IJESRT
INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

RISK ASSESSMENT OF LEAD IN TILAPIA NILOTICA FISH FROM DIFFERENT ENVIRONMENTS OF EGYPT

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ABSTRACT
Lead is among the heavy metals and it is one of the highly toxic metals, which is recognized in most countries. Lead accumulates in fish. The present investigation provides information about the concentrations of lead in Tilapia nilotica fish meat from industrial, heavy traffic and rural areas in Egypt. Results indicated that lead concentrations in different samples are quite variable among the collected samples from areas under investigation. These results were confirmed by statistical analysis, which highly significant differences ($p \leq 0.05$) of lead content were observed between the concentration of lead in different samples from industrial and traffic areas compared with rural areas. On the other hand, no significant differences ($p \geq 0.05$) were observed between the lead levels in fish samples from industrial areas and traffic areas. The highest mean levels of lead, 2.6782 and 2.6596 mg/kg were detected in industrial and traffic areas, respectively. However, the lowest mean lead level (1.4811 mg/kg) was detected in rural areas samples. It can be recommended that monitoring and evaluation of lead levels in fish at regular intervals and maintaining data base is very important.

KEYWORDS: Heavy metals, Lead, Fish, Environments.

INTRODUCTION
Fish is an important source of food for humans and is a key component in many natural food webs. The nutritional benefits of fish are mainly due to the content of high quality proteins (15-24%), carbohydrate (1-3%), lipid (0.1-22%), water (66-84%) and other essential nutrients, whereas fatty fish possess also a high content of omega-3 polyunsaturated fatty acids (PUFAs), some of which have shown to have protective effects in preventing cardiovascular health disease among other important health benefits (Kris-Etherton *et al*., 2002). Fish is also an excellent source of micronutrients (trace elements, vitamins or provitamins) and long poly-unsaturated fatty acids (LPUFAs). The LPUFAs promote the improvement of membrane fluidity, decreased blood platelet aggregation and consequently less cardiovascular disease, increased immune resistance and resistance to carcinogenesis.

Heavy metals are widely dispersed in the environment and may causing contamination of foods (Enb, *et al*., 2009**;** *Abou-Arab et al*., 2008; Abou-Arab and Abou-Donia, 2001; 2002 and Soliman *et al*., 1997). Recent years have witnessed significant attention to the problems of heavy metals contamination which have been broadly studied (Abou-Arab *et al*., 2014; Abou Donia, *et. al*., 2014; Turkmen *et al*., 2009; Tuzen and Soylak, 2007; Mokhles, *et al*., 2006 and Ansari *et al*., 2005; Abou-Arab and Abou Donia, 2002; 2001). Heavy metals discharged into the marine environment can damage both marine species diversity and ecosystem due to their toxicity (Turkmen *et al*., 2009). Fish are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water (Mansour and Sidky, 2002; Abou-Arab, *et al*., 1996 and Gomaa, *et al*., 1995). Furthermore, fish is one of the most sensitive indicators of trace metals pollution and risk potential of human consumption (Lavilla *et al*., 2008; Ashraf, 2005 and Papagiannis *et al*., 2004). They enter the human body through food chain causing different diseases and damages to the humans (Tuzen and Soylak, 2007 and Yilmaz *et al.*, 2007). Lead is known to be responsible for many human health problems. Contaminated foods are major source of such heavy metals to man (Ward, 1995). Contamination of food usually takes place during transportation, storage, processing, preparation and environmental conditions. Most heavy metals are non biodegradable and their bioavailability and long biological half-life accounts for their bioaccumulation. The toxicity of metals induced by excessive levels of some these elements, such as lead is well known (Llobet *et al*., 2003). The toxicity of lead is attributed to the fact that it interferes with the normal function of enzymes. Bipolar lead forms strong bonds with enzymes bearing sulfhydryl groups thus inhibiting their action. Lead is toxic to the blood and the nervous, urinary, gastric and genital systems. Furthermore, it is also implicated in causing carcinogenesis, mutagenesis and teratogenesis in experimental animals (Pitot and Dragan, 1996). Accumulation of lead produces damaging effects in the hematopoetical, hematic, renal and gastrointestinal systems (Correia *et al*., 2000). Toxicity of lead is closely related to age, sex, route of exposure, level of intake, solubility, metal oxidation state, retention percentage, duration of exposure, frequency of intake, absorption rate and mechanisms and efficiency of excretion (Mertz, 1986). The inhalation of lead can permanently lower intelligence quotient (IQ), damage emotional stability, cause hyperactivity, poor school performance and hearing loss (Goyer, 1996).

Although human bodies have got homeostatic mechanisms that enable them to tolerate small fluctuations in the intake of heavy metals, the intake of such metals above or below certain permissible or recommended levels have devastating acute and chronic health effects (WHO, 1998; WHO, 1996 and WHO, 1995).

The aim of the present study was to evaluate the concentrations of lead in Bolti fish (*Tilapia nilotica*), which is commonly consumed as a popular diet in Egypt. Also, the investigation provided information about the concentrations of lead in three main areas represent ecosystem in Great Cairo, i.e., industrial, heavy traffic and rural areas.

MATERIALS AND METHODS

Materials

Chemicals and reagents

Stock standard solution (1000 mg/L) of lead (pb) was purchased from Merck (Merck, Darmstadt, Germany). Concentrated nitric acid at high grade (BDH chemical LTD) was also purchased. De-ionized water from a Milli Q water purification was used.

Fish samples

A total of 180 Bolti fish (*Tilapia nilotica*) samples were randomly collected from three main models represent different environments in Great Cairo (Egypt), i.e., industrial (Shoubra El-Khaima and Helwan), heavy traffic (Faysal), and rural (near cultivated lands) areas during the period of 1/5/2010 to 1/11/2012. The collected (60 sample) numbers from each area during this period were 5 samples during the period of 1/5/2010 to 26/10/2010, 15 samples during the period of 1/11/2010 to 30/4/2011, 15 samples during the period of 1/5/2011 to 20/10/2011, 10 samples during the period of 1/11/2011 to 30/4/2012 and 15 samples during the period of 1/5/2012 to 1/11/2012. The samples (3kg for each fish sample) were quite representative since the districts from where foodstuffs were scattered throughout the different environments in Great Cairo, Egypt. Sub-samples (1kg, each) were taken at random from the composite sample and processed for analysis by the dry ashing method. Lead contents were determined in all sample parts without any processes as washing.

Methods and Procedures

Test principle

Lead is extracted from different commodities according to the methods of AOAC (2000). A dry ashing method is used for the destruction of organic matter. The ashed samples are dissolved in acidic de-ionized water and lead contents are recorded by atomic absorption spectrophotometer at maximum absorbance obtained at wavelength 217.0 nm from the cathode lamp.

Sample preparation

The collected samples are homogenized separately and 3-5 g of the fresh homogenate is weighed into crucibles and dried in an oven at 100-110°C overnight (~16h.). Dried samples are ashed in a muffle furnace at 500-550°C. The ashed samples are cooled to room temperature and dissolved in 1mL 10% (v/v) nitric acid and then completed to a definite volume with de-ionized water.

Determination (Instrumentation)
The sample solutions were subsequently measured for lead as wet weight basis using PG-990 atomic absorption spectrophotometer (PG Instruments LTd) with flame atomization (air-acetylene), equipped with a 10 cm burner and a deuterium lamp for back ground correction. Maximum absorbance was obtained by adjusting the cathode lamp at the proper wavelength (217.0 nm). The other analytical parameters were; bandwidth, 0.4 nm; filter factor, 1.0; lamp current, 5.0 ma; integration time, 3.0 sec; background, D2/SR and flame setting, oxidizing blue.

Method's validity
Quality assurance
Quality assurance procedures and precautions were carried out to ensure reliability of the results. All materials used for processing are screened for possible lead contamination. Acidic-cleaned volumetric flasks and other glassware are socked in a soapy solution (2% solution isoclean detergent) for 24hr., then rinsed and soaked in 10-15% nitric acid for 48hr., then rinsed with ultrapure water and dried under clean conditions. De-ionized water was used throughout the study. The samples were generally carefully handled to avoid contamination. Procedural blanks were analyzed in every five samples. Quality control samples made from standard solutions of lead (Pb) was analyzed in every five samples to check for the metal recoveries.

Recovery and Detection Limit
Recovery results refer to complete method as reported before with different concentrations (0.1, 0.2 and 0.4 mg/kg) of lead in fish meat (muscle) were studied. The recoveries of lead in fortified samples were ranged between (94.0 to 96.2%). Detection limit was calculated and recorded which was 0.012mg/L.

Statistical analysis
The data obtained from this study was statistically subjected to analysis of variance (ANOVA) and means separation was by Snedecor and Cochran (1980). The least significant difference (L.S.D) value was used to determine significant differences between means and to separate means at $p \leq 0.05$ using SPSS package version 15.0.

RESULTS AND DICUSSION
RESULTS
Lead levels in fish meat (muscle) samples which collected from industrial, traffic and rural areas were determined and results obtained are presented in Table 1. Data indicated that lead concentrations in different samples are quite variable among the collected samples from industrial and traffic areas compared with rural areas. These results were confirmed by statistical analysis. Highly significant differences ($p \leq 0.05$) of lead content were observed between the concentrations of lead in different samples from industrial and traffic areas compared with rural areas. On the other hand, no significant differences ($p \geq 0.05$) observed between the lead levels in fish samples from industrial areas and traffic areas. The highest mean levels of lead were, 2.6782 and 2.6596 mg/kg in industrial and traffic areas, respectively. Regarding to the rural area's samples, data indicated that mean lead level was 1.4811 mg/kg.

Table 1. Lead contents (mg/kg) in fish muscle samples from industrial, traffic and rural areas collected during the period of 1/5/2010 to 1/11/ 2012.

Area	Concentrations (mg/kg)	
	Mean± SD	Range
Industrial areas	$2.6782^{a} \pm 0.03$	0.3300- 5.0572
Traffic areas	$2.6596^{a} \pm 0.03$	0.0572- 5.1156
Rural areas	$1.4811^{b} \pm 0.02$	0.1510- 3.5665
LSD at 5%	0.34	-

-All values are means of samples number determinations in each area ± standard deviation (SD).
-Means within rows with different letters are significantly different ($p \leq 0.05$).

Lead levels in muscles were determined among five periods of samples collection during the period of 1/5/2010 to 1/11/2012 (about 6 months in each period). Data proved that the highest mean levels of lead in muscle samples were detected in the three areas, i.e., industrial (3.1985 mg/kg), traffic (3.0584 mg/kg) and rural (2.2051 mg/kg) during the period of 1/11/2010 to 30/4/2011, 1/5/2011 to 20/10/2011 and 1/5/2012 to 1/11/2012, in the same order (Table 2). Analysis of variance indicated that significant differences ($p \leq 0.05$) were observed between lead contents in most periods of sample's collection from the three areas. On the other hand, no significant differences ($p \geq 0.05$) were detected between lead levels in the period of 1/11/2010 to 30/4/2011 and 1/5/2011 to 20/10/2011 in the samples of industrial areas, 1/11/2010 to 30/4/2011, 1/5/2011 to 20/10/2011 and 1/5/2012 to 1/11/2012 in the samples of traffic areas and between 1/5/2011 to 20/10/2011 and 1/11/2011 to 30/4/2012 in the samples of rural areas.

Table 2. Mean lead contents (mg/kg) in fish muscle samples from industrial, traffic and rural areas collected during the periods of samples collection (1/5/2010 to 1/11/ 2012).

Areas	**Mean concentrations (mg/kg) ± SD**					**LSD at 5%**
	1	**2**	**3**	**4**	**5**	
Industrial	1.5122^{c}±0.06	3.1985^{a}±0.03	2.7014^{b}±0.04	2.7198^{b}±0.05	2.4729^{b}±0.02	0.82
Traffic	2.5134^{b}±0.06	2.7479^{b}±0.03	3.0584^{a}±0.05	2.2539^{b}±0.03	2.4917^{b}±0.03	0.87
Rural	0.3202^{c}±0.06	1.5218^{b}±0.03	1.0482^{b}±0.03	1.4535^{b}±0.03	2.2051^{a}±0.04	0.39

-All values are means of samples number determinations in each period from each area ± standard deviation (SD).
-Means within columns with different letters are significantly different (p≤0.05).
1:1/5/2010 to 26/10/2010 **2:** 1/11/2010 to 30/4/2011 **3:** 1/5/2011 to 20/10/2011
4:1/11/2011 to 30/4/2012 **5:** 1/5/2012 to 1/11/2012

The previous periods of samples collection were in summer and winter seasons. Comparing lead levels in the samples collection from the three areas among the two seasons (Table 3), data proved that highest mean value of lead (2.7377 mg/kg) in fish muscle from traffic areas was detected during summer season. However, the highest levels of lead in industrial and rural areas were detected in winter season, which recorded 3.0207 and 1.4909, respectively. Analysis of variance proved that significant differences (p≤0.05) between the samples collection from industrial and traffic areas were observed. However, insignificant differences were reported between lead levels in summer and winter samples collected from rural areas. Analysis of variance proved that significant differences (p≤0.05) between the samples collection from industrial and traffic areas were observed. However, insignificant differences were reported between lead levels in summer and winter samples collected from rural areas.

Table 3. Mean lead contents (mg/kg) in fish muscle samples from industrial, traffic and rural areas during summer and winter collection.

Areas	**Mean concentrations (mg/kg) ±SD**		**LSD at 5%**
	Summer	**Winter**	
Industrial	2.4356^{a} ±0.07	3.0207^{a}±0.04	-
Traffic	2.7377^{a}±0.03	2.5168^{b}±0.03	0.61
Rural	1.4716^{a}±0.03	1.4909a±0.03	0.39

-All values are means of samples number determinations in each area ± standard deviation (SD).
-Means within columns with different letters are significantly different (p≤0.05).

DISCUSSION

The growing human population has increased the need for food supply. Worldwide, people obtain about 25% of their animal protein from fish and shellfish (Bahnasawy *et al*., 2009). In 2004, about 75% (105.6 million tones) of estimated world fish production was used for direct human consumption (FAO, 2006). It has been predicted that fish consumption in developing countries will increase by 57 percent, from 62.7 million tons in 1997 to 98.6 million in 2020 (Retnam and Zakaria, 2010). The nutritional benefits of fish are mainly due to the content of high quality proteins, carbohydrate, lipid and other essential micronutrients as trace elements, vitamins or provitamins (Kris-Etherton *et al*., 2002).

Fish muscle is commonly analyzed to determine contaminant concentrations and to assess the health risks because it is the main part consumed by humans. Fish can be considered as one of the most significant indicators in freshwater systems for the impact of metal pollution (Huang, 2003). The commercial and edible species have been widely investigated in order to check for those hazardous to human health (Mansour and Sidky, 2002).

In the last decades, contamination of aquatic systems by heavy metals has become a global problem. Heavy metals as lead may enter aquatic systems from different natural and anthropogenic (human activities) sources, including industrial or domestic wastewater, application of pesticides and inorganic fertilizers, storm runoff, leaching from landfills, shipping and harbour activities, geological weathering of the earth crust and atmospheric deposition (Yilmaz, 2009). In fish, which is often at the higher level of the aquatic food chain, substantial amounts of metals may accumulate in their soft and hard tissues (Mansour and Sidky, 2002). Pollutants enter fish through a number of routes: via skin, gills, oral consumption of water, food and non-food particles. Once absorbed, pollutants are transported in the blood stream to either a storage point (i.e bone) or to the liver for transformation and/or storage (Obasohan, 2008). Fish accumulate different amounts of metals depending on many factors such as physiological needs, feeding habits and genetic composition, sex of each fish species and the biochemical significant role of each metal (Huang, 2003 and kamaruzzaman *et al*., 2010). High levels of lead in fish may be attributed to the high levels of such pollutants in the fishing area and which accumulated in the fish. Accumulation patterns of metals in fish are dependent on uptake and elimination rates. The uptake metals are influenced by fish species and various environmental factors such as pH and temperatures (Hakanson, 1984). The physiological process depended on growth, salinity, age, sex, position relative to shoreline, water depth and pollutant interactions (Philips, 1980).

In the present investigation, mean lead concentrations of fish muscles collected from industrial, heavy traffic and rural areas were determined. It was found that lead concentrations in muscle samples were higher than those the limits of EUROPA (2004), Turkish Food Code (TFC, 2002), Food Standards Australia New Zealand (FSANZ, 2002), European Union (EU, 2001), United States Environmental Protection Agency (USEPA, 2000) and Food and Agriculture Organization (FAO/WHO, 1983). The corresponding levels were 1.00, 0.40, 0.20, 0.40, 0.491 and 0.5 mg/kg, respectively. On the other hand, lead levels in muscle samples from industrial or traffic areas were higher than the limits of Malaysian Food Regulation (MFR, 1985) and the permissible limits (2.0 mg/kg) reported by FAO/WHO (1983). According to Hong Kong Environmental Protection Department (HKEPD, 1987), limits (6.0 mg/kg) of lead in muscle fish mean lead levels in the samples collection from the three Egyptian areas were lower.

Comparing lead levels in the present investigation with the earlier reports in Egypt, data proved that it was higher than those detected by Abou-Arab and Abou-Donia (2001) and Gomaa *et al.*,(1995). They reported that mean lead values were 0.46 and 0.29 mg/kg in canned tuna and salmon, as well as 0.149 mg/kg in Bolti fish, respectively. On the other hand, mean lead levels in the present study were lower than those reported in some other Egyptian reports. Yacoub and Gad (2012), reported that lead levels in the *Oreochromis niloticus* **(***O. niloticus)* samples collected from the stations of Aswan Reservoir, Chema, Com Ombo, Naga Hammadi, Qus, El-Menia and ElHawamdia were (11.5 and 9.7), (14 and 13), (20 and 12), (19 and 9), (18 and 9), (17 and 11) and (19 and 14) µg/g dry wt., in summer and winter samples, respectively. Also, fish samples (*Mugil cephalus* and *Liza ramada*) were collected from five sites in Lake Manzala, Egypt by Bahnasawy *et al*., (2009). They reported that mean concentrations of lead varied from 1.43 to 2.43 µg/g dry weights in muscle. In January, 2003 concentrations of lead were determined in imported frozen fish namely Mackerel (*Scomber scolnbrus*), Striped red mullet (*Mullus surmuletus*), Groster argentine (*A rgentina silus*), Commen pandora (*Pagellus erytlvinus*) and Atlantic horse mackerel (*Trachurus trachurus*) from fish market in Alexandria, Egypt (El Nemr, 2003).The author reported that lead concentrations were ranging between 4.75 and 23.86 mg/kg with average concentration of 12.85 mg/kg dry weight. In 1996 imported fish samples (Sardine and Mackerel) were collected randomly from Great Cairo Governorates, Egypt to assess the levels of lead (Abou-Arab *et al*., 1996). The authors reported that fish samples had higher levels of lead (mean values of 11.1 and 12.6 ppm, in Sardine and Mackerel, respectively) than the permissible limits proposed by FAO/WHO (1983).

Similar results reported by Milam *et al.*, (2012) who reported that lead concentrations in catfish (*Clarotes laticeps*) in Kiri Dam in the Guyuk local government area, Adamawa State, Nigeria was 0.480 mg/kg in the muscles. On the other hand, Armelle *et al*. (2012) reported that mean lead level of three fish species collected from Lake Nokoué in the surroundings of lacustrial village of Ganvié was 26.80 ppm. Also, lead concentrations were determined in muscle of two fish species (tilapia fish and cat-fish) collected from Tiga Dam Kano, Nigeria during October, 2010 (Sani, 2011). Mean concentration of lead was ranged between 7.60 to 2.33 mg/kg. Tabinda *et al*., (2010) showed that mean concentration (µg/g wet weight) of Pb in the muscle of fish from Keti Bunder Thatta District, Sindh., *Pakistan was ranged between 0.001* to 3.600. Al Othman, (2010) reported that mean level of lead was 0.068 in selected fish samples collected from Riyadh City market. The average mean concentrations (ng/g wet wt.) of lead in 66 fish samples of four different species collected from three different sites on the Gulf coast of Saudi Arabia where there are agricultural, municipality and petroleum industrial activities was 20.0 ng/g wet wt. (Al-Saleh and Shinwari, 2002). In 2000, samples of the most commonly consumed Adriatic fish were collected at the Zagreb market were analyzed for lead levels (Juresa and Blanua, 2000). The authors reported that lead concentrations obtained in the fish muscle ranged from 9.6 to 44.1 µg/kg wet weights. Also, Szefer and Falandysz (1983) found that mean of lead level was 0.09 ppm in herring and sprat samples. On the other hand, Lowe *et al*., (1985) reported that lead level was 6.73 in the collected fish during 1979.

In the present study, data proved that lead levels were variable during the different periods of samples collection and the two seasons (summer and winter). This variation may be depends on regional differences and feeding. Seasonal variations in the concentrations of lead (Pb) were determined in gills, skin and muscles of two fish species (*Mugil cephalus and Liza ramada*) from five locations in Lake Manzala by Bahnasawy *et al*., (2009). They reported that statistical analysis revealed a significant effect of seasons, locations and fish tissues for metal measured. The highest values of the metal were recorded in hot seasons (summer and spring). The seasonal variations of heavy metals in fish were also reported by Ibrahim *et al*., (1999a & b) and Khallaf *et al*., (1998).

In addition, the results of Rizk and Khoder, (2001) confirmed that lead concentrations not stable in the atmosphere. The environmental contamination by lead in different areas leads to the contamination of foods at variable levels due to the seasonal variation. The seasonal mean variation of lead concentrations in the atmospheric city centre of Cairo and Dokki areas during the period from winter (1998) up to winter (1999) were studied (Rizk and Khoder, 2001). It reveals that the maximum mean concentration of lead were 2200 and 1700 ng/m^3 in winter, while the minimum were 1300 and 1100 ng/m^3 during the summer season at the city centre and Dokki sites, respectively. These levels decreased to 455 ng/m^3 in summer and 664 ng/ m^3 in winter during the period of 2001 and 2002 in the atmosphere in Faysal area (Shakour *et al.*, 2006). The seasonal variations of lead concentrations may occur under the effect of seasonal meteorological variations. The temperature inversion layers within the atmosphere are more persistent in winter than in summer for producing the seasonal variations of lead concentrations (Shakour *et al.*, 2006).

Although atmospheric lead originates from a number of industrial sources, leaded gasoline appears to be a principal source of general environmental leaded pollution (Rizk and Khoder, 2001). Tetraethyl lead was introduced as an antiknock agent in gasoline in the 1920s (EPA, 1986). And since then has played an increasingly important role as a pollutant of the general atmosphere. In the present investigation samples were collected from the residential area of Faysal which characterized by heavy traffic, many new buildings under construction and unpaired roads. It also located south west the city center of Cairo City, so the main source of lead was the heavy traffic emission and that transported by winds from the city center. Atmospheric lead concentration differs from one country to another. It depends on motor vehicle density and efficiency of efforts to reduce the lead content of gasoline (WHO, 1987 and 1989). It has been estimated that vehicles contribute 80-96% of all lead emissions to the atmosphere (Moriber, 1979). It has been shown that 70-80% of the lead intake to the motor is expelled to the atmosphere whereas the remaining 20-30% accumulates in the exhaust system and in the lubricating oil (Turk *et al.*, 1973). Recently, the government of Egypt introduced measures to reduce the lead concentration in the environment. These include the use of natural gas as fuel in houses and in some vehicles, as well as, the establishment of a long net underground metro in Cairo City. Beginning from the year 1991, the Egyptian authorities reduced significantly the lead content of gasoline sold in Cairo, where the lead problem had been the most serious. It was planned that in a five-year period by 1996, the gasoline sold must be completely unleaded (Rizk and Khoder, 2001). The future population exposure to air lead is expected to fall further. However, concentrations of lead in different food items including are expected to remain at the present level if produced grown in contaminated areas.

CONCLUSION AND RECOMMENDATION

From the available data, it could be concluded that lead concentration in muscle fish is relatively high. Levels of lead in different samples from industrial areas are much higher as compared to those from rural areas. The elevated levels of lead in the people bodies may result in various health and developmental problems. It can be recommended that monitoring and evaluation of lead levels in foods at regular intervals and maintaining data base is very important, put plans by specified organizations for preventing exposure through controlling or eliminating lead sources, careful washing of fish before processing, careful consumption of fish muscle from industrial areas and control of discharge of heavy metals and other toxic chemicals to the environment.

ACKNOWLEDGEMEN

The team work of the project wishes to express his deepest appreciation to Science and Technology Development Fund team for funding and the continuous guidance and supporting of this work.

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ISSN: 2277-9655
(I2OR), Publication Impact Factor: 3.785

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