

PRELIMINARY ASSESSMENT OF THE CONTAMINATION OF THE MARINE
WATER AND FISH BY TRACE METALS IN COTONOU (BENIN)

A. P. EDORH*, E. MONTCHO**, K. GNANDI**, P. GUÉDÉNON****, L.
KOUMOLOU****, M. BOKO****, A. BIGOT**, B. RIHN***** & E. E. CREPPY*****

*Department of Biochemistry and Cellular Biology, University of Abomey-Calavi
(UAC), 03 PO. BOX 0994 Cotonou, Benin - E-mail: patrickedorh@yahoo.fr

**UFR of Pharmacy, Faculty of Science Health, University of Abomey-Calavi
(UAC), 01 PO. BOX 526 Cotonou, Benin

***Laboratory GTVD (Management, Treatment and Valorization of Waste),
University of Lome, Faculty of Science, PO. BOX 1515, Lome, Togo

****Interfaculty Centre of Training and Research in Environment for Sustainable
Development (CIFRED), Laboratory of Toxicology and Environmental Health,
University of Abomey-Calavi (UAC), 03 PO. BOX 1463, Jéricho, Cotonou, Benin

*****Department of Physiology/Pharmacology, Faculty of Science, University of
Lome (UL), PO. BOX 1515, Lome, Togo

*****Unit Inserm U525, Faculty of Pharmacy, 30 street Lionnois, 54000 Nancy,
France

*****Laboratory of Toxicology and Applied Hygiene/UFR of Pharmaceutical
Sciences, 146, Street Léo Saignat, 33076 Bordeaux Cedex France

ÉVALUATION PRÉLIMINAIRE DE LA CONTAMINATION DE L'EAU ET DES
POISSONS MARINS PAR LES MÉTAUX LOURDS À COTONOU (BÉNIN).

RÉSUMÉ

Face au problème mondial de la pollution des écosystèmes aquatiques, une première contamination de l'environnement marin a été évaluée sur les échantillons d'eau et de poissons venant de l'Océan Atlantique, sur les côtes de Cotonou. L'aluminium (Al), le cadmium (Cd), le chrome (Cr), le cuivre (Cu) et le plomb (Pb) ont été quantifiés par spectrophotomètre d'absorption atomique électrothermique (Varian A 300 avec correction Zeeman), dans le Laboratoire de Toxicologie et d'Hygiène Appliquée/UFR des Sciences Pharmaceutiques (Bordeaux-France). Les résultats montrent que les limites acceptables sont dépassées pour le cadmium et le chrome dans les poissons. L'aluminium, le plomb et le cuivre devraient être surveillés. Tout ceci montre l'évidence de la pollution de l'environnement marin par les métaux toxiques le long de la côte béninoise. Cette étude nous a permis aussi de conclure que certains produits de mer, si importants dans l'alimentation des populations au Bénin, sont malheureusement impropres à la consommation et par conséquent, pourraient être à l'origine des problèmes de santé publique.

In the natural aquatic ecosystems, metals are in weak concentrations, generally of the order of the nanogram or of the microgram per litre (Calamari & Naeve, 1994). But nowadays, the presence of heavy contaminants metals is noticed in concentrations superior to the natural loads (Ademoroti, 1996). This situation presents a real danger for the aquatic fauna and the flora as well as for the man because of the phenomena of bioaccumulation in the food chain (CEDA, 1997). It is necessary to impute these environmental problems to the fast population growth, the rapid and great urbanization, the expansion of the industrial activities and the intensive exploitation of resources.

But this demographic and industrial bang did not spare Benin cities in particular the capital Cotonou that is the economic coastal and major city situated at the edge of the Atlantic Ocean.

As a result, the natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities (Conacher *et al.*, 1993 ; Velez & Montoro, 1998). Heavy metals contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005 ; Vosyliene & Jankaite, 2006 ; Farombi *et al.*, 2007). Among animal species, fish are the inhabitants that cannot escape from the detrimental effects of these pollutants (Forstner & Wittmann, 1981 ; Clarkson, 1998 ; Dickman & Leung, 1998 ; Miquel, 2001 ; Olaifa *et al.*, 2004). Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems (Yousuf & El-Shahawi, 1999 ; Farkas *et al.*, 2002). The toxic effects of heavy metals have been reviewed, including bioaccumulation (Aucoin *et al.*, 1999 ; Rasmussen & Anderson, 2000 ; Usha Rani, 2000 ; Adami *et al.*, 2002 ; Waqar, 2006). The purpose of this research is to quantify the accumulation of heavy metals in marine water and various fish from Cotonou.

MATERIALS AND METHOD

Study area

The setting of our study is the marine environment of Cotonou.

Geographic situation

Cotonou is located on the Spit between Lake Nokoué and the Atlantic Ocean. The city is bisected by a canal, the lagoon Cotonou, dug by the French in 1855. Three bridges operate between the two sides of Cotonou. It covers an area of more than 7600 ha. Because of its geographical location, Cotonou develops trade with neighbouring countries. The city is endowed with aerial, fluvial and maritime outdoors towards Porto-Novo and terrestrial lines that serve the whole of the sub-region: Burkina Faso, Togo, Niger and Nigeria (Figure 1).

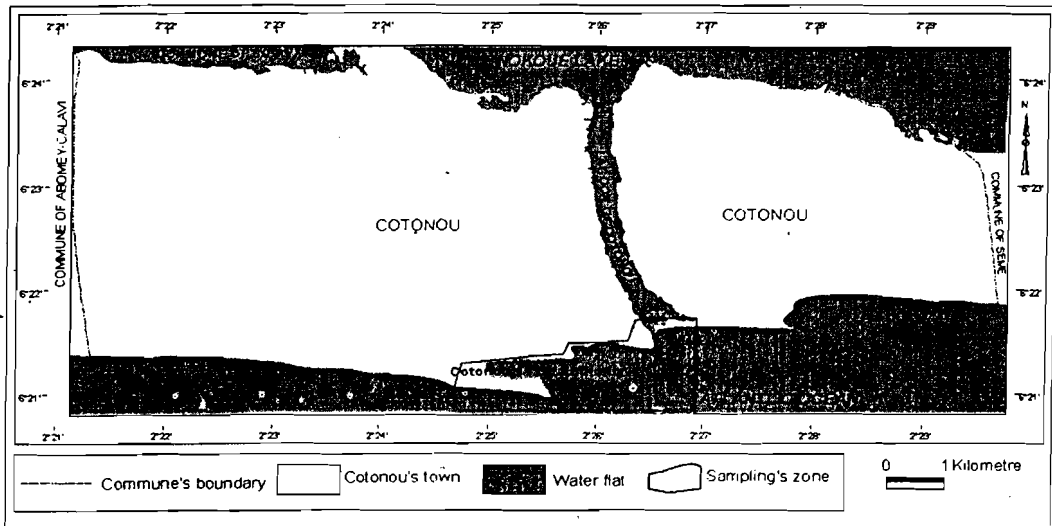


Figure1. Map of the study area (Cotonou)

Urbanization and demographic evolution of the town

The town of Cotonou has known an intense demographic development regular enough : the growth rate, between 1961 and 1979 was 8.26 %. In 2003, the current population of Cotonou is estimated at about 1.100.000 inhabitants (INSAE, 2003). With this demographic growth, the town quickly increased its commercial activities with the development urban traffic, the enlargement of the port space and the intensification of marine traffic. These factors had an impact on marine pollution as in the majority of the african countries (Calamari & Naeve, 1994). Added to this, all sorts

of marine fishing are practised in its marine environment (small-scale and industrial).

Materials

The materials used in this study are marine water and fish.

Marine water

We have taken four water samples: (i) in the port dock at 300 m from the coast and at 6 m deep, (ii) in the port area at about 5 km from the coast and at 16-18 m deep, (iii) at the level of the mouth between Cotonou lagoon and the ocean near the intertidal area at about 25 m from the coast and at 4 m deep, (iv) in the estuary at about 30 m from the coast and at 3 m deep. The Table 1 shows the sampling of water.

Table 1. Sampling of water in the study area

Number of water samples	Sampling zone	Distance from the coast	Deep
1	Port dock	300 m	6 m
1	Port area	5 km	16-18 m
1	Grand-Popo	25 m	4 m
1	Estuary	30 m	3 m

Marine fish

The six species of fish were all taken randomly in the Port Zone and at Grand Popo. But the identification of fish in Carnivores and herbivores by the Laboratory of Zoology of the University of Abomey-Calavi and by Bernard & Pierre (1990) and Murai *et al.* (2003), directed the research in purpose to obtain some elements indicative of the bioaccumulation in the trophic chain. In the laboratory, the flesh was identified, weighted, measured and stored at -20°C until analysis. The length and the diameter of fish are variable. A Table 2 shows the characters and the number of sampling for the appreciation of the representativeness of sampling.

Table 2. Numbers and characters of the fish sampled

	Characters (Murai <i>et al.</i> 2003)			Number of samples
	Scientific Name	Diet food	Dimensions (measured)	
Estuary	<i>Acanthurus monroviae</i>	Herbivores	L: 32 cm; ϕ : 27 cm	1
	<i>Pseudupeneus prayensis</i>	Herbivores	L: 20 cm; ϕ : 10 cm	1
Port Dock	<i>Sphyracna afra</i> (Adulte)	Carnivores	L: 87 cm; ϕ : 26 cm	1
	<i>Sphyracna afra</i> (Jeune)	Carnivores	L: 40 cm; ϕ : 14 cm	1
Port Zone	<i>Elops lacerta</i>	Carnivores	L: 40 cm; ϕ : 18 cm	1
	<i>Lutjanus goreensis</i>	Carnivores	L: 34 cm; ϕ : 23 cm	1
Grand Popo	<i>Decapterus rhonchus</i>	Carnivores	L: 29 cm; ϕ : 17 cm	1
L = Length ϕ = Diameter				

Method

Treatment and analysis

The fish were collected and washed immediately with the river water to remove mud. The samples were labelled and placed in air-tight plastic containers and packed in box with liquid nitrogen and transferred, for heavy metals analysis, to the Laboratory of Toxicology and Applied Hygiene, University Victor Segalen Bordeaux 2 in France.

2 g of each sample were digested into a flask of 150ml with 10ml of 25N HNO₃ and 4ml of 20N H₂SO₄ (Merck, France) during 24 h and mineralized by using Maxidigested MX 350 equipment (Prolabo, France). Water samples were not subjected to further treatment and were analysed as fish samples, by atomic absorption spectrophotometer (Anane *et al.* 1995 ; Vaidya & Rantala 1996 ; Anane *et al.*, 1997 ; Mensi *et al.*, 2008). Values of heavy metals are expressed in mg/kg for fish and μ g/L for water.

Statistical analysis

Tests of significance were carried out using the statistical analysis system (SAS), 1989. The "Proc means" procedure has been used to calculate the mean concentrations.

The General Linear Model (GLM) procedure has been used for variance analysis. The means have then been compared with the Student test t.

RESULTS AND DISCUSSIONS

*Presentation of the results**Marine water*

The different concentrations and mean concentrations of the trace metals in marine water are presented in Table 3.

Table 3. Toxic heavy metals contents in the samples of marine water

Metals ($\mu\text{g/L}$)	Port Dock	Port Zone	Estuary	Grand- Popo	Means	SD
Al	0.135	0.149	0.154	0.83	0.317	0.342
Cd	0.07	0.09	0.07	0.06	0.073	0.013
Cr	3	2.6	0.7	0.15	1.613	1.399
Cu	2.125	1.125	0.375	0.425	1.013	0.817
Pb	0.24	0.16	0.95	0.165	0.379	0.383

a) SD = Standard Deviation

b) In the ascending order Cr comes before Cu, Pb, Al and Cd

Marine fish

The flesh, the grills and the liver of each fish species were analyzed, but only the average of the contents was retained. For example, Table 4 shows toxic heavy metals content in the organs of *Elops lacerta* but in Table 5, only the mean concentration for this organs is retained. Table 5, then, gathers together all the concentrations of toxic heavy metals in the different fish species.

Table 4. Toxic heavy metals content in the organs of *Elops lacerta*

Métaux ($\mu\text{g/g}$)	Flesh	Gills	Liver	Mean	SD
Al	4.455	7.1237	4.125	5.235	1.644
Cd	0.0875	0.025	0.0125	0.042	0.040
Cr	0.775	0.335	0.9875	0.699	0.333
Cu	1.5	1.379	1.225	1.368	0.138
Pb	0.0125	0.05	0.1625	0.075	0.078

a) SD = Standard Deviation

b) For the means, in the ascending order Al comes before Cu, Cr, Pb, and Cd.

Table 5. Toxic heavy metals concentration in the different fish species

Fish species	Al (mg/Kg)		Cd (mg/Kg)		Cr (mg/Kg)		Cu (mg/Kg)		Pb (mg/Kg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Elops lacerta	5.23*	1.64	0.04*	0.04	0.70#	0.33	1.37#	0.14	0.07*#	0.08
Acanthurus monroviae	0.75#	0.09	0.06*	0.02	1.35*	0.14	1.47*#	0.03	0.0#	0
Lutjanus goreensis	3.65*#	2.34	0.04*	0.01	0.77#	0.26	2.03*	0.07	0.02*#	0.02
Pseudupeneus prayensis	4.70*	0.50	0.08*	0.03	1.06*#	0.41	1.18#	0.05	0.14*	0.02
Sphyræna afra (adult)	1.95#	1.17	0.06*	0.03	0.73#	0.10	1.37#	0.26	0.07*#	0.07
Sphyræna afra (young)	3.30*#	2.66	0.06*	0.04	0.71#	0.22	1.60*#	0.59	0.07*#	0.06
Decapterus rhonchus	1.15#	0.79	0.07*	0.05	0.83#	0.09	1.15#	0.14	0.03#	0.03

a) SD = Standard Deviation.

b) If two means of the same column are together followed at least by a same symbol (* or #), they are not significantly different at the threshold of 5 % ($p > 0.05$ %). otherwise, they are.

Table 6 shows the comparative study of toxic heavy metals content between herbivores and carnivores. This comparison is doing in order to obtain some elements indicative of the bioaccumulation in the trophic chain.

Table 6. Toxic heavy metals concentration between carnivores and herbivores fish

Fish' food mode	Al (µg/g)		Cd (µg/g)		Cr (µg/g)		Cu (µg/g)		Pb (µg/g)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Carnivores	2,23	0,87	0,07	0,01	0,90	0,12	1,16*	0,13	0,07	0,03
Herbivore	3,03	0,55	0,06	0,01	0,81	0,70	1,53*	0,08	0,06	0,02

a) SD = Standard Deviation.

b) The mean of the same column followed by * are significantly different at the threshold of 5% ($p > 0.05$ %). Otherwise, they are not.

DISCUSSION

Marine water

The mean concentration of Al in the marine water samples is $0.32\mu\text{g/L}$. This value is below the permissive limit $1\mu\text{g/L}$ (ATSDR 1999). The content in Cr ($1.40\mu\text{g/L}$) also, is between the acceptable limit (1 to $10\mu\text{g/L}$) fixed by CE (2006). And the mean concentrations of Cd, Cu and Pb (respectively 0.07, 1.01 and $0.38\mu\text{g/L}$) are all below those obtained in the Lagos for the same metals which are respectively 2, 3 and $9\mu\text{g/L}$ (Okoye, 1991b). This shows that marine heavy metals contamination is differentiated and

depends certainly on the level on pollution in the regions. However, the concentration in Cd ($0.07\mu\text{g/L}$) is between 0.05 and $1.2\mu\text{g/L}$ in marine water. With these rates, Galaf and Ghannan (2003) have observed on crustacean grubs in Morocco, some respiratory disruptions, enzyme stimulation and an inhibition of the phytoplankton growth. These contents prove that, in spite of these relatively low contents, the ecosystem ocean in Benin is already polluted at such point that it can potentially be dangerous for the watery species.

Marine fish

According to the results, all the fish samples are contaminated. But the highest concentrations in Al have been obtained with *Elops lacerta* and *Pseudupeneus prayensis* with the respective contents of 5.23 and 4.70 mg/kg. These concentrations are significantly higher than those obtained with *Acanthurus monroviae*, *Sphyraena afra* (adult) and *Decapterus rhonchus* ($P < 0.05$). On the other hand, *Lutjanus goreensis* and *Sphyraena afra* (young) have a concentration in Al similar to the one of the other fish species.

The concentrations in Cd are similar to the level of all the fish species sampled with the value from 0.04 up to 0.07 mg/kg respectively for *Elops lacerta* and *Decapterus rhonchus*.

The highest concentration in Cr has been obtained with *Acanthurus monroviae* with the value of 1.35 mg/kg. This value is above the permissive limit of 0.3 mg/kg (CE, 2006) and also significantly higher than those obtained with *Elops lacerta*, *Lutjanus goreensis* (adult and young), *Sphyraena afra* and *Decapterus rhonchus* ($P < 0.05$). On the other hand, *Pseudupeneus prayensis* has a concentration in Cr similar to those of the other species.

The highest concentration in Cu has been obtained with *Lutjanus goreensis* with a content of 2.03 mg/kg. This concentration is lower than that obtained by Okoye (1991a, 1991b) and Okoye *et al.* (1991) in Nigeria (11.3 mg/kg) but higher than the one obtained by the Institute of Aquatic Biology (1990) in Ghana (0.46 mg/kg). This concentration is significantly higher than those obtained with *Elops lacerta*, *Pseudupeneus prayensis*, *Sphyraena afra*, (adult), and *Decapterus rhonchus* ($P < 0.05$).

The highest concentration in Pb (0.14 mg/kg) obtained with *Pseudupeneus prayensis* is nevertheless under the maximum limit of 0.2 mg/kg (CE 2006) and inferior to those obtained by Okoye (1991a, 1991b), Okoye et al. (1991) in Nigeria (2.28 mg/kg) and the Institute of Aquatic Biology (1990) in Ghana (0.36 mg/kg). This concentration is significantly higher than those obtained with *Acanthurus monroviae* and *Decapterus rhonchus* ($P < 0.05$).

All these results show that bioaccumulation of heavy metals in the marine fish would depend on several factors such as the bioavailability of the metals, the age, the species and the intrinsic nature of the bioaccumulator (Luoma, 1983).

Concerning the comparison between herbivores and carnivores, we notice that, put aside the contents of Cu which are significantly different, all the contents are roughly the same for Al, Cr, Cd and Pb. These results let believe that the food mode of fish would not influence the bioaccumulation. But, if herbivorous and carnivorous fish are contaminated, their predatory in the food chain will be contaminated. They will attend to a bioamplification of heavy metals in the entire ecosystem (Ramade, 1992).

However, it was shown on several occasions that instead of bioamplification, they attend to a dilution of heavy metals between the primary and secondary trophic levels (Casas, 2005).

CONCLUSION

This study has allowed measuring the pollution level in our marine environment by some toxic heavy metals (Al, Cd, Cr, Cu, Pb) in water and fish. For marine fish species, the limit values of some heavy metals are already exceeded. For the other heavy metals, although the concentrations are still low, they are worrying. These different indicators show not only the necessity to prevent the toxicity risks for the marine environment and human on average and long terms. But it is also necessary to carry out studies of impact on health because according to the alimentary habits and the quantities of fish consumed, the acceptable daily intake could be exceeded.

The data on Pb constitute an initial state of pollution by this heavy metal just before its prohibition in fuel in 2005 in Benin. These data are very precious to follow the evolution of the concentration in Pb in the coming years.

With the probable intensification of urbanization and sociodemographic activities, we shall have to identify the sources and quantify the toxic heavy metals discharges into the marine environment. Then, important measures to be taken to fight against pollution. Those measures should involve legislation, norms and criteria to minimize waste production and effluents processing.

REFERENCES

- ADAMI G. M., BARBIERI P., FABIANI M., PISELLI S., PREDONZANI S. & REISENHOFER E. 2002. Levels of Cd and zinc in hepatopancreas of reared *Mytilus galloprovincialis* from the Gulf of Trieste (Italy). *Chemosphere*. 48 (7): 671-677.
- ADEMOROTI C. M. A. 1996. *Standard Methods for Water and Effluent Analysis*. Foludex Press Ltd. Ibadan. p182.
- ANANE R., BONINI M., GRAFEILLE M. J. & CREPPY E. E. 1995. Bioaccumulation of water soluble Al chloride in the hippocampus after transdermal uptake in mice *Arch. Toxicol.* 69: 568-571.
- ANANE R., BONINI M. & CREPPY E. E. 1997. Transplacental passage of aluminum from pregnant mice to fetus organs after maternal transcutaneous exposure. *Hum Exp Toxicol.* 16(9): 501-504.
- ASHRAJ W. 2005. Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microdon* fish from the Arabian Gulf. *Environ. Monit. Assess.* 01(1-3): 311-316.
- ATSDR (Agency for Toxic Substances and Disease Registry). 1999. Easily accessible, current information for every environmentalist: Risks, limits and health effects for aluminium. 40p.
- AUCOIN J., BLANCHAND R. & BILLIOT C. 1999. Trace metals in fish and sediments from Lake Boeuf, South Eastern Louisiana. *Micro. Chem. J.* 2 (2): 299-307.
- BERNARD S. & PIERRE O. 1990. *Poissons de mer de l'Ouest Africain tropical*. [In french]. ORSTOM. Paris. 450p.
- CALAMARI D. & NAEVE H. 1994. *Revue de la pollution dans l'environnement aquatique africain*. [In french]. Document technique du CPCA, 25, Rome, FAO. 129p.
- CASAS S. 2005. *Modélisation de la bioaccumulation de métaux Traces (Hg, Cd, Pb, Cu et Zn) chez la moule, mytilus Galloprovincialis, en milieu méditerranéen*. [In french]. Thèse de doctorat. 363p.
- CE (Communautés Européennes). 2006. *Règlement CE No 1831/2006 : Fixation de teneurs maximales pour certains contaminants dans les denrées alimentaires*. [In french]. Journal officiel de l'Union européenne. L 364: 5-24.
- CEDA: Centre pour l'Environnement et le Développement en Afrique. 1997. [In french]. Profil environnemental de la zone côtière. Côte d'Ivoire. p43.
- CLARKSON T. W. 1998. Human toxicology of mercury. *J. Trace. Elem. Exp. Med.* 11 (2-3): 303-317.

- CONACHER H. B., PAGE B. D. & RYAN J. J. 1993. Industrial chemical contamination of foods [Review]. *Food. Addit. Contam.* 10 (1):129-143.
- DICKMAN M. D. & LEUNG K. M. 1998. Mercury and organochlorine exposure from fish consumption in Hong Kong. *Chemosphere.* 37(5): 991-1015.
- FARKAS A., SALANKI J. & SPECZIAR A. 2002. Relation between growth and the heavy metal concentration in organs of bream *Abramis brama* L. populating Lake Balaton. *Arch. Environ. Contam. Toxicol.* 43 (2): 236-243.
- FAROMBI E. O., ADELOWO O. A. & AJIMOKO Y. R. 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish (*Clarias gariepinus*) from Nigeria Ogun river. *Int. J. Environ. Res. Public Health.* 4 (2): 158-165.
- FORSTNER U. & WITTMANN G. T. W. 1981. *Metal Pollution on the Aquatic Environment*, Springer-Verlag, Berlin, Heidelberg, New York. p486.
- GALAF F. & GHANNAM S. 2003. Pollution du milieu marin. [In french]. Institut Agronomique Vétérinaire Hassan II, Rabat (Maroc). 115p.
- INSAE (Institut National de la Statistique et de l'Analyse Economique). 2003. [In french]. 3^{ème} Recensement Général de la Population et de l'Habitat (RGPH3), Bénin.
- INSTITUTE OF AQUATIC BIOLOGY. 1990. Summary of trace metals analyses. WACAF/2 Phase1 (1985-89). Accra, Institute of Aquatic.
- LUOMA S. N. 1983. Bioavailability of trace metals to aquatic organisms - a review. *Sci. Total. Environ.* 28: 1-22.
- MENSI G. S., MOUKHA S., CREPPY E. E. & MAAROUFI K. 2008. Metals accumulation in marine bivalves and seawater from the Lagoon of Boughrara in Tunisia (North Africa). *International Journal of Environmental Research*, 2(3): 279-284.
- MIQUEL G. 2001. Les effets des métaux lourds sur l'environnement et la santé. [In french]. Rapport : Office Parlementaire d'évaluation des choix scientifiques et technologiques, Sénat (261) : Assemblée Nationale (2979), Paris (France). 360p.
- MURAI T., D'ALMEIDA A. F. M. & SOHOU Z. 2003. Atlas des poissons et crustacés du Bénin. [In french]. Eaux marines, Direction des pêches.188p.
- OLAIFA F. G., OLAIFA A. K. & ONWUDE T. E. 2004. Lethal and sublethal effects of Cu to the African Cat fish (*Clarias gariepinus*). *Afr. J. Biomed. Res.* 7: 65-70.
- OKOYE B. C. O. 1991a. Nutrients and selected chemical analysis in the Lagos lagoon surface waters. *Int. J. Environ. Stud.* 38: 131-136.
- OKOYE B. C. O. 1991b. Heavy metals and organisms in the Lagos lagoon. *Int. J. Environ. Stud.* 37: 285-292.
- OKOYE B. C. O., AFOLABI O. A. & AJAO E. A. 1991. Heavy metals in the Lagos lagoon sediments. *Int. J. Environ. Stud.* 37: 35-41.
- RAMADE F. 1992. Précis d'écotoxicologie. [In french]. Ed. Masson/Paris, 300 pages.
- RASMUSSEN A. D., ANDERSON O. 2000. Effects on Cd exposure on volume regulation in the lugworm, *Arenicola marina*. *Aquat. Toxicol.* 48: 151-164.
- USHA RANI A. 2000. Cd induced bioaccumulation in tissue of freshwater teleost *Oreochromis mossambicus*. *Ann. N. Y. Acad.* 919(1): 318-320.

- VAIDYA C. O. & RANTALA T. T. R. 1996. A comparative study of analytical methods determination of heavy metal in mussels (*Mytilus edulis*) from eastern Canada. *Int. Environ. Anal. Chemistry*. 63 (3): 179-185.
- VELEZ D. & MONTORO R. 1998. Arsenic speciation in manufactured seafood products: *J. food. Protect.* 61(9): 1240-1245.
- VOSYLIENE M. Z., JANKAITE A. 2006. Effect of heavy metal model mixture on rainbow trout biological parameters. *Ekologija*. 4: 12-17.
- WAQAR A. 2006. Levels of selected heavy metals in Tuna fish. *Arab. J. Sci. Eng.* 31(1A): 89-92.
- YOUSUF M. H. & EL-SHAHAWI A. 1999. Trace metals in *Lethrinus lentjan* fish from Arabian Gulf: Metal accumulation in Kidney and Heart Tissues. *Bull. Environ. Contam. Toxicol.* 62(3): 293-300.