DETECTION THE ACCUMULATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN TISSUES OF *ARABIBARBUS GRYPUS* COLLECTED FROM TIGRIS RIVER , IRAQ

Raghad A.Mustafa[1] A. J.Al-Rudainy[1] L .M. Abbas [2]
Researcher Prof. Chief Sci. Res
[1]Coll.Vet. Med.,University Baghdad [2]Anim. Fish Reso.Cen.
Email:alrudainy612003@yahoo.com

ABSTRACT

The present study was aimed to detect the accumulative effect of polycyclic Aromatic Hydrocarbon (PAHs) in *Arabibarbus grypus* collecting along the Tigris River Southern of Baghdad City. A total of 130 specimens were taken from three sites in Al-Zafaranyia City from the period between Summer 2020 until Spring 2021.Results of statistical analysis for total PAHs at S3 showed a significant increase ($P \leq 0.05$) compared to S1 and S2 for all seasons. Results revealed that the total concentrations of PAHs in both muscles and gills in *A.grypus* were 57.79, 86.50, 76.90 and 58.20 µg /g during summer, autumn, winter and spring respectively at S1, while at S2 were 83.60, 121.18, 166.90 and 119.90 µg /g during summer, autumn, winter and spring respectively, as well as in S3 the total concentration of PAHs were 139.08, 140.40, 192.70 and 158.80 µg /g during summer, autumn, winter and spring respectively.The highest values of PAHs were recorded during winter at all sites of collection sample either in water or fish, while the lowest values of PAHs were recorded during summer. It can be concluded that the higher concentration of PAHs in fish gills and muscle was recorded at S3 which could be due to its richness of human activities (e.g. industrial, agriculture, urban),have accumulated in sediments which high PAHs compared to S1 and S2.

Keyword: Pollution, Fish, Gills, Bioaccumilation

التحري عن تراكم الهيدروكربونات العطرية متعددة الحلقات (PAHs) في أنسجة *Arabibarbus grypus* المصيدة من نهر دجلة، العراق

رغد عادل مصطفى عبد المطلب جاسم الربيعي لؤي محمد عباس
باحث استاذ رئيس باحثين

المستخلص

اجريت الدراسة الحالية للتحري عن تراكيز مركبات الهايدروكاربونات العطرية متعددة الحلقات في انسجة اسماك الشبوط *Arabibarbus grypus* جمعت من نهر دجلة جنوب مدينة بغداد.تم جمع 130 عينة اخذت من ثلاث مواقع مختلفة في مدينة الزعفرانية للفترة من صيف 2020 حتى ربيع 2021. أظهرت نتائج التحليل الإحصائي لمجموع الهيدروكربونات العطرية متعددة الحلقات في الموقع الثالث زيادة معنوية ($P\leq 0.05$) مقارنة مع الموقعين الثاني والثالث.اظهرت نتائج الدراسة الحالية ان مجموع تراكيز المركبات العطرية في كل من العضلات وغلاصم اسماك الشبوط للموقع الاول هي 57.79 و 86.50 و 76.90و 58.20مايكروغرام / غرام في الصيف والخريف والشتاء والربيع على التوالي, بينما كانت تراكيز نفس المركبات للموقع الثاني هي 83.60 و121.18 و 166.90 و 119.90 مايكروغرام /غرام في الصيف والخريف والشتاء والربيع على التوالي. اما الموقع الثالث فقد كانت التراكيز كالتالي 139.08 و 140.40 و158.8 192.70 مايكروغرام /غرام في الصيف والخريف والشتاء والربيع على التوالي. بينت النتائج ان اعلى تركيز لمركبات الهايدروكاربونية المتعددة الحلقات سجل خلال فصل الشتاء في جميع المواقع سواء في المياه او الاسماك ، بينما اقل تركيز لمركبات الهايدروكاربونية المتعددة الحلقات سجل في فصل الصيف . يمكن ان نستنتج بأن التركيز الأعلى للهيدروكربونات العطرية متعددة الحلقات في عضلات وغلاصم أسماك الشبوط سجل في الموقع الثالث وقد يكون بسبب وجود الانشطة البشرية (مصانع، مزارع، نشاط عمراني) تراكمت في القاع مقارنة مع الموقعين الاول والثاني.

الكلمات المفتاحية: تلوث، اسماك، غلاصم، تراكم حيوي.

Received:14/5/2021, Accepted:29/8/2021

INTRODUCTION

Tigris River is considered one of the Rivers that suffer from the effect of conservative pollutants due to different agricultural drainages, industrial discharge and domestics disposal during its passing in different cities in Iraq (6,7,18,19). The concentration of petroleum products and PAHs in water of Tigris River was studied by Al- Khafajy (2) and Hameed (14). Also the effects of discharged petroleum products and PAHs into Iraqi water bodies were investigated by Farid et al. (13) who made a comparison study of the presence, origin, types and distribution of PAHs in sediments of Shatt Al-Arab River and showed that there was a significant relationship between the concentration of PAHs and Total Organic Carbon (TOC). Shabout *Arabibarbus grypus* is a species that can be founded in Rivers also can be founded in estuaries, getting a maximum size of approximately two meters and more than 50kg(20), is belonging to euryhaline and eurytherme species , nutritionally omnivorous and extensively spread in Iran, Turkey, Syria and Iraq (10). Hydrocarbon compounds such as naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, benz (a)anthracene, chrysene, benzo (b) fluoranthene, benzo(k)fluoranthene, benzo (a) pyrene, benzo (ghi) perylene dibenz (a,h)anthracene and indeno(1,2,3-cd)pyrene .These compounds enter to fish tissues either during direct contact ,contaminate their gills or via food contamination, also PAHs and their intermediate degradation products have the potential to generate toxic or mutagenic effects in fish (16) and humans (21). Reaching higher levels in Rivers ,therefore this can affect human health in areas that have commercial fisheries (21). Based on carcinogenic properties of some PAHs, WHO (24) sets out guideline value for carcinogenic compounds belong of PAHs in water concentrations equal to 0.7 µg/l, which corresponds to lifetime cancer risk of $10^{-5,}$ while in fish tissue the maximum concentrations were 5.0 lg/kg wet weight (24). Seven PAH compounds have been classified as probable human carcinogens: benz (a) anthracene, benzo(a)pyrene, benzo (b) fluoranthene, benzo (k) fluoranthene, chrysene, dibenz (ah) anthracene, and indeno (1,2,3-cd) pyrene. Chronic exposure to some chemicals found in oil may cause genetic abnormalities or cancer in sensitive species or suffering from changes in heart beats, respiratory rate, enlarged liver, reduce growth, fin erosion, a variety of biochemical and cellular changes, reproductive and behavioral responses (12). Fish eggs and larvae are generally very sensitive to oil pollution, whereas adult fish may have the possibility to avoid the oil contaminated areas, eggs or fish larvae do not have this option and oil may be toxic to fish larvae at low concentrations (11). Okenyi et al.(20) stated that fishes are quite sensitive to PAHs, leading to several specific and non-specific response by the immune system. The specific responses may involve the production of antibodies ,while unspecific responses may involve the effect on increased activities such as lysozyme and/or phagocytosis, these responses may depend on concentrations of PAHs compound and the route of exposure (5,15). The aim of current study is to detect the concentration of PAHs in gills and muscles of *A.grypus* collected from Tigris River Southern of Baghdad City.

MATERIALS AND METHODS

Study area: The current study was conducted in three sites at the Tigris River southern of Baghdad-Iraq for the period from September 2020 until March 2021 ,as the first (S1) extend away on 1 Km with Diyala River near Animal and Fish Resource Center /Ministry of Science and Technology in Al-Zaafarania City, second (S2) was located in Tigris River is about 5 Km before Diyala River joined with Tigris River ,while the third (S3) was located in the second substation of Tigris River about 5 Km (Fig.1).

Samples collection A total of 130 specimen of *A.grypus* (weight 317-805g , length 27-46 cm) were collected from Tigris River in the early morning using gill nets,then specimens were placed in ice box and transported directly to the Animal and Fish Resource Center / Ministry of Science and Technology in Al-Zaafarania City for analysis.

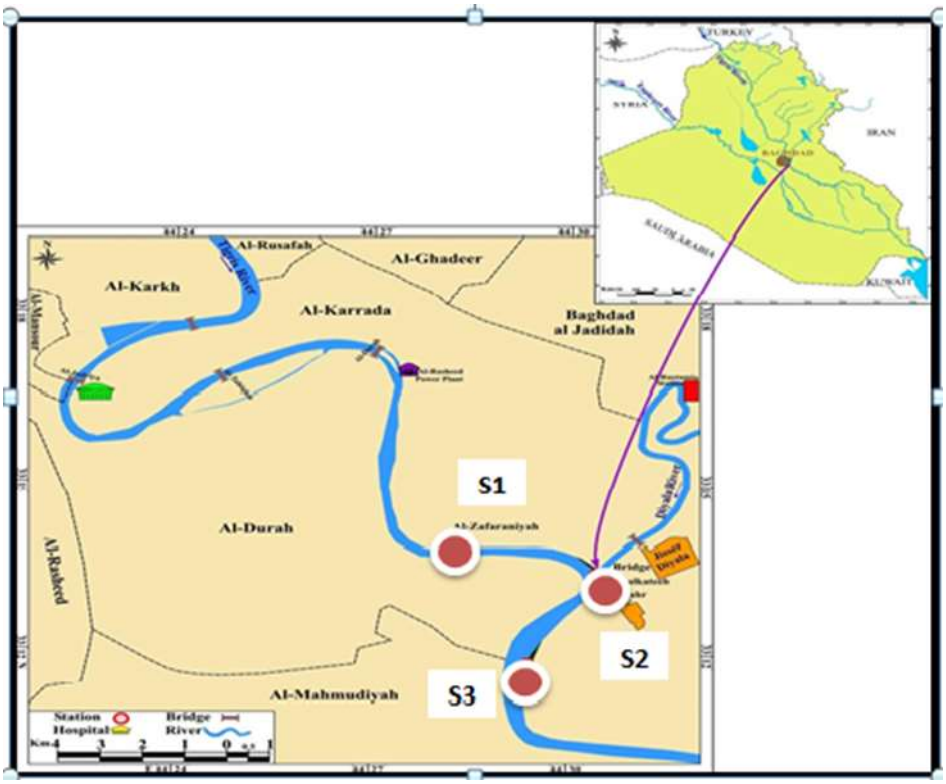

Figure 1. Study area showing fish samples collection locations at Tigris River

Extraction for determination of PAHs in water : Water samples were filtered through 0.45 mmicro porous filter membrane under vacuum in order to remove algae, zooplankton and suspended particles,there different extraction PAHs from water sample one of the method Liquid –liquid Extraction (LLE) is a method to separate compound based on their relative solubility in different immiscible liquid . In the LLE procedure , the water sample poured in to separator funnel and the mixture of 100 ml n-hexane and dichloromethane (1:1 v/v) was added and shaken for 2 min.The water phase was drained and then the organic phase was poured in to a glass funnel containing 20g of anhydrous sodium sulfate and re-extracted with 50 ml of the same solvent mixture , The extract was concentrated prior for PAHs analysis.

Extraction for determination of PAHs in *A.gyrpus*

Preparation of samples: Fish samples were dissected (gills and muscles), dried by using oven (40^{o}C).Then,these samples homogenized and 3g taken from homogenate and put into centrifuging and add a 5:5 (v/v)acetone /n-hexane (20 ml).Blank was prepared following the same procedure without adding sample. All samples were vortexed for 1min and the mixture was subjected to ultrasonic treatment for 15 min prior to extraction of Poly Aromatic Hydrocarbons. After that ,samples were centrifuged at 4000 rpm for 10 min until the obtained of organic layer containing the extracted compound and then siphoned out with pipette. Finally samples were re-extracted twice with 2:2 (v/v)acetone =hexane (10 ml) Hydrocarbon compounds named : naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a) pyrene, benzo(ghi)perylene dibenz(a,h)anthracene andindeno(1,2,3-cd)pyrene.

Statistical analysis

The Statistical Analysis System (23). Program was used to detect the effect of different factors in study parameters, Least significant difference-LSD test.Two way analysis of variance (ANOVA) to determine the significant differences between variables. A probability level equal or less than 5% ($P \leq 0.05$) were considered significantly different.

RESULTS AND DISCUSSION

Some physiochemical properties of water: Water temperature ranged from 10.20 ^{o}C at S2 in winter to 28.70 ^{o}C at S3 in summer. Salinity showed slight variations during study period which ranged between 0.34 – 0.45g/l at

S2 and S3 in winter and summer respectively. The values of PH ranged between 6.12 – 7.23 at S3 in spring and autumn respectively. Result of dissolved oxygen showed that the lowest values recorded at S3 reached 5.30 mg/l in summer and the highest values noticed at S2 attained 8.80 mg/l in winter.

Total concentration of PAHs in water

Results of Tab.1 showed that the lowest value of total PAHs recorded 3.00 mg/l at S2 in summer , whereas the highest value of total PAHs registered at S3 attained 16.20 mg/l in winter. The values of total PAHs at S1 ranged between 5.0mg/l in autumn , winter and spring to 8.5 mg/l in summer, while the values of total PAHs at S2 ranged between 3.30mg/l in summer to 3.8mg/l in autumn , winter and spring.Finally the values of total PAHs at S3 ranged between 10.10mg/l in summer , autumn and spring to 16.2mg/l in winter. Generally the results of statistical analysis for total PAHs at S3 showed a significant increase ($P \leq 0.05$) compared to S1 and S2 for all seasons (Tab.1).

Table 1. Total concentration of PAHs mg/l in water

Season	Sites			LSD
	S1	S2	S3	
Summer	8.50±0.53aA	3.30 ±0.26aB	10.10 ±1.4bA	4.51 *
Autumn	5.00±0.48bB	3.80 ±0.44aB	10.10 ±0.62bA	3.47 *
Winter	5.00±0.51bB	3.80 ±0.41aB	16.20± 0.74aA	3.08 *
Spring	5.00±0.55bB	3.80 ±0.38aB	10.10±0.62bA	3.15 *
LSD	2.77*	1.09 NS	3.69 *	---

Small alphabetic letters indicate significant difference within the same column at (p≤0.05)
Capital alphabetic letters indicate significant difference within the same row at (p≤0.05)

Total concentration of PAHs in *A. gyrpus*

The total concentrations of PAHs in both muscles and gills of *A.gyrpus* were registered 57.79, 86.50, 76.90 and 58.20µg/g during summer, autumn, winter and spring respectively at S1. While, concentrations at S2 were reported 83.60, 121.18, 166.90 and 119.90 µg/g during summer, autumn, winter and spring respectively, Whereas at S3 the total concentrations of PAHs were recorded 139.08, 140.40, 192.70 and 158.80 µg/g during summer, autumn, winter and spring respectively (Tab.2). The highest values were recorded during winter in all sites of samples collection , while the lowest values were recorded during summer in all sites. Result of the statistical analysis of S1 and S3 showed no significant differences ($p>0.05$) among seasons. Result of S2 which showed a significant difference ($p\leq0.05$) in winter compared to summer, there were no significant at S2 among summer, autumn and spring months. Results of summer showed a significant difference ($p\leq0.05$) of S3 compared to S1 but there were no significant differences ($p\leq0.05$) in autumn in all sites. In winter S3 showed a significant increase ($p\leq0.05$) compared to S1, in spring S3 showed a significant increase ($p\leq0.05$) compared to S2.

Table 2. Total concentration of PAHs (µg/g) in muscle and gills samples of *A.gyrpus*

Season	Sites			LSD
	S1	S2	S3	
Summer	57.79±4.2aB	83.60±6.2bAB	139.08±7.2aA	76.26*
Autumn	86.50 ±2.9aA	121.18±3.8abA	140.40 ±8.1aA	55.82NS
Winter	76.9±3.0aB	166.9±7.5abA	192.7 ±8.6aA	83.19 *
Spring	58.2±2.6aB	119.9±5.7ab	158.8 ±6.9aA	78.25 *
LSD	41.77NS	70.63*	64.39 NS	---

Small alphabetic letters indicate significant difference within the same column at (p≤0.05)
Capital alphabetic letters indicate significant difference within the same row at (p≤0.05)

PAHs are widely spread within marine and coastal environments. According to the International Agency for Research on Cancer, more than half of PAHs are potentially carcinogenic to humans (21). Long-term exposure to high level of polycyclic aromatic hydrocarbons is associated with cancers and adverse health problems. In the present study the seasonal variations in PAHs concentration may be due to the differences in fats content, abundant of food, habitat, food habit, size, weight, age, sex and ecological conditions such as temperature, dissolved oxygen (3,9).Temperature also has a considerable effect on the ability of microorganisms to degrade PAHs(20). In addition to that oxygen

solubility decreases with increasing temperature, which will reduce the metabolic activity of aerobic microorganisms (19). Low concentration of PAHs in summer could be due to the warm climate of Iraq in Summer , Whereas high temperatures cause PAHs to evaporate from water (8). High temperature also stimulate microorganisms to break down these compounds, especially low molecular weights of PAHs (11), and the process of optical oxidation is even greater because of the length of the day and intensity of brightness of solar radiation(4). The results of present study are in agreement with Jazza et al.(16)who recorded that the higher concentrations of PAHs in winter, whereas the lower concentrations in summer at Al-Kahlaa River in Missan Province, Iraq. Also these findings in agreement with Al-Hijaj et al.(3)who reported that the highest concentration of PAH compounds in water was 31.254 mg/l during winter and the lowest concentration was 3.62 mg/l during summer at northern part of Shatt-Al-Arab River, Iraq. In contrast the high concentrations of PAHs in winter is probably due to the increase in burning of fuel and wood used in heating (5). Furthermore it can be noticed that the levels of PAHs compounds in the muscles of *A.gyrpus* were less than their levels in other fishes collected from other regions of Iraq, but these species contain the dangerous compounds in their muscle tissues like Pyrene, Carbazole, Anthracene+ phenthrathrene, Benzo(b+k) fluornanthene and Benzo(a) anthracene because many studies reported that these compounds have a high carcinogenic activity for human and other living organisms (4). The contamination of fish with PAHs could occur through direct contact with polluted water and then absorption and accumulation in fish tissue or during cooking process especially smoked fish. Okenyi et al. (20) reported that the total PAHs concentration were 2.86 ± 0.10 mg/l and 11.00 ± 0.20 µg/g for River water and fresh fish, respectively while the total carcinogenic PAHs were 1.94 ± 0.10 mg/l and 11.00 ± 0.20 µg/g for the River water and fresh samples of *A.grypus* respectively. As well as the residual concentrations of PAHs in fish collected from Ghana and Alaska was high that could be due to higher water contamination (21). In a study conducted in Reynaud Deschaux (22), the PAHs concentrations in fresh fish samples which were used as control samples were all below the detection limits(1.00–2.00 g/kg)excepted for mackerel while the mean total PAH concentrations in the smoked fish samples ranged from 250.59 to 1143.51 g/kg for 2h of smoke curing, 595.33–1315.66 g/kg for 4h and 574.97–1376.09 g/kg for 8h smoke curing(11). It can be concluded that the higher concentration of PAHs in fish gills and muscle was recorded at S3 may be due to presence of urban activities like factory waste and another population activities comparing with S1 and S2. Same phenomena found in AlGharraf River/ThiQar(1) and Tigris river (7,17) . This indicated that the study area was generally contaminated with PAHs and continuous consumption of food from this area may pose public health hazards, therefore should be preventing the various kinds of industrial and domestic contaminants to be discharged into the River.

REFERENCE

1**.** Al-Aboudi ,H.J. ,A.J. Alrudainy and A.A. Maktoof. 2022. Accumilatir of lead and cadmium in tissues of *Cyprinus carpio* collected from cages of AlGharraf River / ThiQar / Iraq. Iraqi J.Agric.Sci.3(4):819- 824

2. Al- Khafagy, T. Y. F. 2005. Oil Pollution in Tigris River (Baghdad) and Effect of Gas Oil ToxiCity on Some Biological and Pathological Indicators for Common Carp Fish *Cyprinus carpio* L. Ph.D. Thesis,College of Agriculture,University of Baghdad.145p

3. Al-Hijaj, H., M. A. Talal, and A. A. Hantoush.2019. Polycyclic Aromatic Hydrocarbons (PAHs) in waters from northern part of Shatt Al-Arab River, Iraq. Marsh Bull. 14(1): 11–21.

4. Al-Khatib, F. M. 2008. Determination the Concentrations, Origin and Distribution of Hydrocarbon Compounds in Water, Sediments and Some Biota of Hor Al-Howaiza South of Iraq and Their Sources. Ph.D. Thesis, College of Science, University of Basrah.228p

5. Al-Khion, D. D. 2012. Distribution of Polycyclic Nuclear Compounds in Iraqi Coast Regions Ph.D, Thesis, College of Agriculture, University of Basrah. 171P.

6. Al-Marsoumi, A. M. H., K. M. Al-Bayati, and E. A. Al-Mallah. 2006. Hydrogeochemical

aspects of Tigris and Euphrates Rivers within Iraq: A comparative study. Rafidain J. Sci.17(2): 34-49

7. Al-Rudainy A. J. and S. M. Al- Samawi. 2017. Determination of lead concentration in water and in different organs of *Carrasobarbus luteus* and *Cyprinus carpio* in Tigris River. The Iraqi J. Vet. Med. 41(1):43-48

8. Al-Shwafi, N. A. A. 2008. Total petroleum hydrocarbon carcinogens in commercial fish in the Red Sea and Gulf of Aden – Yemen. J. King Abdul Aziz University. Mar. Sci.19 (1): 15-28

9. Al-Timari, A. A. K. 2000.Oil pollution in Shatt Al-Arab water studying the monthly variations of polycyclic aromatic hydrocarbons(PAHs).Mar. Mesopot. 15(2):535-548

10. Coad,B. W. 2010. Freshwater Fishes of Iraq. Pensoft Publisher. 274p

11. Deb, S. C., T. Avaki and T. Fukushima. 2000.Polycyclic aromatic hydrocarbon in fish organs. Mar. Poll.Bull.40(10):882-885

12. Dragsund, E.,V.H. Nilssen, T.Soma, A.Mikkelsen, A. Rudberg and O.A. Listerud. 2004**.**SkipstrafikklangsNorskekysten-AnalyseavMilj.Risiko, 2004-0778. Oslo: DNV. 117:2-19

13. Farid, W.A., H.T. Al-Saad and A.H.Y.A. Adhub. 2010. Toxicity of aromatic hydrocarbons to several species of mollusks from Shatt Al- Arab River. Marsh Bull. 5(1): 103- 117

14. Hameed, M. S. 2014. Determination of Some Aromatic Hydrocarbon in Tigris River Near Dora Refinery. M.Sc. Thesis, College of Science, University of Baghdad. 98p

15. Hussain, A. A. 2009. Monthly changes of some physiochemical parameters for Tigris River- Baghdad between (2002-2003) Eng. Tech. J. 27(2): 64- 70

16. Jazza, S. H., Y. A. Al-Adhub and T. Al-Saad. 2015. Polycyclic Aromatic Hydrocarbons (PAHs) in muscles of two commercial fish species from Al-Kahlaa River in Missan Governorate, Iraq. Ilum Kelautan IJMS. 20(3):121-126

17. Meshhadani, Y. D. and A. A. Jassim. 2012. Study of some characteristics of Tigris River between Mosul City and Hamam Al–Aleel Provice. Rafidain J. Sci. 23(4): 56-67

18. Mustafa, S., A. J. Al-Rudainy and S. M. Al – Samawi. 2020. Histopathological and level of bioaccumulation of some heavy metals in fish *Carabarbus luteus* and *Cyprinus carpio* tissues caught from Tigris river , Baghdad . Iraqi J. Agric. Sci.1(2):698-704

19. Nasir, A. M. 2007. Seasonal Variations of The Level of Petroleum Hydrocarbons, Nickel and Vanadium Metal in Water ,Sediments, Some Fishes and Shrimp from Iraqi Marine Water .Ph.D. Thesis, College of Science, University of Basrah.154p

20. Okenyi, A. D., C. S. Ubani, O. A. Oje and N. E. Onwurah. 2016. Levels of Polycyclic Aromatic Hydrocarbon (PAH) in freshwater fish dried with different drying regimes. J. Food Measur.Charact. 10 (2):405–410

21. Oliva, A.L.,N.S. Colla, A.H. Arias, G.E. Blasina, A.Lopez and J.E. Marcovecchio. 2017. Distribution and human health risk assessment of PAHs in four fish species from a SW Atlantic estuary. Environ. Sci. Poll. Res. 24 (23):18979–18990

22. Reynaud, S. and P. Deschaux. 2006.The effects of polycyclic aromatic hydrocarbons on the immune system of fish: A review. Aqua. Toxicol. 77(2):322-236

23. 24. SAS.2010. SAS/STAT. User Guide for Personal Computer. Release 9.13. SAS Institute, Inc., Cary, N.C., USA

24. WHO. 2011. Guidelines for Drinking-Water Quality. 4th edition. World Health Organization, Geneva, Switzerland.