DOI: 10.1590/1980-5497201600010002

ORIGINAL ARTICLE / *ARTIGO ORIGINAL*

The intake of fish and the mercury concentration of fishing families at the city of Imperatriz (MA), Brazil

A ingestão de pescado e as concentrações de mercúrio em famílias de pescadores de Imperatriz (MA)

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ABSTRACT: ***Introduction:*** Significant levels of mercury exposure associated with fish intake have been demonstrated in riverine populations living in areas of mineral exploration as the basin of Tapajós and Madeira. In the Tocantins region, although few studies, there is no evidence of human exposure through food. ***Objective:*** To assess the levels of exposure to mercury in resident fishermen families in the riverside area of the middle Tocantins and to quantify the levels in fish consumed by these families. ***Methods:*** We conducted a cross-sectional study involving families of Beira Rio community fishermen, located on the Tocantins riverbanks in the city of Imperatriz, Maranhão. Brazil. Data were collected from socio-demographic and food profile, as well as samples of fish and hair, which were analyzed by atomic absorption spectrophotometry. ***Results:*** The socio-demographic profile of families studied was common to the local population located in other basins. The food profile did not run the default rule, with the fish being the primary dietary protein source. The species of piscivorous and zooplancton habits had the highest mercury concentrations, and the mean values were 0.2775 μg/g in fish-dog and 0.1360 μg/g in mapará. Among the 25 families evaluated, the lowest average concentration of family was 0.186 ± 0.043 μg/g and the higher was 5.477 ± 2.896 μg/g. ***Conclusion:*** Fishing families have low mercury exposure levels in the same order of magnitude, probably because of the food consumption of fish, including piscivorous species, which were found to be below the safe upper limit for human consumption established by Brazilian standards. This serves as a reference for other studies.

Keywords: Mercury. Mercury poisoning. Environmental exposure. Environmental pollution. Contamination. Toxicity.

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Conflict of interests: nothing to declare – **Financial support:** *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), Universal project, Public notice no. 14/2012, protocol no. 79624/2012-7.

RESUMO: ***Introdução:*** Níveis importantes de exposição ao mercúrio associados à ingestão de pescado têm sido demonstrados em populações ribeirinhas residentes em áreas de exploração mineral, como na bacia do Tapajós e do Madeira. Na região do Tocantins, apesar de poucos estudos, não há evidência de exposição humana através da alimentação. ***Objetivo:*** Avaliar os níveis de exposição ao mercúrio em famílias de pescadores residentes em área ribeirinha do médio Tocantins, além de quantificar os níveis no pescado consumido por essas famílias. ***Método:*** Realizou-se um estudo transversal envolvendo famílias de pescadores da comunidade Beira Rio, localizada às margens do Rio Tocantins no município de Imperatriz, Maranhão. Foram coletados dados de perfil sociodemográfico e alimentar, além de amostras de pescado e de cabelo, que foram analisadas através da espectrofotometria de absorção atômica. ***Resultados:*** O perfil sociodemográfico foi comum ao da população ribeirinha situada em outras bacias. O perfil alimentar não fugiu à regra do padrão, sendo o pescado a principal fonte de proteína da dieta. As espécies de hábitos piscívoro e zooplâncton apresentaram as maiores concentrações de mercúrio, sendo os valores médios do peixe-cachorro 0,2775 μg/g e do mapará 0,1360 μg/g. Dentre as 25 famílias avaliadas, a menor concentração média total/família foi 0,186 ± 0,043 μg/g e a maior foi 5,477 ± 2,896 μg/g. ***Conclusão:*** Famílias de Imperatriz possuem baixos níveis de exposição em virtude do consumo alimentar de peixes com baixos níveis de contaminação, incluindo as espécies piscívoras, que se encontravam abaixo do limite máximo de segurança para consumo humano estabelecido pelas normas brasileiras, servindo de referência para outros estudos.

Palavras-chave: Mercúrio. Intoxicação por mercúrio. Exposição ambiental. Poluição ambiental. Contaminação. Toxicidade.

INTRODUCTION

Mercury (Hg) is recognized as a toxic agent to living organisms and is responsible for damage to health, especially to the human nervous system. Records indicate that Hg was already used for religious, decorative, and cosmetic purposes since prehistoric times, being used by the Greek and Roman peoples. However, over the centuries, studies have been credited to Hg and its compounds responsibility for several episodes of intoxication, and, currently, there are various regulatory agencies that impose severe restrictions on its use[1].

The problem of ecosystem contamination by metals such as Hg reaches large territorial extensions and affects river, estuarine, and maritime systems, being observed in different parts of the world as a result of industrial expansion[2,3]. Episodes of poisoning with epidemic characteristics were associated mainly to organic Hg compounds, some of them arising from the consumption of fish contaminated with methylmercury (MeHg)[1].

In Brazil, Hg has long been used widely in industry and mining activities, and it was particularly in the Amazon region that the use of Hg in gold mining contributed to the contamination of fish and human exposure to MeHg through diet. High concentrations of Hg were found in fish caught in areas impacted by gold mining activities, including the region

of Madeira[4] and Tapajós[5] rivers. On the other hand, there are reports of fish contamination in the Rio Negro region, where there is no evidence of mining extraction activities[6], being attributed to the action of natural Hg.

In addition to the occupational exposure of workers involved directly with the extraction of gold, the Indians, fishermen, and locals, including children, have been affected by unsafe levels of Hg acquired through a diet containing contaminated fish[7-11].

Levels of exposure to Hg associated with the consumption of fish capable of causing damage were found in the riverside population of Tapajós[11-13]. Moreover, Dórea et al.[6] found them in the basin of Madeira. In the Tocantins Basin, studies show the lowest Hg concentrations ever found in the Amazon[14].

Riverside communities with high consumption of fish in their diet, located in the municipality of Imperatriz, Maranhão State, in middle Tocantins, had not yet been evaluated, considering that its location is influenced by other environmental contaminants factors, including hydroelectric activities and possible forest fires in this region.

Assessment of the impact of fish contamination on the exposure of fishing families through diet deserves attention, because most of the studies conducted involved nonfamiliar clusters, which justifies this proposal, which will enable knowledge of the risks of this form of domestic exposure in the Tocantins Basin.

METHODS

The study was conducted in the Beira Rio community, located in the Bacuri district, in the city of Imperatriz, Maranhão. The area is located on the southwest side of the city of Imperatriz (5°31'39.54''S / 47°29'39.74''W), bounded by the banks of the Tocantins River. The site is monitored by the Family Health team of Beira Rio, who provide care and counseling services in health through the Family Health Strategy (FHS).

Currently, there are 54 families living in this community, among which 25 survive directly from fish. The sample population consisted of 100% of the fishing families in the Beira Rio community. Children under 14 years of age were excluded.

DATA COLLECTION

The demographic and epidemiological data (age, sex, time of local residence, occupational activity, lifestyle, frequency, and species of the fish most consumed in the diet) were obtained between April and June 2012, through a form previously applied in other studies in the Tapajós region[14] after subjects signed the informed consent. Additional information was obtained from the report issued by the family health team of Beira Rio.

COLLECTION AND ANALYSIS OF HAIR SAMPLES

Approximately, 10 to 20 mg of hair was obtained from each participant, cut close to its insertion on the scalp, with stainless steel scissors, especially from the neck region. Each sample was packed in paper envelopes, identified by a coding system set up for each family and later directed for analysis of total mercury content (HgT), in Laboratório de Toxicologia Humana e Ambiental do Núcleo de Medicina Tropical (NMT) of Universidade Federal do Pará (UFPA)[15].

The hair samples were first subjected to washing with detergent, followed by two rinses with acetone and drying at room temperature. Next, with the aid of stainless steel scissors, the samples underwent multiple tiny cuts until a sample in powder form was obtained. Approximately, 10 mg was used between calcium carbonate, calcium hydroxide, and aluminum hydroxide layers to be analyzed by atomic absorption spectrophotometry with amalgamation in a gold sheet, using an automatic Hg gauge commercially referred to as Mercury Analyzer (MA), SP 3D model by Nippon Corporation, Japan[15].

All of these steps were carried out strictly following the equipment manufacturer's recommendations (NIC Corporation), whose techniques were also used by Khoury et al.[9], Corvelo et al.[16], Pinheiro et al.[17], and Pinheiro[18].

The accuracy of the HgT analysis was determined by quantification in duplicate and the accuracy established by the international reference standard called IAEA 085[15]. The results were expressed in μg/g (ppm).

FISH SPECIES SELECTED FOR STUDY

To determine the HgT concentrations in fish, four different species were selected, based on the frequency of weekly intake by the community during the study period: payara (*Hydrolycus scomberoides*), black prochilodus (*Prochilodus nigricans*), threespot leporinus (*Leporinus friderici*), and highwaterman catfish (*H. edentatus*). Each species was represented with 20 copies, except for *P. nigricans* (black prochilodus), of which, owing to the scarcity of the species in the period, only 13 copies were obtained. The weight of each fish species ranged from 300 to 400 g, and the size ranged from 30 to 50 cm.

Payara is a predator fish species belonging to the *Hydrolycus* genus, Cynodontidae family, which is easily identified by the oblique mouth, large canine teeth, and relatively long pectoral fins[19]. Threespot leporinus is a member of the Anostomidae family. This species has herbivorous and omnivores habits, basically consuming fruits, seeds, roots, insects, and other aquatic invertebrates. It has a medium size, reaching 30 to 40 cm and a total of 1.5 kg[20]. Black prochilodus belongs to the Prochilodontidae family member, and the species' feeding habits are detritivorous, consuming particulate organic matter, algae, and periphyton[20]. *P. nigricans* is a mid-sized species reaching up to 45 cm in total length. They are migratory fish, form schools, and perform movements within the aquatic ecosystem with trophic

and reproductive purposes[21]. The highwaterman catfish belongs to the Pimelodidae family. This species is medium-sized, reaching up to 58 cm in length, 1.3 kg in weight, and has zooplankton-feeding habits[22].

HGT DETERMINATION IN THE MUSCLE TISSUE OF FISH

All fish samples were caught on the Tocantins River by fishermen living in the community and during the visit. After measuring weight (grams) and length (cm), a portion of 20 g of muscle from the ventral region of the fish was obtained, which was then wrapped in plastic bags and recorded, coded, and stored in a cooler transported to the Laboratory of Human and Environmental Toxicology (NMT)[23].

After microfragmentation with the aid of a scalpel, a sample weighing about 0.5 g was obtained, which was transferred to a 50 mL volumetric flask. To each volumetric flask was added 1 mL of distilled water, 2 mL of nitric acid with perchloric acid (HNO_3 $HClO_4$) at a ratio of 1:1 (one to one), and 5 mL of sulfuric acid (H_2SO_4), remaining in contact with the sample for 12 hours. The next morning, the samples were subjected to acid digestion process in a heater plate at a temperature of 210°C for 30 minutes. Then, when the samples were in room temperature, distilled water to a final volume of 50 mL was added to the volumetric flasks[23].

For the preparation of the standard solutions concerning the calibration curve of the HgT analysis, a standard MeHg solution of 100 ppm, a standard MeHg 1 ppm solution (1 mL of the 100 ppm solution), and an L-Cysteine 0.01% solution had to be prepared[23].

Analyses of HgT in fish samples were carried out by atomic absorption spectrophotometry using a semiautomatic Hg analyzer Model 201-Hg from the Laboratory of Human and Environmental Toxicology (NMT), using the methodology developed by Suzuki et al.[23].

The method involves reduction and atomic absorption spectrophotometry by cold vapor and includes reduction of Hg^{2+} ions in the sample solution with stannous chloride to generate elemental mercury vapor (Hg0) and the introduction of mercury vapor in the photoabsorption cell for absorbance measurement at 253.7 nm[23].

FREQUENCY OF FISH CONSUMPTION

The estimated frequency of fish consumption was based on the study by Brune et al.[24], which considers five categories of fish consumers:

- category I (no fish consumption);
- category II (< two fish meals/week);
- category III (two to four fish meals/week);
- category IV (> four fish meals/week);
- category V (unknown consumption).

STATISTICAL ANALYSIS

HgT concentrations in fish and hair samples were presented in mean value, standard deviation, and minimum and maximum values. To compare the means of the Hg concentrations obtained from each species of fish studied, the one-way ANOVA variance test was applied, using Tukey's test for correction. To confront the average HgT between genera, we applied Student's *t*-test. The frequency of fish consumption and HgT levels in the population were analyzed using the Kruskal–Wallis test.

The BioEstat software version 5.0 was selected for testing. Differences were considered significant when $p < 0.05$[25].

ETHICAL ASPECTS

The study was conducted according to the rules of Resolution 196/1996 of the National Health Council, Ministry of Health, Brazil. It was approved by protocol number 044/2011 CEP NMT/UFPA at a meeting of the Research Ethics Committee of the Tropical Medicine Center of Universidade Federal do Pará, held on September 6, 2011.

RESULTS

The study population was represented by 81% of nonwhite people, 56% being female, 26% in the range of 14 to 20 years, and 64% married or with a steady partner. On the educational level, 59% had not finished elementary school.

In relation to the socioeconomic aspects, 76% had a piped water supply. However, only 12% were using treated water for consumption. With regard to electricity, 100% of households had that service.

As for sanitation, only 4% had a biological septic tank as their sanitary installation. Most (92%) of the families owned their own homes and had electricity.

The frequency of the dietary fish consumption and Hg exposure levels are shown in Table 1. About 90% of these riverside families had more than two, and over 60% had more than four weekly fish meals, most with a daily consumption, with a median concentration of HgT recorded at 0.736 μg/g and maximum at 8.79 μg/g.

Four species of fish that were common in the diet of families were analyzed for HgT concentrations: payara (*H. scomberoides*, piscivorous), black prochilodus (*P. nigricans*, detritivorous), threespot leporinus (*L. friderici*, herbivorous/omnivorous) and highwaterman catfish (*H. edentatus*, zooplankton-feeding). The average, minimum, and maximum HgT concentrations in each species are shown in Table 2.

The piscivorous species showed significant difference compared with the other species studied ($p < 0.01$). The zooplankton-feeding species showed significant difference from the herbivorous/omnivorous and detritivorous species ($p < 0.01$).

There was no significant difference between the herbivorous/omnivorous and detritivorous species studied ($p > 0.05$).

Twenty (80%) fishing families showed concentrations lower than 2.0 μg/g. One family showed a maximum concentration over 6.0 μg/g. The distribution of the families according to the range of concentrations and the variation of the maximum concentration of HgT is shown in Table 3.

Table 1. Estimated fish consumption frequency among fishermen family members in Imperatriz, MA, according to the classification by Brune[24].

Consumption frequency/diet	No. of people	%	HgT (μg/g)
			Median (min–max)
Category I – No fish consumption	1	1.7	0.088
Category II – Less than two fish meals/week	5	8.5	0.264 (0.083 – 0.825)
Category III – Two to four fish meals/week	20	33.9	0.813 (0.152 – 4.674)
Category IV – Greater than four fish meals/week	33	55.9	0.736 (0.000 – 8.790)
Category V – Unknown consumption	–	–	–

HgT: total mercury.

Table 2. Average concentration of HgT in different fish species consumed by the fishermen families in Imperatriz, MA.

Species code	Species	Eating habits	Number of samples	Mean ± SD (μg/g)	Min–Max (μg/g)
1	*H. scomberoides (payara)*	Piscivorous	20	0.2775 ± 0.0551	0.20 – 0.36
2	*L. friderici (threespot leporinus)*	Herbivorous/ omnivorous	18	0.0506 ± 0.0183	0.02 – 0.08
3	*P. nigricans (black prochilodus)*	Detritivorous	12	0.0308 ± 0.0108	0.02 – 0.06
4	*H. edentatus (highwaterman catfish)*	Zooplankton-feeding	20	0.1360 ± 0.0985	0.00 – 0.40

SD: standard deviation.

Table 3. Variations of HgT levels in hair samples in fishermen families in Imperatriz, MA, 2012.

HgT concentration (μg/g)	Number of families	%	Maximum variation HgT
0.0 – 2.0	20	80.0	0.152 – 1.827
2.0 – 6.0	4	16.0	2.387 – 5.182
6.0 – 10.0	1	4.0	3.423 – 8.790

HgT: total mercury.

In relation to average HgT concentrations according to sex, the average concentration in the male group was 1.01 ± 1.97 μg/g, whereas in female subjects 0.69 ± 0.82 μg/g. There was a significant difference between genders (Student's *t*-test; $p < 0.05$).

DISCUSSION

Frequent consumption of food containing MeHg has been identified as a risk factor for health. The most common source of exposure to this compound is the ingestion of fish and seafood[1].

Riverside communities of the Amazon region have the feeding habits of consuming fish from the region. However, exposure levels vary according to geographical areas. In areas affected by Hg from gold mining activities, as in the basin of the Rio Tapajós[26] and Madeira[11], human exposure levels to Hg are higher than in areas not impacted, as in the lower Tocantins[14]. In this study, we evaluated the levels of exposure by measuring the concentration of Hg in accordance with the number of fish meals per week, with no significant difference between the groups with less and more weekly fish meals, suggesting that the population in the lower Tocantins is consuming fish with low Hg content. In the group that consumed fish daily, 27.2% showed HgT concentrations greater than 1 μg/g, ranging from 1.04 to 8.79 μg/g. Among those who consume more than two and less than four meals per week, 40% showed HgT levels higher than 1 μg/g, ranging from 1.072 to 3.80 μg/g. According to the WHO[27], the normal level of Hg in hair is 1 to 2 μg/g for nonfish consumers, although people who eat fish once or more per day may present concentrations of Hg in hair exceeding 10 μg/g. The reference dose recommended by the United States Environmental Protection Agency is approximately 1 μg/g of Hg in hair for people with low fish consumption in their diet[1].

Regarding the frequency of consumption in Brazil, the maximum allowed by the Ministry of Health for food intake is 400 g of fish with a Hg concentration of 0.5 μg/g per adult weekly[28]. It is observed, therefore, that the study population represents a group with high fish intake, but with low Hg exposure levels and, thus, lower risk for damage from the ingestion of MeHg through fish in the diet.

The fish are used to support the estimate of the ecosystems' pollution level owing to the presence of anthropogenic activities, which is why they are considered indicators of the quality of the aquatic environment[5]. It is also admissible that, in the Amazon, fish is the primary route of human exposure, especially for riverside communities, where fish is an important source of protein in the diet[13].

In this study, all fish species studied showed HgT concentrations within the limits established by current Brazilian law of 0.5 μg/g for nonpredator fish (*L. friderici, P. nigricans,* and *H. edentatus*), and 1.0 μg/g for predator fish (*H. scomberoides*). These values are based on data recommended by the Codex Alimentarius Commission as an acceptable tolerance limit for human consumption[1]. Ordinance no. 685/1998 of the National Health Surveillance

Agency (ANVISA) has also set these limits. However, they refer to inorganic Hg in food and not to contamination by MeHg[28].

According to studies conducted in different ecosystems of the Amazon, Hg contamination is comprehensive and does not reach only areas under the influence of gold mining[4]. Contamination of fish with Hg coming from rivers of the Amazon without a history of gold mining activity among its tributaries was recorded by Dórea et al.[6], who found high concentrations of HgT in some species of fish caught in the Rio Negro basin. These concentrations have shown to be unfit for human consumption. However, in the Tocantins Basin, specifically in the geographical area of this study, HgT levels presented by the region's riverside population were low, similar to those found in a previous study conducted in the lower Tocantins[14], suggesting that, during the study period, there was little influence of the contaminant factors as observed in other ecosystems.

Nonpiscivorous species should be recommended for those who have a high frequency of fish consumption, considering that, in different environments, both contaminated and noncontaminated by Hg, low Hg concentrations are observed in the edible part of these species.

Kitahara et al.[29], studying samples of black prochilodus (*P. nigricans*) obtained in the Madeira River region, showed HgT concentrations ranging from 0.01 to 0.39 μg/g, lower contamination levels than 0.5 μg/g, established as the tolerance level for human consumption[1]. Likewise, other nonpiscivorous species show lower concentrations in other studies carried out in areas contaminated by Hg[4,5]. The habit of eating the types of fish in question could explain the similarity of the results and can serve as a reference for safe consumption of riverside communities.

The piscivorous species *H. scomberoides* (payara) presented lower levels of HgT than those dictated by regulatory agencies (0.2771 ± 0.0551 μg/g). These results are not explained on the theory laid out by Dórea[6], which proposes that the concentration of Hg in fish is species-specific, reflecting changes in the feeding behavior of the fish prone to bioaccumulation during periods of flooding. The types of fish analyzed in this study were caught in high-tide period, corresponding to the period of floods in Tocantins. It is possible that the local lack of natural Hg of gold mining spots and of other industrial activities explain the low contamination levels of the fish in this area of the Tocantins.

Low HgT levels in piscivorous fish species were also found by Kitahara et al.[29], who studied the species Trahira (*Hoplias malabaricus*) and Dorado. Hg concentrations varied from 0.26 a 0.39 μg/g, similar to the levels found in this study's predator species.

The species *H. edentatus* (highwaterman catfish), even though a nonpredator species and presenting low level of Hg contamination, showed significant difference from the HgT contents of the other two nonpredatory species *L. friderici* (threespot leporinus) and *P. nigricans* (black prochilodus). The food habit of the highwaterman catfish may explain the difference found. It is a zooplankton-feeding species that feeds on the lime and particulate matter deposited at the bottom of rivers, which, by deposition, may contain higher Hg loads[22].

Determination of HgT in hair samples is an important indicator in estimating the degree of exposure to Hg through diet and has been used in the exposure assessments in riverside and/or of fishing communities in the Amazon. Most studies evaluated the exposure

to unfamiliar clusters, located near gold mining areas. In the region of the Madeira River, Boischio and Barbosa[30] found very high concentrations of Hg in a family with five members, ranging from 90.6 to 303.1 μg/g. However, the study did not include only fishing families.

This study evaluated the exposure to fishing families in an area not affected by gold mining, and among the 25 families evaluated, the lowest average concentration of HgT/family was 0.186 ± 0.043 μg/g and the highest 5.477 ± 2.896 μg/g. Although they included piscivorous species, most households reported frequent consumption of nonpredatory species at the end of the rainy season, which may explain the results. In the region of the Tapajos, where there is an influence of the mining activity, Dolbec et al.[31] found that riverside communities ate more herbivorous species at the end of the rainy season, justifying the low Hg exposure levels.

In a study conducted at the lower Tocantins, Pará State, riverside communities showed HgT concentrations below 10 μg/g[14]. Although this study has been carried out on the river flood period, this research did not involve fishing families, but riverside dwellers individually, who reported frequent consumption of the fish in the area. The average concentrations of HgT found in that study, as in this study, suggest that riverside communities in the lower and middle Tocantins are consuming fish with low Hg levels, which is consistent with that expected in unexposed populations, whose reference levels must be less than[27].

In this study, only two participants exceeded this estimate, one with 8.79 μg/g (the largest value found in this study) and the other with 5.18 μg/g; both were male fishermen and from the same family.

Studies in gold mining areas in the Amazon region revealed a different reality from that found in the Tocantins Basin. On the Madeira River Basin, riverside communities situated close to mining areas, with diet based on the consumption of fish, showed critical levels of Hg exposure[32]. Pinheiro et al.[14] showed that Hg levels of the population of the Rio Tapajós region, specifically in the communities near the city of Itaituba, ranged on average from 14.1 to 20.8 μg/g with a maximum value of 62.9 μg/g. These and other studies in areas of gold mining activity confirmed the great influence of mining on environmental contamination and exposure to Hg in the local resident population. More recent data showed a reduction of exposure levels in the Tapajós region. However, these levels still pose risk to the onset of Hg poisoning symptoms[14].

Hg levels in hair samples considered safe in the long-term exposure have not yet been well-defined. The evaluation for maternal risk is always considered, and studies carried out in last decades in already contaminated areas proposed Hg levels of 6 μg/g, and above that in maternal hair could be associated with changes in the brain function of the conceptus[33].

Even within acceptable limits, the median concentration of HgT presented by women was lower than that of men ($p < 0.05$) and lower than that observed in a study in the area near to a gold mining activity area, where the men showed higher Hg concentrations than women[34]. The higher consumption of fish by men in this study is based on their permanence in the river for many days to catch fish, lacking any other protein sources in the period of occupational activity, while women have easy access to other sources of protein for being close to the municipality.

CONCLUSION

In general, fishing families in the community studied have the fish in the region as the primary protein source in their diet, feeding on predator and not predator species with low levels of contamination, which is below the safety limit for human consumption set by Brazilian standards, and could serve as a reference for other studies.

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Received on: 05/22/2015
Final version presented on: 09/28/2015
Accepted on: 12/14/2015