applied optics

Laser-induced breakdown spectroscopy determination of toxic metals in fresh fish

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A method based on laser induced breakdown spectroscopy (LIBS) for monitoring lead and copper accumulation in edible fish, particularly "tilapia del Nilo" (*Oreochromis niloticus*) is presented. The capability of this analytical method is compared with results obtained by atomic absorption spectrometry. Detection limits by LIBS are 25 parts per million (ppm) for Pb and 100 ppm for Cu, values that are below the maximum permissible levels of some international standards. Application of LIBS detection allows the development of portable instruments for contamination control of edible fish.

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1. INTRODUCTION

Nile tilapia is a teleost fish native of Africa, belonging to the *Cichlidae* family. It inhabits most of the tropical regions where conditions are favorable for reproduction and growth. Its cultivation began in 1820 in Africa and from there spread to the rest of the world. This fish withstands adverse environmental conditions, tolerates low oxygen concentrations, and can be grown in ponds and cages. For these reasons the consumption of tilapia has extended worldwide [1]. Since it is one of the most accepted species in world aquaculture, it is a fact that the natural habitat of the Nile tilapia is being polluted by the increasingly intense development of industrial technological processes that release untreated toxic waste into seas, rivers, lakes, and also into artificial harvesting pools [2]. This increases the chances of encountering heavy metals like copper and lead as contaminants in this fish [3].

Considering their biological function, heavy metals are classified as essential for life, as is the case of Fe, Cu, Zn, and Sn, and nonessential, like Hg, Pb, and Cd [4]. The latter are considered toxic to the organism in any level of concentration, although there are maximum permissible levels, regulated by international standards.

In this regard, Parks and Rose [5] have addressed the question of fish content; they analyzed the content of Cu in 20 different species of fishes. They used a colorimetric method to determine the content of Cu [3,5]. Their finding is that the content of Cu in all the species analyzed varies between 1.6 and 4.1 mg/kg. The lead content of uncontaminated fish is supposed to be under the limit of detection of any analytical technique known to date.

Meanwhile, Cu and Pb are bioaccumulative elements in plant and animal species whose presence is particularly serious for the Nile tilapia, since this fish is very low in the natural trophic chain because its diet is based on algae, decomposing matter, and plankton [3]. It should be noted that, in most cases, high concentrations of chemical substances do not change the appearance of food; therefore, the contamination is not apparent to the eye and will go unnoticed [4].

Because of the dangerous effects of heavy metals for human health, there is a need for fast and portable analytical techniques for food control. Edible fish also requires techniques for controlling *in situ* the presence of these contaminants when they are above the levels allowed by international standards. There are several analytical techniques employed to analyze heavy metals on the environmental matrix. Among them are flame atomic absorption spectroscopy (FAAS), atomic absorption spectroscopy with graphite furnace (AAS-GF), inductively coupled plasma with mass spectrometry (ICP-MS) or optical detection (ICP-AES), and laser ablation inductively coupled plasma with mass spectrometry (LA-ICP-MS). All the aforementioned analytical techniques have truly good detection limits, and particularly low levels of contamination can be detected

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with the techniques based on ICP. In particular, ICP-MS has limits of detection as low as parts per billion (μg/kg) for heavy metals like Cr, As, or Pb. However those techniques have several drawbacks, such as the cost of the equipment and the cost of operation; in the case of ICP, the plasma is generated with a flux of analytical grade argon, which is not a cheap gas. Another disadvantage of those techniques is that in all cases the sample preparation is usually a complicated step. Finally, none of the mentioned techniques is capable of field analysis.

Laser-induced breakdown spectroscopy (LIBS) is an emergent technique that allows an immediate assessment of the elemental composition of materials [6]. It has significant advantages over other conventional analytic methods. Among them is the fact that the sample requires no previous preparation and it can have any size and shape [7]. Also, the complexity of the measuring process is considerably reduced [8]. Recently, several reports of LIBS applied to determination of heavy metals, such as Pb, Cd, Hg, and Cr, in food products, vegetables, and fruits were published [9–14].

In this paper, LIBS was applied to determine the presence of Pb and Cu in Nile tilapia (*Oreochromis niloticus*) muscle. Calibration curves obtained by using atomic absorption spectroscopy (AAS) measurements as a reference allows determining LIBS limits of detection for these elements.

2. MATERIALS AND METHODS

A. Sample Preparation

Experiments were performed in muscle samples of Nile tilapia fishes (*O. niloticus*) from the Central Market of Altamira City, Mexico. Specimens with weights between 300 and 400 g and lengths of 20–25 cm were selected, deposited immediately in a container with ice, and transferred to the laboratory for analysis.

The muscle was prepared according to the Mexican standard of service [15] and it was cut into pieces 2 cm wide and 2.5 cm long, with thickness of about 0.5 cm and weight of approximately 30 g [16]. Prior to analysis, the samples were treated by drying in a microwave oven (950 W, 2450 MHz) for 1 min. This was done to eliminate the presence of water, which causes a strong electronic emission in the LIBS spectra. After that, the samples were contaminated with Pb and Cu by introducing them in salt solutions of copper and lead, dissolved in deionized water at concentrations of 10,000, 1000, 200, 100, 50, and 20 parts per million (ppm) for 1 h at 35°C temperature. In order to homogenize the sample contamination throughout its volume, an ultrasonic cleaner was used for 30 min, at a vibration frequency of 60 Hz. Finally, the samples were dried again in the microwave for 1 min. The concentrations of Pb and Cu in the fish samples were determined by AAS with a 200 Perkin Elmer Analyst device [17]. To obtain mineralization [15], the samples were calcined using a wet process following the procedure described in International Standard-SSA1-1994 NOM-117 [18].

Standard high-purity samples of Cu and Pb were used as references for identification of characteristic lines in the LIBS spectra, obtained in the same experimental conditions used for the fish sample analysis.

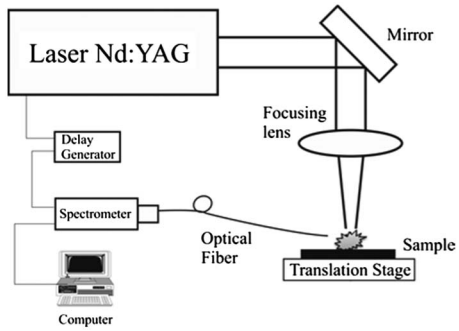

Fig. 1. Experimental setup.

B. Experimental Setup

Figure 1 show the experimental setup used. The irradiation source was a *Q*-switched Nd:YAG laser (Continuum Surelite II) with pulse duration of approximately 7 ns (full width at half-maximum, FWHM) operating at 1064 nm. The laser beam was focused on the sample using a 20 cm focal lens. The energy of the laser pulse was controlled to have laser fluences of the order of 20 J/cm^2. To collect the radiation emitted by the plasma, a fused silica optical fiber (0.5 mm opening), was used. Detection and analysis were made by using an Ocean Optics HR2000+ spectrometer (spectral range 250–1100 nm, resolution 1.5 nm). To avoid bremsstrahlung emission, an electronic delay system was used.

LIBS spectra were obtained by averaging in each measurement 10 laser shots. For each sample, 20 sets of spectra were obtained, each corresponding to a different region of the sample. The set of 20 spectra were averaged to obtain a final value with its dispersion. Each spectrum was integrated over 3 ms. Data were processed with the Origin8 program, and line emissions were identified by using data from the NIST database [19].

3. RESULTS

A. Optimization of the LIBS Measurements

Figure 2 shows two LIBS spectra corresponding to a dry and a wet sample of the muscle of tilapia. It can be seen that the presence of water causes an increase in the electron emission, which is reflected in a higher background of the spectrum. Taking into account this result, measurements were performed always on dry samples.

As is well known in LIBS measurements, the spectra registered immediately after excitation is affected by bremsstrahlung radiation, a continuum emission produced by deceleration of electrons [20,21]. To obtain the best signal to background (S/B) ratio, a delay time between excitation and acquisition is necessary. To obtain the best S/B ratio in the fish spectra contaminated with Cu and Pb, the S/B ratio of two characteristic lines of Pb (405.78) and Cu (324.74 nm) were measured at different delay times between excitation and acquisition.

Figure 3 shows the S/B ratio as a function of the delay time for the Cu line at 324.74 nm. The hatched region in the figure corresponds to the most convenient time delay range for measurements. As can be seen, the best S/B ratio is obtained

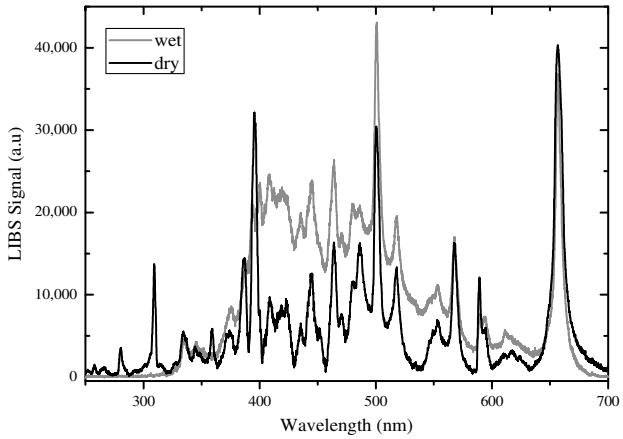

Fig. 2. LIBS spectra of wet and dry samples of tilapia fish.

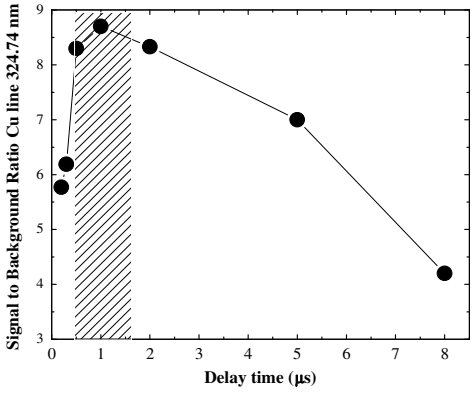

Fig. 3. S/B ratio versus the delay time between the laser shot and the acquisition, for the Cu line at 324.74 nm.

between 500 ns and 1.5 μs. A similar result was obtained for the Pb line.

Taking into account this result, the LIBS spectra were measured in all cases by using a delay time of 1 μs between the laser shot and the acquisition.

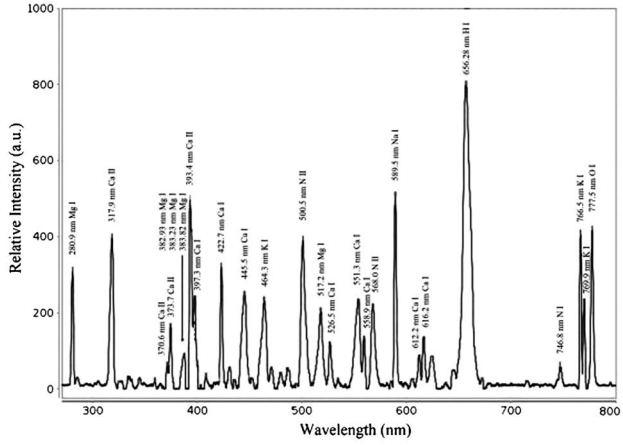

Fig. 4. LIBS spectrum of a dry muscle of Nile tilapia fish.

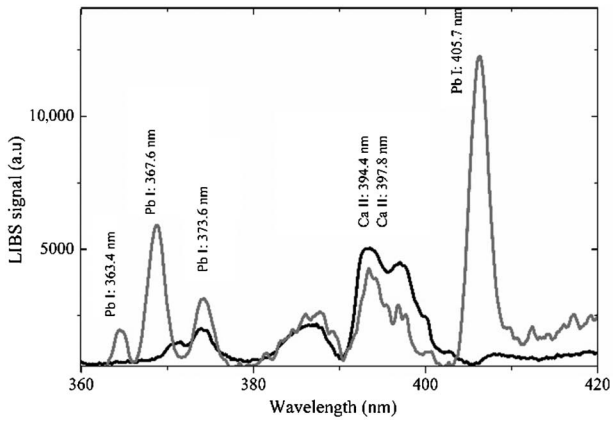

Fig. 5. LIBS spectrum of a dry muscle of Nile tilapia fish, contaminated with lead (gray line), compared to the spectrum of an uncontaminated sample (black line).

B. LIBS Measurements

Figure 4 shows a typical multiline LIBS spectrum of the dry muscle of Nile tilapia fishes. Mg, Ca, Na, and K characteristic lines are clearly observed. These elements are the most common minerals that can be found in freshwater fish. Because water is 70% of the muscle of fish, even in dry samples *O* and *H* elements are also present.

Figure 5 shows a spectrum in the region 360–420 nm of a dry muscle of Nile tilapia fish contaminated with lead (gray line), compared to the spectrum of a noncontaminated sample (black line). As is clearly seen, the characteristic lines of Pb I (363.4, 367.6, 373.6, 405.7 nm) are present.

Figure 6 shows a spectrum in the region 300–540 nm of a dry muscle of Nile tilapia fish contaminated with Cu (black line) compared to the spectrum of a noncontaminated sample (gray line). The characteristic lines of Cu I (324.75, 327.40, 510.55, 515.32, 521.82 nm) are present.

C. Calibration Curves

Quantifying the concentration of a given element in a sample by using calibration curves is a standard procedure in analytical techniques [8], particularly in LIBS [21].

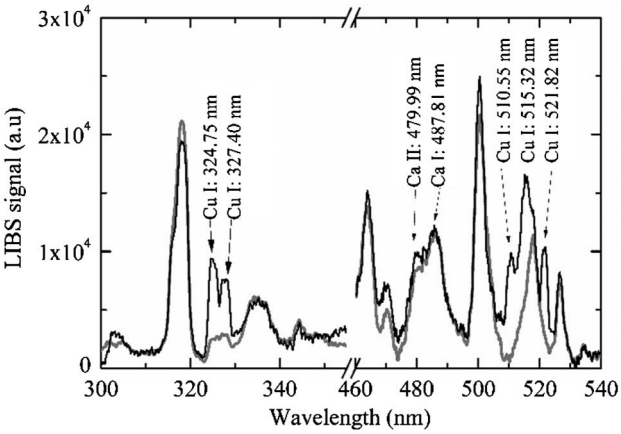

Fig. 6. LIBS spectrum of a dry muscle of Nile tilapia fish contaminated with copper (black line), compared to the spectrum of an uncontaminated sample (gray line).

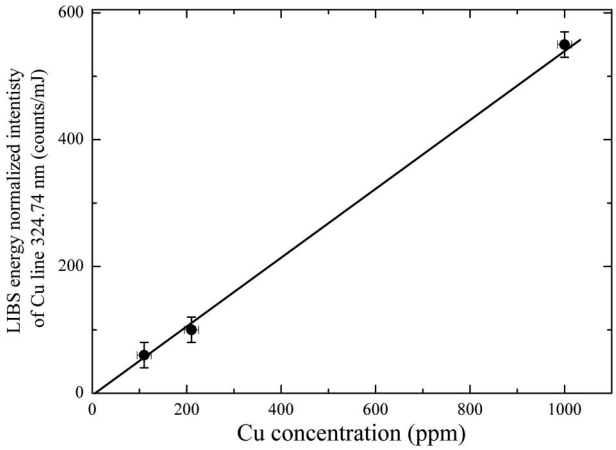

Fig. 7. Calibration curve for Cu determination in tilapia fish.

By using samples of dry muscle of tilapia fish contaminated with different concentrations of copper and lead, calibration curves for the determination of the presence of these elements were obtained. For each sample, the concentration of the element was measured by AAS following the procedure descripted in [17], and the energy normalized emission intensity of a characteristic line of Cu and Pb (324.74 and 405.78 nm, respectively) was measured by LIBS.

The criterion used to determine the detection limit of each element with LIBS was that the intensity of the reference line has a value 3 times greater than background.

Figure 7 shows the calibration curve obtained for samples contaminated with Cu. The X axis shows the result obtained by AAS and the Y axis represents the energy normalized intensity of the LIBS signal. The curve was fitted using the least-squares method.

For concentrations of less than 100 ppm, the LIBS signal intensity is below the criterion used for the detection limit and, therefore, the technique is not sensitive enough to detect these concentrations. Figure 8 shows the calibration curve obtained for samples contaminated with Pb. In this case the detection limit criterion allows measurement of a concentration of 25 ppm.

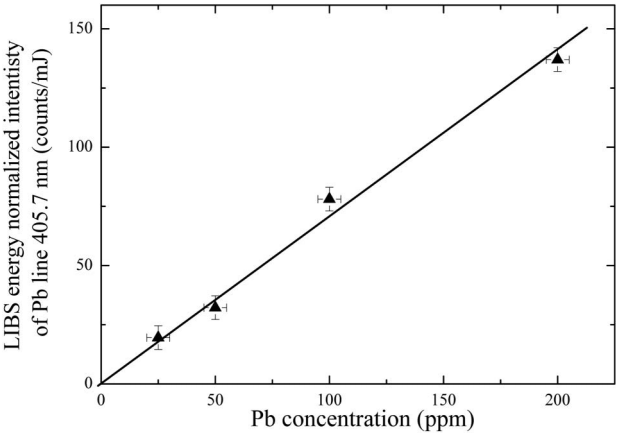

Fig. 8. Calibration curve for Pb determination in tilapia fish.

Table 1. LIBS Detection Limits of Cu and Pb on Tilapia Fish[a]

Element	Reference Line (nm)	LIBS Detection Limit (ppm)	LIBS Detection Range (ppm)	Calibration Factor(ppm mJ/counts)
Cu	324.74	100	100–1000	1.87
Pb	405.78	25	25–200	1.40

[a]The valid detection range and the calibration factor are also presented.

Table 1 shows the characteristic line used in the measurements for each element, the detection limit in ppm, the range of concentration (in ppm) for which the calibration curve is valid, and the calibration factors in ppm mJ/counts, obtained from the slope of the curves of Figs. 7 and 8.

According to international standards, the maximum permissible levels of Pb and Cu in the edible parts of fish and aquaculture products, expressed in wet mass, varies between 20 and 120 ppm for Cu, and between 5 and 40 ppm for Pb, depending on the country and agency [22–24]. As shown in Table 1 the method developed in this work allows the detection of both metals in this range and may be a suitable alternative for the control of these products in different countries.

4. CONCLUSIONS

A method based on laser induced plasma spectroscopy for monitoring lead and copper accumulation in edible fish, particularly tilapia del Nilo, was developed. The detection capability of this analytical method was checked by using independent measurements performed with atomic absorption spectrometry. Results show that the detection limits by LIBS are 25 ppm for Pb and 100 ppm for Cu, values that are below the maximum tolerable levels of some international standards, particularly those set by countries like Mexico, which is a major consumer of this type of food. The measurements do not require special preparation and can be performed by a compact portable instrument that can determine *in situ* and in real time whether the product is fit for human consumption.

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