

## Research Article

# Detection of Pb, Ba, and Sb in Blowfly Larvae of Porcine Tissue Contaminated with Gunshot Residue by ICP OES

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Lead (Pb), barium (Ba), and antimony (Sb) concentrations were monitored in flies larvae (immature *Chrysomya albiceps*) contaminated with gunshot residue (GSR) from .40 caliber Taurus pistols during the period of 2 to 12 days after the death of a female pig in decomposition, during the winter, under the influence of rain and high relative humidity. The samples were also analyzed by the colorimetric test using sodium rhodizonate (Feigl-Suter reaction). It was possible to detect and quantify the three metals of interest by inductively coupled plasma-optical emission spectrometry (ICP OES), where the concentrations of all three elements kept practically constant during the putrefaction stage. Minimum ([Pb] = 382.26  $\mu\text{g L}^{-1}$ ; [Ba] = 140.50  $\mu\text{g L}^{-1}$ ; [Sb] = 39.18  $\mu\text{g L}^{-1}$ ) and maximum ([Pb] = 522.66  $\mu\text{g L}^{-1}$ ; [Ba] = 190.30  $\mu\text{g L}^{-1}$ ; [Sb] = 56.14  $\mu\text{g L}^{-1}$ ) concentrations were found during the third and fifth days after death, respectively. ICP OES presented higher sensitivity in metals determination when compared to the conventional colorimetric test, which showed negative result for the GSR extracts obtained from the immature *Chrysomya albiceps*.

## 1. Introduction

Violence directly affects civil society. Firearms are used in 71% of all homicides in Brazil, corresponding to approximately 35000 homicides per year. In this context, Forensic Ballistic takes on a role of great legal and social relevance, acting as a legal instrument to elucidate the dynamics and authorship of crimes involving the use of firearms. Forensic Ballistics is a branch of criminology that studies firearms, ammunition, and the effects produced by them, whenever they are directly or indirectly related to criminal offenses, aiming to clarify and prove by a technical way its occurrence [1].

In Forensic Sciences, Entomology has been highlighted in studies of insects and arthropods associated with criminal matters [2, 3]. Entomological evidence in corpses has helped to elucidate murders, such as postmortem interval that contributes to the location and time of death in a homicide

or suicide. Thus, in many cases, Ballistic and Forensic Entomology may be associated, increasing crime solution.

In Forensic Ballistic, during the firing, a considerable amount of material in the gaseous or solid aerosol phase is produced and expelled along the projectile. Part of the gaseous material solidifies, producing gunshot residues (GSR). GSR is composed of lead, barium, and antimony elements [4], which can be found primarily deposited on the shooter's hands, face, and clothes; on people close to the firearm discharge; and even on the victim [5]. In this way, the elements Pb, Ba, and Sb are the major chemical markers present in inorganic GSR released after one shot and the identification of these metals is one of the practices carried out by forensic laboratories.

Characteristics of gunshot wounds can vary greatly based on the type of firearm, firing distance, type of ammunition, and location of the wound. This variability is further

influenced by many postmortem factors, including bodily decomposition, burial, and insect activity in and around the wound tract. Decomposition and burial can obscure obvious GSR tattooing or stippling while insect activity can create new tracts, obscure existing tracts, and subsequently change the morphology of the wound. Hence, identification of gunshot wounds, particularly in decomposing corpses, is complicated, and the ability to chemically detect and identify GSR around a suspected gunshot wounds would be a valuable tool [6].

In this context, the use of analytical techniques in forensic analysis to GSR identification is becoming increasingly important in the replacement of traditional qualitative methods as colorimetric assays [7–12]. The inductively coupled plasma-optical emission spectrometry technique (ICP OES) [13, 14] becomes promising in this sense because it has good sensitivity in the analysis of metals in addition to being multielement, simple, quick, and cheap when compared with other techniques such as scanning electron microscopy with X-ray energy dispersive detector (MEV/EDX) [12, 15–17] and inductively coupled plasma mass spectrometry (ICP-MS) [18–20].

In 2003, Roeterdink et al. [21] investigated the Pb, Ba, and Sb detection by ICP-MS in flies larvae from pieces of beef contaminated with GSR, using controlled conditions in a closed environment. In 2009, LaGoo et al. [6], based on the work of Roeterdink and collaborators, altered the analysis place and held the experiment outdoors and under the influence of seasonal variations (summer and winter). They have used pigs killed by firearm and roast beef contaminated with GSR. The detection of GSR was conducted by ICP-MS. In 2012, Taborelli et al. [22] used the technique of SEM/EDX for Pb, Ba, and Sb identification of GSR in a pilot study of nine samples of pig tissue wounded by firearm and skeletonized by four years. In this work, ICP OES technique is applied to evaluate the Pb, Ba, and Sb quantifications of GSR produced in immature flies of *Chrysomya albiceps* collected in a cadaver of an animal simulating a real case, in a period of 2 to 12 days after death, using a Taurus .40 caliber pistol.

## 2. Experimental

**2.1. Materials and Reagents.** GSR collection was performed in the Center for Development and Improvement of the Military Police of Espírito Santo State, Brazil. The firearm used was a Taurus .40 caliber pistol and a CBC .40 S&W.

Nitric acid ( $\text{HNO}_3$ ) of suprapure quality (65%, Merck Química Brasil, Brazil), ultrapure water ( $18.2 \text{ M}\Omega\text{-cm}$ ), prepared by a reverse osmosis system (PURELAB Mk2 Ultra, UK), hydrogen peroxide (Cromoline Fine Chemicals, Brazil), and ethyl alcohol (95%, Vetec Química Fina, Brazil) were used for sample preparation. All reagents and solvents were used as received. A stock multielemental solution containing  $1000 \mu\text{g L}^{-1}$  of the standards Ba, Sb, and Pb (Sigma-Aldrich, Switzerland) was serially diluted (100, 200, 300 to  $500 \mu\text{g L}^{-1}$ ) to form the calibration curve. All standard solutions were acidified with 2%  $\text{HNO}_3$  [13, 14].

**2.2. Instrumentation.** An ICP OES (PerkinElmer, Model Optima 7000, USA) was used for the quantification of

TABLE 1: Main ICP OES parameters.

RF power (W)	1350		
Coolant gas ( $\text{L min}^{-1}$ )	15		
Auxiliary gas ( $\text{L min}^{-1}$ )	0.2		
Nebulizer gas ( $\text{L min}^{-1}$ )	1.2		
Sample uptake ( $\text{mL min}^{-1}$ )	1.0		
Viewing	Axial		
	Pb	Ba	Sb
Analyte wavelength (nm)	220.353	233.527	206.826
Limit of detection ( $\mu\text{g L}^{-1}$ )	1.49	0.15	4.79
Limit of quantification ( $\mu\text{g L}^{-1}$ )	4.97	0.50	15.97

Pb, Ba, and Sb. A SeaSpray U-Series concentric nebulizer and cyclonic spray chamber with peristaltic pumping were used for introducing the samples into the plasma torch. The operating parameters were optimized using a central composite design [23]. The optimized operating parameters, as well as the values of the limit of detection (LOD), limit of quantification (LOQ), and correlation factor of linear curve for the analytes Pb, Ba, and Sb, are shown in Table 1, as proposed by Vanini et al. (2013 and 2015) [13, 23]. After the collection step, the samples were digested in a microwave (CEM, Model Xpress, USA).

**2.3. ICP OES Analysis of Larvae Samples.** Entomological step was carried out “*in situ*” in a forest area with clay soil at the Center for Training and Improvement of Military Police of Espírito Santo State, Brazil.

The animal selected for study was a female pig of the species *Sus scrofa*, weighing approximately 15 kg, which shows internal anatomic similarity to humans, when compared to the size of the thoracic cavity and the amount of hair [24]. The animal was placed in a metal cage, a rectangular shape measuring  $90 \times 70 \times 50 \text{ cm}^3$ .

Three shots were made in the animal within walking distance (25–40 cm, two in the head and one in the abdominal region) and immature flies of *Chrysomya albiceps* were collected in the pig in decomposition. For this step a .40 caliber pistol, by Taurus model 940, and ammunition .40 caliber S&W, by CBC, were used.

Larvae were collected, following the classification of Oliveira-Costa [24], who reported that there exist five decomposition stages: (i) initial, which occurs in the first two days; (ii) putrefaction (2nd to 12th day); (iii) black rot (12th to 20th day); (iv) butyric fermentation (20th to 40th day); and (v) dry (the 40th day onwards). In this work between 20 and 80 immature larvae from stages (i) (initial) and (ii) (putrefaction) were collected, which were stored in polypropylene tubes containing 10 mL of 70 v/v% ethanol organic matter preservation. Then the samples were macerated and 50 mg was transferred to perfluoroalkoxy (PFA) tubes to be subjected to pretreatment by microwaves. In each tube 6 mL of concentrated  $\text{HNO}_3$  and 4 mL of  $\text{H}_2\text{O}_2$  30% (w/w) were added. The microwave heating program is described in Table 2. Finally, the samples were transferred to

TABLE 2: Main microwave operating conditions.

Parameters	Stage 1	Stage 2	Stage 3
Temperature (°C)	100	100	
RF power (W)	800	800	Cooling
Time (min)	10	5	



FIGURE 1: Adult and immature *Chrysomya albiceps* flies are attracted to the animal carcass in decomposition nearby the regions where the perforation by caliber .40 S&W projectiles occurred.

polypropylene tubes, 5 mL of ultrapure water was added, and the samples were analyzed by ICP OES.

**2.4. Colorimetric Test.** The sensibility of ICP OES was compared to colorimetric assays, which were performed according to Feigl-Suter reaction [7]. Initially, a buffer solution containing a mixture of sodium bicarbonate and tartaric acid (being prepared with approximately ca. 1.9 g of sodium tartrate and 1.5 g of tartaric acid dissolved in 100 mL of ultrapure water) of  $\text{pH} \cong 3$  was sprayed on the surface of the cotton containing GSR extract produced by the immature flies of *Chrysomya albiceps*. After that, a solution of 0.2% (w/v) of sodium rhodizonate reagent was also sprayed. Positive results are obtained only for Pb through a visual detection of a red-pink color [13, 14].

### 3. Results and Discussion

**3.1. ICP OES Analysis of Larvae Samples.** Figure 1 shows the shooting area over the female pig tissue (*Sus scrofa*) from a Taurus pistol. The image shows adult and immature *Chrysomya albiceps* flies, attracted to the animal carcass in decomposition nearby the regions where the perforation by caliber .40 S&W projectiles occurred. Adult and immature flies when in contact with the shooting area end up being contaminated, thereby ingesting the GSR from the initiator mixture contained in the weapon fuse.

Immature flies' samples in the interval of 2 to 12 days after the pig death were collected. After the 12th day no samples were collected because the animal was in an advanced decomposition stage, the black rot stage. Table 3 shows the temperature and humidity values during the collection days, and the sampling period was in the Brazilian winter (July to August) with minimum and maximum temperatures ranging from 19 to 30°C.

TABLE 3: Minimum and maximum temperature (°C) and relative humidity during the period of 2 to 12 days after the death of a female pig in decomposition, during the winter.

Decomposition time	Temperature		Relative humidity	
	Minimum	Maximum	Minimum	Maximum
48 hours (2nd day)	23.4°C	30.2°C	46.0%	77.0%
72 hours (3rd day)	23.8°C	29.8°C	47.0%	87.0%
96 hours (4th day)	24.2°C	30.6°C	52.0%	80.0%
120 hours (5th day)	23.9°C	29.6°C	63.0%	82.0%
144 hours (6th day)	24.2°C	26.3°C	69.0%	83.0%
168 hours (7th day)	21.2°C	21.8°C	72.0%	89.0%
192 hours (8th day)	19.4°C	21.6°C	78.0%	91.0%
216 hours (9th day)	19.2°C	22.4°C	74.0%	89.0%
240 hours (10th day)	20.6°C	22.6°C	69.0%	82.0%
264 hours (11th day)	20.4°C	23.2°C	69.0%	86.0%
288 hours (12th day)	21.2°C	22.6°C	78.0%	82.0%

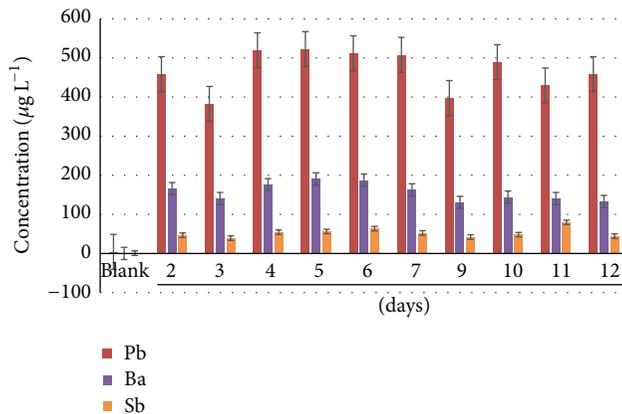


FIGURE 2: Concentrations of Pb, Ba, and Sb from GSR produced in larvae digests ( $n = 3$ ) during the period of 2 to 12 days after the death of a female pig in decomposition.

Figure 2 shows the concentrations and standard deviations of Pb, Ba, and Sb found in immature *Chrysomya albiceps* flies. In general, a maximum variation of 36, 46, and 100% at concentrations of Pb, Ba, and Sb, respectively, was observed during the collection time (2 to 12 days). This variation is related to temperature and relative humidity in the collection environment, which helped preserve and not disperse the shot residue, allowing a constant metal accumulation in the immature flies. Temperature showed a minimum of 19.2°C on the 9th day and a maximum of 30.6°C on the 4th day. Relative humidity remained high, with a minimum of 77% on the 2nd day and a maximum of 91% on the 9th day. Pb was the metal found in highest concentration, followed by Ba and Sb, corroborating with the literature [6, 13, 14].

In the interval from the 4th to the 7th day a trend of maximum concentrations were observed at which the highest values were detected in the 5th day ([Pb] = 522.66  $\mu\text{g L}^{-1}$  or 156.80  $\mu\text{g g}^{-1}$ ; [Ba] = 190.30  $\mu\text{g L}^{-1}$  or 57.09  $\mu\text{g g}^{-1}$ ; and [Sb] = 56.14  $\mu\text{g L}^{-1}$  or 16.84  $\mu\text{g g}^{-1}$ ). In the 4th, 5th, and 6th days, there was a rainy period in the experiment, which led to

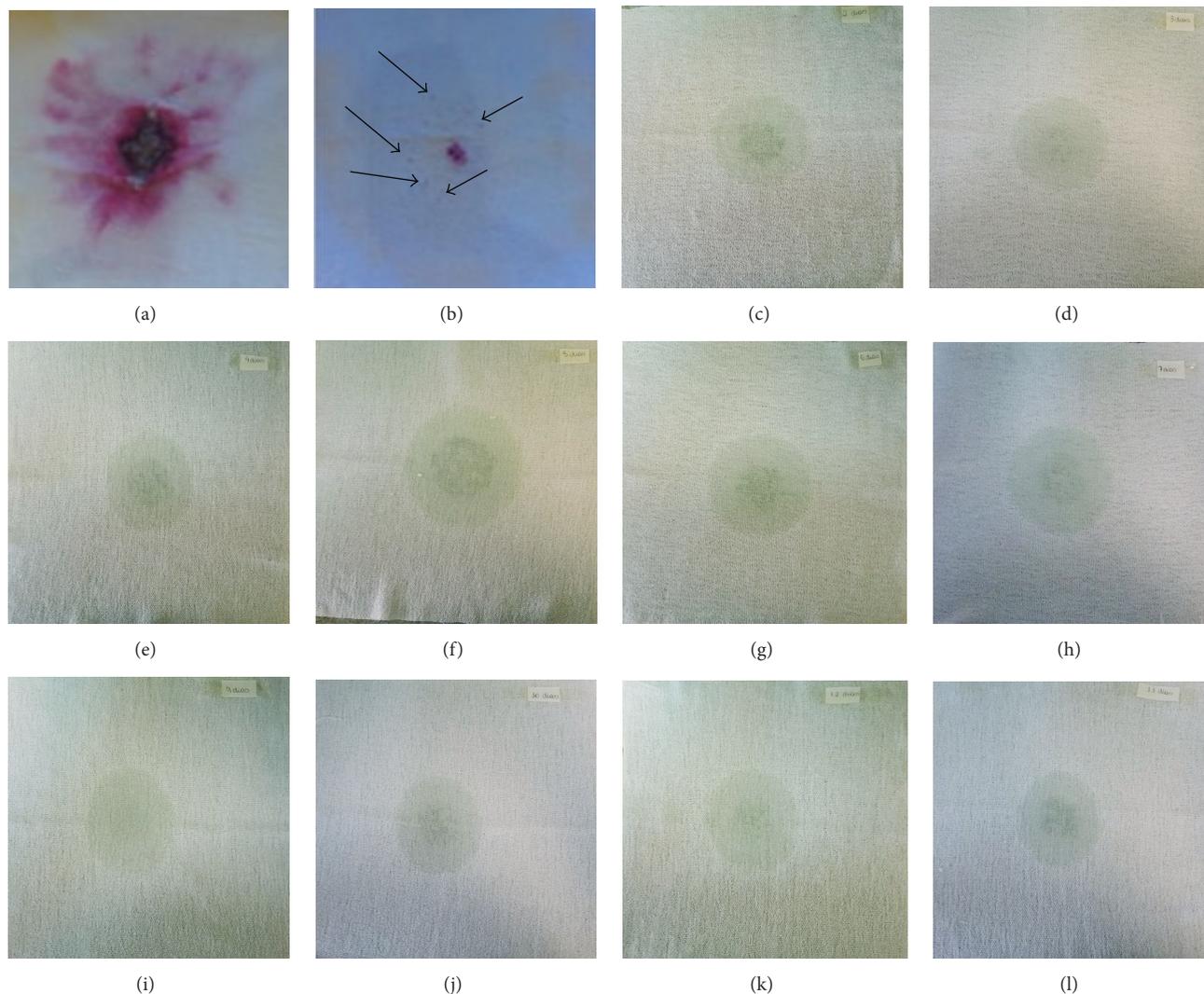


FIGURE 3: Colorimetric assay using sodium rhodizonate on white cotton containing GSR from one shot at (a) 0 cm and (b) 50 cm; and on solutions of the collected immature flies in the period of 2–12 days (c–l).

a more humid environment and therefore more favorable for the immature flies' proliferation, which also justifies the higher humidity value found on the 9th day soon after the rainy period. In addition, small accumulations of water in the shot perforations helped the accumulation of shot residue around the particle dispersion area, justifying a higher concentration of Pb, Ba, and Sb in these days. On the 2nd day ( $[Pb] = 458.20 \mu\text{g L}^{-1}$ ;  $[Ba] = 165.56 \mu\text{g L}^{-1}$ ;  $[Sb] = 46.50 \mu\text{g L}^{-1}$ ) and 12th day ( $[Pb] = 458.50 \mu\text{g L}^{-1}$ ;  $[Ba] = 132.43 \mu\text{g L}^{-1}$ ;  $[Sb] = 44.14 \mu\text{g L}^{-1}$ ) the concentrations of Pb and Sb were very similar. The presence of the analytes in the sample blank was not detected, proving that the detected concentrations of Pb, Ba, and Sb came from the GSR. The blank was measured by analyzing one immature flies' specimen grown in the laboratory.

In 2010, LaGoo et al. [6] used ICP-MS to monitor the concentrations of Pb, Ba, and Sb in GSR in periods of summer and winter, collected in pig larvae and over the animal's

wound. Similar to our work, during the winter, the three elements were detected in the tissue samples at relatively constant significant levels. LaGoo et al. found maximum and minimum concentrations, respectively, of  $500 \mu\text{g g}^{-1}$  to  $81.3 \mu\text{g g}^{-1}$  for Pb;  $126 \mu\text{g g}^{-1}$  to  $34.9 \mu\text{g g}^{-1}$  for Ba; and  $35.6 \mu\text{g g}^{-1}$  to  $5.23 \mu\text{g g}^{-1}$  for Sb in the GSR collected over the animal's wound. For the GSR collected from the larvae during the summer, the three elements were detected only on the 3rd and 4th days after death; however, they were detected at significant levels in tissue samples throughout the entire sampling period (60 days after death).

**3.2. Colorimetric Tests.** In order to evaluate and compare the sensitivity of the colorimetric test with the ICP OES technique in GSR detection in immature larvae, colorimetric tests were conducted with cotton fabric containing GSR from a firearm shot (blank) at 0 cm and 50 cm, Figures 3(a) and 3(b), respectively, and for the immature flies' samples

(Figures 3(c)–3(l)). In the case of immature flies' samples, the cotton fabric was wetted with the microwave pretreated solutions corresponding to each day of data collection (2–12 days). It can be seen that the red-pink color is evident only in cotton fabrics that have received the shot, wherein the GSR released at 50 cm occupied a smaller radius and a smaller proportion (red-pink color points are indicated by arrows) when compared to the shot at 0 cm. The presence of red-pink color is an indicator of the formation of the lead rhodizonate complex,  $[\text{PbC}_6\text{O}_6]$ , in acid medium from sodium rhodizonate solution (yellow color) and the  $\text{Pb}^{2+}$  ions [25]. The colorimetric assay was tested in the immature flies' solution, where negative results were observed for all analytes, showing that the colorimetric test does not show sufficient sensitivity to cases where the shot residue is collected in decomposition stages of the victim, either initial or putrefaction stage.

When compared with the colorimetric results (that provided positive result only for Pb containing  $\approx 3000 \mu\text{g L}^{-1}$  on white cotton from one shot at 0 and 50 cm) [14], ICP OES technique proved to be more sensitive and effective in identifying Pb, Ba, and Sb in entomological researches throughout the collection period (2–12 days) in the Brazilian winter and was able to identify inorganic markers even after the beginning of putrefaction stage and climate change, as the presence of rain, reaching a minimum concentration of  $382 \mu\text{g L}^{-1}$  for Pb.

#### 4. Conclusion

Inductively coupled plasma-optical emission spectrometry (ICP OES) is a powerful tool for GSR analysis, providing multielemental quantification of lead (Pb), barium (Ba), and antimony (Sb) with limits of detection and quantification of  $1.49$  and  $4.97 \mu\text{g L}^{-1}$  for Pb;  $0.15$  and  $0.50 \mu\text{g L}^{-1}$  for Ba; and  $4.79$  and  $15.97 \mu\text{g L}^{-1}$  for Sb. It was possible to determine the concentrations of Pb, Ba, and Sb throughout the collection period (2–12 days) with a maximum on the 5th day ( $[\text{Pb}] = 522.66 \mu\text{g L}^{-1}$ ;  $[\text{Ba}] = 190.30 \mu\text{g L}^{-1}$ ;  $[\text{Sb}] = 56.14 \mu\text{g L}^{-1}$ ) and a minimum on the 3rd day ( $[\text{Pb}] = 382.26 \mu\text{g L}^{-1}$ ;  $[\text{Ba}] = 140.50 \mu\text{g L}^{-1}$ ;  $[\text{Sb}] = 39.18 \mu\text{g L}^{-1}$ ). Pb was the inorganic marker found in higher abundance, followed by Ba and Sb. When the sensitivity of ICP OES is compared to conventional colorimetric test (Feigl-Suter reaction) to identify GSR as a function of decomposing time of porcine (immature flies) in Brazilian winter, a better sensitivity was observed for the ICP OES technique.

#### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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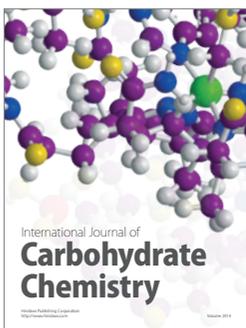
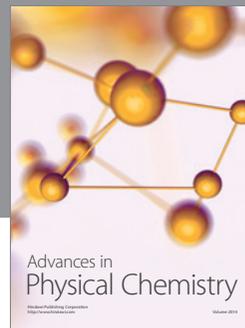
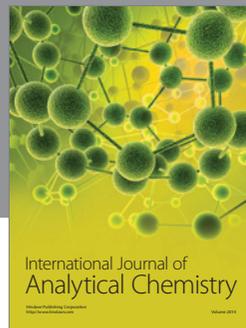
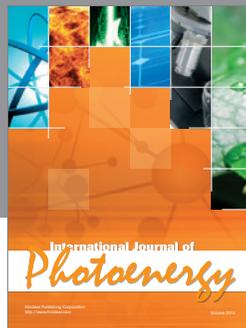
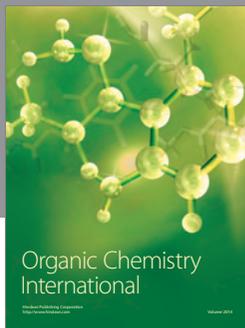
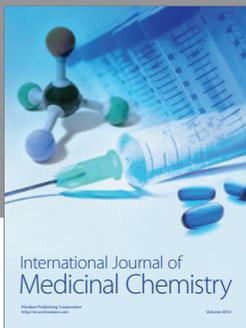
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