

Chapter 5

Metallomics in Fish



Camila Pereira Braga, Jiri Adamec, and Pedro de Magalhães Padilha

Abstract Metallomics allows the integration of traditionally analytical studies with inorganic and biochemical studies. The study of metallomics in living organisms allows us to obtain information about how the metal ion is distributed and coordinated with proteins, the essentiality and/or toxicity, and the individual concentrations of metal species, thus contributing to elucidation of the physiological and functional aspects of these biomolecules. In this context, several lines of research have appeared in the literature with different terms and approaches. For example, *metallomic*, which deals with the characterization of the total metal/metalloid species present in an organism; *metalloprotein*, which deals with the characterization of the total elements present in a specific site of an organism (cellular behavior, protein, metalloprotein); and *metallomic*, which deals with a more in-depth study of metalloids. In this area, information is sought on the interactions and functional connections of metal/metalloid species with genes, proteins, metabolites and other biomolecules of the organism and, therefore, the elucidation of the biological role exerted by the metal ions bound to the biomolecules. In this chapter, we will describe techniques used in animal studies.

Keywords Animal · Fish · Metallomic · Metalloprotein

C. P. Braga · J. Adamec

Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, USA

P. de Magalhães Padilha (✉)

Department of Chemistry and Biochemistry, Institute of Bioscience, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

e-mail: padilha@ibb.unesp.br

Abbreviations

2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
AAS	Atomic absorption spectrometry
ESI	Electrospray ionization
ESI-MS/MS	Electrospray ionization tandem mass spectrometry
FAAS	Flame atomic absorption spectrometry
GFAAS	Graphite furnace atomic absorption spectrometry
ICP-MS	Inductively coupled plasma source mass spectrometry
IEF	Isoelectric focusing
LC	Multidimensional liquid chromatography
MALDI	Matrix assisted laser desorption ionization
MALDI-TOF/MS	Time-of-flight mass spectrometry coupled to laser-assisted matrix
Mm	Molecular mass
MS	Mass spectrometry
PAGE	One-dimensional polyacrylamide gel electrophoresis
pI	Isoelectric point
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC	size-exclusion chromatography

5.1 Introduction

Metal ions are important in biological processes by stabilizing proteins and nucleic acids and participating in enzymatic reactions (Szpunar 2004; Barnham and Bush 2014). Metal deficiencies result in anemia (iron [Fe] and copper [Cu]), bone deterioration (calcium [Ca]), growth retardation (zinc [Zn]), skin changes (Zn), and brain and heart diseases (Cu); some metals are considered carcinogens (arsenic [As], cadmium [Cd], chromium and nickel); and the presence and or excessive presence of metals can be associated with nephrotoxicity (Cd and uranium) and neurotoxicity (aluminum, mercury [Hg] and manganese [Mn]) (Lippard 1994; Szpunar 2004). Therefore, elucidation of the biological essentiality and toxicity of metals has become important in varied fields of research (Haraguchi 2004).

The aim of metallomics is to verify the distribution of metal and/or metalloid and elucidate the physiological and functional aspects of proteins, genes and metabolites containing metals of biological interest (Haraguchi 2004). Therefore, in metallomics, metallomes are defined as metalloproteins, metalloenzymes and metal-containing biomolecules representing the main target of metallomics studies (Haraguchi 2004). Metallomics information can be classified as qualitative (identification of individual metal species), quantitative (determination of concentrations of these species), and comparative (monitoring changes of the metallomes of a given organism under the influence of external stimulus) (Haraguchi 2004).

Metalloproteins are characterized by high affinity of the metal–protein interaction. They are also known as metalloenzymes when they exert catalytic activity using the metal incorporated into their active site as part of the catalytic process, such as alcohol dehydrogenase, catalase, carbonic anhydrase, DNA polymerase, ferritin, transferrin and RNA polymerase (Haraguchi 2004; Dydio et al. 2016). In an attempt to protect organisms from metal toxicity, some metalloenzymes—such as superoxide dismutase, glutathione peroxidase, glutathione reductase, glutaredoxins, thioredoxins and peroxiredoxins—are induced so they can be used as biomarkers of metal toxicity, thus contributing to elucidation of the physiological and functional aspects (López-Barea and Gómez-Ariza 2006). Metalloproteomic studies consist of a selectivity component (separation technique to isolate the target species from matrix), a sensitivity component (sensitive detector to quantify the elements), and a structural component (specific detector for molecules) (Gao et al. 2003; da Silva et al. 2010).

The most used techniques in the fractionation/purification stage of the biomolecules are one- (PAGE) or two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and multi-dimensional liquid chromatography (LC). In the step of quantitative qualitative determination and speciation analysis, techniques employed include x-ray fluorescence with synchrotron radiation (SR-XRF), flame atomic absorption or graphite furnace atomic absorption spectrometry (FAAS/GFAAS), and inductively coupled plasma source mass spectrometry (ICP-MS). To characterize the biomolecules (metalloproteins and/or metal-binding proteins), the most commonly used techniques are time-of-flight mass spectrometry coupled to laser-assisted matrix desorption ionization (MALDI TOF MS) and electrospray ionization tandem mass spectrometry (ESI-MS/MS).

5.2 Metallomic Studies in Fish

Fish live in different habitats and can adapt to changes in the environment; they are also an important source of proteins, minerals and vitamins (Beyer et al. 1996). Through ingestion, biomagnification and bioconcentration, fish can accumulate chemicals (Van der Oost et al. 2003), and thus they provide information about environmental contamination by known toxic or new chemicals (Beyer et al. 1996; Van der Oost et al. 2003). In fish, metal-bound proteins can influence total metal levels, thus reflecting toxic exposure (Hauser-Davis et al. 2012). Thus, metalloproteins in fish have been used as biomarkers for environmental contamination showing ecological importance. In addition, another group of small proteins that show ecological importance, known as metallothioneins, are important in the detoxification of essential (Zn and Cu) and toxic metals (As, Cd and Hg) (Sun and Chai 2010). Cysteine residues present in metalloproteins and metallothioneins have a high affinity to bind with heavy metals, such as Pb^{2+} , Hg^{2+} , Zn^{2+} , Cd^{2+} , $\text{Fe}^{2+/3+}$, As^{3+} , Cu^+ and Ag^+ (Huang et al. 2016).

Mercury (Hg) is one of the main toxic metals involved in environmental contamination. It is a naturally occurring metal found in the earth's crust and, in small quantities, in air, soil and water, where it assumes various chemical forms, such as

metallic or elemental Hg (Hg^0); inorganic metal as mercuric salts (HgCl_2 , HgS) and mercurous chloride (Hg_2Cl_2); and organic Hg bound to carbon radicals (methylmercury [MeHg] and ethylmercury [EtHg]) (Clarkson and Magos 2006). Due to Hg's toxic effects on the environment and health (loss of skin sensitivity, loss of muscle coordination, deafness and death), countries around the world have been acting to minimize the risks of Hg contamination.

5.2.1 The Problem of Hg in the Amazon Region

Past gold-mining activity in the Amazon region is associated with Hg contamination. Therefore, the development of hydroelectric complexes in the Madeira River basin has aroused scientific discussions about the possible remobilization and bio-availability of Hg species in reservoirs in the Amazon region. However, with the construction and implementation of hydroelectric complexes in the Madeira River basin, the Hg dynamics in this environment can be altered due to environmental changes (Bastos et al. 2006). Hg concentrations have remained within natural levels in Amazonian rivers; however, it is known that the transformations and processes of remobilization of previously unavailable mercurial species can change their chemical form, thus making them available to aquatic biota (Pfeiffer et al. 1993; Bastos et al. 2006).

Bioaccumulation along the trophic chain is responsible for the high Hg content in Amazonian fish. In the algae and aquatic plants of Amazonian rivers, Hg concentrations are greater than those in the rivers themselves; therefore, fish that feed exclusively on these algae and plants have greater concentrations of Hg (Akagi et al. 1995; Aula et al. 1995). This happens along the trophic chain through to top predatory fish, which have concentrations 1 million times greater than those found in river waters. Among the species of Amazonian fish, some are of great commercial and ecological interest. In addition, fish have a great importance for human consumption because fish is the main source of protein and represents the daily food of the riverine population.

In this context, the identification of biomarkers of Hg toxicity and Hg-species toxicity applicable to environmental and human health surveillance in the areas of direct and indirect influence of hydroelectric complexes is important in order to contribute to estimation of the magnitude of total or partial Hg exposure.

5.2.1.1 Fish Studies: Biomarkers of Hg Toxicity in the Amazon Region

Studies are being conducted to study possible biomarkers of Hg toxicity in fish using 2D-PAGE, analytical technique for the quantification of elements (atomic absorption spectrometry [AAS]), and MS.

In 2D-PAGE under denaturing conditions, only stable links of proteins having metal ions are uncompromised due to protein conformation. Proteins exhibit func-

tional groups with sulfur atoms (thiol groups) in their peptide fragment—which have characteristics of soft bases and bind preferentially to metals and metalloids with characteristics of soft acids, such as Hg in the form $R\text{-Hg}^+$ —even when they are submitted to protein-denaturing conditions, under which such metal–protein structure is maintained. The connections in the case of metal-binding proteins should also be stable because the denaturing conditions did not break these connections. In this case, Hg can be bound to nitrogen (N), which is an intermediate base and can form stable bonds with an element having the characteristics of either a hard acid or a soft acid (in this case, Hg).

5.2.1.2 Protein Fractionation Using 2D-PAGE

2D-PAGE is an extremely powerful technique that allows simultaneous separation and resolution of several proteins in two dimensions according with the isoelectric point (pI) and the molecular mass (Mm) (O'Farrell 1975). 2D-PAGE consists of five main steps: sample preparation, first dimension, second dimension, protein detection, scanning and image processing (Gorg et al. 2000).

For each spot on the gel to represent one or more proteins, the proteins in the sample must be denatured, disaggregated, reduced and solubilized. The first dimension is isoelectric focusing, in which the proteins are separated according to their pI in strips containing gels with a pH gradient. In the second dimension, which involves sodium dodecyl sulfate–PAGE, the proteins are separated based on their molecular mass. Each spot on the gel can be considered as an orthogonal coordinate of a protein that migrated specifically as a function of its pI (x-axis) and its Mm (y-axis). The appearance or disappearance of spots may provide information about stage-specific proteins, whereas spot intensity provides quantitative information regarding differential protein expression.

After the second-dimension step is performed, the gels are stained and scanned using a specific scanner, and the intensity of each spot is converted into an image using software to analyze the gels images obtaining the number of spots present in the gel as well as pI and Mm information from each spot.

5.2.1.3 AAS

AAS is a technique in which free gaseous atoms absorb electromagnetic radiation at a specific wavelength, thus producing a corresponding measurable signal that is proportional to the concentration of the free atoms present in the optical pathway (Garcia and Baez 2012). AAS consists of a source of radiation, a sample-introduction system, an atomization system, a monochromator, a detection system, and a reading; all of these are connected to a computerized system for the control and processing of data (Jenniss et al. 1999).

The two types of atomizers most used in AAS are FAAS and GFAAS. FAAS is used for elemental analyzes at mg L^{-1} levels (using some milliliters of the sample),

whereas GFAAS is used at low concentrations, e.g. $\mu\text{g L}^{-1}$ (using microliters of the sample) (Garcia and Baez 2012). Analysis of biological samples can pose some problems: The analyte may be present in different oxidation states; the analyte may be combined with different anions or bound to proteins or other organic binders; and the organic portion of the sample may cause large problems. To minimize interferences in the analysis of biological samples, the procedure commonly performed is wet digestion/mineralization of the organic portion of the sample using strong acids.

5.2.1.4 MS

Mass spectrometers consist of an ion source, a mass analyzer, an ion detector, and a data-acquisition component. It is an analytical instrument capable of converting neutral molecules into ions in gaseous form and separating them according to their mass/charge ratio using electromagnetic fields. The most commonly used ionization techniques in protein analysis are MALDI and ESI (Aebersold and Mann 2003).

5.2.1.5 Studies

A preliminary study developed by Moraes et al. (2012), using 2D-PAGE and SR-XRF to study fish from the Madeira River Basin in the Amazon region of Brazil, found a protein spot with Hg in muscle samples of dourada (*Brachyplatystoma rousseauxii* [predator species]) and muscle samples of pacu (*Mylossoma* and *Myleus* spp. [omnivorous with a tendency to herbivory]) (Moraes et al. 2012).

The same group continuing the metallomic study in fish from Madeira River Basin in the Amazon region of Brazil developed a method to determine Hg concentration using GFAAS involving acid mineralization, a chemical modifier (copper nitrate) and a permanent modifier (sodium tungstate) (Moraes et al. 2013). Using those methods, the investigators found Hg muscle concentrations (mg kg^{-1}) in dourada, pacu and jaraqui of 0.331 ± 0.007 , 0.081 ± 0.002 , and 0.132 ± 0.004 , respectively (Moraes et al. 2013).

In studies published in 2015, the results of protein fractionation were shown with posterior analysis of the presence of Hg in muscle samples of dourada (*Br. rousseauxii*) and in muscle and liver samples of tucunaré (*Cichla* spp.) from the Madeira River, Brazil (Braga et al. 2015; Vieira et al. 2015). The methods used were 2D-PAGE to determine protein fractionation, GFAAS to determine Hg concentration and ESI-MS/MS to determine protein characterization (Braga et al. 2015; Vieira et al. 2015). The study of dourada identified parvalbumin (isoforms: alpha and beta), protein NLRC5, 39S ribosomal protein L36 mitochondrial, N-alpha-acetyl transferase 20, Mth938 domain-containing protein, ubiquitin-40S ribosomal protein S27a as potential candidates for biomarkers of Hg toxicity (Braga et al. 2015), whereas in tucunaré (*Cichla* spp.), seven proteins with characteristics of Hg biomarkers were found, including parvalbumin and its isoforms, ubiquitin-40S and ribosomal-S27, zinc fin-

ger, BTB domain containing protein 24, and protein of double-specificity phosphatase 22-B (Vieira et al. 2015).

Vieira et al. (2017), using the same methodology, analyzed liver tissue samples of dourada (*Br. rousseauxii*) and pacu (*Mylossoma duriventre*) and identified possible biomarkers of Hg toxicity. In this study, proteins were found, including isoforms of parvalbumin, ubiquitin-40S ribosomal protein S27a, brain-specific angiogenesis inhibitor 1-associated protein, 2-like protein 2, and betaine-homocysteine S-methyltransferase 1 exhibited by Hg-associated proteins.

5.2.1.6 Other Studies Involving Hg Toxicity

Korbas et al. (2008), using zebrafish (*Danio rerio*), a common model employed to study toxicology, investigated organic Hg exposure at the body and molecular level using larval stage. SR-XRF mapping was used to generate the image with metal localization, and the results showed that the accumulation of MeHg and EtHg directly affected ocular tissue. In addition, compared with other tissues analyzed, the investigators found four times higher Hg in the layer of the eye lens ($1 \mu\text{g}/\text{cm}^2$) (Korbas et al. 2008). This was associated with organic Hg being able to generate visual defects with partial or complete loss of vision.

In a preliminary study performed by Kutscher et al. (2012)—using a method combining size-exclusion chromatography, LC coupled to ICP-MS, and ESI-MS/MS—investigated MeHg-binding proteins in certified tuna fish muscle (BCR-464) and their association with Hg toxicity (Kutscher et al. 2012). This study found a high molecular-weight protein as MeHg binding and skeletal muscle myosin heavy chain; using LC-ICP-MS, it was possible to detect Hg-containing peptides (Kutscher et al. 2012).

5.2.2 Other Studies in Fish

A preliminary study using liver tissue of Nile tilapia (*Oreochromis niloticus*) employed 2D-PAGE electrophoresis; SR-XRF to map proteins spots with Ca, Fe and Zn; and FAAS to quantify Ca, Fe and Zn (Lima et al. 2010). The results showed a range in protein spots of Ca, Fe and Zn of 1.08 to 5.80, 2.02 to 8.03, and 1.60 to 8.55 mg g^{-1} , respectively (Lima et al. 2010). Studying the same species and using similar methodology (in this studying using GFAAS) to analyze Mn and Zn in the protein spots, the investigators found a range from 0.8 to 2.6 mg g^{-1} and 1.0 to 6.3 mg g^{-1} , respectively (Santos et al. 2011).

In study published in 2015 involving muscle of Nile tilapia (*Or. niloticus*), 2D-PAGE was used for protein fractionation, FAAS and GFAAS to determine Ca, Cu, Zn and Mn concentration, and ESI-MS/MS for protein characterization (Cavecci et al. 2015). The results showed non-specific binding—such as the Zn found in parvalbumin-2—which can be a non-specific binding protein that forms a

metal-binding protein rather than a metal cofactor. The Ca and Zn detection in hemoglobin subunit alpha was also associated with non-specific binding regarding the terminal N atoms present in the hemoglobin chains (Cavecci et al. 2015).

5.3 Metallomic Studies in Rat

To understand the mechanisms of pathogenesis and the complications of some diseases, such as diabetes mellitus type 1 (DM1), animal models have been extensively used. Effective methods for inducing experimental diabetes, such as the administration of β -cytotoxic chemical agents like aloxane and streptozotocin (STZ), are effective ways to promote DM1 (Rees and Alcolado 2005). It is suggested that STZ acts by stimulating the production of reactive oxygen species (ROS) along with increased lipid peroxidation and decreased activity of antioxidant enzymes. In addition, one of the major toxic effects of STZ involves alteration of the DNA structure, which is fragmented by ROS, thus compromising insulin biosynthesis and secretion (Xiang et al. 2010).

Studies were developed using plasma and liver from DM1 animals, 2D-PAGE, GFAAS and FAAS (for quantitative determination of Cu, Mg, Se and Zn in the spots that showed differences of expression), and protein characterization by ESI/MS-MS. In total, 35 proteins were found to be differently expressed in the plasma, indicating (1) alpha-1-macroglobulin and haptoglobin as potential biomarkers of controlled type 1 diabetes (i.e. insulin treated); and (2) 2'-deoxynucleoside 5'-phosphate N-hydrolase 1, transmembrane protein 11, serum amyloid P-component, vitamin D-binding protein and biliverdin as possible candidates for biomarkers of uncontrolled type 1 diabetes (Braga et al. 2017). In addition, these studies showed the presence of Cu, Mg, Se and Zn in spots relating some metal-binding interactions that can be involved in the disease progression and complications in cases of DM1 (Braga et al. 2017).

5.4 Metallomic Studies in Bovines

Different factors—such as color, water-retention capacity, palatability and nutritional value—vary between bovine breeds and species, and these influence the quality of the meat. Meat quality has a direct relation with its degree of tenderness, which is of extreme importance because it leads to an increase in consumption and its commercialization. Studies have been developed to understand what leads to variation in meat tenderness.

In this context, using muscle tissue from Nellore breed (*Bos indicus*) and methodologies, such as 2D-PAGE, SR-XRF to map protein spots with Ca, and ESI-MS/MS to characterize protein, the investigators found two proteins, which were

detected as Ca, pyruvate kinase and albumin with differences in animals having tough meat, animals having tender meat, and Piedmontese breed animals (*B. taurus*) used as comparative model (Baldassini et al. 2015).

References

- Aebersold R, Mann M (2003) Mass spectrometry-based proteomics. *Nature* 422:198–207
- Akagi H, Malm O, Kinjo Y et al (1995) Methylmercury pollution in the Amazon, Brazil. *Sci Total Environ* 175:85–95
- Aula I, Braunschweiler H, Malin I (1995) The watershed flux of mercury examined with indicators in the Tucuruí reservoir in Pará, Brazil. *Sci Total Environ* 175:97–107
- Baldassini WA, Braga CP, Chardulo LAL et al (2015) Bioanalytical methods for the metalloproteomics study of bovine longissimus thoracis muscle tissue with different grades of meat tenderness in the Nellore breed (*Bos indicus*). *Food Chem* 169:65–72
- Barnham KJ, Bush AI (2014) Biological metals and metal-targeting compounds in major neurodegenerative diseases. *Chem Soc Rev* 43:6727–6749
- Bastos WR, Gomes JPO, Oliveira RC et al (2006) Mercury in the environment and riverside population in the Madeira River basin, Amazon, Brazil. *Sci Total Environ* 368:344–351
- Beyer J, Sandvik M, Hylland K et al (1996) Contaminant accumulation and biomarker responses in flounder (*Platichthys flesus* L.) and Atlantic cod (*Gadus morhua* L.) exposed by caging to polluted sediments in Sorfjorden, Norway. *Aquat Toxicol* 36:75–98
- Braga CP, Bittarello AC, Padilha CCF et al (2015) Mercury fractionation in dourada (*Brachyplatystoma rousseauxii*) of the Madeira River in Brazil using metalloproteomic strategies. *Talanta* 132:239–244
- Braga CP; Vieira JC; Leite AL et al (2017) Metalloproteomic and differential expression in plasma in a rat model of type 1 diabetes. *Int J Biol Macromol* 104:414–422.
- Braga CP, Vieira JCS, Grove RA et al (2017) A proteomic approach to identify metalloproteins and metal-binding proteins in liver from diabetic rats. *Int J Biol Macromol* 96:817–832
- Cavecci B, De Lima PM, De Queiroz JV et al (2015) Metalloproteomic profile determination of muscle samples from Nile tilapia (*Oreochromis niloticus*) using AAS and ESI-MS/MS after 2D-PAGE separation. *J Braz Chem Soc* 26:239–246
- Clarkson TW, Magos L (2006) The toxicology of mercury and its chemical compounds. *Crit Rev Toxicol* 36:609–662
- da Silva MAO, Garcia JS, GHMF S et al (2010) Evaluation of sample preparation protocols for proteomic analysis of sunflower leaves. *Talanta* 80:1545–1551
- Dydyo P, Key HM, Nazarenko A, et al (2016) An artificial metalloenzyme with the kinetics of native enzymes. *Science* (80-) 354:102–106
- Gao Y, Chen C, Zhang P et al (2003) Detection of metalloproteins in human liver cytosol by synchrotron radiation X-ray fluorescence after sodium dodecyl sulphate polyacrylamide gel electrophoresis. *Anal Chim Acta* 485:131–137
- Garcia R, Baez AP (2012) Atomic absorption spectrometry (AAS). In: *At. Absorpt. Spectrosc.*, pp 1–12
- Gorg A, Obermaier C, Boguth G et al (2000) The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 21:1037–1053
- Haraguchi H (2004) Metallomics as integrated biometal science. *J Anal At Spectrom* 19:5
- Hauser-Davis RA, De Campos RC, Zioli RL (2012) Fish metalloproteins as biomarkers of environmental contamination. *Rev Environ Contam Toxicol* 218:101–123
- Huang S, Liu X, Wang D et al (2016) Structural basis for the selective Pb(II) recognition of Metalloregulatory protein PbrR691. *Inorg Chem* 55:12516–12519

- Jenniss SW, Katz SA, Lynch RW (1999) Applications of atomic spectrometry to regulatory compliance monitoring. *ACH-Models Chem* 136:55–68
- Korbas M, Blechinger SR, Krone PH et al (2008) Localizing organomercury uptake and accumulation in zebrafish larvae at the tissue and cellular level. *Proc Natl Acad Sci U S A* 105:12108–12112
- Kutscher DJ, Sanz-Medel A, Bettmer J (2012) Metallomics investigations on potential binding partners of methylmercury in tuna fish muscle tissue using complementary mass spectrometric techniques. *Metallomics* 4:807–813
- Lima PM, Neves RDCF, Dos Santos FA et al (2010) Analytical approach to the metallomic of Nile tilapia (*Oreochromis niloticus*) liver tissue by SRXRF and FAAS after 2D-PAGE separation: preliminary results. *Talanta* 82:1052–1056
- Lippard SJ (1994) Metals in medicine. In: *Bioinorganic chemistry*. University Science Books, Mill Valley, pp 505–584
- López-Barea J, Gómez-Ariza JL (2006) Environmental proteomics and metallomics. *Proteomics* 6(Suppl 1):S51–S62
- Moraes PM, Santos FA, Padilha CCF et al (2012) A preliminary and qualitative metallomics study of mercury in the muscle of fish from Amazonas, Brazil. *Biol Trace Elem Res* 150:195–199
- Moraes PM, Santos FA, Cavecci B et al (2013) GFAAS determination of mercury in muscle samples of fish from Amazon, Brazil. *Food Chem* 141:2614–2617
- O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 250:4007–4021
- Pfeiffer WC, Lacerda LD, Salomons W, Malm O (1993) Environmental fate of mercury from gold mining in the Brazilian Amazon. *Environ Rev* 1:26–37
- Rees DA, Alcolado JC (2005) Animal models of diabetes mellitus. *Diabet Med* 22:359–370
- Santos FA, Lima PM, Neves RCF et al (2011) Metallomic study on plasma samples from Nile tilapia using SR-XRF and GFAAS after separation by 2D PAGE: initial results. *Microchim Acta* 173:43–49
- Sun H, Chai Z-F (2010) Metallomics: an integrated science for metals in biology and medicine. *Annu Reports Sect "A" Inorganic Chem* 106:20–38
- Szpunar J (2004) Metallomics: a new frontier in analytical chemistry. *Anal Bioanal Chem* 378:54–56
- Van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ Toxicol Pharmacol* 13:57–149
- Vieira JCS, Cavecci B, Queiroz JV et al (2015) Determination of the mercury fraction linked to protein of muscle and liver tissue of Tucunaré (*Cichla* spp.) from the Amazon region of Brazil. *Arch Environ Contam Toxicol* 69:422–430
- Vieira JCS, Braga CP, de Oliveira G et al (2017) Identification of protein biomarkers of mercury toxicity in fish. *Environ Chem Lett* 15:717–724
- Xiang F, Lu X, Strutt B et al (2010) NOX2 deficiency protects against streptozotocin-induced β -cell destruction and development of diabetes in mice. *Diabetes* 59:2603–2611