min; 650 W for 5 min; ventilation for 25 min. The bombs were left to cool at room temperature. The resulting solutions were diluted to 15

mL with HPW. The digested solutions were freshly prepared for analysis with each technique.

For DMA the analysis was performed directly on the freeze-dried powder, since no other samplepretreatment is required.

All the experiments were performed in duplicate and blanks were simultaneously run.

201

202 2.2.2 Deposition of gold nanoparticles on the glassy carbon electrode

Modification with gold nanoparticles layer was performed following the procedure described in our
 previous work (Abollino, Giacomino, Malandrino, Piscionieri, & Mentasti, 2008)

205 The presence of AuNPs was confirmed by SEM analyses and by cyclic voltammetry (see below).

206 The latter was performed varying the potential from 0 to 1.3 to 0 V in 0.5 M H_2SO_4 .

207 An estimation of the roughness of the obtained gold layer was obtained by AFM (see below).

208

209 2.2.3 Solid gold electrode pretreatment

The SGE was polished and activated following the procedure reported in our previous paper(Giacomino, Abollino, Malandrino, & Mentasti, 2008).

During all the analyses hereafter described there was no need to renew electrode surface withalumina powder.

214

215 2.2.4 Determination of mercury by SW-ASV

Aliquots of 4 or 2 ml of digested solutions were transferred into the voltammetric cell and diluted to
20 ml with 0.06 M HCl or 60 mM NaCl, in the case of SGE or AuNPs-GCE respectively.

7

218 The following procedures were the same for the two electrodes.

After 120 s of deposition at 0 V, a voltammetric scan was performed, adopting the parameters,
previously optimised in our laboratory (Giacomino, Abollino, Malandrino, & Mentasti, 2008).

The medium exchange technique was adopted for the analysis: after the electrodeposition step from the sample solutions, the potential was maintained at 0 V with the optional function "Hold" of the instrument and the sample solution cell was replaced by a solution of 0.06 M HCl or 0.06 M NaCl in which the stripping step was then performed (Abollino, Giacomino,Malandrino, Piscionieri, & Mentasti, 2008).

After each determination the electrode was maintained in a mixture of 0.2 M HClO₄/3 mM NaCl/1 mM NaEDTA for 30 s at 0.80 V, to remove residues of mercury from its surface (Metrohm, Application Bulletin No 96/4e). The presence of EDTA, probably thanks to its complexing properties, favoured this removal.

After recording the voltammogram of the sample solutions, aliquots of Hg were added and the corresponding signals were recorded. The standard addition method was adopted for the evaluation of the concentration of mercury in all investigated samples. We obtained well defined peaks by subtracting the blank signal from the voltammograms of the sample solutions.

Each sample was analysed in duplicate.

235

236 2.2.5 Determination of mercury by CV-AAS and DMA80

DMA-80 was used to determine the concentration of total mercury, as reported in U.S. EPA Method 237 238 7473 (EPA, Method 7473, 2007). By injecting increasing volumes of standard into quartz sample boats, calibration graphs of 0-20 ng and 20-500 ng of mercury were created using the 0.1 and 1 239 240 mg/l standards, respectively. The analyses were carried out using the following parameter: drying temperature/time: 90 s to 200°C; decomposition ramp: 120 s to 750°C; decomposition hold: 90 s at 241 242 750°C; catalyst temperature: 600°C; purge time: 60 s; 12 s at 900°C; recording time: 60 s; oxygen flow: 120 mL/min Aliquots of freeze-dried tuna (about 0.1 g) were transferred into the sample boats 243 244 and directly analysed.

For CV-AAS 1 ml of sample solution was diluted to 15 ml with 1,5% HNO₃ directly in the MHS vessel. A few drops of KMnO₄ solution were added to monitor the reaction of reduction (the colour of the solution changes as a consequence of the reduction $MnO_4 \rightarrow MnO_2$). The method of external calibration was adopted for the evaluation of mercury content in the sample solutions. Blank solution and five standards were prepared for the instrument calibration (40, 50, 70, 100 and 200 ngof Hg).

251

252 **3 Results and discussion**

253 3.1 ASV analysis

The performances of both the SGE and the AuNPs-GCE for the determination of Hg in aqueous 254 solutions were shown in our previous works (Abollino, Giacomino, Malandrino, Piscionieri, & 255 Mentasti, 2008; Giacomino, Abollino, Malandrino, & Mentasti, 2008), which report the 256 repeatability, linearity, accuracy, detection limit of the procedure and the interferences of other 257 cations and of anions. Briefly, the height of Hg peak increased with increasing deposition time; a 258 value of 120 s was found to be suitable for concentrations down to 50 μ g/L. In synthetic solution, 259 the limit of detection (estimated as LOD= $3\sigma B/slope$) for SGE was 0.40 µg/l and sensitivity was 260 1.71 μ A/ μ gl⁻¹; the relative error for the determination of 1 μ g/l of Hg was -1%. The LOD and 261 sensitivity for AuNPs-GCE were 0.15 ng/l and 3.5 μ A/ μ gl⁻¹ respectively. A concentration as low as 262 10 ngl^{-1} could be quantified with a relative error of - 0.8%. The interference of several metal ions 263 (As(V), Bi(III), Cd(II), Co(II), Cr(III), Cu(II), Fe(II), Mn(II), Ni(II), Pb(II), and Se(IV)) and some 264 anions (ClO₄⁻, SO₄²⁻, S²⁻, PO₄³⁻, HCOO⁻, BO₃³⁻, IO₃⁻, CH₃COO⁻, CO₃²⁻, ClO₂⁻, F⁻, I⁻, NO₃⁻ and NO₂⁻) 265 on the mercury stripping signal was investigated using both the electrodes in our previous works 266 267 (Abollino, Giacomino, Malandrino, Piscionieri, & Mentasti, 2008; Abollino, Giacomino, Ginepro, Malandrino & Zelano, 2012). The voltammogram of a solution with 5 µg/l of Hg was recorded in 268 the presence of each ion (added into the vessel in 1:1; 1:10 and 1:100 concentration ratios with 269 270 respect to the analyte). For all considered cations, no interference was observed on the Hg quantification and the linearity of the calibration curve of Hg was maintained, in good agreement 271 272 with literature data reported in literature (Augelli, Munoz, Richter, Gouveia & Angnes, 2005; Okcu, Ertas, Gokcel & Tural, 2005). Among the considered anions, no interference was observed with the 273 exception of Br⁻, I⁻ and S²⁻. These ions are known to adsorb strongly on gold surfaces, and among 274 the halogen ions iodides are those with the highest affinity for gold. They have been found to shift 275 276 analyte signal to more positive potentials as reported by Tamer et al. (Tamer,U., Oymak T., & Ertas N., 2007). The presence of Br⁻ interfered with the quantification of Hg for [Hg]< 3µg/l or in 277 presence of a ratio Hg:Br \geq 1:100. The presence of I⁻ and S²⁻ caused a decrease of the background 278 current, a sharp decrease of the mercury signal and a loss of linearity, which hindered the 279 quantification of the analyte. To minimize the excessive adsorption effect a negative potential of -280

281 0.80 V was applied for 30 s between the deposition step and the stripping step. At this potential Γ , 282 S²⁻ and Br⁻ and the other cited anions do not adsorb on the AuNPs-GCS surface. This procedure 283 permits to have well defined voltammetric peaks and a stable voltammetric baseline (Salaun P., & 284 van den Berg C.M.G. 2006).

Considering the low analyte concentrations expected in sample solutions, the medium exchange technique was adopted to eliminate the effect of components in the sample matrix that might cause interferences in the stripping step (Detcheva & Grobecker, 2006). Using this method, we observed an improvement of Hg recovery (about + 8%) in the reference materials for both the electrodes.

289 3.2 Experiments with SGE

Using SGE, the stripping step was conducted in a solution of either 60 mM HCl or 60 mM NaCl. In our previous work, the measurements in HCl showed higher sensitivity in comparison with NaCl; however, in the case of real sample solutions containing HNO₃ we chose to work in NaCl to avoid the formation of traces of *aqua regia* which could damage the Au surface. In the present study, we have checked the response of the SGE using both the supporting electrolytes.

All the results are reported in Table 2. We can observe as, using NaCl instead of HCl, the recoveries increase from 89.6% to 94.4 % for *BCR 643* and from 80.2 % to 99.0 % for *Ispra T22*.

As to CT solutions, we found higher concentrations using NaCl in comparison with HCl. This is due to the fact that, using HCl, the baselines of the voltammograms related to each sample (i.e. blank, sample solution before and after standard additions) were not perfectly overlapped: this behavior caused a systematic underestimation of the mercury concentration in all CTs. Using NaCl, the baselines for each sample are exactly overlapped.

The LOQ in the considered matrix (computed as the minimum amount determined with a good accuracy) was 0.7 μ g/l Hg, corresponding to 0.4 mg/kg in the dried sample.

304 Using SGE, no detectable mercury peaks were observed for samples 3, 4 and 8.

305

306 *3.3 Experiments with* AuNPs-GCE

All the analyses were repeated using AuNPs-GCE adopting the best condition found for SGE, that is NaCl 60 mM as supporting electrolyte (Table 2). In this conditions, we obtained recoveries of 96.8 % and 99.8% for *BCR 643* and *Ispra T22* respectively. Using AuNPs-GCE, it was possible to quantify mercury also in the CTs characterised by the lowest analyte concentrations. The LOQ in the investigated matrix is $0.1 \mu g/l$ Hg, corresponding to 0.06 mg/kg in the dried sample. These results confirm that the large surface area of the deposited gold nanoparticles permits an improvement of sensitivity in comparison with a traditional solid surface. As-expected, we obtained similar values of concentration for all the considered samples with both electrodes (see section 3.7 for statistical comparison).

316

317 *3.4 Effect of supporting electrolyte*

In our previous study we demonstrated that the performance of HCl and NaCl as supporting 318 electrolytes with AuNPs-GCE is the same. This finding is in agreement with EPA Method 7472, 319 based on ASV with a gold film electrode (GFE), which states that the solutions can be rendered 320 321 electrically conductive by adding HCl or NaCl indifferently. These reagents permit to obtain the 322 best results, since the formation of complexes between Hg and chloride ions enhances the sensitivity of the stripping signal (Metrohm, Application Bulletin No 96/4e). On the other hand, the 323 324 two electrolytes have a different behaviour with SGE, as shown above, probably because of the formation of traces of aqua regia, and consequently of nitrosyl chloride, which attacks the gold 325 326 surface forming a passivating layer. In the case of AuNPs-GCE (Abollino, Giacomino, Malandrino, Piscionieri, & Mentasti, 2008) or GFE (EPA, Method 7472, 1996) this effect is not appreciable due 327 328 to the renovation of active surface The fact that NaCl provides more stable signals with SGE confirms the observations reported by Bonfil (Bonfil, Brand, & Kirowa-Eisner, 2000), who 329 330 suggested a mixture of NaCl and HNO₃ as the best supporting electrolyte for the determination of metals using this kind of electrode. 331

Figure 1 shows the voltammograms obtained with SGE using HCl (a) and NaCl (b) as supporting 332 electrolytes for the analysis of BCR 643; the baseline considered for the evaluation of peaks height 333 is also reported. As we can see, in the case of NaCl the baseline is well defined by the points in 334 which the voltammograms cross, while in the case of HCl a shift of the baseline on the left of the 335 336 peaks seems to cause an underestimation of the mercury concentration. For comparison Fig.1c reports the voltammograms recorded at AuNPs-GCE for the same sample: as specified in 337 "Procedure" section in the case of SGE the sample was diluted in the electrolyte with a ratio 1:5, 338 while in the case of AuNPs-GCE the ratio is voltammetric cell is 1:10; even if the concentration in 339 340 cell is lower, the voltammograms reported in Fig.1 show the higher sensitivity obtained by the 341 nanostructured electrode. The coefficient of correlation obtained was greater than 0.9950 for all the considered samples. Figure 1d reports the voltammograms obtained analysing CT3 with AuNPs 342 343 using NaCl as electrolyte. In all CT-voltammograms it is possible to see another peak at 0.38 V, caused by the presence of traces of Cu (Abollino, Giacomino, Malandrino, Piscionieri, & Mentasti, 344 345 2008), present in tuna as a micronutrient; a fraction of this metal might derive from the metallic package, as found by Buculei et al. (Buculei, Amariei, Oroian, Gutt, Gaceu, & Birca, 2014). 346

347

348 *3.5 Monitoring of the electrodes surface*

The deposition of gold nanoparticles is well detectable through a colour change of the glassy carbon surface from black to red-orange. The status of the gold nanoparticles layer was valued with SEM. From SEM images (Fig. S1, Supplementary material), it is possible to see the presence of a homogeneous gold layer composed of particles, having an average diameter of approximately 100 ± 25 nm.

- A very good repeatability in the morphology of the nanostructured layer was obtained starting from different GCEs and different brands of Au salts. A drawback of SEM technique as method to monitor the surface is that once a metal nanoparticles-modified electrode is analysed with SEM, the metal particles are charged by the beam and they could coalesce and form an "Au film", so another method has to be used for the daily monitoring of the goodness of the active surface.
- A fast and easy method to do this is cyclic voltammetry (CV). Fig. 2a and 2b show voltammograms
 recorded in 0.5 M H₂SO₄ with SGE and AuNPs-GCE respectively.
- 361 The shapes of the voltammograms reported as "line 1" are well known in literature and identify a 362 solid (Fig 2a) or nanostructured (Fig 2b) gold surface (Salaun, Planer-Friedrich, & van den Berg, 2007). The anodic peak at 1.25 V is due to oxide formation, probably consisting of hydrated oxides 363 or Au(OH)n_{ads}. In the CV voltammogram for AuNPs-GCE, we can observe a "shoulder" before the 364 oxidation peak, typical of a nanostructured gold surface, caused by the formation of different multi-365 366 oxide species for the gold nanoparticles (Priano et al., 2008). The cathodic peak at +0.90 V, present in both the voltammograms, can be attributed to the reduction of the gold oxide formed during the 367 368 anodic cycle. The charge recorded under the reduction peak is generally used to characterize and 369 monitor the electroactive sensor area, since it is proportional to the amount of deposited Au (Dai, 370 Nekrassova, Hyde, & Compton, 2004). As expected, a higher signal is obtained in the case of AuNPs-GCE in comparison with SGE, due to the presence of nanoparticles that increase the 371 electrode surface. 372
- The voltammogram reported as "Line 2" in Fig.2a represents the CV recorded after a prolonged use of the electrode surface, that causes a worsening in the analytical response. In the case of SGE, the CV reported in Fig. 2a shows additional peaks in comparison to CV obtained from an optimal surface. These peaks are due to the electrochemistry of gold, since are caused by the formation of multilayers of oxides in which gold can exist in different oxidation states (Au0/AuI/AuIII) (Giacomino, Abollino, Lazzara, Malandrino, & Mentasti, 2011); the presence of these layers causes a worsening of the electrode performance. After the treatments with NaOH, ethanol and water a

cyclic voltammogram like that reported in Fig. 2a-line1 is again obtained. We suppose that in the presence of NaOH the surface is covered with a layer of hydroxides, that replace completely or in part the hydration layer; ethanol removes this deposited layer and water removes the traces of ethanol.

Regarding AuNPs-GCE, in Fig. 2b-line2 we can observe i) a shift of the anodic peak to more positive potential contemporary to a reduction of the peak height and to the disappearance of the shoulder and ii) a drastic reduction of the cathodic peak due to a loss of available active surface. In this condition the electrode can be used as "Au film electrode", but it presents a lower sensitivity. With this kind of electrode the treatment of the surface is usually not performed, as it is more convenient to dissolve the gold layer and deposit a new one.

Daily, we recorded ten CV-scans in H_2SO_4 to check the repeatability of the electrode response and, consequently, the goodness of the electrode surface. Many researchers use this CV-treatment as an activation step for solid electrodes: for our experience, we suggest to make, after the treatment in H_2SO_4 , a further activation step by applying a potential of 0.60 V for 60 s in 0.06 M HCl before mercury determination, in order to maintain the electrode surface active and reproducible and consequently to enhance the quality and reproducibility of the mercury signal.

An estimation of the roughness of the obtained gold layer was obtained by AFM. Unfortunately, 396 397 AFM instruments are not suitable to analyse this type of electrode, because of its size (height = 5cm). For this reason this evaluation was performed with the aid of a glassy carbon plate (height = 3398 399 mm), onto which the same procedure of surface cleaning and deposition adopted for GCE was 400 followed. Obviously, the conditions in which the deposition occurs were different because i) during 401 the deposition the electrode is rotated, while it is not possible to move the GC plate: so, in this case, 402 the solution was maintained in agitation with a magnetic stirrer; and ii) the sizes of the GC surfaces are different: the electrode surface can be estimated as $\approx 3.14 \text{ mm}^2$ (as calculated from the radius of 403 the active surface = 1 mm \pm 0.01, provided by the producer), while the plate has an area \approx 2,5 cm² 404 (manually estimated by measuring the total surface in contact with Au solution). In any case, AFM 405 analysis (Fig. S2, Supplementary material) confirmed the presence of a homogeneous gold layer: in 406 particular the profile reported in Fig.S2-c shows the regularity of the obtained surface. 407

408

409 *3.6 Atomic Absorption Spectroscopy Analysis*

410 In the last decade, direct mercury analysers demonstrated to be a reliable alternative to wet 411 chemistry techniques. The DMA automatically performs thermal decomposition, catalytic reduction, amalgamation, desorption and AAS detection to rapidly treat and analyse solid or liquid samples for mercury, with an output result for mercury content in about 5 min (per sample) with no pre-treatments required and no waste generation. For the considered matrix, the limit of quantitation (LOQ) for detection of mercury by means of this method is 0.037 mg/kg (wet weight). The obtained calibration curve, $y = -3.06 \ 10^{-7}x^2 + 1.10x \ 10^{-3}x - 5.5x 10^{-3}$ shows a good linearity, $R^2 = 0.9998$

417 (Squadrone, Benedetto, Brizio, Prearo, & Abete, 2015).

The results are reported in Table 3: as we can observe, a quantitative recovery was obtained 418 analysing BCR material. For this reason, and taking into account that the use of DMA for mercury 419 determination is well-established, we considered the results obtained using this technique as 420 "reference values" to evaluate the data obtained with the other methods. Moreover, since the 421 process does not require the conversion of mercury to mercuric ions, both solid and liquid matrices 422 can be analysed without the need for acid digestion or other sample preparation steps. Therefore, 423 the comparison with DMA results permitted us to quantify the possible losses through volatilization 424 or incomplete digestion which may take place with the other investigated analytical techniques 425 426 (Haynes, Gragg, Johnson, Robinson, & Orazio, 2006).

Table 3 shows also the results obtained using conventional CV-AAS, preceded by sample digestion in microwave oven. The method has a good linearity, as shown by the calibration curve obtained (y = 0.0012x - 0.0011, R² = 0.9981).

The concentrations measured in the certified materials show a loss of mercury of about - 20 ± 5 % that can be ascribed to a partial volatilization of the metal during the digestion step and/or during the conversion to elemental Hg and transfer to the measurement cell. Then, the analysis of the CTs was carried out: the quantification of Hg concentration was possible in all the samples with the only exception of CT3, in which the signal was too low. The results obtained with CV-AAS were surely negatively influenced by the absence of the gold trap required for determination of Hg at ultratrace level by this technique.

437

438 *3.7 Comparison among the techniques*

We compared the results obtained with the considered techniques to understand the realperformance of ASV determination, in particular using AuNPs-GCE.

441 Considering the DMA results ([Hg_{DMA}]) as "true", the concentrations in CTs found using SGE with

442 NaCl, AuNPs-GCE and CV-AAS were expressed as percentages of recovery (Table 4) with respect

443 to $[Hg_{DMA}]$.

As it can be seen, the values range between 79% and 112%. The median values increase in the order
CVAAS < SGE < AuNPs-GCE.

Figure 3 reports scatter plots comparing [Hg]_{DMA} with the concentrations measured with SGE in 446 NaCl (3a), AuNPs (3b) and CV-AAS (3c), the equations of the lines so obtained and the 447 corresponding values of R². For SGE only the samples containing higher concentrations (six CTs) 448 were considered, while for CV-AAS it was possible to compare the results obtained for all the 449 samples with the exception of sample CT3. The comparison indicate a good correlation between the 450 mercury amounts measured in the samples with different techniques. The results obtained by ASV 451 demonstrated the highest correlation with [Hg]_{DMA}; in particular using AuNPs-GCE a correlation of 452 0.9889 was obtained considering the whole data set. 453

A statistical comparison among all the results obtained was also made using ANOVA (level of probability = 95%) considering the concentrations found using the different techniques for each sample. For all the considered samples, the Hg concentrations measured with DMA were not significantly different from those found by ASV using AuNPs-GCE. As to the other techniques, among the quantifiable samples, no significant differences were observed from [Hg]_{DMA} with the only exception of CT3 in the case of CV-AA.

It is very important to underline the key role of analysing freshly prepared sample solutions (maximum one week), since losses of 10-30 % were observed in solutions analysed two weeks after the digestion. Good repeatability was observed among sample solutions prepared from different aliquots of the same lyophilized tuna demonstrating the homogeneity of the sample.

464 Finally, Table 5 summarizes the analytical performance found in this study for each adopted465 technique.

Both voltammetric and spectroscopic approaches can be considered suitable techniques to monitor Hg concentration in fish, since they allowed to reach accurate Hg quantification at concentration values lower than the maximum admissible level of this element in the considered matrix (1 mg/kg_{ww}) (Commission Regulation (EC) No. 629/2008).

470 Considering the two investigated spectroscopic techniques, we can conclude that DMA performs
471 better than conventional CV-AAS and can be considered a fast, accurate and reliable alternative to
472 wet chemistry techniques. Its main advantage is the high sample throughput, as no sample

preparation is required The technique permits to determine Hg with high accuracy and precision. Itsmain drawback is that it is applicable only to the determination of this analyte.

Electrochemical techniques are sensitive, relatively inexpensive and they also enable the 475 determination of a number of inorganics and organics at trace or ultratrace concentrations. The 476 drawbacks are the longer time required in comparison with DMA, in particular for the wet digestion 477 step, with possible loss of analyte by vaporisation, and the production of waste solutions. In the case 478 of in-cell concentrations higher than 0.7 µg/l both SGE and AuNPs-GCE provide very accurate 479 determinations. The presence of the nanostructured active surface in the latter permits to quantify 480 481 lower concentrations with a LOQ comparable to that of DMA. Another great advantage of the electrochemical approach is the possibility to carry out speciation studies: in the case of mercury, it 482 483 is possible to distinguish between inorganic Hg and methylmercury, the most harmful specie for 484 humans (Abollino, Giacomino, Malandrino, Marro, & Mentasti, 2009)

485

486 *3.8 Evaluation of Hg in examined canned tunas*

Since the main purpose of our study is to verify the applicability of AuNPs-GCE for the 487 quantification of Hg in fish in routine controls, we compared the concentrations obtained with the 488 European Legislation limit for this analyte: the maximum admissible level for mercury in fishery 489 products and fish tissues is 0.5 mg/kgww for most fish species, and 1 mg/kgww for tuna and some 490 others species (Commission Regulation (EC) No. 629/2008). During the lyophilisation step, the 491 CTs lost about 30 ± 5 % of weight, so we calculated the final concentrations in wet fish. All the 492 examined samples contained Hg concentrations lower than the admissible value (Table S1, 493 494 Supplementary material).

495

496 **4 Conclusions**

In this work the suitability of ASV with gold electrodes for the determination of mercury in fishwas demonstrated.

499 Under the optimized conditions, for both the electrochemical approaches, the oxidative current 500 exhibited a linear dependence on Hg(II) ion concentration in wide dynamic ranges of 0.2-100 μ g/l 501 (R²= 0.9891) and 0.010-100 μ g/l (R² = 0.9922) with detection limits of 0.02 and 0.001 μ g/l for SGE 502 and AuNPs-GCE respectively.

The main advantage of the AuNPs-GCE with respect to SGE is the improvement of the analytical 503 504 performance, thanks to the increase of active surface of the gold nanoparticles. In particular the proposed sensor shows high sensitivity (even with short deposition time, 120 s), good selectivity, 505 long-term stability, great repeatability and a limit of quantification of 0.1 μ g/l for Hg in fish matrix. 506 The comparison between DMA and conventional CV-AAS shows that the former has better 507 508 performances in terms of accuracy, precision and analysis times, owing to the integration of sample pretreatment and analyte detection. The results obtained by DMA and ASV with AuNPs-GCE are 509 not significantly different, confirming that the latter is an effective alternative to other more 510 511 common techniques for the determination of mercury in fish.

The choice of the most suitable analytical method for the determination of Hg is influenced by the 512 number of samples, by the available time and by the number of considered analytes: for a fast 513 determination of Hg in a lot of samples, DMA seems to be the best solution; ASV is particularly 514 suitable for a relatively low sample number in which also speciation studies or the measurement of 515 516 other (inorganic or organic) species is required, The proposed technique has the main advantages of electrochemical methods i.e. versatility, sensitivity, low costs of instrument purchase and 517 518 management, availability in most analytical laboratories and the possibility of using portable devices for on-site and in situ measurements. The ease of the procedures requested for the electrode 519 520 modification could offer a simple, inexpensive and rapid methods for the routine determination of mercury in food samples. The good accuracy and precision demonstrated by ASV with AuNPs-521 GCE show as this technique can be fruitfully used in ring tests. 522

523

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530 **References**

- Abollino, O., Giacomino, A., Malandrino, M., Piscionieri, G., & Mentasti, E. (2008). Determination
 of mercury by anodic stripping voltammetry with a gold nanoparticle-modified glassy
 carbon electrode. *Electroanalysis*, 20, 75-83.
- Abollino, O., Giacomino, A., Malandrino, M., Marro, S., & Mentasti, E. (2009). Voltammetric
 determination of methylmercury and inorganic mercury with an home-made gold
 nanoparticle electrode. *Journal of Applied Electrochemistry*, 39, 2209-2216.
- Afkhami A., Bagheri H., Khoshsafar H., Saber-Tehrani M, Tabatabae M, & Shirzadmehr A.,(2012).
 Simultaneous trace-levels determination of Hg(II) and Pb(II) ions in various samples using a
 modified carbon paste electrode based on multi-walled carbon nanotubes and a new
 synthesized Schiff base. *Analytica Chimica Acta* 746, 98–106.
- Afkhami A., Madrakian T., Sabounchei S.J., Rezaei M., Samiee S., & Pourshahbaz M., (2012)
 Construction of a modified carbon paste electrode for the highly selective simultaneous
 electrochemical determination of trace amounts of mercury (II) and cadmium (II) *Sens*, *Actuators* B, 161, 542-548.
- Afkhami A., Madrakian T., Ghaedi H., Rezaeivala M. (2013). Fabrication and application of a new
 modified electrochemical sensor using nano-silica and a newly synthesized Schiff base for
 simultaneous determination of Cd2+, Cu2+ and Hg2+ ions in water and some foodstuff
 samples. *Analytical Chimica Acta*, 771, 21-30.
- Augelli M.A., Munoz R.A.A., Richter E.M., Gouveia A.J., Angnes L.(2005). Chronopotentiometric
 Stripping Analysis Using Gold Electrodes, an Efficient Technique for Mercury.
 Quantification in Natural Waters. *Electroanalysis* 17, 755-.761.
- Bagheri, H., Afkami, A., Saber-Tehrani, M., & Khoshsafar H., (2012). Preparation and
 characterization of magnetic nanocomposite of Schiff base/silica/magnetite as a
 preconcentration phase for the trace determination of heavy metal ions in water, food and
 biological samples using atomic absorption spectrometry. *Talanta*, 97, 87-95.
- Bagheri H., Afkhami A., Khoshsafar H, Rezaei M., Shirzadmehr A., (2013). Simultaneous
 electrochemical determination of heavy metals using a triphenylphosphine/MWCNTs
 composite carbon ionic liquid electrode. *Sensors and Actuators B* 186, 451–460.
- Bonfil, Y., Brand, M., & Kirowa-Eisner, E. (2000) Trace determination of mercury by anodic
 stripping voltammetry at the rotating gold electrode, *Analitica Chimica Acta*, 424, 65-76.

- Buculei, A., Amariei, S., Oroian, M., Gutt, G., Gaceu, L., & Birca. (2014). A. Metals Migration
 between Product and Metallic Package in Canned Meat LWT. *Food Science and Technology*, 58, 364-374.
- 564 Clevenger W.L., Smith B.W. & Winefordner J.D. (1997). Trace Determination of Mercury: A
 565 Review. Critical Reviews in Analytical Chemistry, 27, 1-26.
- Commission Regulation (EC) No. 629/2008 of 2 July 2008 amending Regulation (EC) No.
 1881/2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union L*, 173, 6–9.
- Dai, X., Nekrassova, O., Hyde, M.E., & Compton, R.G. (2004). Anodic stripping voltammetry of
 arsenic (III) using gold nanoparticle-modified electrodes. *Analytical Chemistry*, 76, 5924571 5929.
- 572 Detcheva, A., & Grobecker, K.H.(2006). Analytical Note Determination of Hg, Cd, Mn, Pb and Sn
 573 in Seafood by solid sampling zeeman atomic absorption spectrometry. *Spectrochimica Acta*574 *Part B*, 61, 454-459.
- EPA, Mercury in acqueous samples and extracts by anodic stripping voltammetry, Method 7472,
 1996.
- 577 EPA, Mercury in solids and solutions by thermal decomposition, amalgamation and atomic 578 absorption spectrophotometry, Method 7473, 2007.
- European Ufficial Gazzette. 2011. Regulation (EU) No 420/2011 of the European Parliament and of
 the Council of April 29th 2011 on "The Definition of Maximum Values of Some
 Contaminants in Food Products". European: Amending Regulation (EC) No 1881/2006 of
 the European Parliament.
- Fricke, I., Götz, R., Schleyer, R., & Püttmann, W. (2015). Analysis of Sources and Sinks of
 Mercury in the Urban Water Cycle of Frankfurt am Main, Germany. *Water*, 7, 6097-6116.
- Giacomino, A., Abollino, O., Malandrino, M., & Mentasti, E. (2008). Parameters affecting the
 determination of mercury by anodic stripping voltammetry using a gold electrode. *Talanta*,
 75, 266-273.
- Giacomino, A., Abollino, O., Lazzara, M., Malandrino, M., & Mentasti, E. (2011). Determination
 of As(III) by anodic stripping voltammetry using a lateral gold electrode: Experimental
 conditions, electron transfer and monitoring of electrode surface. *Talanta*, 83, 1428-1435.
- Ginsberg, G.L.,& Toal B.F. (2009). Quantitative approach for incorporating methylmercury risks
 and omega-3 fatty acid benefits in developing species-specific fish consumption advice.
 Environmental Health Perspectives, 117, 267-275.

- Has-Schon, E., Bogut, I., & Strelec, I. (2006). Heavy metal profile in five fish species included in
 human diet, domiciled in the end flow of River Neretva (Croatia). Archives Of *environmental Contamination And Toxicology*, 50, 545-551.
- Haynes, S., Gragg, R.D., Johnson, E., Robinson, L., & Orazio, C.E. (2006). An evaluation of a
 reagentless method for the determination of total mercury in aquatic life. *Water Air and Soil Pollution*, 172, 359-374.
- Inskip, M.J., & Piotrowsiki, J.K. (1995). Review of the health effects of methyl mercury. *Journal of Applied Toxicology*, 5, 113-133.
- Jayaratna, H.G. (1997). Determination of trace mercury (Hg(II)) by anodic stripping voltammetry at
 a gold electrode, Bioanalytical Systems, Inc., USA. *Current Separations*, 16, 93-98.
- Kurmaz V.A. & Gultyai V.P. (2010). Electrode reactions and electroanalysis of organomercury
 compounds. *Russian Chemical Reviews*, 79, 307-350.
- Martín-Yerga D., González-García M.B., Costa-García A. (2013). Electrochemical determination of
 mercury: A review. *Talanta*, 116, 1091–1104
- Metrohm, Stripping Voltammetry Analysis of Mercury, Application Bulletin No 96/4e, Herisau,
 Switzerland.
- Meucci V., Laschi S., Minunni M., Pretti C., Intorre L., Soldani G., & Mascini M. (2009).
 Talanta77(2009)1143
- Okcu, F., Ertas, F.N., Gokcel, I.J., & Tural, H. (2005). Anodic stripping voltammetric behavior of
 mercury in chloride medium and its determination at a gold film electrode, *Turkish Journal of Chemistry*, 29, 355-366.
- Priano, G., González, G., Günther, M., & Battaglini, F. (2008). Disposable Gold Electrode Array
 for Simultaneous Electrochemical Studies. *Electroanalysis*, 20, 91-97.
- Radulescu M.C., & A.F. Danet. (2008). Mercury Determination in Fish Samples by
 Chronopotentiometric Stripping Analysis Using Gold Electrodes Prepared from Recordable
 CDs. *Sensors*, 8, 7157-7171.
- Ramezani S., Mashhadizadeh M.H., Jalilian S., Aghili M. (2015). Structure-switching of an
 organothiol neutral carrier by gold nanoparticles decorated on SHMWCNTs for ultra-trace
 voltammetric assay of Hg(II) using a carbon paste electrode. *Analytical Methods*, 7, 7765–
 7775.
- Salaun P., & van den Berg C.M.G. (2006). Voltammetric detection of mercury and copper in
 seawater using a gold microwire electrode. Analytical chemistry, 78, 5052-50-60.

- Salaun, P., Planer-Friedrich, B., & van den Berg, C.M.G. (2007). Inorganic arsenic speciation in
 water and seawater by anodic stripping voltammetry with a gold microelectrode. *Analytica Chimica Acta*, 585, 312–322.
- Sasmaz, M., Akgül, B., Yıldırım, D., & Sasmaz, A. (2016). Mercury uptake and phytotoxicity in
 terrestrial plants grown naturally in the Gumuskoy (Kutahya) mining area, Turkey.
 International Journal of Phytoremediation, 18, 69-76.
- Souza-Araujo, J., Giarrizzo, T., & Lima, M.O. (2015). Mercury concentration in different tissues of
 Podocnemis unifilis (Troschel, 1848) (Podocnemididae: Testudines) from the lower Xingu
 River Amazonian, Brazil. *Brazilian Journal of Biology*, 75, 106-111.
- Squadrone, S., Benedetto, A., Brizio, P., Prearo, M., & Abete, M.C. (2015). Mercury and selenium
 in European catfish (Silurus glanis) from Northern Italian Rivers: Can molar ratio be a
 predictive factor for mercury toxicity in a top predator? *Chemosphere*, 119, 24-30.
- Tamer,U., Oymak T., & Ertas N. (2007). Voltammetric Determination of Mercury(II) at Poly(3 hexylthiophene) Film Electrode. Effect of Halide Ions. *Electroanalysis* 19, 2565 2570.
- 640 Widmann, A., & van den Berg, C.M.G. (2005). Mercury detection in seawater using a
 641 mercaptoacetic acid modified gold microwave electrode. *Electroanalysis*, 17, 825-831.