

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

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### 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 particular, 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).

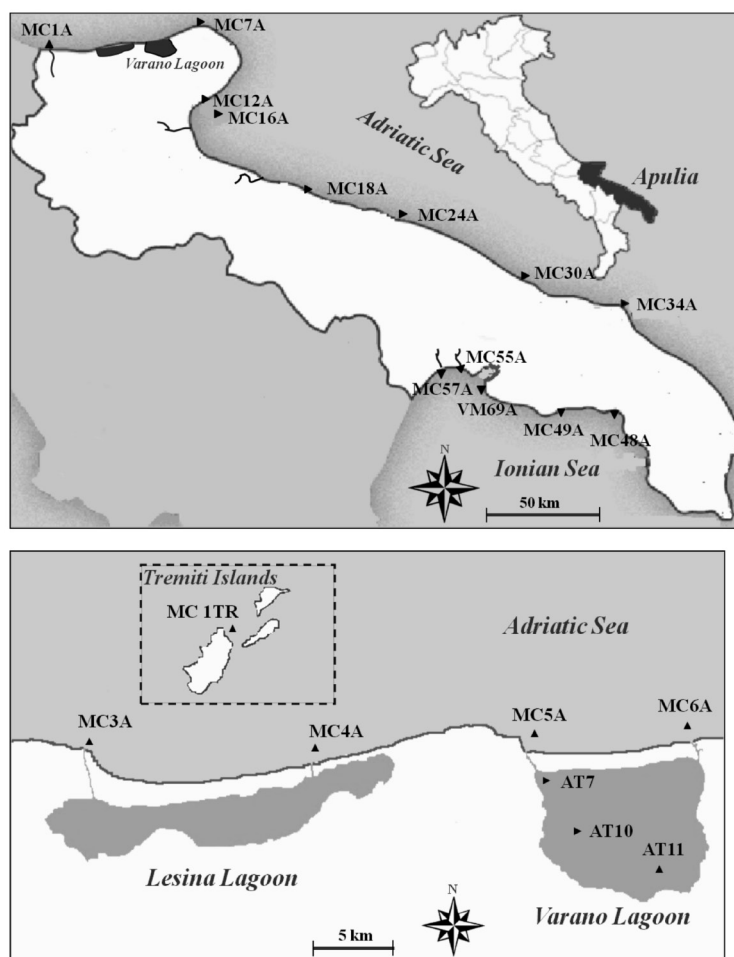
For these reasons, state monitoring programmes, known as "Mussel Watch", have been applied to monitoring the environmental situation of different coastal regions (Kimbrough *et al.*, 2008).

As intertidal filter feeders, mussels accumulate heavy metals in their tissues in proportion to the degree of environmen-

tal 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
	AT7	Varano Lagoon	cultured	41°54'03"N	15°41'10"E
	AT10	Varano Lagoon	cultured	41°53'00"N	15°43'08"E
Adriatic Sea	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 Capioale 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<sub>3</sub> (70% v/v) and 1 ml H<sub>2</sub>O<sub>2</sub> (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<sub>3</sub> 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  $\pm$  standard deviation.

Element	Certified	Measured <sup>a</sup>
Arsenic	6.07 $\pm$ 0.13	6.12 $\pm$ 0.19
Cadmium	0.348 $\pm$ 0.007	0.343 $\pm$ 0.018
Copper	9.45 $\pm$ 0.13	9.52 $\pm$ 0.41
Chromium	0.78 $\pm$ 0.06	0.75 $\pm$ 0.09
Lead	2.00 $\pm$ 0.04	2.09 $\pm$ 0.13
Mercury	0.196 $\pm$ 0.009	0.188 $\pm$ 0.016
Zinc	83.1 $\pm$ 1.7	82.7 $\pm$ 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 Pate-misco 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 Pate-misco 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 <sup>a</sup>	As	Cd	Cr	Cu	Hg	Ni	Pb	V
MC 55A	4.66	25.93 $\pm$ 2.11	0.38 $\pm$ 0.04	9.46 $\pm$ 0.91	7.90 $\pm$ 0.81	0.10 $\pm$ 0.01	15.14 $\pm$ 1.66	2.32 $\pm$ 0.27	3.80 $\pm$ 0.39
MC 57A	5.27	28.21 $\pm$ 2.88	0.38 $\pm$ 0.04	5.50 $\pm$ 0.33	7.00 $\pm$ 0.67	0.10 $\pm$ 0.01	5.94 $\pm$ 0.47	2.16 $\pm$ 0.19	4.60 $\pm$ 0.43
MC 48A	4.52	25.7 $\pm$ 2.17	1.14 $\pm$ 0.16	3.56 $\pm$ 0.33	6.05 $\pm$ 0.47	0.12 $\pm$ 0.01	3.28 $\pm$ 0.29	1.07 $\pm$ 0.13	2.20 $\pm$ 0.17
MC 49A	6.36	60.36 $\pm$ 7.21	1.10 $\pm$ 0.11	2.50 $\pm$ 0.21	7.62 $\pm$ 0.63	0.31 $\pm$ 0.03	3.71 $\pm$ 0.32	1.57 $\pm$ 0.17	3.33 $\pm$ 0.23
VM69A	6.33	34.22 $\pm$ 3.11	0.48 $\pm$ 0.04	1.81 $\pm$ 0.17	5.89 $\pm$ 0.35	0.16 $\pm$ 0.01	1.02 $\pm$ 0.09	1.12 $\pm$ 0.11	1.40 $\pm$ 0.10
AT 7	6.09	7.06 $\pm$ 0.55	2.54 $\pm$ 0.39	2.58 $\pm$ 0.19	7.72 $\pm$ 0.61	0.63 $\pm$ 0.04	2.74 $\pm$ 0.26	0.83 $\pm$ 0.05	1.34 $\pm$ 0.11
AT 10	4.55	6.35 $\pm$ 0.34	1.32 $\pm$ 0.15	2.14 $\pm$ 0.14	5.26 $\pm$ 0.48	0.16 $\pm$ 0.01	2.08 $\pm$ 0.18	1.21 $\pm$ 0.08	1.32 $\pm$ 0.16
AT 11	6.19	24.95 $\pm$ 1.97	1.54 $\pm$ 0.17	3.07 $\pm$ 0.32	6.73 $\pm$ 0.59	0.14 $\pm$ 0.01	5.21 $\pm$ 0.52	1.50 $\pm$ 0.11	2.66 $\pm$ 0.21
MC 1A	5.06	17.10 $\pm$ 1.36	1.10 $\pm$ 0.13	2.35 $\pm$ 0.37	10.99 $\pm$ 0.98	0.81 $\pm$ 0.06	5.94 $\pm$ 0.55	1.21 $\pm$ 0.11	3.05 $\pm$ 0.29
MC 3A	3.79	22.92 $\pm$ 2.05	0.56 $\pm$ 0.04	2.76 $\pm$ 0.44	7.90 $\pm$ 0.45	0.20 $\pm$ 0.01	6.22 $\pm$ 0.61	1.21 $\pm$ 0.16	3.02 $\pm$ 0.26
MC 4A	7.63	18.61 $\pm$ 1.44	0.74 $\pm$ 0.07	2.22 $\pm$ 0.32	6.32 $\pm$ 0.43	0.66 $\pm$ 0.04	3.76 $\pm$ 0.29	1.89 $\pm$ 0.17	2.22 $\pm$ 0.19
MC 5A	7.38	9.32 $\pm$ 0.91	1.84 $\pm$ 0.17	2.78 $\pm$ 0.38	7.76 $\pm$ 0.55	0.62 $\pm$ 0.04	3.48 $\pm$ 0.33	3.25 $\pm$ 0.28	2.48 $\pm$ 0.18
MC 6A	4.42	6.46 $\pm$ 0.77	1.54 $\pm$ 0.11	0.96 $\pm$ 0.07	5.62 $\pm$ 0.49	0.32 $\pm$ 0.03	1.12 $\pm$ 0.09	0.37 $\pm$ 0.32	0.94 $\pm$ 0.09
MC 7A	4.94	17.27 $\pm$ 1.53	0.58 $\pm$ 0.06	4.14 $\pm$ 0.32	7.14 $\pm$ 0.53	0.23 $\pm$ 0.01	5.06 $\pm$ 0.41	1.67 $\pm$ 0.18	4.96 $\pm$ 0.48
MC12A	4.36	17.60 $\pm$ 1.08	0.85 $\pm$ 0.07	3.60 $\pm$ 0.26	6.78 $\pm$ 0.59	0.13 $\pm$ 0.01	2.76 $\pm$ 0.31	1.19 $\pm$ 0.15	3.68 $\pm$ 0.37
MC 16A	4.57	17.12 $\pm$ 1.03	0.52 $\pm$ 0.05	2.68 $\pm$ 0.17	8.59 $\pm$ 0.86	0.14 $\pm$ 0.02	1.90 $\pm$ 0.17	1.29 $\pm$ 0.16	2.50 $\pm$ 0.33
MC 18A	4.48	21.54 $\pm$ 1.76	0.81 $\pm$ 0.06	3.44 $\pm$ 0.29	7.10 $\pm$ 0.66	0.13 $\pm$ 0.01	2.56 $\pm$ 0.27	1.02 $\pm$ 0.09	2.30 $\pm$ 0.19
MC 24A	4.63	76.17 $\pm$ 5.32	0.72 $\pm$ 0.05	1.78 $\pm$ 0.08	19.22 $\pm$ 1.54	0.26 $\pm$ 0.02	2.16 $\pm$ 0.18	2.23 $\pm$ 0.25	3.38 $\pm$ 0.31
MC 34A	5.44	23.12 $\pm$ 2.83	0.52 $\pm$ 0.05	3.00 $\pm$ 0.29	4.66 $\pm$ 0.32	0.24 $\pm$ 0.02	2.50 $\pm$ 0.17	1.15 $\pm$ 0.12	1.16 $\pm$ 0.11
MC 30A	5.87	26.31 $\pm$ 2.05	0.73 $\pm$ 0.08	2.12 $\pm$ 0.18	6.42 $\pm$ 0.54	0.12 $\pm$ 0.01	2.53 $\pm$ 0.21	1.12 $\pm$ 0.08	1.75 $\pm$ 0.16
MC 1 TR	6.55	35.21 $\pm$ 2.98	1.04 $\pm$ 0.17	8.19 $\pm$ 0.65	6.74 $\pm$ 0.55	0.13 $\pm$ 0.01	6.15 $\pm$ 0.54	1.93 $\pm$ 0.18	5.52 $\pm$ 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:

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

where  $C_m$  represents metal concentration in fish or seafood ( $\mu\text{g/g w.w.}$ );  $IR$  the ingestion rate ( $\text{g/d}$ ) of fish or seafood and  $B_w$  represents body weight ( $\text{kg}_{bw}$ ). Dietary metal exposure is expressed as  $\mu\text{g/kg}_{bw}$  day. In order to calculate total daily exposure, average seafood consumption of  $70 \text{ g/d}$  was considered (weekly consumption of  $490 \text{ g}$ ). This value is  $10 \text{ g}$  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  $0.015 \text{ mg/kg}$  (WHO, 2011) of body weight per week, or  $129 \mu\text{g/day}$  for a subject of  $60 \text{ kg}$ . 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 ( $0.015 \text{ mg/kg}$ ). 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  $0.007 \text{ mg/kg bw}$  ( $0.42 \text{ mg}$  per week for a  $60\text{-Kg}$  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  $3.5 \text{ mg/kg bw/week}$  (equivalent to  $210 \text{ mg/week}$  for a  $60 \text{ Kg-adult}$ ) and at  $7.0 \text{ mg/kg bw/week}$  (equivalent to  $420 \text{ mg/week}$  for a  $60 \text{ Kg-adult}$ ), 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 Izmir 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 increase blood pressure and cardiovascular diseases in adults (EC, 2001). The PTWI for Pb, established by the Joint FAO/WHO (2000) is  $0.025 \text{ mg/kg bw/week}$  (equivalent to  $1.5 \text{ mg/week}$  for a  $60 \text{ Kg-adult}$ ).

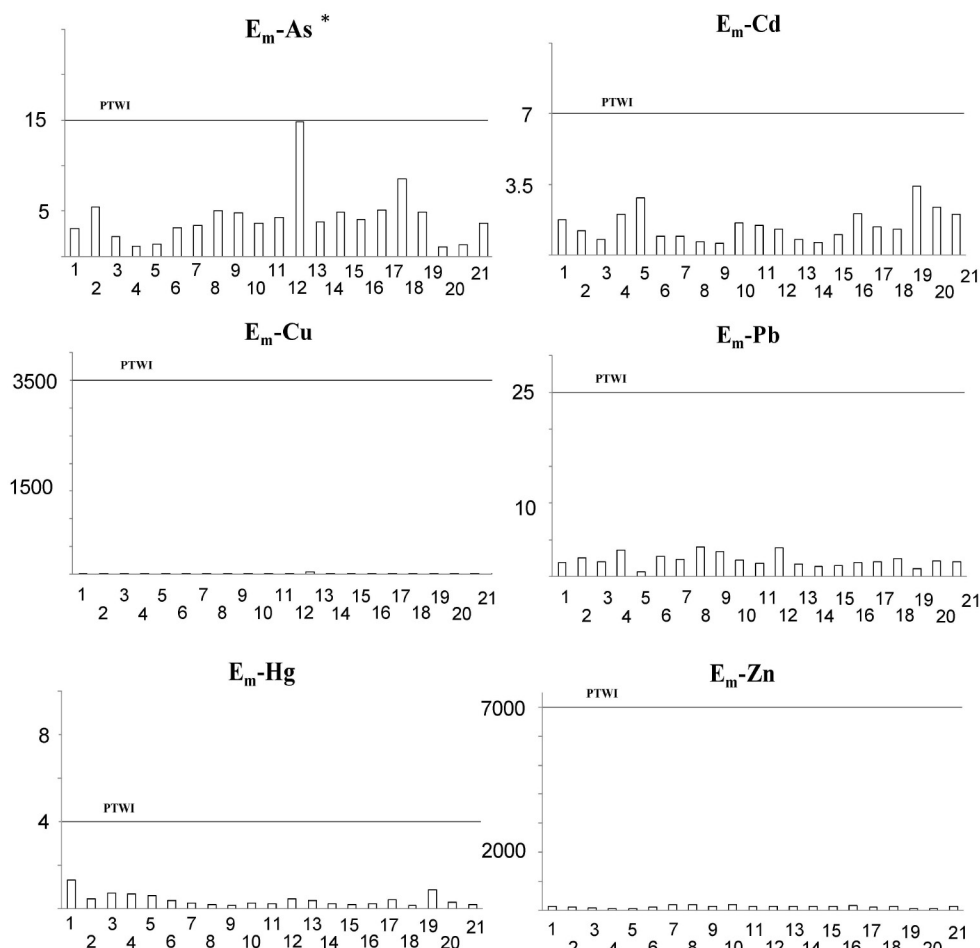
As shown in Figure 2, the estimated PTWIs of Pb for Apulian Sea mussels were below the established PTWI. Pb levels of this study were lower than those reported for the Croatian Coast (Kljakovic-Gašpic *et al.*, 2010; Orescanin *et al.*, 2006), Balearic Islands (Deudero *et al.*, 2009) and Venice Lagoon (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  $25\text{--}40\%$  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  $0.004 \text{ mg/kg bw}$  (WHO, 2011). The previous PTWI of  $0.005 \text{ mg/kg bw}$  for total Hg ( $0.3 \text{ mg/week}$  for a  $60 \text{ Kg-adult}$ ), 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



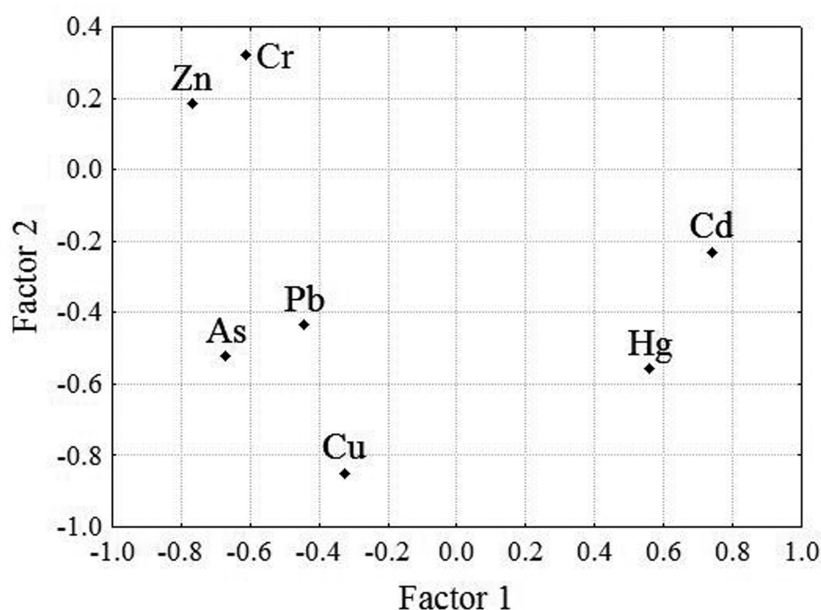
\*The  $E_m$ -As has been calculated considering the 11% (Munoz *et al.*, 2000) of the total concentration of As.

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  $\mu\text{g/kg}$  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 <i>et al.</i> , 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 <i>et al.</i> , 2004
Mar Piccolo, Taranto (Ionian Sea)	dry					0.63			Spada <i>et al.</i> , 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 <i>et al.</i> , 2004
Izimir Bay (Aegean Sea)	dry		0.02	0.14	4.48	0.02	27.70	0.24	Kucuksezgin <i>et al.</i> , 2008
Croatian Coasts (Adriatic Sea)	dry		0.99	1.72	10.53	0.25	158.00	4.21	Kljakovic-Gašpic <i>et al.</i> , 2010
Croatian Coasts (Adriatic Sea)	dry	15.2		1.85	6.1			4.05	Orescanin <i>et al.</i> , 2006
Montenegro Coasts (Adriatic Sea)	dry			3.04	8.26		119.60		Joksimovic <i>et al.</i> , 2011
Baleari Islands (Western Mediterranean)	dry	9.05	2.83	0.53	4.76	0.2	234.16	2.48	Deudero <i>et al.</i> , 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 from 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

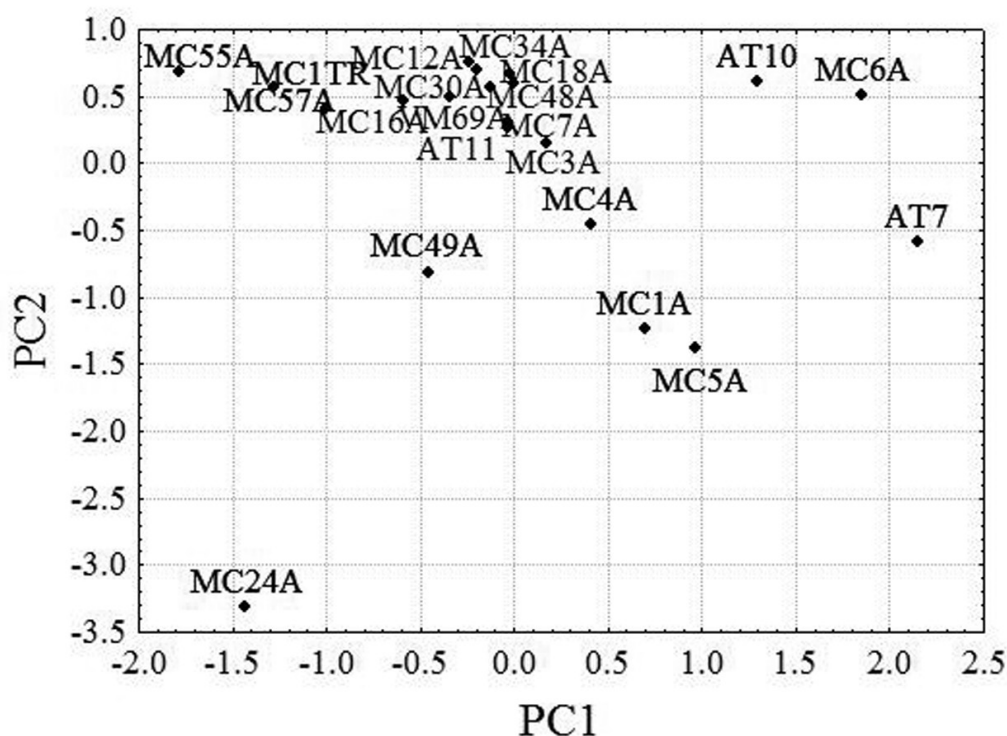


Fig. 4: Scatter plot of the scores for the first two principal components.

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.

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