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Trace element concentrations (Zn, Cu, Pb and Cd) in the Mediterranean mussel *Mytilus galloprovincialis* from Oran Harbour (Oran Bay, Algerian west coast)

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ABSTRACT

A study was conducted to determine the contamination of Mediterranean mussels (*Mytilus galloprovincialis*) by some essential and non-essential heavy metals collected from the large Oran Harbour (Oran Bay, Algerian west coast) in November (fall) 2009 and 2010. A total of fifty individuals of mussels, *Mytillus galloprovincialis* were sampled and metals such as Zinc (Zn), Copper (Cu), Lead (Pb) and Cadmium (Cd) concentration were determined using a flame atomic absorption spectrophotometer equipped with a graphite furnace.
The observed mean concentration of Zn, Cu, Pb and Cd in the soft tissues of mussels was respectively, 95.24±1.85, 5.27±0.18, 16.74±1.74 and 0.66±0.04 µg/g dry weight in samples of 2009 and 83.35±2.19, 1.15±0.12, 11.65±0.55 and 0.68±0.04 µg/g dry weight in those of 2010. These concentrations were relatively low compared to those found in mussels of other parts of the world and below certified safety guidelines except the Pb concentrations which exceeded the permissible limits and may lead to adverse health effects of consumers.

KEYWORDS: Contamination, heavy metals, mussels, Oran Harbour, Algeria, public health.

INTRODUCTION

Marine mussel from Mytilidae family is a sessile bivalve and has both ecological and economic importance. Mussels have a wide distribution which extends over the Atlantic coast from the Bay of Agadir to the British Isles and also includes the entire Mediterranean basin, South Africa, New Zealand and California (Mc Doneld et Koehn, 1991). They are commonly found in the littoral and sublittoral zones and in polyhaline portions of the coastal waters (Sahin *et al.*, 2011).

The marine mussels are also very important seafood source around the world and provide cheap source of protein for human consumption (Culha, 2008; Sahin *et al.*, 2011). In 2008, annual world mussel production was approximately 1.7 mt and about 95% of them came from aquaculture (Sahin *et al.*, 2011). The quality requisites of bivalve molluscs are primarily dependent on the quality of the aquatic environment, assuring a healthy product and a safe consumption (Karakoltsidis *et al.*, 1995; Orban *et al.*, 2002). They are frequently consumed by man, and play an important role in the transfer of chemical contaminants along food chains. One of the main environmental problems with toxic metals is their capacity to accumulate in the tissues of living organisms, particularly bivalve molluscs collected for human consumption. The absorption of large amounts of metal by edible species could ultimately be harmful to man at the upper end of the food chain (Chafik *et al.*, 2001).

Mytilus galloprovincialis, this species present along Algerian coast, is appreciated by consumers for its organoleptic properties and for the competitive price if compared with other bivalves. However, the natural mussel beds are scarce and mussel farming is not developing along the Algerian coastline, which incite local people and fisherman to exploit wild mussel beds, located in polluted sites such as harbour areas which receive a large amount of urban and industrial untreated wastewater. These mussels are collected and commercialized without any sanitary control which may lead a hazard on health of the Consumer.

This study aims to evaluate the concentrations of heavy metals i.e. Zn, Cu, Pb, and Cd in edible soft tissues of Mediterranean mussels, *Mytilus galloprovincialis*, from Oran Harbour (Oran Bay, Algerian west coast) which is commercialized and consumed by oranian people. Further, their hazardous levels were compared with available certified safety guidelines proposed by World Health Organization (WHO) and U.S Food and Drug Administration (FDA) for human consumption.

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MATERIALS AND METHODS

Sample collection and preparation

Mussels samples were collected from the large Oran Harbour (35°42'31.07''N, 0° 38'26.76''W), located in Oran Bay (Algerian west coast) (Fig. 1), into which a large amount of urban and industrial untreated wastewater were discharged by the Oran metropolis and many industrial units.

A total of Fifty (50) Mussels *(M. galloprovincialis)* were collected in November (fall) of 2009 and 2010 according to techniques recommended by the program QUASIMEME (1992). The specimens were washed at each site with sea water to eliminate encrusted organisms and were immediately transported under at +4°c to the laboratory. Upon arrival, mussels were inspected and dead animals discarded. The whole soft body tissues of mussels were grouped in ten pools (five individuals each of similar size) as replicates and dried at 70°c for 48h for chemical analyses of trace metals.

Fig.1: Map showing sampling location: Oran Harbour (Oran Bay, Algerian west coast).

Tissue digestion

The concentration of zinc (Zn), copper (Cu), lead (Pb) and cadmium (Cd) were measured in whole soft tissues of mussels after hot mineralization samples, following the method of Amiard *et al.*, (1987). Approximately 0.2 g of dry weight of was digested in 4 ml of concentrated nitric acid (Merck Suprapure) at 95 ° C for 1 hour. The metal contents in acid solutions were determined by using a flame atomic absorption spectrophotometer equipped with a graphite furnace (Perkin Elmer AAnalyst-100 - Version 1.10). Quality assurance and quality control was assessed by processing blank samples and reference standard material (Mussel Tissue Standard Reference Material (SRM 2976), National Institute of Standards and Technology). Concentrations obtained for standard reference materials were always within the 95% confidence interval of certified values (Table 1).

Table 1: Observed and certified values of heavy metal concentrations (µg/g dry wt.) in the standard reference material (SRM 2976).

	Zn	Cu	Pb	Cd
Measured value	128±8.00	3.96±0.08	1.15±0.04	0.78±0.03
Certified value	137±13.00	4.02±0.33	1.19±0.18	0.82±0.16

Statistical Analysis

The data are presented as the mean ± standard error of the mean. One-way analyses of variance (ANOVA) were used to compare means of heavy metal concentrations between years. When ANOVA was significant ($p<0.05$), post-hoc comparison of means was done using Duncan's test. Statistical analysis was performed using the software STATISTICA (Statsoft STATISTICA version 6.1.478.0).

RESULTS

The annual variations of heavy metal concentrations (Zn, Cu, Pb, Cd) in *M. galloprovincialis*, collected in Oran Harbour in November 2009 and 2010, were shown in Table 2.

The concentrations of Zn, Cu, and Pb recorded in 2009 were significantly higher compared to those of 2010 ($p<0.05$). However, no significant difference was recorded for Cd which the concentrations remain stable during the two years. The concentrations of the investigated metals in the mussel soft tissue decreased in the following order: Zn>Pb>Cu>Cd.

The concentration of Zinc (Zn) in the mussel ranged between 74.51-102.70 µg/g with the mean concentration of 95.24±1.85 µg/g in 2009 and 83.35±2.19 µg/g in 2010.

Copper (Cu) concentration in the edible soft tissues of *M. galloprovincialis* ranged between 0.72-5.80 µg/g. The mean concentrations of this essential metal reached 5.27±0.18 µg/g in 2009 and 1.15±0.12 µg/g in 2010.

The mean concentrations of Lead (Pb) were relatively high, reaching 16.74±1.74 µg/g in mussels collected in 2009 and 11.65±0.55 µg/g in those of 2010.

Cadmium (Cd) was accumulated in lower concentration in the soft edible tissue of *M. galloprovincialis* with the mean concentrations which did not exceed 0.68 µg/g.

Table 2: Variation of heavy metal concentrations (µg/g dry wt.) in soft tissues of mussels, *Mytilus galloprovincialis*, from Oran Harbour. Results are expressed as mean ± SE, limit values are in brackets.

	Zn	Cu	Pb	Cd
November 2009	95.24±1.85[a] (87.90-102.70)	5.27±0.18[a] (4.45-5.80)	16.74±1.74[a] (10.02-24.20)	0.66±0.04[a] (0.54-0.81)
November 2010	83.35±2.19[b] (74.51-88.04)	3.91±0.50[b] (0.72-1.63)	4.60±0.42[b] (9.71-13.59)	0.68±0.04[a] (0.50-0.88)

[a, b] Different letters indicate significant differences (Duncan's test, $p<0.05$) between years.

The heavy metal concentrations in *M. galloprovincialis*, collected in Oran Harbour were compared with other studies and guideline values (Table 3). The levels of Zn, Cu and Cd recorded in this Mollusc collected from Oran Harbour were relatively lower than those of mussels from the Canadian, Portuguese, Moroccan coasts and the Baltic Sea. However, it should be noted that the mean levels of Pb recorded in our samples were significantly higher than those found at Bay of Izmir in Turkey (1.36 µg/g), Casablanca coast in Morocco (7.10 µg/g) and Malaysian coasts of Pekan, Pahang (0.47 µg/g). These hazardous levels exceeded the Maximum Permissible Limits (MPLs) recommended by World Health Organization (WHO) and U.S Food and Drug Administration (FDA) for human consumption.

Table 3: Comparison of heavy metals concentrations (µg/g dry wt.) in soft tissues of mussels, *Mytilus galloprovincialis*, from Oran Harbour with other sampling locations from different localities in the world.

Sampling area	Zn	Cu	Pb	Cd	Authors
Izmir Bay (Turkey)	279	6.92	1.36	1.1	Tuncer and Yaramaz (1982)
Canadian coast	197	9.65	—	3.94	Lobel *et al.* (1991)
Portuguese coast	542	13.4	—	1.25	Coimbra *et al.* (1991)
Baltic Sea	269	13.4	5.22	—	Pempkowiak *et al.* (1999)
Casablanca (Moroccan coast)	—	17.33	7.1	0.78	Bouthir *et al.* (2004)
Pekan, Pahang (Malaysian coast)	45.54	19.05	0.47	0.3	Kamaruzzaman *et al.* (2011)
MPL/WHO	100	10	5	2	WHO (1982)
MPL/FDA	150	100	1.5	0.2	FDA (2001)
Oran Harbour (Oran bay, Algeria)	89.29	3.63	10.67	0.67	Present study

DISCUSSION

The Algerian west coast is relatively polluted. All the indicator marine species, whether benthic or pelagic, present high concentrations of pollutants which vary among individuals and sampling sites which presents a hazard for consumers of fish and seafood (Boutiba *et al.*, 2003). The present study revealed that the mussel *Mytilus galloprovincialis* from Oran Harbour accumulate Zn, Cu, Pb and Cd in its soft tissues. The results obtained confirm also that the site is polluted by these metals. This enrichment in metallic elements could come from the inputs of urban and industrial sewage of Oran city, which discharge at the two main outfalls of Harbour. The high load of organic particles in these effluents could be a privileged vector for metals and could accumulate indirectly in the tissues of aquatic organisms (Coimbra *et al.* 1991; Wang *et al.*, 1996; Ozdemir *et al.*, 1998). In addition, it is well

known that mussels are able to filter very important volumes of water (between 0.2 to 5 liters/hour) to satisfy their nutritional and respiratory requirements (Monfort, 2006). Thus, these bivalves can concentrate highly in their digestive tracts and tissues, chemical contaminants present in the water or food (phytoplankton), including heavy metals without lethal effects (Prueli *et al.*, 1987; Livingstone, 1991).

It should be noted that bioaccumulation of heavy metals by the mussel *Mytilus galloprovincialis* from the port of Oran presented a very clear inter-annual variations. Thus we observed that heavy metal concentrations recorded in 2009 were significantly higher compared to 2010. This decrease in the concentrations may be due to the closure of one of two sewage outfalls discharging at the port. The annual changes in concentrations of different metals in mussel tissues could result from a interaction of biological factors such as the cycle of reproduction, metabolism, development and age (Amiard-Triquet and Caurant 1994; Kamaruzzaman, 2011) as well as environmental (exogenous) factors such as temperature, salinity, food availability, modification of biogeochemical cycle and bioavailability of metals (Casas and Bacher, 2006; Sara and Pesceddu, 2008).

Globally, the Zn was the metal with the highest concentration in mussel tissues as compared with the other metals. Similar results have been already observed by other authors, in the bivalve mollusks (Boutier, 1982 ; Abada 1996; Chafik *et al.*, 2001). The elevated levels of zinc can be found in port and marina waters, because of the leaching from the antifouling paints and the anodes used to reduce corrosion of boat hulls (AERMC, 1997).

It was demonstrated that the mussels could be contaminated by Zn through the dissolved and suspended forms present in the water and through the food chain (Wang *et al.*, 1996).

Zinc is an essential trace element for both animals and humans. The recommended daily intake is 10 mg/day for children and 15 mg/day for adults (Sivaperumal *et al.*, 2007). Zinc appears to have a protective effect against the toxicities of both cadmium and lead (Malik *et al.*, 2010).

The concentrations of copper in the analyzed samples of *M. galloprovincialis* were low compared to Zn. Cu is an essential part of several enzymes and is necessary for the synthesis of hemoglobin (Sivaperumal *et al.*, 2007). However, a high intake of Cu has been recognized to cause adverse health problems (Gorell *et al.*, 1997). Some authors reported that chronic exposure to Zn and Cu is associated with Parkinson's disease (Gorell *et al.*, 1997) and that these elements might act alone or together over time to induce the disease, (Prasad, 1983).

Cadmium (Cd) was accumulated in lower concentration in the soft tissue of *Mytillus galloprovoncialis* from Oran Harbour. Cd is a toxic metal accumulated naturally by marine organisms but as yet has no known metabolic role. It poses risks for consumers of seafood. Even at low concentrations, it tends to accumulate in the kidneys. It most frequently results in kidney damage (necrotic protein precipitation) and metabolic anomalies caused by enzyme inhibitions (Gazza, 1990). JECFA (Joint Expert Committee for Food Additives) Joint FAO / WHO recommended in humans a Tolerable Weekly Intake (TWI) of 7 micrograms of cadmium per kilogram of body weight and per week (JECFA, 1993).

It was noteworthy that the levels of Pb were very high in 2009 and 2010. The accumulation of this toxic metal could be related to an increase of urban population, leading to the more significant urban wastewater discharges as well as the higher levels of atmospheric Pb, due to a more intense traffic (Igwegbe, 1992). In the latter case, the contamination is both direct by atmospheric deposition and indirect by the leaching of roads by rainwaters (Conor 1980, Goody *et al.* 1995). Lead has been recognized to be potentially toxic within specific limiting values; a considerable potential hazard exists for human nutrition. Basically, as a result of their comparatively high affinity for proteins, the lead ions consumed bond with the haemoglobin and the plasma protein of the blood. This leads to inhibition of the synthesis of red blood cells and thus of the vital transport of oxygen (Gazza, 1990).Tolerable Weekly Intake (PTWI) of Pb is 25 micrograms per kilogram of body weight and per week (JECFA, 1993).

When compared with previous study and the Maximum Permissible Limit (MPLs), concentrations of Zn, Cu and Cd found in soft of *Mytilus galloprovincialis* collected from Oran Harbour were relatively low compared to those found in other parts of the world and below the FDA, WHO and EC limits (WHO, 1982; FDA, 2001; EC, 2001). These concentrations showed that the mussels were harmless and did not appear to present any danger for consumers. However, the Pb concentrations exceeded the recommended level by WHO (1982), FDA (2001) and the EC Regulation 221/2002 (EC, 2001). The high concentrations of this toxic metal found in edible soft tissues of mussels may lead to adverse health effects of consumers of this bivalve. Indeed, lead, like Cadmium, is as a cumulative poison, i.e. the danger lies primarily in the regular consumption of foodstuffs with low contamination (Gazza, 1990).

Conclusion

The present study revealed that the mussels *Mytilus galloprovincialis* collected from the large Oran Harbour (Oran Bay, Algerian west coast) accumulate the heavy metal in their soft tissues. The results obtained confirm also that the site is polluted by these metals and mussels were exposed to this pollution. Zinc, copper, and cadmium concentrations were generally found low; even though Lead concentrations were usually found high.

These results showed that the mussels from Oran Harbour were harmless in terms of zinc, copper, and cadmium for public health and environment, but it may be harmful to people consumed these organisms and environment in terms of Lead levels. Therefore, it is important to control periodically the concentrations of heavy metals in seafood consumed by people for public health.

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