J. Mater. Environ. Sci. 5 (2) (2014) 581-586
ISSN : 2028-2508
CODEN: JMESCN

Assessment of organochlorine pesticides contamination of Oued Souss estuary (South of Morocco): Seasonal variability in sediment and a detritivore annelid *Neries diversicolor*.

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Received 26 Sept 2013, Revised 20 Dec 2013, Accepted 20 Dec 2013.
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Abstract
The Oued Souss river drains an agricultural region with intense use of pesticides. In order to assess the organochlorine pesticides (OCPs) contamination in Oued Souss estuary, surface sediment and *Neries Diversicolor* samples are collected, seasonally, between July 2007 and June 2008. Ten OCPs residues are detected in this ecosystem: Endosulfan A, Endosulfan B, Endosulfan S, Dichlofluanide, Fenarimol , Tetradifon, Lambda-Cyalothrin, vinclozolin, Lindane and Dichlofluanide. Total OCPs in sediment oscillate between 82.6 and 4058.70 ng/g dw (dry weight). In *Neries Diversicolor* , the values are situated between 192.68 and 6949.68 ng/ g dw. Seasonal variations are noted in the surface sediment as well as in the *Neries Diversicolor*. These variations of contaminant concentrations can be explained by the reproductive cycle and also by the seasonal use of pesticides in agriculture. In fact these products are used mainly in spring.

Keywords: Neries Diversicolor - Sediment - Organochlorine - Pesticide - Oued Souss Estuary

Introduction
Oued Souss, Main River of southern Morocco, has an important ecological and socio-economical role [1]. Moreover, its estuary constitutes an important biotope for avifauna, particularly migratory birds and is included in the list of wetlands protected under the Ramsar Convention. It is also a part of the Souss Massa National Reserve and contents a lot of endemic flora and fauna [2]. However, Souss river crosses one of the more modern and productive agriculture areas in the Morocco, whose products are destined to a large market [3]; [4]. Considering that this activity use a lot of pesticides in different cultures, Souss river drains probably a part of these chemicals to the estuary and marine ecosystems. The present study propose to evaluate the level contamination of organochlorine pesticides (OCPs) in Oued Souss estuary ecosystems. These chemicals are typical toxic and bioaccumulative persistent organic pollutants (POPs). The coastal environment is particularly under the risk from the effects of contamination by these pollutants [5]. They are degraded with difficulty and can be transported for long distances [6], and may be associated with organic matter of sediments [7]; [8]. Consequently, the marine sediment is one of the most important reservoirs of these contaminants [9]; [10], what constitutes a risk to organisms of this ecosystems especially those living buried in the sediment. These organisms possess, in diverse degrees, the property to absorb weak tracks of pesticides contained in water and sediment. They are accumulated in diverse organs, especially in liver and fatty tissues of carnivores situated at the top of the trophic webs [11].

Aiming to evaluate the concentration levels of organochlorine pesticides in Oued Souss estuary, we use in the present study, two principal compartments of this ecosystem: sediment and a polychaete, *Nereis diversicolor*, who is detritivorous and feeding from sediment. This specie is perfectly adapted to estuarine ecosystem [12] and plays a very important role in estuarine food webs, it is a principal prey species for crustaceans, fishs and birds [13], [14]. It is characterized by its capacity to tolerate high salinity variations and to resist to stress. Many monitoring programs of chemical pollution are using *N. diversicolor* as bioindicator particularly for the assessment of pesticides pollutants effects in estuarine ecosystems [15].

This work is also undertaken to complete the investigations of our laboratory in Oued Souss estuary which is an ecosystem of highly ecological and socio-economic importance. In fact, our precedent studies in this ecosystem concerned the physico-chemical of water and sediment, [16]; [17]; [18]; [19], Microbiological pollution [20], macroinvertebrates community structure [21], Biology and population dynamics of some species [19]. The present study constitutes a contribution in the pesticides contamination assessment of the ecosystem.

2- Materials and Methods

2.1. Study area and sampling

All samples of sediments and *Neries diversicolor* were collected from the estuary of Oued Souss between July 2007 and June 2008. The location site (30°21,858'N ; 009°35,303'W), situated at two kilometres from the Atlantic Ocean, is shown in the Figure 1. Collected samples were stored in a cooler box with ice and immediately transported to the laboratory, where they were frozen at -20 C° until extraction.

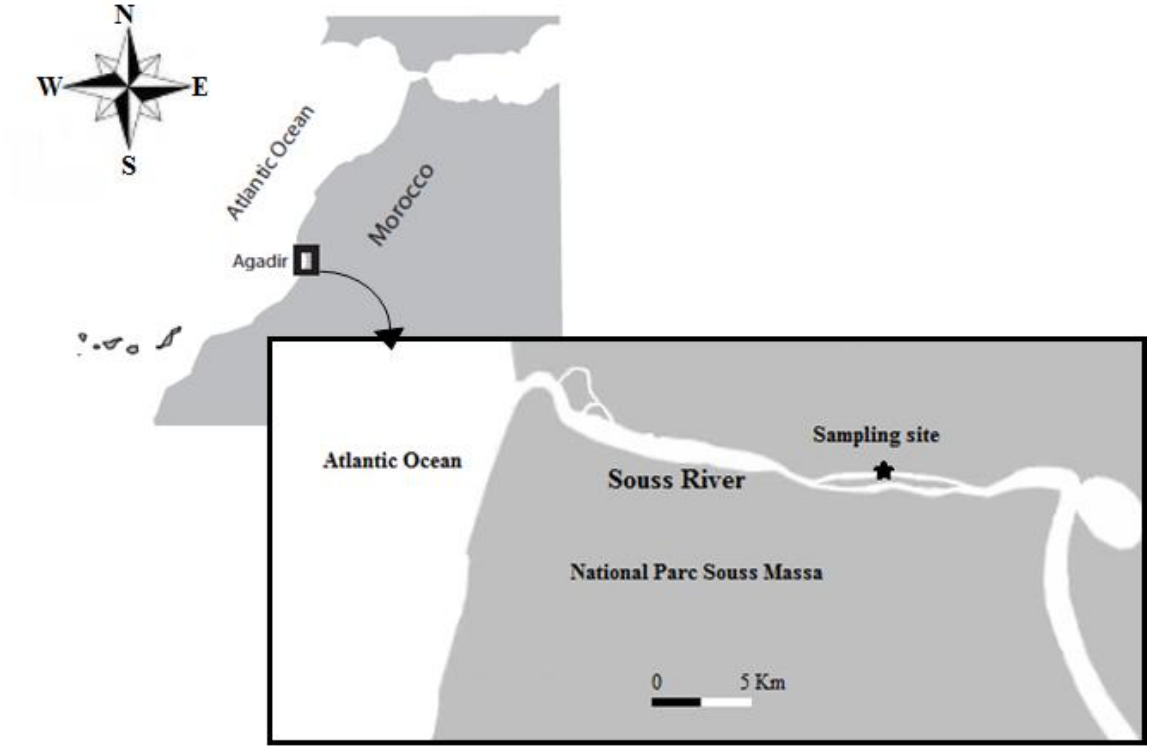

Figure 1: Sampling stations of Oued Souss estuary

2.2. Extraction and cleanup

2.2.1 Organisms sample

All samples of *Neries diversicolor* were freeze dried. Approximately 10 g (dry Weight) were Soxhlet-extracted for 8 hours, using 250 ml of n-hexane. The Soxhlet extracts containing organochlorine residues were concentrated to 5 ml volume on a rotary evaporator at 40 C° and treated with concentrated sulfuric acid (H_2SO_4 at 40%). The extracts were subjected to clean-up procedures in glass-wool plugged columns of height 60 cm and 1 cm in diameter containing 18 g of florisil, topped with a 2 cm layer of anhydrous sodium sulphate (Na_2SO_4). The first fraction was obtained by eluting the sample with 70 ml of hexane; this fraction contained mainly PCBs, HCB, DDE and Aldrin. The second fraction was obtained with 50 ml of a freshly prepared mixture of 70:30 hexanes: Dichloromethane (DCM) and it contained DDD, DDT, and HCH. The third fraction containing Endrin and Dieldrin was eluted with 40 ml of DCM and this fraction was recovered only from the aliquot of the sample extract not treated with concentrated sulphuric acid, since H2SO4 destroys Dieldrin and Endrin. All fractions were further concentrated to about 1 ml and analyzed by gas chromatography equipped with an electron capture detector (GC-ECD). Lipid content was determined according to the method of Marsh and Weinstein [22].

2.2.2 Sediment sample

Sediment samples were homogenized and freeze-dried. Approximatively 20 g of dried and homogenized sediment samples were extracted for 8 h in a Soxhlet apparatus with 250 ml of n-hexane. The extract was concentrated up to a volume of about 2–3 ml and on a rotary evaporator at 40 °C, the mercury were added to the collection flask to remove elemental sulfur. A florisil column was used to clean up and fractionate the extract, and the OCPs were eluted with 70 ml of n-hexane, the second fraction with 50 ml of hexane/ dichloromethane (2:1). All fractions were further concentrated to about 1-2 ml with a rotary vacuum evaporator and at 1ml under a gentle gas stream of purified nitrogen and analyzed by gas chromatography (GC-ECD). 10g of dried sediments were heated at 500°C for about 6 h to determine organic matter.

2.3. Compounds Identification

An HP7890 gas chromatograph equipped with two Ni electrons captures detectors (GC-ECD) was used for detecting the organochlorine levels. 1µL of each samples and a blank extract were injected, separately, into the GC. For every five injections made, the GC programmed conditions were calibrated by injecting 1µL of hexane. Quantification was achieved by comparing the peak areas of sample injections with those of the 17 standards analyzed under the same conditions. ANOVA analysis was performed with the Statistica Software, version 6.0©.

3. Results and discussion

3.1. Concentrations of pesticides residues and organic matter in sediment:

Since the hydrophobic properties of the organochlorine pesticides (OCPs) and the influence of the organic matter on the accumulation in the sediment of these compounds [23], we measure the organic matter content for each sediment sample.

The residual concentrations of the organochlorine pesticides (OCPs) and organic matter in sediments are summarized in Table 1. The total concentration of organochlorine pesticides in sediments has a wide range of fluctuations, from 82.6 ng/g dry weight in summer 2008 to 4658.70 ng/g dry weight in autumn 2007. Among organochlorine pesticides the Tetradifon is the most abundant residue in sediment samples, with concentrations varying between 82.6 - 213,07 ng/g dw followed by Fenarimol (nd - 11.30 ng / g dw), vinchlozolin (nd – 206,41 ng/g dw) and lambda Cyalothrin (nd – 56.4 ng/ g dw). Lindane is detected at the highest level at Autumn 2007 (3834.33ng/g dw). Other OCPs, like dichlofuanide and endosulfans (A, B and S), are not detected. An ANOVA analysis shows statistically significant differences ($P < 0.05$) between summer, winter and spring 2008 data compared to Autumn value. The organic matter content in sediment showed a variation along the sampling period. The highest value is found at winter 2008 (15.18%). While the lowest is found at summer 2007 (8 .58%).

Table 1 : Concentrations of OCPs and organic matter contents in surface sediments of Oued Souss estuary

Pesticides (ng g^{-1}dw)	**Summer 07**	**Autumn 07**	**Winter 08**	**Spring 08**	**summer 08**
Dichlofluanide	ND	ND	ND	ND	ND
Endosulfan A	ND	ND	ND	ND	ND
Endosulfan B	ND	ND	ND	ND	ND
Endosulfan S	ND	ND	ND	70.00	ND
Fenarimol	78.50	11.30	ND	ND	ND
Lindane	ND	3834.33	290.72	ND	ND
Tetradifon	34.70	213.07	10.36	11.80	82.60
Vinclozolin	ND	ND	ND	206.41	ND
Lambda-Cyalothrin	ND	ND	ND	56.40	ND
Total pesticides (ng g^{-1}dw)	113.20	4058.70	301.08	344.61	82.60
Organic matter (%)	8 .58	12.61	15.18	14.67	13.82

Nd : not detected

3. 2. Organochlorine pesticides and content of lipid in Nereis diversicolor

As the lipid content play an important role in the bioaccumulation of organic contaminants such as the organochlorine pesticides [11], we determined the lipid concentration in each *N. diversicolor* sample.

The table 2 illustrates the concentrations of OCPs and content of lipid in *Nereis diversicolor*. Total concentrations of OCPs ranged from 192.68 to 6949.68 ng/ g dw. The highest concentrations are found at spring 2008 (6949.68 ng/g dw). While the lowest concentration is found at summer 2008 (192.68 ng/g). Dichlofluanide is detected at the highest level (nd-3850.27 ng g^{-1} dw), followed by Vinchlozolin (nd – 2934,72 ng g^{-1} dw), Endosulfan S (nd – 1996,75 ng g^{-1} dw), Lindane (52.3 - 1264.02 ng g^{-1} dw), Tetradifon (nd – 977.20 ng g^{-1} dw), and Endosulfan A (11.3 - 829.90 ng g^{-1} dw). Fenarimol and endosulfan B, are respectively detected only in winter and spring (127.62 ng g^{-1} dw and 16.14 ng g^{-1} dw). ANOVA analysis shows that OCPs concentrations are significantly higher ($P<0.05$) in spring 2008. Lipid concentrations indicate seasonal fluctuations, the maximum concentration are observed in spring 2008 (0.318 g g^{-1}dw) although the minimum concentrations are found at autumn 2008 (0.026 g g^{-1}dw).

Our study has shown that the Oued Souss estuary is contaminated by organochlorine pesticides throughout the whole study period. The analysis of *N. diversicolor* samples reveals that OCPs concentrations are higher during spring. While, in estuary sediment, the highest concentrations are observed in autumn compared to other seasons.

Table 2 : Lipid contents and OCP residues in *N. diversicolor* of Oued Sous estuary .

Pesticides (ng g^{-1}dw)	**Summer 07**	**Autumn 07**	**Winter 08**	**Spring 08**	**Summer 08**
Dichlofluanide	ND	267.71	3850.27	ND	37.78
Endosulfan A	829.90	17.3	30.02	740.05	11.3
Endosulfan B	ND	ND	127.62	ND	ND
Endosulfan S	224.08	ND	36.20	1996.75	ND
Fenarimol	ND	ND	ND	16.14	ND
Tetradifon	888.38	26.66	977.20	ND	42.6
Vinclozolin	1073.64	ND	170.39	2932.72	34.3
Lindane	448.25	52.3	60.33	1264.02	66.7
Total pesticides (ng g^{-1}dw)	3464.25	363.97	5252.03	6949.68	192.68
Lipid(g g^{-1}dw)	0.109	0.053	0.111	0.318	0.026

(ND : not detected; DW: dry Weight)

The high concentrations of pesticides in the sediment surface are caused by the input from agricultural activity in Souss Massa region and the discharged of wastewater in this estuary. The accumulation of OCPs in sediments is a complex process influenced by chemical properties of the matrix, the partition coefficients of individual compounds, and the organic content of sediment particles [24]. In fact, the organic carbon concentration may modified the capacity of sediments to absorb the lipophilic organochlorine [7,8]. The high concentrations of lindane recorded in autumn and winter reflect the recent use of lindane. Compared to other regions of the world, the lindane concentrations in Souss river estuary sediment is higher (319.5 ng g^{-1}dw) than those from Wu-shi estuary (3.78 ng g^{-1}dw) [25], Quanzhou Bay (1.63 ng g^{-1}dw) [26], Ulsan Bay (0.64 ng g^{-1}dw) [27] and Qiantang River (44.10 ng g^{-1}dw) [28]. The presence of fenarimol could be attributed to the use of this matter against the oïdium (disease affects the tomato) product to treat tomatoes belated during the period of strong heat July and August (summer) [29]. Lambda-Cyalothrin is used during periods between March and April. The presence of the tetradifon during all sampling season could be related to the most intense use of this product in Souss Massa region.

The higher concentrations were observed at *Nereis diversicolor* in spring 2008. It was the results of the increased agricultural runoff which characterized Souss Massa region and the persistence of these pesticides in the environment and the consequence of their chemical stability. Seen their liposolubility, they have a big tendencies to accumulate in lipid tissue of the aquatic species [30]. The higher concentrations of OCP residues are accompanied by higher lipid contents (Table 2). These lipids play a key role in the bioaccumulation depending on the age, the affinity of the chemical pollutant for tissues of the organisms [11]. Concentrations of lindane were higher detected because of its higher tendencies to accumulate in fatty tissue. For samples of *N.*

diversicolor from Souss river estuary, the means concentration of lindane (365,9 ng g^{-1}dw) is higher than those measured in bivalves of other regions in the world (Quanzhou Bay : 0.19 ng g^{-1}dw [31] ; 29 ng g^{-1}dw [32].

The obtained result seems to be correlated with the cycle of reproduction because the pesticides are detected simultaneously with the spawning period (spring) determined for this specie by Ait Alla *et al*., [19]. Besides the biological or ecological characteristics of the organisms examined, the physicochemical properties of certain compounds, may also contribute to OCPs bioaccumulation in aquatic organisms [11,33,34]. The occurrence of pesticides should be related to the application period and raining conditions and the most intense use of OCPs in the Souss Massa region is in March to July for tomatoes pest control. Other applications are given out on citrus plantations during spring and summer. Some pesticides are determined in the tissues of *Neries diversicolor* and not in the sediment; this may be explained by the aquatic organism capacity to absorb weak traces of pesticides contained in water and to concentrate them in their tissues [35].

Conclusion

This work provided the first systemic data on the contamination status of OCPs in Oued Souss estuary. The analyses of contamination of OCPs in surface sediments and *Neries diversicolor* show high concentrations residues of OCPs in Oued Souss estuary. This is due essentially to the excessive agricultural use of pesticides. The seasonal variation distributions of OCPs in this ecosystem are generally influenced by the hydrological characteristic of Souss River, the biological cycle of the studied organism. The present study reveals the link between the use of pesticides in Souss Massa region and the contamination of coastal ecosystems nearby the agricultural areas. This monitoring study contributes to enhance the scientific information about the organochlorine pollutants concentrations in Atlantic coastline. Our results show the necessity to establish an assessment of the estuarine biological resources, in order to evaluate the bioaccumulation of OCPs in the food web and the associated risks to the Oued Souss estuary ecosystems and human health.

References

1. Saidi M., *Bulletin de l'Association des Géographes Français*. 1 (1994) 94- 106.
2. Qninba A, Dakki1 M, Benhoussa A, El Agbani M., *Ostrich.*78 (2) (2007) 489–493
3. Baroud A., *Terre et Vie*, 55 (2002) 1- 4
4. Bensalk S, Bignebat C, El Hadad-Gauthier F, Perrier-Cornet Ph., INRA-SFER-CIRAD – Agro Campus-Ouest, Rennes, France. (2009) PP 1- 26
5. Wade H F, York A.C, Elizabeth Morey A., Padmore J.M, Rudo K.M., *Journal of Environmental Quality*. 27 (5) (1998) 1018-1026.
6. Guzzella L., Rosscioli C., Vigano L., Saha M, Sarkar S.K, Bhattacharya A., *Environment International.* 31 (4) (2005) 523-534.
7. Miglioranza K.S.B., Moreno J.E.A., Moreno V. J., *Environmental Toxicology and Chemistry*. 22 (2003) 712–717.
8. Bakan G., Ariman S., *Marine Pollution Bulletin.* 48 (2004) 1031–1039.
9. Voorspoels S., Covaci A, Maervoet J, De Meester I, Schepens P., *Marine Pollution Bulletin*. 49 (2004) 393–404.
10. Yang R. Q., Lv A. H., Shi J. B., Jiang G. B., *Chemosphere*. 61 (2005) 347–354.
11. Oliveira Ribeiro C.A., Vollaire Y., Sanchez-Chardi A., Roche H., *Aquatic Toxicology.* 74 (2005) 53–69
12. Fidalgo E., Costa P, Oliveira R.F, Cancela Da Fonseca L., *Pan-American Journal of Aquatic Sciences.* 1 (2) (2006), 114-126.
13. Heip C., Herman R., Estuarine *Coastal and Marine Science*. 8 (1979) 297-305.
14. Arias A. M, Drake P., *Cahier de Biologie Marine*. 36 (1995) 201-210
15. Scaps P., Demuynck S, Descamps M, Dhainaut A., *Archives of Environmental Contamination and Toxicology.* 33 (1997) 203–208.
16. Bergayou H., Mèmoire d'habilitation, université Ibn Zohr, Agadir. (2006) 160P. unpublished.
17. Moukrim A., Kaaya A., Najimi S., Romeo M., Gnassia-Barelli M., Narbonne J. F., *Bulletin of Environmental Contamination and Toxicology*. 65 (2000) 478–485.
18. Mouneyrac C., Pellerin J., Moukrim A., Ait Alla A., Durou C., Viault N., *Ecotoxicol Environ Safe*. 65 (2005) 181-187

19. Ait Alla A., Mouneyrac C, Durou C., Moukrim A., Pellerin J., *Comparative Biochemistry and Physiology, Part C.* 143 (2006) 23–29
20. Mimouni R., Ait Alla A., Anajjar E.M., Finance C., Moukrim A., *Journal Européen d'Hydrobiologie.* 33 (1) (2002) 115-123.
21. Gillet P, Gorman E., Tallec P., Moukrim A., Mouloud M., Ait Alla A., Bergayou H., Kaaya A., *Journal de Recherche Océanographique*. 20 (2003)39 - 44.
22. Marsh J. B. and Weinstein D. B., *Journal of Lipid Research*. 7 (1966) 574-576.
23. Leppänen M., *Annales Zoologici* Fennici. 32 (1995) 247-255.
24. McIntyre J. K., Beauchamp D. A., *Science* of the *Total Environment*. 372 (2007) 571–584.
25. Doong R. A., Peng C K, Sun Y C, Liao P L., *Marine Pollution Bulletin*. 45 (2002) 246 –253.
26. Gong X., Qi S., Wang Y, Julia E B, Chunling Lv., *Marine Pollution Bulletin.* 54 (2007) 1434–1440.
27. Khim J. S., Lee K. T., Kannan K., Villeneuve D. L., Giesy J. P., Koh C. H. *Archives Environmental Contamination and Toxicology*. 40 (2001) 141–150.
28. Zhou R., Zhu L, Yang K, Chen Y., *Journal of Hazardous Materials A.* 13 (2006) 68–75
29. El fadl A., Chtaina., Office National de la Sécurité Sanitaire des Produits Alimentaires, Maroc. (2010)
30. Abarnou A., and Loizeau V., *Océanis.* 20 (1994) 29-45.
31. Yatawara M., Qi S., Owago O. J., Zhang Y., Yang D., Zhang J., Burnet J. E., *Journal of Environmental Sciences.* 22 (2) (2010) 314–320.
32. Otchere F. A., *Science of the Total Environment.* 348 (2005) 102–114
33. Fisk A.T., Hobson K.A., Norstrom R. J., *Environmental Science & Technology*. 35 (2001) 732–738.
34. Pastor D., Sanpera C., Gonzalez-Solis J., Ruiz X., Albaiges J., *Chemosphere.* 55 (2004) 567–576.
35. Zhao Z., Zhang L., Wu J., Fan Ch., *Chemosphere.* 77 (2009) 1191–1198