Study of metallic trace elements and pesticides in the Louisiana crawfish (*Procambarus clarkii* Girard, 1852) in the Gharb and the Low Loukkos regions, Morocco

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Abstract. In both Gharb and Low Loukkos regions, no trace of pesticide was detected. Regarding the metallic trace elements, the highest content of Hg (0.003 mg kg^{-1} of dry weight) has been measured around the irrigation grid of Allal Tazi, and the lowest value (0.001 mg kg^{-1} of dry weight) has been detected in the Low Loukkos marshes and the Nador Canal. In the Low Loukkos marshes, samples showed the highest values of Pb (1.038 mg kg^{-1} of dry weight), Cd (0.066 mg kg^{-1} of dry weight) and Cr (1.162 mg kg^{-1} of dry weight), whereas the lowest values have been measured in the Gharb rice field (Pb: 0.872 mg kg^{-1} of dry weight; Cd: 0.029 mg kg^{-1} of dry weight; Cr: 1.003 mg kg^{-1} of dry weight). On all samples of all sites, the heavy metals have accumulated in the same order: Cr > Pb > Cd > Hg. The results of this study match with those by El Qoraychi in 2015 in the Nador Canal of the Gharb region and in Merja Zerga. No relation has been highlighted between the metallic trace elements quantities in the sediments and in the crawfish flesh. Indeed, the sites exhibiting the highest concentrations of metallic traces in sediments are not necessarily the same as the sites showing higher concentration of metallic traces in the crawfish flesh. The crawfish flesh that was sampled in the Gharb and the Low Loukkos regions shows a satisfying sanitary level; no trace of pesticides was detected and the concentrations of metallic traces elements that were measured are below the maximal limits defined by the European norms.

Key Words: *Procambarus clarkii*, metallic trace elements, pesticides, Gharb, Low Loukkos marshes.

Introduction. The Louisana crawfish *Procambarus clarkii*, originally from the United States of America and the north of Mexico, is today the most produced freshwater crawfish species in the world (FAO 2017). A quantity of 919 887 tons (FAO 2019) of *P. clarkii* was produced in 2016, which is more than 30% of the world inland waters crustacean production, which was estimated at 3 033 000 tons the same year (FAO 2018).

In Louisiana, U.S.A., *P. clarkii* represents a several million dollars industry, with more than 50 000 hectares of culture (Gutierrez-Yurrita & Montes 1999). In Europe, its introduction has been particularly profitable for Spain, since it created a thriving industry which revitalized some business sectors (Ackefors 1999). *P. clarkii* has been a commercial success in all Europe because of its adaptability (Lindqvist et Huner 1999). Indeed, *P. clarkii* can live in a large variety of freshwater habitats, it is very tolerant and can adapt to a wide range of aquatic conditions, such as low salinity, low oxygen level, extreme temperatures and pollution (Gherardi & Panov 2006; Cruz & Rebelo 2007).

Illegally introduced to Morocco, *P. clarkii* populations have developed and colonized almost all water bodies in the Gharb plains and the Low Loukkos marshes, with an estimated stock of 2 603 500 crawfishes (equivalent to 60.39 tons) in the Gharb and 2 121 000 crawfishes (equivalent to 30.33 tons) in the Low Loukkos marshes (Ben

Yahkoub et al 2019). In Morocco, there is no *P. clarkii* production or commercialization, the wild crawfish being rarely and exclusively used for a few fishermen's own consumption. In certain regions where *P. clarkii* is present, a few inhabitants have unsuccessfully tried to commercialize small amounts in the cities around. Currently, the authorities are working on a legislative frame to recognize, regulate and manage the crawfish industry. On that purpose, this study aims to evaluate the sanitary conditions of Louisiana crawfish *P. clarkii,* by analyzing the content of metallic trace elements and pesticides in crawfish flesh, inside the Gharb region (rice fields, irrigation grid, Nador canal) and in the Low Loukkos (Low Loukkos marsh).

Material and Method

Description of the studied sites. This study has been carried out for a period of 7 months, from May 1st to November 30th 2017 in two areas of the North-West of Morocco (Figure 1): the Gharb plain and the Low Loukkos. For the first one, the sampling has been done on three points, as shown below:

- point 1 - Nador canal along Ould Mesbah town, on coordinates 34°48'48.8"N and 6°17'54.7"W (Figure 2);

- point 2 - Gharb paddy fields near Allal Tazi town, on coordinates 34°33'19.7"N and 6°18'58.9"W (Figure 2);

- point 3 - irrigation canal which provides water to paddy fields near Allal Tazi town, on coordinates 34°32'35.9"N and 6°19'25.0"W (Figure 2).

Concerning the second area, the sampling point was located in Low Loukkos marsh near Larache province, between Ain Chouk town and Dhiria, on coordinates 35°08'12.2"N and 6°06'30.5"W (Figure 3). This area is part of a wet areas complex belonging to the largest wet area in Ain Chouk, Baggara and Boucharene.

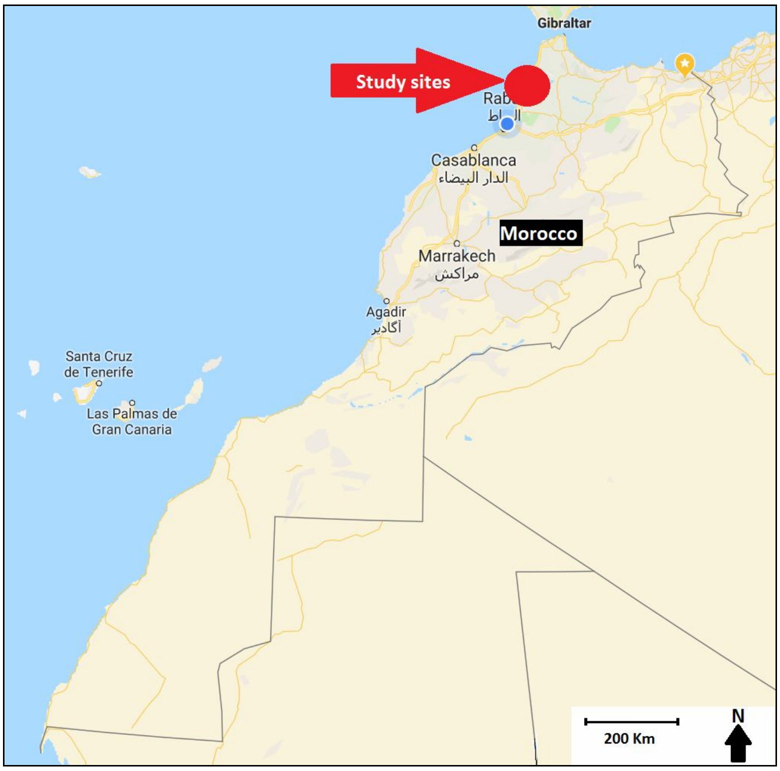

Figure 1. Localization of the study sites in Morocco (Google maps 2019).

Figure 2. Localization of the sampling points in the Gharb plain (Google maps 2019).

Figure 3. Localization of the sampling points in the Low Loukkos marshes (Google maps 2019).

Sampling and analysis methodology. The samples used to control metallic trace elements and pesticides in the crawfish flesh and sediments were taken in compliance with the methods described by the European commission and the international organisms. Any contamination or deterioration of the samples was avoided at all stages.

The general instructions that were made for the treatment and expedition of the samples are the following:

- labelling all samples with additional information on any relevant parameter (for example: possible contamination by other pesticide);
- the samples were put on PET bags for metallic trace elements (MTE) analysis, and in aluminum boxes for pesticide analysis. All these bags and boxes are new and were not re-used;
- the samples were transported in refrigerating suitcases, conserved in fridge and analyzed during the following 24 hours by the Laboratory of Toxicology of the National Hygiene Institute (Rabat, Maroc).

The crayfish was captured using an 80 cm long, 25 cm high and 25 cm wide cage, made of 5 mm mesh. The inlet is a cone of 15 cm long and 6 cm diameter at the smallest side (Figure 4); as bait, local fish or sardines (depending on availability) were used in each trap.

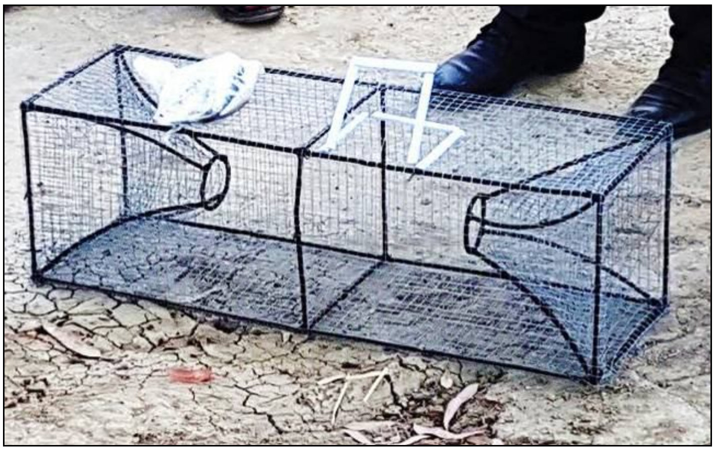

Figure 4. Cage used as a trap for crayfish.

The sediments have been collected on the superficial level of the sampling points. They were stocked in plastic bags, oven-dried in the laboratory during 48 hours at 80°C and screened with a 100 µm mesh.

The analysis of MTE and pesticide residues were carried out in compliance with the following norms:

- NF EN 1528 Research and concentration analysis of PCBs and organophosphorus pesticides in fat aliments (products of animal origin);

- method used for MTE concentration analysis in the pollution control program MED POL (Auger 1989; Chiffoleau & Truquet 1994).

In brief, these methods consist in solvent extraction, liquid-liquid partition, drying, purification and GM-MS analysis for pesticides, hot mineralization by acids and spectrophotometry for MTE.

Results and Discussion. Pesticide analysis on the crawfish showed that on the four sites and on all tested samples, the residues of organophosphorus pesticides and carbamates are inferior to the detection limit (no residue), which is paradoxical because the Gharb and the Low Loukkos regions have an intensive agriculture that often uses pesticides (ONSSA 2015).

Schematically, the presence of pesticide residues in the aquatic organisms is due to the pollution of water, sediments and aliments on the one hand, and on the other hand it depends on the organism’s own capacity to accumulate or eliminate the substance. High concentrations can be found in aquatic organisms because of the bioaccumulation in some species. According to the available data on pesticide contamination in aquatic organisms, organochlorine insecticides are the most frequently detected and quantified pesticides. Contamination of fishing and aquaculture products by pesticides other than organochlorine is not well-documented. A study carried-out in Taiwan (ANSES 2010) analyzed 91 pesticides on 920 samples of fish, cephalopod and crustacean taken out from the markets of three big cities between 2001 and 2003, and showed that 11.4% of fish and 1% of crustacean contain organophosphorus pesticides, mostly at low concentrations. This can explain the absence of pesticides in the samples of crawfish that were analyzed in this study.

The analysis of metallic trace elements shows a variable presence depending on the site and the element (Table 1).

Table 1

Metallic concentration in mg kg^{-1} of the crawfish's dry weight

Origin	*Hg*	*Pb*	*Cd*	*Cr*
Allal Tazi irrigation grid	0.003	0.995	0.040	1.155
Gharb rice fields	0.002	0.872	0.029	1.003
Nador Canal	0.001	1.025	0.044	1.160
Low Loukkos marshes	0.001	1.038	0.066	1.162

The highest content of mercury has been recorded around the irrigation grid of Allal Tazi, and the lowest value has been found in the Low Loukkos marshes and the Nador Canal. In the Low Loukkos marshes, the samples have the highest values of lead, cadmium and chrome, whereas the lowest values have been measured in the Gharb rice fields. On all the samples of all sites, the heavy metals have accumulated in the same order: Cr > Pb > Cd > Hg.

Industrial and agricultural activity is the main source of metallic pollution in the lower part of Oued Sebou's lake (Fekhaoui et al 1996). In this study, the sites of the Gharb plains are located upstream from Kenitra's industrial zone. Regarding the Low Loukkos sites in the region of Larache, there is no industrial activity. Thus, metallic trace elements contamination is more probably due to the use of phytosanitary products, especially since the sites are located in regions with high agricultural activity (ONSSA 2015). Indeed, a recent study (Defarge et al 2018) has revealed the presence of heavy metals – including arsenic, chrome, cobalt, lead and nickel – in 22 pesticide formulations. In Morocco, there are 1 291 formulations based on 377 active substances (ONSSA 2019). This strengthens the hypothesis that the use of phytosanitary products is probably the main source of heavy metals detected in the crawfish flesh.

The Table 2 below compares the concentration of metallic trace elements found in the crawfish flesh in this study and the ones found in literature. The values that were detected in the present study are relatively similar to those published in 2015 by El Quoraychi in the same region, for two elements: chrome and lead. Cadmium content is up to three times higher in the Nador canal.

Table 2

Data comparison between crawfish metallic concentrations (in mg kg^{-1}) found in literature

Country	*Reference*	*Hg*	*Pb*	*Cd*	*Cr*
Spain - DW	Diaz-Mayans et al (1986)	-	-	0.020	-
USA - FW	Finerty et al (1990)	1.37	10.69	3.55	
USA	Madden et al (1991)	2.0	5.0	0.33-0.73	0.46-0.51
USA	Hothem et al (2007)	1.10	0.19	0.03	0.43
Egypt	Abd-Allah & Abdallah (2006)	-	15.93	1.97	5.03
Czech Republic (Boskovice reservoir) - DW	Kuklina et al (2014)	1.18	-	-	4.19
Czech Republic (Nová Říše reservoir) - DW	Kuklina et al (2014)	0.39	-	-	3.79
Morocco (Merja Zerga) - DW	El Qoraychi et al (2015)	-	1.247	0.0128	1.167
Morocco (Nador canal) - DW	El Qoraychi et al (2015)	-	1.461	0.0124	1.127
Morocco (Allal Tazi irrigation grid) - DW	Present study (2017)	0.003	0.995	0.040	1.155
Morocco (Gharb rice field) - DW	Present study (2017)	0.002	0.872	0.029	1.003
Morocco (Nador canal) - DW	Present study (2017)	0.001	1.025	0.044	1.160
Morocco (Low Loukkos marshes) - DW	Present study (2017)	0.001	1.038	0.066	1.162

DW - dry weight; FW - fresh weight.

All metallic trace elements concentrations that were measured in this study are lower than the literature values in the United States of America, Egypt, as well as Czech Republic, except:

- Holthem et al (2007) and Madden et al (1991), USA. The first study has the lowest values of Pd, Cd and Cr, and the second one has the lowest value of Cr;
- Diaz-Mayans et al (1986), Spain, acknowledges the lowest value of Cd.

The Table 3 below compares the concentration of metallic trace elements found in the crawfish flesh in this study, with the upper limits fixed by the European norms CE 466/2001. To carry out the comparison, the dry weight (DW) concentrations have been converted to fresh weight (FW), in accordance with the study by Pattee (1968). This study determined that the fresh weight of the crustacean is 3 to 5 times higher than the dry weight, and then concentration in fresh weight is 3 to 5 times lower than concentration in dry weight (due to water dilution).

Table 3

Comparison of crawfish metallic concentrations in mg kg^{-1} (fresh weight) with the limits defined by the European norms

Studied sites	*Hg*	*Pb*	*Cd*	*Cr*
Allal Tazi irrigation grid	0.0006-0.001	0.199-0.332	0.008-0.013	0.231-0.385
Gharb rice field	0.0004-0.0006	0.174-0.291	0.006-0.010	0.201-0.334
Nador canal	0.0002-0.0003	0.205-0.342	0.009-0.015	0.232-0.387
Low Loukkos marshes	0.0002-0.0003	0.208-0.346	0.013-0.022	0.232-0.387
European norm CE 466/2001 (mg kg^{-1})	0.500	0.500	0.500	-

This comparison allows to affirm that the crawfish from the studied regions has lower concentrations in metallic trace elements than the maximal limits defined by the European norm.

The comparison of metallic trace elements in the sediments and in the flesh of the crawfish (Table 4) demonstrates that, for the Louisiana crawfish, there is no relation between the metallic residues in the sediments and in the flesh. The sites with highest concentrations of metallic traces in sediments are not necessarily the same as the sites with higher concentration in the crawfish flesh. This is probably due to the carapace of the crustacean which prevents the metallic elements to enter into the muscles flesh, even though the crawfish has a digging behavior and often digs into the sediment to hide, both in reproduction and drought seasons. Thus, the metallic trace elements detected in the crawfish flesh have probably accumulated through feeding and breathing. Indeed, several studies (Madigosky et al 1991; Bennet-Chambers & Knott 2002; Mackevičienė 2002; Bruno et al 2006; Kouba et al 2010) carried out on different species of crawfish have proved that metallic concentrations are very different between the hepatopancreas and the flesh, and in some cases, the concentrations were higher in hepatopancreas than in flesh.

Table 4

Comparison of metallic concentrations in mg kg^{-1} (dry weight) between crawfish flesh and sediments

Studied sites	*Hg*		*Pb*		*Cd*	
	Sd	*Ec*	*Sd*	*Ec*	*Sd*	*Ec*
Allal Tazi irrigation grid	0.002	0.003	3.860	0.995	0.660	0.040
Gharb rice field	0.001	0.002	5.450	0.872	1.130	0.029
Nador canal	0.001	0.001	4.441	1.025	0.820	0.044
Low Loukkos marsh	0.001	0.001	6.381	1.038	0.066	0.066

Sd - sediments; Ec - crawfish flesh.

Conclusions. Even if the studied areas have a strong agricultural activity using phytosanitary products, no trace of pesticides was detected in the crawfish flesh during this study. Paradoxically, these areas do not have industrial activities but mercury, lead, cadmium and chrome contents were detected. This can be explained because the crustaceans accumulate less pesticides than other organisms such as fishes. Regarding the metallic trace elements, they probably come from the phytosanitary products used in agriculture. For this reason it would be interesting to study the content of metallic trace elements in pesticides in Morocco. The content of metallic trace elements measured in the crawfish flesh in this study is close to those published in 2015 by El Qorayshi for the Nador Canal and the Merja Zerga.

The comparison of metallic trace elements proved that there is no link between the MTE concentration in the sediments and in the flesh. Indeed, the sites with highest concentrations of metallic traces in sediments are not necessarily the same as the sites with higher concentration of metallic traces in the crawfish flesh.

The crawfish flesh that was sampled in the Gharb and the Low Loukkos regions shows a satisfying sanitary level; no trace of pesticides was detected and the concentrations of metallic traces elements that were measured are below the maximal limits defined by the European norm.

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