Mediterranean Marine Science

Vol 14, No 1 (2013)

Heavy metals monitoring in mussels Mytilus galloprovincialis from the Apulian coasts (Southern Italy)

L. SPADA, C. ANNICCHIARICO, N. CARDELLICCHIO, S. GIANDOMENICO, A. DI LEO

doi: 10.12681/mms.323

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
http://dx.doi.org/10.12681/mms.323

Heavy metals monitoring in the mussel *Mytilus galloprovincialis* from the Apulian coast (Southern Italy)

L. SPADA, C. ANNICCHIARICO, N. CARDELLICCHIO, S. GIANDOMENICO and A. DI LEO

C.N.R. - Institute for Coastal Marine Environment, Operative Unit of Taranto, via Roma 3, 74123 Taranto, Italy

Corresponding author: lucia.spada@iamc.cnr.it

Handling Editor: Silvano Focardi

Received: 19 September 2012; Accepted: 19 November 2012; Published on line: 26 February 2013

Abstract

Concentrations of six heavy metals (Cd, Cr, Cu, Hg, Pb and Zn) and one semi-metal (As) were determined in tissues of the mussel *Mytilus galloprovincialis* collected along the Apulian coast (Mediterranean Sea). This project, executed in 2009, focused on the assessment of the levels and spatial distribution of metals in the environment, in order to evaluate coastal water quality, using mussels as bioindicators, and the health risk for mussel consumers. The concentrations (mg/kg d.w.) of these metals ranged from 6.35 to 76.17 for arsenic (As), 0.38 to 2.54 for cadmium (Cd), 0.96 to 9.46 for chromium (Cr), 5.26 to 19.22 for copper (Cu), 0.10 to 0.81 for mercury (Hg), 25.00 to 110.51 for zinc (Zn) and 0.37 to 3.25 for lead (Pb). These levels were lower than the permissible limits set by the European Commission and FAO, with the exception of Cr at three sampling stations. Evaluation of the public health risk associated with mollusc consumption indicates that there is no evident risk for a moderate *Mytilus g*. consumer; however, heavy metal concentrations must be monitored periodically and carefully with respect to consumer health.

Keywords: Biomonitoring, heavy metals, *Mytilus galloprovincialis*, health risk.

Introduction

National and regional biomonitoring represents a useful tool for quantifying and clarifying the current status of environmental health, and indirectly assessing coastal water quality and changes in the environment, generally due to anthropogenic causes. In addition, biomonitoring plays a key role in the formulation of control and protective measures for specific and vulnerable marine ecosystems, such as coastal and estuarine areas, transitional waters, influenced by various anthropic activities.

Monitoring programmes use a great number of bioindicators - "sentinels" - to detect temporal and spatial variation of chemical pollutants and to contribute to the knowledge of trends trend in marine contamination (Carro *et al.,* 2004). Different organisms, both animal and vegetal species, have been proposed as biomonitors and bivalve molluscs, mussels in partcular, are largely utilized as bioindicators because of their wide geographical distribution, easy sampling, sedentary nature, resistance to stress and sessile lifestyle (Cardellicchio *et al.,* 2008; O'Connor, 2002; Ozlem & Gorkem, 2004; Tenabe, 2000).

For these reasons, state monitoring programmes, known as "Mussel Watch", have been applied to monitoring the environmental situation of different coastal regions (Kimbrough *et al.,* 2008).

As intertidal filter feeders, mussels accumulate heavy metals in their tissues in proportion to the degree of environmental contamination. Thus, heavy metal concentration in tissues reflects environmental levels (Fang *et al.,* 2003). Moreover, mussels not only serve as bioindicators, but also raise public health concerns because, whether cultivated or wild, they are consumed as seafood (Cardellicchio *et al.,* 2010).

Heavy metals occur naturally in the marine environment but during the last decade, anthropogenic activities have increased the natural flux of these elements to levels that are hazardous for marine ecosystems. Their inputs include urban run-off, industrial effluents, anti-fouling paints for boats, mining operations and atmospheric depositions, and are in either particulate or dissolved form. Although many elements are essential in small quantities, they can be toxic to organisms, above certain threshold concentrations, and for the protection of aquatic biota it is important that these limits are not exceeded in aquatic environments (Brown & Depledge, 1998).

Metals can be accumulated by marine organisms and their concentrations provide a time-integrated measure of metal supply, over long periods of time (weeks, months or even years) depending on the species (Rainbow, 1995). These pollutants can cause long-term effects on marine organisms, even if their impact has no visible influence compared to other pollutants. Their capacity to bind with short carbon chains can be dangerous for marine organisms, since they continuously accumulate and concentrate metals in their tissues (Shahidul-Islam & Tanaka, 2004).

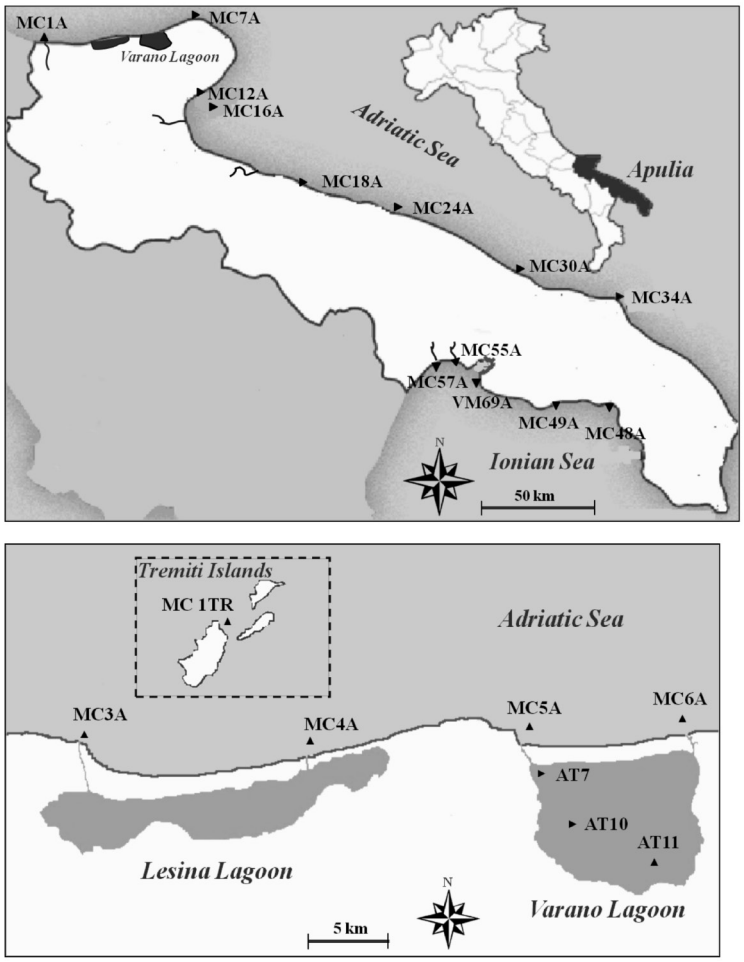


Fig. 1: Location of the sampling stations along the Apulia Region coast.

Mytilus spp. mussels, being sedentary filter-feeders, are known to be good bioaccumulators of certain trace elements and for this reason are widely used as sentinel species for monitoring coastal environments (Box *et al.,* 2007; Deudero *et al.,* 2007; Rainbow & Dallinger, 1993; Saavedra *et al.,* 2004; Santovito *et al.,* 2005). In these organisms metals are probably absorbed both from water and from ingested phytoplankton and other suspended particles (George, 1980).

Considering the growing concern over marine coastal environmental quality, the main purpose of this study was to identify the levels and spatial distribution of metal pollution in *Mytilus galloprovincialis* sampled at twenty-one sites, all along the Apulian coast, in areas characterized by different levels of anthropogenic loadings.

Results were also compared with data from previous studies and checked in order to evaluate compliance with the limits laid down in the Regulations of the European Commission and FAO, setting maximum levels for certain contaminants in foodstuffs, including some heavy metals. Moreover, in order to investigate public health risks, associated with consuming seafood harvested from these areas, the weekly intake has been calculated. The obtained weekly intake was compared with the provisional tolerable weekly intake (PTWI), recommended by the FAO/WHO Expert Committee on Food Additives.

Material and Methods

Sampling and sample pre-treatment

Mussels – *M. galloprovincialis* (Lamarck, 1819) were collected manually in 2009 at twenty-one stations distributed along the coastal area of the Apulia Region, Southern Italy (Fig.1). Sampling was performed during the prespawning period (September-October) in order to control seasonal variation due to mussel physiology (Carro *et al.,* 2004). As shown in Figure 1, sixteen stations are located in the Adriatic Sea and five in the Ionian Sea.

Sampling location, sample name and geographical coordinates are shown in Table 1.

Sampling sites were selected by considering different possible anthropogenic activities and unpolluted areas, in particular seven sites were overlooking the mouth of a number of river and channels characterized by agricultural runoff and urban discharge (Mouth of Patemisco, Lenne, Capoiale and Varano Rivers, Saccione River, Schiapparo channel and Punta Pietre Nere near Acquarotta Channel). Three sampling stations were located in Sites of National Interest (SIN) established by the Ministerial Decree of 10.01.2000 (Taranto, Manfredonia and Brindisi); two sites were characterized by harbour activities (Barletta and Bari); three stations were located in the Varano Lagoon, a

Table 1. Samples name, location, geographical coordinates and typology of mussels (wild or cultured) "*M. galloprovincialis"* sampled along the Apulian coastal areas.

	Code	Sampling location		Lat.	Long.
Ionian Sea	**MC55A**	Mouth of Patemisco river	wild	40°31'09"N	17°06'13"E
	MC57A	Mouth of Lenne river	wild	40°30'12"N	17°00'58"E
	MC48A	Porto Cesareo	wild	40°14'40"N	17°53'26"E
	MC49A	Torre Colimena	wild	40°17'46''N	17°44'49''E
	VM69A	Mar Grande, Taranto	wild	40°25'42"N	17°13'36"E
Adriatic Sea	**AT7**	Varano Lagoon	cultured	41°54'03"N	15°41'10"E
	AT10	Varano Lagoon	cultured	41°53'00"N	15°43'08"E
	AT11	Varano Lagoon	cultured	41°51'21"N	15°45'41"E
	MC1A	Mouth of Saccione River	wild	41°55'41''N	15°08'20''E
	MC3A	Punta Pietre Nere	wild	41°55'05''N	15°20'25''E
	MC4A	Mouth of Schiapparo Channel	wild	41°54'36''N	15°30'35''E
	MC5A	Mouth of Capoiale River	wild	41°55'23''N	15°40'00''E
	MC6A	Mouth of Varano River	wild	41°55'20''N	15°47'44''E
	MC7A	Molino di Mare	wild	41°55'50''N	15°54'00''E
	MC12A	Manfredonia	wild	41°37'26"N	15°56'37"E
	MC16A	Saline	cultured	41°37'26"N	15°57'49"E
	MC18A	Barletta Est	wild	41°19'47"N	16°17'18"E
	MC24A	Bari	wild	41°08'50"N	16°50'55"E
	MC30A	Torre Canne	wild	40°50'59"N	17°28'10"E
	MC34A	Brindisi, Capo Bianco	cultured	40°39'46"N	17°58'52"E
	MC1TR	Tremiti Islands	wild	42°07'18''N	15°29'48"E

transitional area of the Gargano National Park. Moreover, different unpolluted areas were selected: Torre Colimena, Torre Canne, Saline and the Marine Protected Area of Porto Cesareo and Tremiti Islands. Among the sampling stations, five sites were from aquaculture farms and the rest were collected from the wild (Table 1).

More than 300 mussel samples were collected at each location, stored in bags, kept in a cooler box with ice and transported to the laboratory. Then, for a 24-h period, molluscs were placed in filtered seawater collected at the corresponding station to allow depuration of particulate matter residues present in the mantle cavity and digestive tract.

After this period, mussels were cleaned, shucked with a Teflon® knife, sorted into pools of similar size (3.50-5.00 cm) with about 150 individuals and then homogenized by an Ultra-Turrax T25 homogeniser. To avoid contamination, all the parts of the homogeniser, which came into contact with the sample, were covered with Teflon® adaptors. The homogenates were lyophilized and stored in polyethylene bottles.

An aliquot of the homogenized sample of mussel tissue was used for dry weight calculation only, by oven drying at 105 °C until constant weight (Table 1).

For metal analysis, a 0.25 g lyophilized sample was mineralized with 9 ml of concentrated HNO_3 (70% v/v) and 1 ml H_2O_2 (30%v/v) (US-EPA Method 3052), in a closed Teflon® vessel using a microwave digestion system (MARS-X CEM Corporation, Matthews, NC). After mineralization, digests were cooled and the resulting solutions were diluted to a known volume (50 ml) with Milli-Q® water and stored in polyethylene bottles, until analysis. A blank digest was performed in the same way.

All the chemicals used in sample treatments were of ultrapure grade (Merck Suprapur, Darmstadt, Germany) and all the glassware was cleaned prior to use by soaking in 10% v/v HNO_3 for 24 h and rinsed with Milli-Q® water. All solutions were prepared daily using ultra-pure deionised water (<0.1 µs at 25 °C), obtained by treating double distilled water in a Milli-Q® system (Millipore, Milford, Ma., USA).

Chemical analysis

As, Cd, Cu, Cr, Hg, Pb, Zn concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS model Elan 6100 DRC Plus, Perkin Elmer, Norwalk, CT, USA). For calibration, all standards were prepared in the same matrix as the one used for mussel analyses. The standard solutions of metals were prepared from stock standard solutions of ultrapure grade supplied by Merck. To check for contamination, procedural blanks were analysed in every five samples. The detection limits (LOD), expressed in mg/kg dry weight, were: As: 0.01; Cd: 0.002; Cr: 0.02; Cu: 0.003; Hg: 0.004; Pb: 0.02; Zn: 0.02.

Analytical quality control and statistical analysis

The accuracy of measurements was tested using Community Bureau of Reference (BCR) certified reference material CRM 278R (mussel tissue). Results were in agreement with certified values and the standard deviations were low, proving good repeatability of the method (Table 2). Recoveries were in the range of 96.1 % (Cr) to 104.5 % (Hg). Each measured and reported value is an average of five determinations.

Table 2. Concentrations of heavy metals (mg/kg dry weight) in CRM 278R (mussel tissue, Mytilus edulis), average ± standard deviation.

Element	Certified	Measured[a]
Arsenic	6.07 ± 0.13	6.12 ± 0.19
Cadmium	0.348 ± 0.007	0.343 ± 0.018
Copper	9.45 ± 0.13	9.52 ± 0.41
Chromium	0.78 ± 0.06	0.75 ± 0.09
Lead	2.00 ± 0.04	2.09 ± 0.13
Mercury	0.196 ± 0.009	0.188 ± 0.016
Zinc	83.1 ± 1.7	82.7 ± 3.31

a = calculate on five replicate analyses

Experimental data were elaborated by multivariate statistical analysis performed using the STATISTICA (StatSoft Inc., Tulsa, OK, USA) software package. The Principal Component Analysis (PCA) technique was used.

Results and Discussion

Metal concentrations in mussels and related risk for human health

The mean concentrations of the investigated metals in soft tissues of the mussel *M. galloprovincialis* and wet/dry ratio are given in Table 3. Considering all metals and all the sampling sites of this work, the obtained mean values decreased in the following order: Zn>As>Cu>Cr>Pb>Cd>Hg.

In comparison with the permissible limits set for Cd (1.0 mg/kg w.w. - EC Regulation n. 1881/2006), Hg (0.5 mg/kg w.w. - EC Regulation n. 1881/2006), Pb (1.5 mg/kg w.w. - EC Regulation n. 1881/2006), Cu (30.0 mg/kg w.w. - FAO, 1983), Cr (1.0 mg/kg w.w. - FAO, 1983) and Zn (30.0 mg/kg w.w. - FAO, 1983) all the mean values (mg/kg w.w.) of analyzed metals were lower than these limits with the exception of Cr levels at the MC55A - mouth of Patemisco River (2.03 mg/kg w.w.), MC1TR – Tremiti Islands (1.25 mg/kg w.w.) and MC57A – Molino di mare (1.04 mg/kg w.w.) stations, where levels were found to be higher or equal to the permissible limits established by FAO (1983) for fishery products. The Cr level in the mussels of the Patemisco River mouth is probably related to discharge form urban areas and small industries flowing into the river. In the other zones, such as the protected marine area of Tremiti Islands, the values exceeding the limits are probably due

Table 3. Mean values of As, Cd, Cr, Cu, Hg, Pb, Zn (mg/kg dry weight), standard deviation and wet/dry ratio in soft tissues of *M. galloprovincialis* collected from the Apulian coasts (*n*=5).

Station	W/D[a]	As	Cd	Cr	Cu	Hg	Ni	Pb	V
MC 55A	4.66	25.93 ± 2.11	0.38 ± 0.04	9.46 ± 0.91	7.90 ± 0.81	0.10 ± 0.01	15.14 ± 1.66	2.32 ± 0.27	3.80 ± 0.39
MC 57A	5.27	28.21 ± 2.88	0.38 ± 0.04	5.50 ± 0.33	7.00 ± 0.67	0.10 ± 0.01	5.94 ± 0.47	2.16 ± 0.19	4.60 ± 0.43
MC 48A	4.52	25.7 ± 2.17	1.14 ± 0.16	3.56 ± 0.33	6.05 ± 0.47	0.12 ± 0.01	3.28 ± 0.29	1.07 ± 0.13	2.20 ± 0.17
MC 49A	6.36	60.36 ± 7.21	1.10 ± 0.11	2.50 ± 0.21	7.62 ± 0.63	0.31 ± 0.03	3.71 ± 0.32	1.57 ± 0.17	3.33 ± 0.23
VM69A	6.33	34.22 ± 3.11	0.48 ± 0.04	1.81 ± 0.17	5.89 ± 0.35	0.16 ± 0.01	1.02 ± 0.09	1.12 ± 0.11	1.40 ± 0.10
AT 7	6.09	7.06 ± 0.55	2.54 ± 0.39	2.58 ± 0.19	7.72 ± 0.61	0.63 ± 0.04	2.74 ± 0.26	0.83 ± 0.05	1.34 ± 0.11
AT 10	4.55	6.35 ± 0.34	1.32 ± 0.15	2.14 ± 0.14	5.26 ± 0.48	0.16 ± 0.01	2.08 ± 0.18	1.21 ± 0.08	1.32 ± 0.16
AT 11	6.19	24.95 ± 1.97	1.54 ± 0.17	3.07 ± 0.32	6.73 ± 0.59	0.14 ± 0.01	5.21 ± 0.52	1.50 ± 0.11	2.66 ± 0.21
MC 1A	5.06	17.10 ± 1.36	1.10 ± 0.13	2.35 ± 0.37	10.99 ± 0.98	0.81 ± 0.06	5.94 ± 0.55	1.21 ± 0.11	3.05 ± 0.29
MC 3A	3.79	22.92 ± 2.05	0.56 ± 0.04	2.76 ± 0.44	7.90 ± 0.45	0.20 ± 0.01	6.22 ± 0.61	1.21 ± 0.16	3.02 ± 0.26
MC 4A	7.63	18.61 ± 1.44	0.74 ± 0.07	2.22 ± 0.32	6.32 ± 0.43	0.66 ± 0.04	3.76 ± 0.29	1.89 ± 0.17	2.22 ± 0.19
MC 5A	7.38	9.32 ± 0.91	1.84 ± 0.17	2.78 ± 0.38	7.76 ± 0.55	0.62 ± 0.04	3.48 ± 0.33	3.25 ± 0.28	2.48 ± 0.18
MC 6A	4.42	6.46 ± 0.77	1.54 ± 0.11	0.96 ± 0.07	5.62 ± 0.49	0.32 ± 0.03	1.12 ± 0.09	0.37 ± 0.32	0.94 ± 0.09
MC 7A	4.94	17.27 ± 1.53	0.58 ± 0.06	4.14 ± 0.32	7.14 ± 0.53	0.23 ± 0.01	5.06 ± 0.41	1.67 ± 0.18	4.96 ± 0.48
MC12A	4.36	17.60 ± 1.08	0.85 ± 0.07	3.60 ± 0.26	6.78 ± 0.59	0.13 ± 0.01	2.76 ± 0.31	1.19 ± 0.15	3.68 ± 0.37
MC 16A	4.57	17.12 ± 1.03	0.52 ± 0.05	2.68 ± 0.17	8.59 ± 0.86	0.14 ± 0.02	1.90 ± 0.17	1.29 ± 0.16	2.50 ± 0.33
MC 18A	4.48	21.54 ± 1.76	0.81 ± 0.06	3.44 ± 0.29	7.10 ± 0.66	0.13 ± 0.01	2.56 ± 0.27	1.02 ± 0.09	2.30 ± 0.19
MC 24A	4.63	76.17 ± 5.32	0.72 ± 0.05	1.78 ± 0.08	19.22 ± 1.54	0.26 ± 0.02	2.16 ± 0.18	2.23 ± 0.25	3.38 ± 0.31
MC 34A	5.44	23.12 ± 2.83	0.52 ± 0.05	3.00 ± 0.29	4.66 ± 0.32	0.24 ± 0.02	2.50 ± 0.17	1.15 ± 0.12	1.16 ± 0.11
MC 30A	5.87	26.31 ± 2.05	0.73 ± 0.08	2.12 ± 0.18	6.42 ± 0.54	0.12 ± 0.01	2.53 ± 0.21	1.12 ± 0.08	1.75 ± 0.16
MC 1 TR	6.55	35.21 ± 2.98	1.04 ± 0.17	8.19 ± 0.65	6.74 ± 0.55	0.13 ± 0.01	6.15 ± 0.54	1.93 ± 0.18	5.52 ± 0.47
Range		6.35-76.17	0.38-1.84	0.96-9.46	4.66-19.22	0.1-0.81	1.02-15.14	0.37-3.25	1.16-5.52

a = wet/dry ratio

to natural sediment enrichment. However,no published data are available about the metal content of sediment or background levels in these areas.

However, the potential hazards of metals being transferred to humans are related to the amount of mussels consumed by an individual. The risk to human health, as a result of consuming fish and seafood - dietary exposure - can be evaluated by calculating daily metal exposure E_m (US-EPA, 1994) as follows:

$$\frac{E_m = C_m \cdot IR}{B_W}$$

where C_m represents metal concentration in fish or seafood (µg/g w.w.); IR the ingestion rate (g/d) of fish or seafood and B_W represents body weight (kg_{bw}). Dietary metal exposure is expressed as $\mu g/kg_{bw}$ day. In order to calculate total daily exposure, average seafood consumption of 70 g/d was considered (weekly consumption of 490 g). This value is 10 g more than the value suggested by ISTAT (2000) for Italy, because the population in Taranto can be considered a high level fish and seafood consumer (Spada *et al.,* 2011).

These current intakes were compared with the respective provisional tolerable weekly intake (PTWI) established by the FAO/WHO Expert Committee on Food Additives.

As is classified as a carcinogenic agent for humans (IARC, 2011; US EPA, 2007). For inorganic As, the PTWI is 0.015 mg/kg (WHO, 2011) of body weight per week, or 129 µg/day for a subject of 60 kg. Literature studies show that most As found in fish and seafood is in the form of organic As (IOM, 2006; Munoz *et al.,* 2000; Penrose *et al.,* 1997; Simonič 2009; Velez *et al.,* 1995), which represents the less toxic form. The percentage of inorganic As has been reported to be between 0.02 and 11% (Munoz *et al.,* 2000) in fish and shellfish, so the inorganic forms usually only contribute a few percentage points (Donohue *et al.,* 1999; Francesconi & Edmond, 1997; Munoz *et al.,* 1999; Francesconi *et al.,* 2002). In this study we analyzed total (inorganic and organic) As, and hypothesizing the maximum percentage (11%); it is clear that the mussels studied in this work are safe for human consumption, as regards the As content (Fig. 2). Only station MC24A – Bari city, located in the Adriatic Sea, showed an E_m value close to the established PTWI for As (0.015 mg/kg). Comparing As concentrations with the literature data (Table 4) it is possible to show that As levels in this study were much higher than the other Mediterranean regions; while As concentrations in the Varano Lagoon were comparable with those reported by Giusti & Zang (2002) for the Venice Lagoon.

Cd absorption constitutes a risk to humans because it may accumulate in the human body inducing skeletal damage, reproductive deficiencies and kidney dysfunction (EC, 2001). High levels of Cd are often associated with human activity (industrial emissions and the application of fertilizer and sewage sludge to farm land) (Jovic *et al.,* 2011). The provisional tolerable weekly intake for Cd was established as 0.007 mg/kg bw (0.42 mg per week for a 60-Kg person) (WHO, 2006) and the Scientific Committee of Food (SCF) recommended greater efforts to reduce dietary exposure to Cd since foodstuffs are the main source of human intake. In this study, the calculated E_m values were below the PTWI at all sampling stations. As shown in Table 4, Cd levels were much higher than those reported for the Tyrrhenian Sea (Amodio-Cocchieri *et al.,* 2003; Conti & Cecchetti, 2003; Licata *et al.,* 2004) and Aegean Sea (Kucuksezgin *et al.,* 2008) but lower than Cd levels reported by Deudero *et al.* (2009), for the Baleari Islands.

Cu and Zn are essential elements for human health, but in excess may be harmful for human health. Regarding Cu and Zn, the Joint FAO/WHO set the PTWI at 3.5 mg/kg bw/week (equivalent to 210 mg/week for a 60 Kg-adult) and at 7.0 mg/kg bw/week (equivalent to 420 mg/week for a 60 Kg-adult), respectively (FAO/WHO, 2004). Figure 2 showed that in this study it might be said that examined mussels are safe for human consumption regarding Cu and Zn. Cu concentrations in this study, were lower than those indicated by Giusti & Zang (2002) for Venice Lagoon while they were twice as high as the concentrations observed in Izimir Bay (Kucuksezgin *et al.,* 2008) and the Balearic Islands (Deudero *et al.,* 2009).

Pb is a toxic, bio accumulative heavy metal with no known biological function (Stankovic *et al.* 2012) and its absorption may constitute a serious risk to public health. Pb may induce reduced cognitive development and intellectual performance in children and increate blood pressure and cardiovascular diseases in adults (EC, 2001). The PTWI for Pb, established by the Joint FAO/WHO (2000) is 0.025 mg/kg bw/week (equivalent to 1.5 mg/week for a 60 Kg-adult).

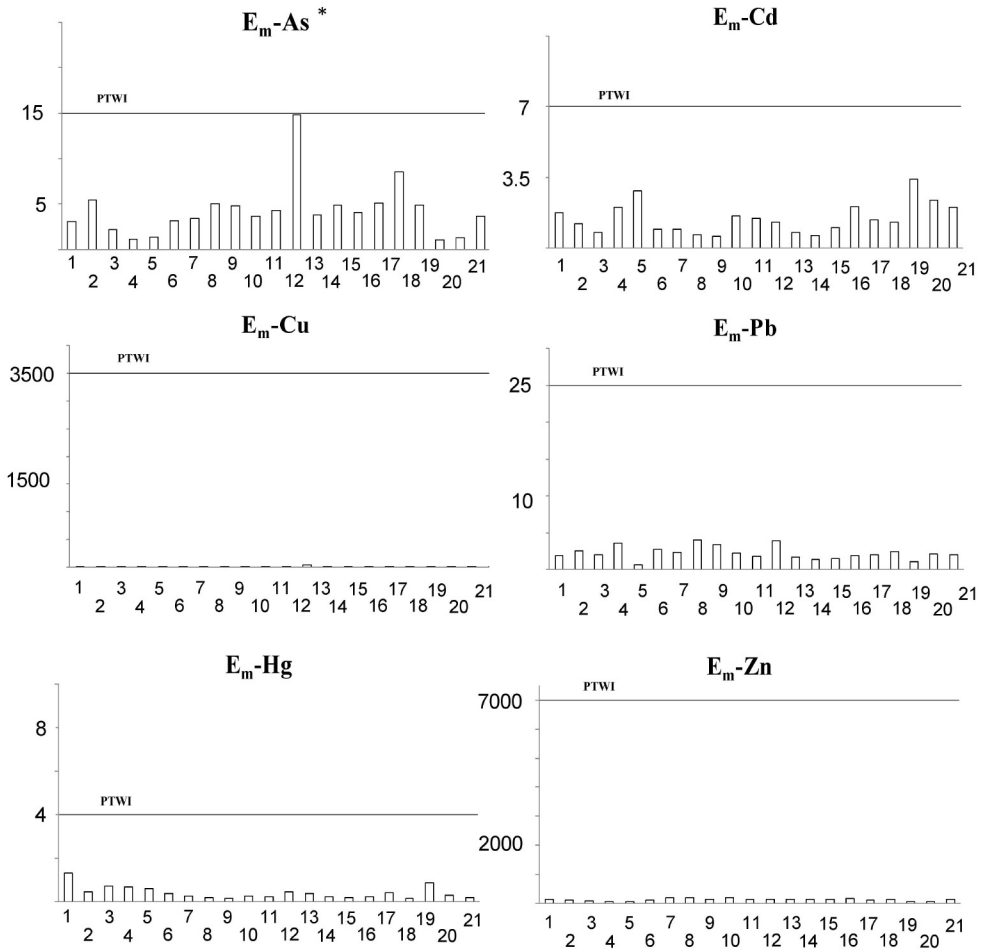
As shown in Figure 2, the estimated PTWIs of Pb for Apulian Sea mussels were below the established PTWI. Pb levels of this study were lower than those reported for the Croatian Coast (Kljakovic-Gašpic *et al.,* 2010; Orescanin *et al.,* 2006), Balearic Islands (Deudero *et al.,* 2009) and Venice Lagoon (Giusti & Zhang, 2002).

Hg contaminates fish and fishery products mainly and its organic form, methylmercury (Me-Hg), may induce alterations in the normal development of the brain of infants and at higher levels defined period of time, the maximum limit laid down may induce neurological changes in adults (EC, 2001). Different literature studies report a Me-Hg to total Hg ratio of about 25-40% in the Mediterranean mussel (Mikac *et al.,* 1996; Berzas Nevado *et al.,* 2003; Spada *et al.,* 2011).

The Committee established a PTWI for inorganic Hg of 0.004 mg/kg bw (WHO, 2011). The previous PTWI of 0.005 mg/kg bw for total Hg (0.3 mg/week for a 60 Kg-adult), established at the sixteenth meeting of the Joint FAO/WHO was withdrawn. The new PTWI set for inorganic Hg was considered applicable to dietary exposure to total Hg from foods other than fish and shellfish (WHO, 2011). Considering the estimated PTWI of Hg calculated in this study it could be stated that examined mussels are safe for human consumption as regards Hg.

By comparing the data from different Mediterranean regions (Table 4) it is possible to note that Hg concentrations observed in this study in the Adriatic Sea were higher than those observed by Kucuksezgin *et al.* (2008), along the Croatian coast, and Licata *et al.* (2004) in Lake Faro (Sicily).

No information is available about tolerable intake for



*The E_m-As has been calculated considering the 11% (Munoz *et al.*, 2000) of the total concentration of As.

Station legend - 1: MC1A; 2: MC3A; 3: MC4A; 4: MC5A; 5: MC6A; 6: MC7A; 7: MC16A; 8: MC55A; 9: MC57A; 10: MC12A; 11: MC18A; 12: MC24A; 13: MC34A; 14: VM69A; 15: MC30A; 16: MC58A; 17: MC49A; 18: MC1TR; 19: AT7; 20: AT10; 21: AT11.

Fig. 2: The estimated weekly intake of As, Cd, Cu, Hg, Pb and Zn from *Mytilus galloprovincialis*, according to the PTWI limits expressed in µg/kg body weight/week (As: 15; Cd: 7; Cu: 3500, Hg: 4; Pb: 25, Zn: 7000).

Table 4. As, Cd, Cu, Cr, Hg, Pb, Zn mean levels (mg/kg) found in mussel *M. galloprovincialis* from different Mediterranean areas.

Location	Weight basis	As	Cd	Cr	Cu	Hg	Zn	Pb	References
Gulf of Naples (Tirrenian Sea)	wet	0.15	0.03	0.15		0.20		0.21	Amodio-Cocchieri *et al*., 2003
Gulf of Gaeta (Tirrenian Sea)	dry		0.33	0.87	9.55		157.33	2.22	Conti and Cecchetti, 2003
Lake Faro, Sicily (Tirrenian Sea)	wet		0.05			0.01		0.07	Licata *et al*., 2004
Mar Piccolo, Taranto (Ionian Sea)	dry					0.63			Spada *et al*., 2011
Venice Lagoon (Adriatic Sea)	dry	14.6		2.28	22.25		164.45	3.15	Giusti and Zhang, 2002
Marmaran Sea	dry		1.91	1.18	7.66		264.13	1.73	Topcuoglu *et al*., 2004
Izimir Bay (Agean Sea)	dry		0.02	0.14	4.48	0.02	27.70	0.24	Kucuksezgin *et al*., 2008
Croatian Coasts (Adriatic Sea)	dry		0.99	1.72	10.53	0.25	158.00	4.21	Kljakovic-Gašpic *et al*., 2010
Croatian Coasts (Adriatic Sea)	dry	15.2		1.85	6.1			4.05	Orescanin *et al*., 2006
Montenegro Coasts (Adriatic Sea)	dry			3.04	8.26		119.60		Joksimovic *et al*., 2011
Baleari Islands (Western Mediterranean)	dry	9.05	2.83	0.53	4.76	0.2	234.16	2.48	Deudero *et al*., 2009
Apulian Coasts (Adriatic Sea)	dry	23.75	0.89	3.08	8.17	0.31	75.27	1.50	Present study
Apulian Coasts (Ionian Sea)	dry	34.88	0.70	4.57	6.89	0.16	87.89	1.65	Present study
Varano Lagoon (Adriatic Sea)	dry	12.79	1.80	2.60	6.57	0.31	54.72	1.18	Present study

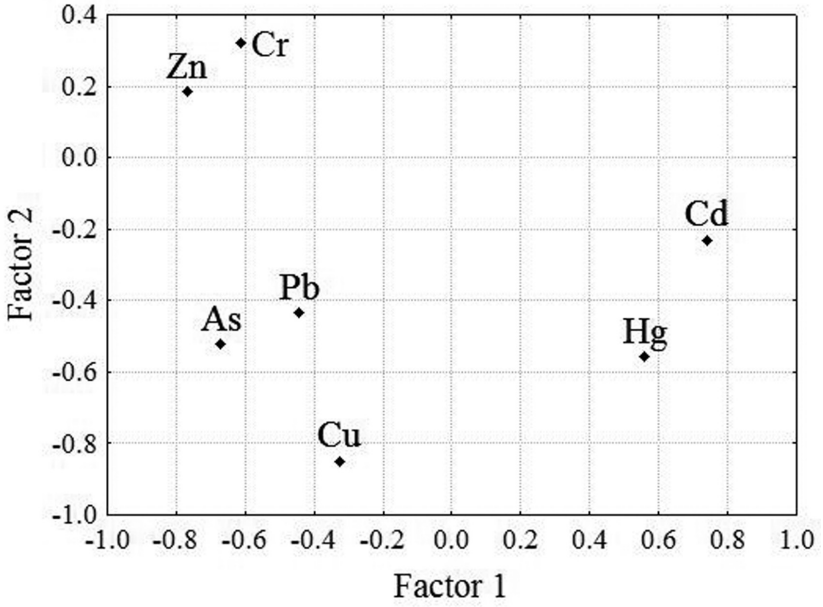


Fig. 3: Loading of the variables on the first two principal components.

Cr concentrations; however comparing data obtained in this work, Cr concentrations were much higher than those reported in other studies, with the exception of mussels collected along the coast of Montenegro (Joksimovic *et al.,* 2011) where Cr values were similar.

PCA was applied using the As, Cd, Cr, Cu, Hg, Pb, Zn mean concentrations in the sampled bivalve molluscs as variables, in order to detect any different contamination levels among sites in the study area. Three main components with eigenvalues >1 were extracted, accounting for 77.6 % of total variance. The first principal component (PC1) accounted for 36.7% and was mainly associated (factor loadings > 0.7) with Cd and Zn, while the second principal component (PC2) was clearly associated with Cu and accounted for 24.2% of data variability (Fig. 3).

The scatterplot of PC1 against PC2 (Fig.4) showed an overlap for most stations, for the metals mentioned above, and there are no statistically significant differences in the element concentrations between the wild and cultivated mussels. However, the MC55A (Mouth of Patemisco River, Ionian Sea) station was characterized by a low concentration of Cd and high concentrations of Zn and Cr compared to the other sampling stations. On the other hand, the AT7 (Varano Lagoon), MC6A (Mouth of Varano River, Adriatic Sea), AT10 (Varano Lagoon) and to a lesser extent the MC5A (Mouth of Capoiale River, Adriatic Sea) and MC1A (Mouth of Saccione River, Adriatic Sea) stations showed high concentrations of Cd and low concentrations of Zn. The high concentration of Cd in mussels collected from the Varano Lagoon was in accordance with an older research study carried out by Storelli & Marcotrigiano (2001) in the same lagoon. This is probably due to the abnormally high Cd concentrations in sediments (mean of 0.95 mg/kg d.w.), as reported by Spagnoli *et al.* (2002). There is insufficient published data about possible sources and Cd concentrations in the lagoon; therefore, the natural or anthropogenic origin of this element remains to be determined (Spagnoli *et al.,* 2002). Compared to other stations, the highest concentration of Cu was found in the MC24A station. This station was also characterized by high concentrations of Zn, which probably originates from antifouling paint associated with Cu oxides, considering the location of this station i.e. in Bari city harbour area.

Conclusions

In general, trace element concentrations found in wild and cultivated mussels from the Apulian coast were below the maximum permissible limits prescribed by international regulations, with the exception of Cr levels at three sampling stations, in particular in the mussels of the Patemisco River mouth. Statistical analysis revealed no statistically significant differences in element concentrations between wild and cultivated mussels. However, mussels collected from the mouth of Patemisco River (Ionian Sea), were characterized by high concentrations of Zn and Cr compared to other sampling stations. These levels are probably related to discharge form urban areas and small industries flowing into the river. On the other hand, higher concentrations of Cd were found at two stations of the Varano Lagoon and one station overlooking the Varano River mouth (Adriatic Sea). This is probably related to the abnormally high Cd concentrations found in sediments by Spagnoli *et al.* (2002), in a previous study of sediment composition in the Varano Lagoon; nevertheless, the natural or anthropogenic origin remains to be determined, because no published data are available.

Moreover, mussels collected from the harbour area of Bari city were characterized by high concentrations of Cu and Zn, probably deriving from antifouling paint associated with Cu oxides. Calculations based on trace element concentrations, present in mussels collected along the Apulian coast, suggest that a large amount of mussels would need

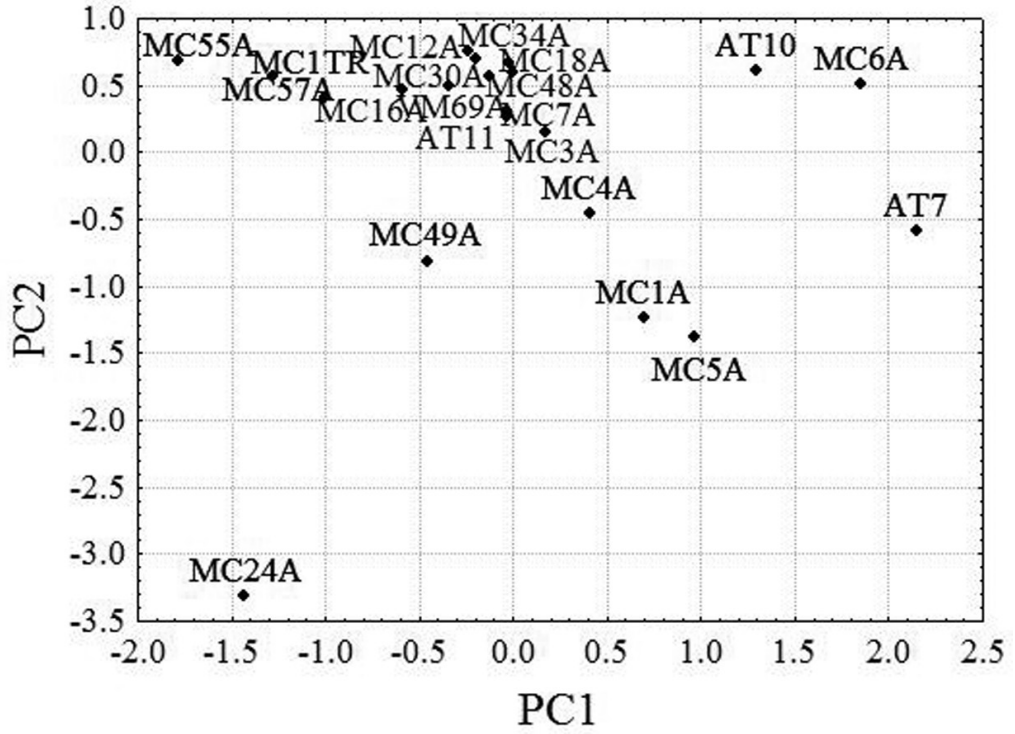


Fig. 4: Scatter plot of the scores for the first two principal components.

to be consumed to exceed the prescribed PTWI values for adults. However, it should be noted that the estimated intake doubles when considering children (with half the weight of adults), especially fishermen's children. Furthermore, pregnant and lactating women should pay particular attention to the amount of seafood and fish introduced in their diets. In addition, the estimated intake does not include the contribution of other foods that may constitute further contamination sources to which the population is also exposed.

It was concluded that *M. galloprovincialis* from the Apulia Region is safe as regards the contaminants studied; however, heavy metal concentrations must be monitored periodically with respect to consumer health. Moreover, these data could allow consumers to make informed decisions about which product to eat in order to reduce the risks of contaminants.

References

Amodio-Cocchieri, R., Amoroso S., Arnese A., Cirillo T., Montuori P. *et al.,* 2003. Pollution by mercury, arsenic, lead, chromium, cadmium, and polycyclic aromatic hydrocarbons of fish and mussels from the Gulf of Naples, Italy. *Bulletin of Environmental Contamination & Toxicology*, 71 (3), 551-560.

Berzas Nevado, J.J., García Bermejo, L.F., Rodríguez Martín-Doimeadios, R.C., 2003. Distribution of mercury in the aquatic environment at Almadén, Spain. *Environmental Pollution*, 122 (2), 261-271.

Box, A., Sureda, A., Galgani, F., Pons, A., Deudero, S., 2007. Assessment of environmental pollution at Balearic Islands applying oxidative stress biomarkers in the mussel *Mytilus galloprovincialis*. Comparative Biochemistry & Physiology Part C: *Toxicology & Pharmacology*, 146 (4), 531-539.

Brown, M.T., Depledge, M.H., 1998. Determinants of trace metal concentrations in marine organisms. p. 186-217. In: *Metal metabolism in aquatic environments*. Langston, W.J., Bebianno, M.J. (Eds). Chapman & Hall, London.

Cardellicchio, N., Annicchiarico, C., Assennato, G., Blonda, M., Di Leo, A. *et al.,* 2010. Preliminary results of pollutants biomonitoring in coastal marine and transitional waters of Apulia Region (Southern Italy). *Fresenius Environmental Bulletin*, 19 (9), 1841-1847.

Cardellicchio, N., Buccolieri, A., Di Leo, A., Giandomenico, S., Spada, L., 2008. Levels of metals in reared mussels from Taranto Gulf (Ionian Sea, Southern Italy). *Food Chemistry*, 107, 890-896.

Carro N., García I., Ignacio M., Mouteira A., 2004. Possible influence of lipid content on levels of organochlorine compounds in mussels from Galicia coast (Northwestern, Spain). Spatial and temporal distribution patterns. *Environment International*, 30 (4), 457-466.

Conti, M.E., Cecchetti, G., 2003. A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. *Environmental Research*, 93 (1), 99-112.

Deudero, S., Box, A., March, D., Valencia, J.M., Grau, A.M. *et al.,* 2007. Organic compounds temporal trends at some invertebrate species from the Balearics, Western Mediterranean. *Chemosphere*, 68 (9), 1650-1659.

Deudero, S., Box, A., Tejada, S., Tintoré, J., 2009. Stable isotopes and metal contamination in caged marine mussel *Mytilus galloprovincialis*. *Marine Pollution Bulletin*, 58 (7), 1025-1031.

Donohue, J.M., Abernathy, C.O., 1999. Exposure to inorganic arsenic from fish and shellfish. p. 89-98. In: *Arsenic Exposure and Health Effects*. Chappell, W.R. Abernathy, C.O., Calderon, R.L. (Eds). Elsevier Science Ltd., Oxford, United Kingdom.

EC, 2001. Commission Regulation N° 466/2001 of 8 March 2001. Setting maximum levels for certain contaminants in

foodstuffs. *Official Journal of the European Communities,* L77, 1-13.

EFSA, 2004. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to mercury and methylmercury in food (Request N° EFSA-Q-2003-030) (adopted on 24 February 2004). *The EFSA Journal*, 34, 1-14.

EPA Method 3052, 1995. Microwave assisted acid digestion of siliceous and organically based matrices. In: *Test methods for evaluating solid waste, physical/chemical methods. SW-846.* U.S. EPA, Washington, D.C.

EU, 2006. Commission Regulation N° 1881/2006 of 19 December 2006. Setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, L364, 5-24.

Fang, Z.Q., Cheung, R.Y., Wong, M.H., 2003. Heavy metals in oysters, mussels and clams collected from coastal sites along the Pearl River Delta, South China. *Journal of Environmental Sciences (China)*, 15 (1), 9-24.

FAO/WHO, 2000. Evaluation of certain food additives and contaminants: fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series, No. 896.* WHO, Geneva, 128 pp.

FAO/WHO, 2004. *Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additive*s (JECFA 1956-2003). ILSI Press International Life Sciences Institute, Washington, DC.

Francesconi, K.A., Edmonds, J.S., 1997. Arsenic and marine organisms. *Advances In Inorganic Chemistry*, 44, 147-189.

Francesconi, K.A., Kuehnelt, D., 2002. Arsenic compounds in the environment. p. 51-94. In: *Environmental Chemistry of Arsenic*. Frankenberger, W.T. (Ed.). Marcel Dekker Inc, New York.

George, S.G., 1980. Correlation of metal accumulation in mussels with the mechanism of uptake, metabolism and detoxification: a review. *Thalassia Jugoslavica*, 16, 347-365.

Giusti, L., Zhang, H., 2002. Heavy metals and arsenic in sediments, mussels and marine water from Murano (Venice, Italy). *Environmental Geochemistry & Health*, 24 (1), 47-65.

IARC, 2012. A review of human carcinogens. Part C: Arsenic, metals, fibres, and dusts. *IARC Monographs on the evaluation of carcinogenic risks to humans. Vol. 100C.* IARC, Lyon, 501 pp.

ISTAT, 2000. *Statistica sulla pesca, caccia e zootecnia*. *Informazione No. 94-2000*. Alba Grafica Spa, Roma. (in Italian).

Joksimovic, D., Tomic, I., Stankovic, A.R., Jovic M., Stankovic, S., 2011. Trace metal concentrations in Mediterranean blue mussel and surface sediments and evaluation of the mussels quality and possible risks of high human consumption. *Food Chemistry*, 127 (2), 632-637.

Jović, M., Stanković, A., Slavković -Beskoski, L., Tomić, I., Degetto, S. *et al.,* 2011. Mussels as a bio-indicator of the environmental quality of the coastal water of the Boka Kotorska Bay (Montenegro). *Journal of the Serbian Chemical Society*, 76 (6), 933-946.

Kimbrough, K.L., Johnson, W.E., Lauenstein, G.G., Christensen, J.D., Apeti, D.A., 2008. An assessment of two decades of contaminat monitoring in the nation's coastal zone. *NOAA Technical Memorandum NOS NCCOS, No 74.* Silver Spring, MD., 105 pp.

Kljaković-Gaspić, Z., Herceg-Romanić, S., Kozul, D., Veza J., 2010. Biomonitoring of organochlorine compounds and trace metals along the Eastern Adriatic coast (Croatia) using *Mytilus galloprovincialis*. *Marine Pollution Bulletin*, 60 (10), 1879-1889.

Kucuksezgin, F., Kayatekin, B.M., Uluturhan, E., Uysal, N., Acikgoz, O. *et al.,* 2008. Preliminary investigation of sensitive biomarkers of trace metal pollution in mussel (*Mytilus galloprovincialis*) from Izmir Bay (Turkey). *Environmental Monitoring & Assessment*, 141 (1-3), 339-345.

Licata, P., Trombetta, D., Cristiani, M., Martino, D., Naccari, F., 2004. Organochlorine compounds and heavy metals in the soft tissue of the mussel *Mytilus galloprovincialis* collected from Lake Faro (Sicily Italy). *Environment International,* 30 (6), 805-810.

Mikac, N., Kwokal, Z., Martincić, D., Branica, M., 1996. Uptake of mercury species by transplanted mussels *Mytilus galloprovincialis* under estuarine conditions (Krka River estuary). *The Science of the Total Environment,* 184 (3), 173-182.

Ministerial Decree of 10/01/2000. Identification of qualifications equivalent to those of a blind switchboard operator, for application of Law n. 113 of 29/03/1999, pursuant to provisions of Art. 45, paragraph 12 of Law n. 144 of 17/05/1999.

Muñoz, O., Devesa, V., Suñer, M.A., Vélez, D., Montoro, R. *et al.,* 2000. Total and inorganic arsenic in fresh and processed fish products. *Journal of Agricultural & Food Chemistry*, 48 (9), 4369-4376.

Muñoz, O., Vélez, D., Montoro, R., 1999. Optimization of the solubilization, extraction and determination of inorganic arsenic [As(iii) + As(v)] in seafood products by acid digestion, solvent extraction and hydride generation atomic absorption spectrometry. *The Analyst*, 124 (4), 601-607.

Nauen, C.N., 1983. Compilation of legal limits for hazardous substances in fish and fishery products. *FAO Fisheries Circular*, No.764. FAO, Rome, 102 pp.

O'Connor, T.P., 2002. National distribution of chemical concentrations in mussels and oysters in the USA. *Marine Environmental Research*, 53 (2), 117-143.

Orescanin, V., Lovrencic, I., Mikelic, L., Barisic, D., Matasin, Z. *et al.,* 2006. Biomonitoring of heavy metals and arsenic on the east coast of the Middle Adriatic Sea using *Mytilus galloprovincialis*. *Nuclear Instruments & Methods in Physics Research*, *Section B*, 245 (2), 495-500.

Otten, J.J., Hellwig, J.P., Meyers, L.D. (Eds), 2006. *Dietary reference intakes: the essential guide to nutrient requirements*. National Academies Press, Washington, D.C., 1330 pp.

Ozlem, S., Görkem, Y., 2004. Determination of trace metal levels in *Mytilus galloprovincialis* collected from Izmir bay, Turkey. *Fresenius Environmental Bulletin*, 14 (9), 777-782.

Penrose, W.R., Conacher, H.B., Black, R., Méranger, J.C., Miles, W., Cunningham, H.M., Squires, W.R., 1997. Implications of inorganic/organic interconversion on fluxes of arsenic in marine food webs. *Environmental Health Perspectives*, 19, 53-59.

Rainbow, P.S., 1995. Biomonitoring of heavy metal availability in the marine environment. *Marine Pollution Bulletin*, 31 (4-12), 183-192.

Rainbow, P.S., Dallinger, R., 1993. Metal uptake, regulation, and excretion in freshwater invertebrates. p. 119-132. In: *Ecotoxicology of metals in invertebrates*. Rainbow, P.S., Dallinger, R. (Eds). Lewis Publishers, Boca Raton.

Saavedra, Y., Gonzalez, A., Fernandez, P., Blanco, J., 2004.

The effect of size on trace metal levels in raft cultivated mussels (*Mytilus galloprovincialis*). *The Science of the Total Environment*, 318 (1-3), 115-124.

Santovito, G., Piccinni, E., Cassini, A., Irato, P., Albergoni, V., 2005. Antioxidant responses of the Mediterranean mussel, *Mytilus galloprovincialis*, to environmental variability of dissolved oxygen. *Comparative Biochemistry & Physiology Part C: Toxicology & Pharmacology*, 140 (3-4), 321-329.

Shahidul-Islam, M., Tanaka, M., 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. *Marine Pollution Bulletin*, 48 (7-8), 624-649.

Simonič, M., 2009. Removal of inorganic As^{5+} from a small drinking water system. *Journal of the Serbian Chemical Society*, 74 (1), 85-92

Spada, L., Annicchiarico, C., Cardellicchio, N., Giandomenico, S., Di Leo A., 2012. Mercury and methylmercury concentrations in Mediterranean seafood and surface sediments, intake evaluation and risk for consumers. *International Journal of Hygiene & Environmental Health*, 215 (3), 418-426.

Spagnoli, F., Specchiulli, A., Scirocco, T., Carapella, G., Villani, P. *et al.,* 2002. The Lago di Varano: hydrologic characteristics and sediment composition. *Marine Ecology*, 23 (Suppl. 1), 384-394.

Stankovic, S., Jovic, M., Stankovic, A.R., Katsikas, L., 2012. Heavy metals in seafood mussels. Risk for human health. p. 311-373. In: *Environmental Chemistry for a Sustainable World. Volume 1: Nanotechnology and Health Risk.* Lichtfouse, E., Schwarzbauer, J., Robert, D. (Eds). Springer, Netherlands.

Storelli, M.M, Marcotrigiano, G.O., 2001. Heavy metal monitoring in fish, bivalve mollusc, water and sediment from Varano Lagoon, Italy. *Bulletin of Environmental Contamination & Toxicology*, 66 (3), 365-370.

Tenabe, S., 2000. Asia-Pacific mussel watch progress report. Editorial. *Marine Pollution Bulletin*, 40 (8), 651.

Topcuoğlu, S., Kirbaşoğlu, C., Yilmaz, Y.Z., 2004. Heavy metal levels in biota and sediments in the northern coast of the Marmara Sea. *Environmental Monitoring & Assessment*, 96 (1-3), 183-189.

U.S. EPA, 1994. *Guidance for assessing chemical contamination data for use in fish advisories. Risk assessment and fish consumption limits.* EPA 823-B94-004. Washington, D.C.

U.S. EPA, 2007. *Toxicity and exposure assessment for children's health. Inorganic arsenic. TEACH Chemical Summary*. http://www.epa.gov/teach/chem_summ/Arsenic_summary.pdf (Accessed on 23 October 2012).

Velez, D., Ybanez, N., Montoro, R., 1995. Percentages of total arsenic represented by arsenobetaine levels of manufactured seafood products. *Journal of Agricultural & Food Chemistry*, 143 (5), 1289-1294.

WHO, 2006. Evaluation of certain contaminants in food: sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series*, *No. 930.* WHO, Geneva, 100 pp.

WHO, 2011. Evaluation of certain contaminants in food: seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series, No. 959*. WHO, Geneva, 105 pp.