SANITARY COMPARATIVE CHARACTERIZATION OF LIPID EXTRACTS FROM MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis* Lmk.) AND HARD-SHELL CLAM (*Rapana venosa*) OF THE BLACK SEA COAST

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ABSTRACT

The paper presents the characterization of the raw material, intermediary substance and obtained products (lipid extracts) from marine mollusks to use them in the pharmaceutical practices.
It is known that for the using in pharmaceutical practices and alimentation these must comply with some sanitary conditions, like heavy metals and pesticides content.
From that reason, the paper presents the analysis of heavy metals and pesticides from the two mollusks.
The obtained data are situated in the normal limits of the national standards, being in correlation with trophic relation of the two species.
The results also showed the valuable composition of the two lipid extracts obtained and the admission of using them in the pharmaceutical purposes.

Keywords: heavy metals, pesticides, sanitary conditions, mollusks

INTRODUCTION

The fact that mollusks accumulate heavy metals that may transiently or permanently be present in their environment has been widely used in marine pollution impact assessments (BELIAEFF *et al.*, 1977; ODZAK *et al*., 1994; STRONKHORST, 1992). Indeed, the importance of mollusks in pollution

impact studies is shown by the magnitude and longevity of the International Mussel Watch (CALOW, 1994), a programme that originated in the late 1960s and continues to maintain momentum today.

Mollusks can play an important role in environments where they are very abundant particularly because of their influence on biogeochemical cycles. For example, the shells are a reserve of calcium carbonate and associated elements (e.g., strontium) which, when the mollusk die, are dissolved in soft water environments in a relatively short time, while in hard water they are preserved for a long time and may give rise to thanatocoenosis. During the mussel's life, there is a continuous flux of elements from the environment (water and food) to the tissues and *vice versa*. There is a huge amount of information on the concentration of a number of elements in marine and freshwater mollusks, but the available information on the element contents in their biomass is unfortunately poor (DAME *et al*., 2002).

Bioaccumulation may have unforeseen effects on the marine environment and, ultimately on human health through consumption in industrial scopes of contaminated mollusks from the sea (AUTUNES, 1997).

Characterization of food lipids is of great interest from a commodity point of view as it involves nutritional, sensory and technological aspects. The knowledge of their compositional characteristics affecting these aspects is important both to modify the composition properly by agronomic, genetic or enzymatic techniques and to identify other species whose components are in better proportions according to a determined aim (DAMIANI, 2003).

The present paper gives an analysis of heavy metals (Cd, Cr, Pb, Cu, Mn and Fe), and pesticides (BHC, lindane, heptachlor, aldrin, dieldrin, endrin, pp'-DDE, pp'-DDD and pp'-DDT) from Mediterranean mussel (*Mytilus galloprovincialis* Lmk.) and hard-shell clam (*Rapana venosa*) mollusks, in scope to obtain the certain qualification of natural products from pharmaceutical point of view.

MATERIAL AND METHODS

Collection and maintenance of mussels

Specimens of *Mytilus galloprovincialis* Lmk. (4,5-5 cm) and *Rapana venosa* (7,5-8 cm) were collected in 2007 summer from Mamaia Bay (Cazino-Mamaia, $44^0$10'N, $28^0$41'E, Romanian sector of the Black Sea). The mussels were kept in well-oxygenated, flow-through, plastic seawater aquaria (75,5 cm×40,5 cm×21 cm) and a glass aquarium (75 cm×45 cm×13 cm). Using a sharp blade, the surfaces of shells were scraped clean of all epiphytes before

being placed in the aquaria. The mollusks were kept for 5 days prior to the commencement of experiments in the aquaria and were supplied with a suspension (7000-27.000 cells/ml) of unicellular alga, *Chlorella* (12 μm). Ambient room and seawater temperatures were recorded daily and found to remain at 23 ± 1^0C, even without temperature control mechanisms. An algae concentration of 4800 cells/ml (500 ml concentrated algae suspension into 100 l seawater) was maintained in both tanks for the duration of the experiment.

Specimen analysis

The two species were separated in three parts:

- the first was kept without any treatment for the analysis (raw material);
- the second was prepared at the dry substance: 2-3 g of sample was dried at 105 ± 2^0C into oven, for 3- 4 hours. If the results obtained from two consecutive dries are not been different from 0,1%, the process is considered over (XFR, 1993);
- from the 3rd part lipids were extracted, according to the Christie method (CHRISTIE, 1982). The tissue was dried at 50^0C, homogenized and the samples subsequently extracted in chloroform : methanol 1:1 (v/v). The mixture was then concentrated on a rotary evaporator under vacuum at room temperature and the brute lipid extract was conserved at -20^0C.

Metal analysis

An amount of 0.1-0.3 g wet biological sample was taken with bi-distillate water into digestion flask. Then it was added 5 mL concentrated HNO_3, the digestion vessels were kept closed and remained at room temperature at least 1 hour. The digestion vessels were then placed on hot plate at 120 ± 2^0C for 5-8 hours. When the mineralization was completed, the sample was cooled at room temperature and after that it was transferred into 100 mL volumetric flasks and brings to the sign with bi-distilled water.

The determination of Cd, Cr, Pb, Cu, Mn and Fe was carried out using the ATI-UNICAM SOLAAR 939Z Atomic Absorption Spectrophotometer. It was used the specifically procedure of heavy metals determination from the Physico-chemical Analysis and Measurements Laboratory from N.I.M.R.D. "Grigore Antipa", Constanza.

A 20 μl quantity of sample was introduced into graphite tube of spectrophotometer and atomizated at very high temperature. A light fascicle was passed through graphite tube, monochromator and detector, which measured the quantity of absorbed light by the atomizated element in tube.

Each metal has characteristic wavelength and for each element it was used as source a specific hollow cathode lamp. The absorbed energy quantity is proportional with the element concentration in the sample.

The heavy metals content from the Mediterranean mussel and hard-shell clam mollusks was compared with the maximum admissible limits established by Ord. M.S. No. 97/2005, The hygienic-sanitary norms for food, as follows: Cd 0.1 mg/kg w.w. and Pb 1.5 mg/kg w.w.

Pesticides analysis

The pesticides extraction from tissue sample (wet/dry) was realised with hexane, in soxhlette devices and from lipid extracts with hexane/ dichloromethane (3:1, v/v) mixture into a separation funnel.

The next manufacture of the sample has followed other stages:

- concentration of the sample at rotary evaporator device,
- treatment of the sample with sulphuric acid for the removing of the lipids excess,
- separation and the concentration of the sample using Kuderna-Denish concentrator and with nitrogen stream.

The data gives by the general rapports from gas-chromatograph computer soft was manufactured for the g of tissue- concentrations or ml-lipid extract.

The determination of pesticides (BHC, lindane, heptachlor, aldrin, dieldrin, endrin, pp'-DDE, pp'-DDD and pp'-DDT) was carried out using the ECD-Perkin ELMER Gas Chromatograph. It was used the specifically procedure of pesticides determination from the Physico-Chemical Analysis and Measurements Laboratory from N.I.M.R.D. "Grigore Antipa", Constanza.

The pesticides content from the Mediterranean mussel and hard-shell clam mollusks was compared with the limits established by Ord. No. 23/2007, The sanitary-veterinary norm for the safety food and the stabilisation of the minim- maxim limits of pesticides from animal origin food. The limits are as follow:

- BHC, heptachlor, aldrin and dieldrin – 0.2 mg/kg;
- lindane – 0.02 mg/kg;
- endrin – 0.05 mg/kg;
- pp' DDE, pp' DDD and pp' DDT – 1 mg/kg.

RESULTS AND DISCUSSION

Heavy metals results are presented in Figures 1 and 2.

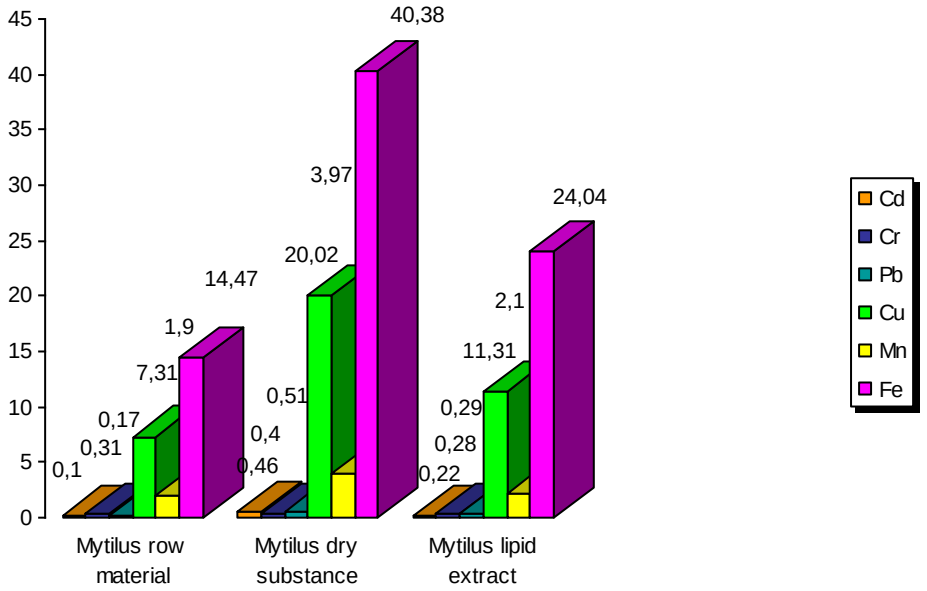

Fig. 1 - Heavy metals concentration in *Mytilus galloprovincialis* Lmk. (raw material, dry substance and lipid extract)

The means and order of magnitude for *Mytilus galloprovincialis* Lmk. are:

- raw material: Fe (14.47 mg/Kg), Cu (7.31 mg/Kg), Mn (1.90 mg/Kg), Cr (0.31 mg/Kg), Pb (0.17 mg/Kg) and Cd (0.10 mg/Kg);
- dry substance: Fe (40.38 mg/Kg), Cu (20.02 mg/Kg), Mn (3.97 mg/Kg), Pb (0.51 mg/Kg), Cd (0.46 mg/Kg) and Cr (0.40 mg/Kg);
- lipid extract: Fe (24.04 mg/Kg), Cu (11.31 mg/kg), Mn (2.10 mg/Kg), Pb (0.29 mg/Kg), Cr (0.28 mg/Kg) and Cd (0.22 mg/Kg).

And for *Rapana venosa* :

- raw material: Cu (10.25 mg/Kg), Fe (7.25 mg/Kg), Mn (1.23 mg/Kg), Cr (0.19 mg/Kg), Pb (0.09 mg/Kg) and Cd (0.065 mg/Kg);
- dry substance: Cu (37.14 mg/Kg), Fe (29.31 mg/Kg), Mn (4.79 mg/Kg), Cr (1.70 mg/Kg), Cd (0.51 mg/Kg) and Pb (0.24 mg/Kg);
- lipid extract: Cu (19.27 mg/Kg), Fe (13.24 mg/Kg), Mn (2.02 mg/Kg), Cr (0.78 mg/Kg), Cd (0.16 mg/Kg) and Pb (0.10 mg/Kg).

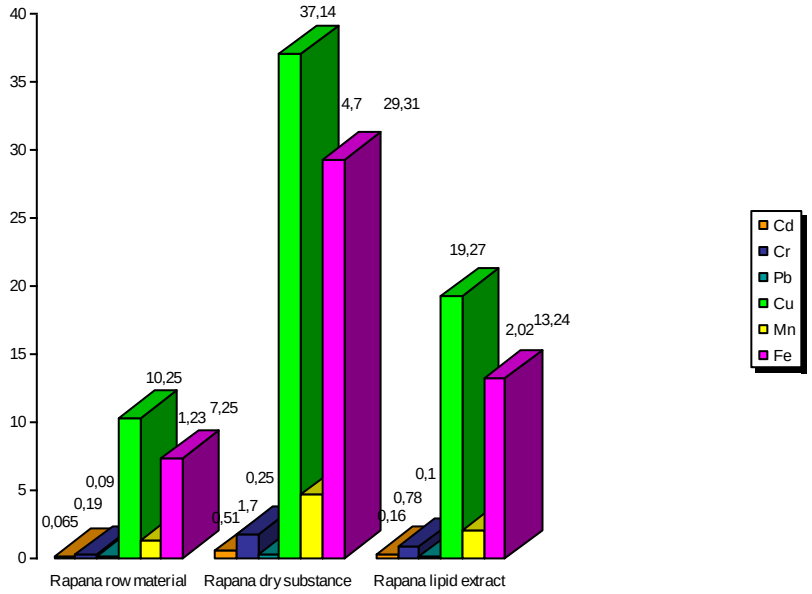

Fig. 2 - Heavy metals concentration in *Rapana venosa*
(raw material, dry substance and lipid extract)

The Cd and Pb concentrations were below maximum admissible limits established by Ord. M.S. No. 97/2005. There are no limits values for Cu, Cr, Mn and Fe established in the national legislation.

Cr toxicity is a function of oxidation state. For example, hexavalent Cr has a high solubility in water and as a consequence, tends to be mobile. While trivalent Cr is relatively innocuous and immobile, hexavalent Cr is actively transported into cells by the sulphate transport system where is capable of causing damage to DNA as well as indirectly generating oxygen radicals (HANSEL, 2003). Mn compounds exist naturally in the environment and Mn is an essential component of over thirty-six enzymes that are used for the carbohydrate, protein and fat metabolism (MOHAN & WILKER, 2004). Fe in the environment is mainly particulate bound with relatively low mobility and bioavailability (MOHAN & WILKER, 2004).

Taking into account the concentrations ranges that were registered in the present study, we may conclude that the analysed samples from the two species are suitable for using in pharmacology scope, from the heavy metals point of view.

Pesticides content results are presented in Figures 3 and 4.

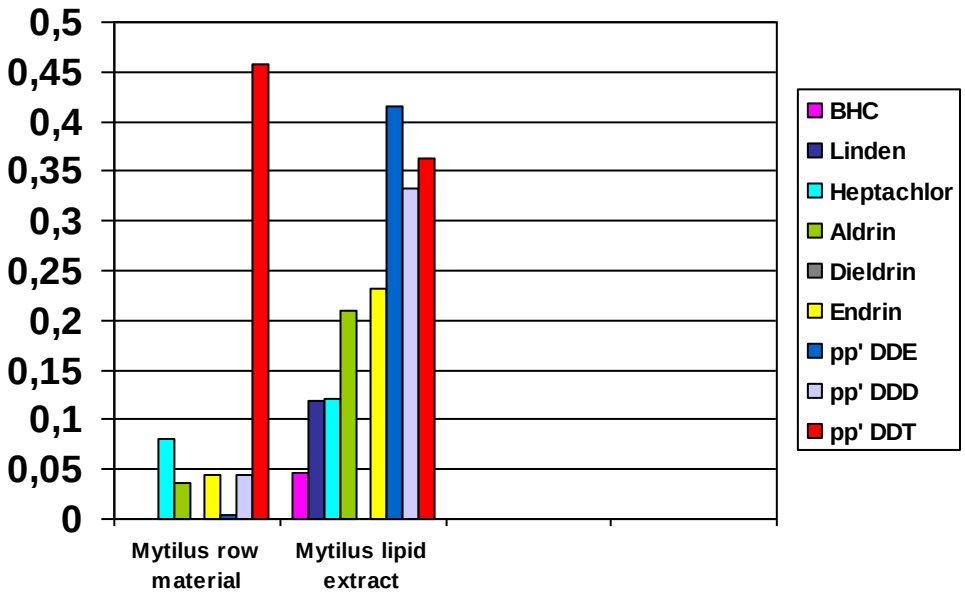

Fig. 3 - Pesticides concentration in *Mytilus galloprovincialis* Lmk.
(raw material, dry substance and lipid extract)

Pesticides, especially the organo-chlorinated ones (BHC, aldrin, endrin, etc.) are known to be persistent, but in dry substance all values were below detection limits in both species.

These chemicals in the two mollusks are in the limits of Romanian STAS for food toxicity with some exception in lipid extracts of *Mytilus galloprovincialis* Lmk. (lindane – 0.1188 mg/kg and endrin – 0.2323 mg/kg) and *Rapana venosa* (lindane – 0.0644 mg/kg) probably caused by the concentration effects which lead to accumulation of this in lipid extracts.

Despite these exceptions, we may resume that the two species are still not representing a danger, for these using on pharmaceutical market.

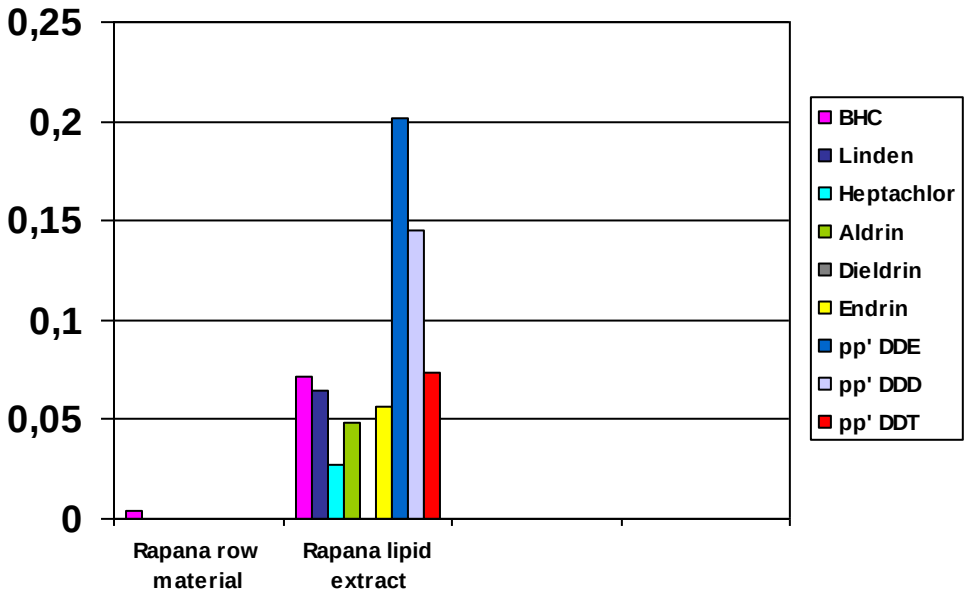

Fig. 4 - Pesticides concentration in *Rapana venosa*
(raw material, dry substance and lipid extract)

CONCLUSIONS

The controls about quality and genuineness of natural food have continuously a much considerable significance that concern more and more to food chemistry. This occurrence is the result of the increased demand of the consumers to dispose of pharmaceutical products with particular attention to their bio-nutritional and hygienic-sanitary appearances, their sensory proprieties and also the respect to relative laws.

The obtained results demonstrated that the content in heavy metals and pesticides of the two mollusks are in generally complying with the Romanian legislation, allowing the use of lipid extracts in pharmaceutical domain.

The toxicology investigations on which the described research is based constitute moreover the point of departure of other studies, regarding the synthesis of new lipid structures, for their possible implications in pharmacological order and the interest like “functional foods”.

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