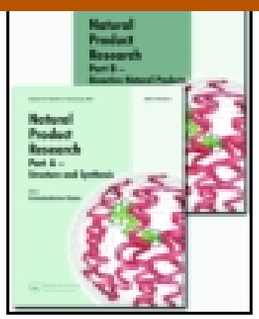
View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Archivio istituzionale della ricerca - Università di Modena e Reggio Emilia



Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

Toxic mineral elements in *Mytilus galloprovincialis* from Sicilian coasts (Southern Italy)

Gaetano Cammilleri, Paola Galluzzo, Andrea Pulvirenti, Innocenzo Ezio Giangrosso, Gianluigi Maria Lo Dico, Giovanna Montana, Nadia Lampiasi, Maria Alessandra Mobilia, Antonio Lastra, Mirella Vazzana, Antonio Vella, Pietro La Placa, Andrea Macaluso & Vincenzo Ferrantelli

To cite this article: Gaetano Cammilleri, Paola Galluzzo, Andrea Pulvirenti, Innocenzo Ezio Giangrosso, Gianluigi Maria Lo Dico, Giovanna Montana, Nadia Lampiasi, Maria Alessandra Mobilia, Antonio Lastra, Mirella Vazzana, Antonio Vella, Pietro La Placa, Andrea Macaluso & Vincenzo Ferrantelli (2019): Toxic mineral elements in *Mytilus galloprovincialis* from Sicilian coasts (Southern Italy), Natural Product Research, DOI: 10.1080/14786419.2019.1610963

To link to this article: https://doi.org/10.1080/14786419.2019.1610963

View supplementary material

Published online: 03 Jul 2019.

Submit your article to this journal

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=gnpl20

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2019.1610963

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Check for updates

Toxic mineral elements in *Mytilus galloprovincialis* from Sicilian coasts (Southern Italy)

Gaetano Cammilleri[a,b], Paola Galluzzo[a], Andrea Pulvirenti[b], Innocenzo Ezio Giangrosso[a], Gianluigi Maria Lo Dico[a], Giovanna Montana[c], Nadia Lampiasi[c], Maria Alessandra Mobilia[d], Antonio Lastra[a], Mirella Vazzana[e], Antonio Vella[a], Pietro La Placa[a], Andrea Macaluso[a] and Vincenzo Ferrantelli[a]

[a]Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy; [b]Dipartimento Scienze della Vita, Università degli Studi di Modena e Reggio Emilia, Modena, Italy; [c]Istituto di Biomedicina ed Immunologia Molecolare "Alberto Monroy", Consiglio Nazionale delle Ricerche, Palermo, Italy; [d]Freelance Medical Doctor, Messina, Italy; [e]Dipartimento STEBICEF, Università degli studi di Palermo, Palermo, Italy

ABSTRACT

We assessed the relationship between V, Cr, Mn, Hg, As, Cd, Sn, Sb and Pb concentrations in *Mytilus galloprovincialis* samples from the coasts of Sicily and the expression of *metallothioneins*. Toxic mineral elements assessment was carried out by A.A. Spectrometry and ICP-MS. The *metallothioneins* expression was performed by q-PCR method. Low metals' levels were found in the mussel samples examined, in comparison with what was reported in literature. The highest mean values of toxic mineral elements were found in Gela (Cr 0.178 ± 0.03 mg/Kg, Mn 4.325 ± 0.012 mg/Kg, As 3.706 ± 0.009 mg/Kg, Sn 0.148 ± 0.014 mg/Kg, Sb 0.009 ± 0.004 mg/Kg e Pb 0.364 ± 0.01 mg/Kg). Significant levels of Hg were found in samples from Catania (0.014 ± 0.005 mg/Kg). Only vanadium and lead concentrations showed significant differences between sampling areas ($p < 0.05$). Molecular analysis verified a basal expression of *Mt1* and the absence of over-expression of *Mt2*, confirming the low mineral's concentrations found in the samples examined.

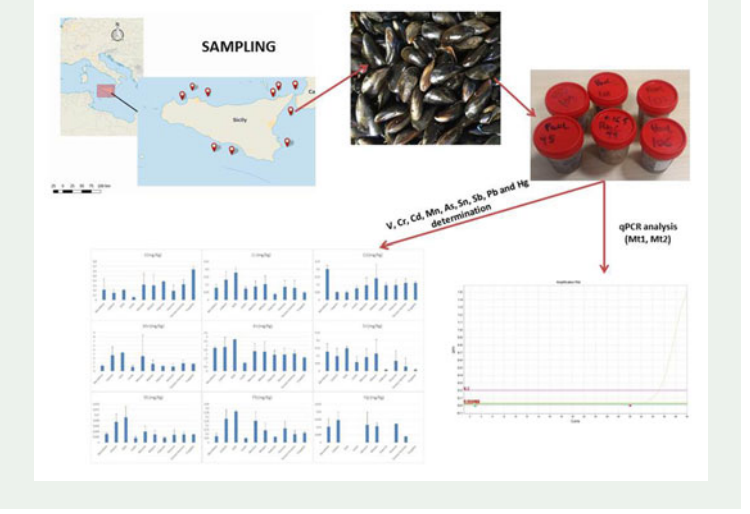
ARTICLE HISTORY
Received 26 March 2019
Accepted 20 April 2019

KEYWORDS
Mussels; biomarkers; toxic mineral elements; metallothioneins

CONTACT Paola Galluzzo paola.galluzzo82@gmail.com

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2019.1610963.

© 2019 Informa UK Limited, trading as Taylor & Francis Group



1. Introduction

Pollution by toxic metals in marine environment increased considerably because of anthropogenic activities, leading to an increase of the number of studies on the presence of these elements in different species of the marine ecosystem (Lo Turco et al. 2013; Di Bella et al. 2015; Naccari et al. 2015; Giangrosso et al. 2016; Cammilleri et al. 2018). Toxic metals bioaccumulates in the aquatic food chain and reach the highest concentration in the upper trophic levels (Di Bella et al. 2010, 2017). At present, many studies have focused on the application of innovative techniques for the detection of organic and inorganic contaminants in the environment for biomonitoring purpose (Campone et al. 2016, 2018a,b, 2019). Biomonitoring, as proved strategy to detect inorganic contaminants in Mediterranean Sea, has widely employed some species representative of different trophic levels (Di Bella et al. 2013, 2018). *Mytilus galloprovincialis* is a bivalve mollusc belonging to the *Mytilidae* family, often used as a sentinel organism to monitor the biological effects associated with the presence of toxic metals (Catsiki and Florou 2006; Fasulo et al. 2008). These contaminants can produce alterations of biochemical and physiological processes that can be quantified by estimating biological parameters whose variations may be related to the physiological state of animals (Bolognesi et al. 1996; Fasulo et al. 2008). Among the detoxification mechanisms, *metallothioneins* (Mt) induction in response to toxic metals exposure is certainly the most well documented (Nordberg 1998; Domouhtsidou et al. 2004). The biological response may also depend from seasonal changes, which in turn can influence the sensitivity to pollutants (Ivanković et al. 2005).

Considering that the Mt production is induced by exposure to toxic metals, the purposes of this work are to estimate a correlation between V, Cr, Mn, As, Cd, Sn, Sb and Pb levels in *M. galloprovincialis* samples from Sicilian coasts and the metallothioneins expression by molecular methods.

2. Results and discussion

The linearity test of the ICP-MS method for V, Cr, Mn, Cu, As, Cd, Sn, Sb and Pb analysis gave satisfactory results ($r^2 > 0.999$). The LOD and LOQ values obtained (Table S3) were in accordance with the regulation (CE) n. 333/2007. Recoveries studies showed values between 96% and 105%. All the results were satisfactory for limit of repeatability (metrological approach). All concentration levels showed Horrat values of repeatability and reproducibility lower than 2 as specified by the Commision Regulation n° 836/2011. The DMA-80 has been used according to the US EPA method 7473 (Di Bella et al., 2018) and the validation results of the Hg determination method are shown in Table S4. The method developed was able to determine the Hg concentration of the samples in a wide range of concentrations (0.050–2.00 mg/kg) with a mean recovery of 103%.

The average toxic mineral elements concentrations obtained from *M. galloprovincialis* samples sorted by sampling area are shown in Figure S1. All the samples analysed showed V, Cr, Mn, Cu, As, Cd, Sn, Sb and Pb values above the limits of the quantification of the method. Whereas, only 29% of the samples examined showed Hg values below the LOQ.

The highest mean values were reached in samples from Trappeto for V (0.635 ± 0.057 mg/Kg), Gela for Cr (0.178 ± 0.03 mg/Kg), Mn (4.325 ± 0.012 mg/Kg), As (3.706 ± 0.009 mg/Kg), Sn (0.148 ± 0.014 mg/Kg), Sb (0.009 ± 0.004 mg/Kg) and Pb (0.364 ± 0, 01 mg/Kg), Barcellona PG for Cd (0.201 ± 0.025 mg/Kg) and Catania for Hg (0.014 ± 0.005 mg/Kg). The highest values were found in Barcelona P.G. for V (0.701 mg/Kg), Catania for Cr (0.178 mg/Kg), Messina for Mn and Pb (13.644 mg/Kg and 0.402 mg/Kg), Catania for As, Cd and Sb (3.706 mg/Kg, 0.05 mg/Kg and 0.03 mg/Kg), Milazzo for Sn (0.222 mg/Kg) and Syracuse for Hg (0.027 mg/Kg). All the *M. galloprovincialis* samples from Gela, Licata, Palermo and Trappeto showed Hg levels below the quantification limit of the method. The Kruskal-Wallis test revealed statistically significant differences between sampling areas ($p < 0.05$) for V and Pb levels, whereas no differences were found between sampling areas ($p > 0.05$) for Mn, Cr, As, Cd, Sn, Sb and Hg levels. Dunn's post-hoc test showed that samples from Palermo and Trappeto contribute predominantly to the differences between data groups for V, whereas Gela and Catania for Cr and Pb. The average values of toxic mineral elements obtained in this study are lower than the limits imposed by the EC Reg. 1881/2006, suggesting the absence of important risks in the sampling areas studied. The values obtained in this study are also significantly lower than those found by Maanan (2007) in mussel samples from Morocco, with average concentrations of Cd, Pb, Hg, Cr and Mn from 7 to 10 times higher than those of our study. Furthermore, our Cd, Pb, Hg, Cr and Mn values are 3 to 10 times lower than what showed by Orescanin et al. (2006) in the Adriatic coasts of Croatia. The samples from Gela revealed the highest levels of chromium, manganese, arsenic, tin and antimony, probably due to the intense industrial activity registered in this area (Ministero dell'Ambiente e della Tutela del Territorio e del Mare et al. 2008).

The highest mercury concentrations were found in mussels samples from Catania coast; these values could be related to the Etna volcanic activity, considered one of the major natural sources of mercury release into the environment (Martin et al. 2012). These considerations are further confirmed by the highest concentrations of mercury in samples of mussels taken along the eastern coast of Sicily (Messina, Milazzo and

Syracuse). The mRNA expression study in mussels examined showed no significant changes in the pollution response genes. Our findings are in contrast to what was found by Fasulo et al. (2008) on the expression of methallothioneins in *M. galloprovincialis* samples from Faro lake (Messina, Southern Italy) probably due to the low concentrations of toxic mineral elements found in our work and the absence of significant differences between sampling areas of the major inductors of metallothioneins expression (Cr, Cd, Pb and Sn). Mussels and other marine invertebrates tend to accumulate pollutants in their tissues (Odžak et al. 1994; Salvo et al. 2016), without showing any apparent harmful effect, probably because they react to the presence of high concentrations of xenobiotics. The results of this study showed an overall absence of risks in the sampling areas examined. As far we know, this is the first report on the presence of V, Cr, Mn, Sn and Sb in *M. galloprovincialis* samples from central Mediterranean sea, suggesting that further studies on the use of stress indicator markers are needed to have a comprehensive evaluation of inorganic pollutants presence in Mediterranean Sea.

3. Experimental

See the supplementary material for the experimental section.

Acknowledgements

This work was financially supported by the Italian Ministry of Health through an Ricerca Corrente project (RC IZS SI 05/14). The authors are grateful to Dr. Ladislao La Scala, Dr. Giovanni Lo Cascio and Mrs. Barbara Randisi for the technical support during the sampling plan and the technical support during the analysis.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Ministero della Salute.

ORCID

Gaetano Cammilleri http://orcid.org/0000-0001-6300-6571
Paola Galluzzo http://orcid.org/0000-0003-1766-5327
Pietro La Placa http://orcid.org/0000-0001-5402-2510

References

Bolognesi C, Rabboni R, Roggieri P. 1996. Genotoxicity biomarkers in *M. galloprovincialis* as indicators of marine pollutants. Comp Biochem Physiol C Toxicol Pharmacol Endocrinol. 113(2): 319–323.

Cammilleri G, Vazzana M, Arizza V, Giunta F, Vella A, Dico GL, Giaccone V, Giofrè SV, Giangrosso G, Cicero N, Ferrantelli V. 2018. Mercury in fish products: what's the best for consumers between bluefin tuna and yellowfin tuna? Nat Prod Res. 32(4):457–462.

Campone L, Piccinelli AL, Celano R, Pagano I, Di Sanzo R, Carabetta S, Russo M, Rastrelli L. 2018. Occurrence of aflatoxin M1 in milk samples from Italy analysed by online-SPE UHPLC-MS/MS. Nat Prod Res. 32(15):1803–1808.

Campone L, Piccinelli AL, Celano R, Pagano I, Russo M, Rastrelli L. 2016. Rapid and automated analysis of aflatoxin M1 in milk and dairy products by online solid phase extraction coupled to ultra-high-pressure-liquid-chromatography tandem mass spectrometry. J Chromatogr A. 1428:212–219.

Campone L, Piccinelli AL, Celano R, Pagano I, Russo M, Rastrelli L. 2018. Rapid and automated on-line solid phase extraction HPLC-MS/MS with peak focusing for the determination of ochratoxin A in wine samples. Food Chem. 244(244):128–135.

Campone L, Celano R, Piccinelli AL, Pagano I, Cicero N, Di Sanzo R, Carabetta S, Russo M, Rastrelli L. 2019. Ultrasound assisted dispersive liquid-liquid microextraction for fast and accurate analysis of chloramphenicol in honey. Food Res Int. 115:572–579.

Catsiki VA, Florou H. 2006. Study on the behavior of the heavy metals Cu, Cr, Ni, Zn, Fe, Mn and 137 Cs in an estuarine ecosystem using *Mytilus galloprovincialis* as a bioindicator species: the case of Thermaikos gulf, Greece. J Environ Radioact. 86(1):31–44.

Commission Regulation (EC). No 1881/06 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

Commission Regulation (EU). No 836/2011, of 19 August 2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.

Commission Regulation (EC). No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.

Di Bella G, Cavallaro N, Lo Turco V, Furci P, Rando R, La Pera L, Dugo G. 2010. Autochthonous clams monitoring of Ganzirri Lake (Sicily). Environ Monit Assess. 171(1-4):281–287.

Di Bella G, Lo Turco V, Potortì AG, Rando R, Licata P, Dugo G. 2013. Statistical analysis of heavy metals in Cerastoderma edule glaucum and Venerupis aurea laeta from Ganzirri Lake, Messina (Italy). Environ Monit Assess. 185(9):7517–7525.

Di Bella G, Potortì AG, Lo Turco V, Bua D, Licata P, Cicero N, Dugo G. 2015. Trace elements in *Thunnus thynnus* from Mediterranean Sea and benefit–risk assessment for consumers. Food Addit Contam Part B Surveill. 8(3):175–181.

Di Bella G, Russo E, Dugo G. 2017. Heavy metals and persistent organic pollutants in marine organisms from two sicilian protected areas: strait of Messina and Cape Peloro Lakes. Curr Org Chem. 21(5):387–394.

Di Bella G, Tardugno R, Cicero N. 2018. Investigation of Hg content by a rapid analytical technique in Mediterranean pelagic fishes. Separations. 5(4):51.

Di Bella G, Pizzullo G, Bua GD, Potortì AG, Santini A, Giacobbe S. 2018. Mapping toxic mineral contamination: the southern oyster drill, *S. haemastoma* (L., 1767), as evaluable sentinel species. Environ Monit Assess. 190(1):7.

Domouhtsidou GP, Dailianis S, Kaloyianni M, Dimitriadis VK. 2004. Lysosomal membrane stability and metallothionein content in *Mytilus galloprovincialis* (L.), as biomarkers Combination with trace metal concentrations. Mar Pollut Bull. 48(5-6):572–586.

Fasulo S, Mauceri A, Giannetto A, Maisano M, Bianchi N, Parrino V. 2008. Expression of metallothionein mRNAs by in situ hybridization in the gills of *Mytilus galloprovincialis*, from natural polluted environments. Aquat Toxicol. 88(1):62–68.

Giangrosso G, Cammilleri G, Macaluso A, Vella A, D'Orazio N, Graci S, Lo Dico GM, Galvano F, Giangrosso M, Ferrantelli V. 2016. Hair mercury levels detection in fishermen from Sicily (Italy) by ICP-MS method after microwave-assisted digestion. Bioinorg Chem Appl. 2016: 5408014. http://dx.doi.org/10.1155/2016/5408014.

Helsel DR. 2005. Nondetects and data analysis: statistics for censored environmental data. Hoboken (NJ): Wiley, p. 48.

Ministero dell'ambiente e della Tutela del Territorio e del Mare. 2008. Interventi di riqualificazioni ambientali funzionali alla reindustrializzazione e infrastrutturazione delle aree comprese nel Sito di Interesse Nazionale di "Priolo". . https://www.minambiente.it/sites/default/files/archivio/allegati/bonifiche/accordo_priolo_07_11_08.pdf.

Ivanković D, Pavičić I, Erk M, Filipović-Marijić V, Raspor B. 2005. Evaluation of the *Mytilus galloprovincialis* Lam. Digestive gland metallothionein as a biomarker in a long-term field study: seasonal and spatial variability. Mar Pollut Bull. 50:1303–1313.

Lo Turco V, Di Bella G, Furci P, Cicero N, Pollicino G, Dugo G. 2013. Heavy metals content by ICP-OES in *Sarda sarda*, *Sardinella aurita* and *Lepidopus caudatus* from the Strait of Messina (Sicily, Italy). Nat Prod Resea. 27(6):518–523.

Maanan M. 2007. Biomonitoring of heavy metals using *Mytilus galloprovincialis* in Safi coastal waters, Morocco. Environ Toxicol. 22(5):525–531.

Martin R, Witt MLI, Sawyera GM, Thomas HE, Watt SFL, Bagnato E, Calabrese S, Aiuppa A, Delmelle P, Pyle DM, et al. 2012. Bioindication of volcanic mercury (Hg) deposition around Mt. Etna (Sicily). Chem Geo. 310:12–22.

Naccari C, Cicero N, Ferrantelli V, Giangrosso G, Vella A, Macaluso A, Naccari F, Dugo G. 2015. Toxic metals in pelagic, benthic and demersal fish species from Mediterranean FAO zone 37. Bull Environ Contam Toxicol. 95(5):567–573.

Nordberg M. 1998. Metallothioneins: historical review and state of knowledge. Talanta. 46(2): 243–254.

Odžak N, Martinčić D, Zvonarié T, Branica M. 1994. Bioaccumulation rate of Cd and Pb in *Mytilus galloprovincialis* foot and gills. Marine Chemistry. 46(1-2):119–131.

Orescanin V, Lovrencic I, Mikelic L, Barisic D, Matasin Z, Lulic S, Pezelj D. 2006. Biomonitoring of heavy metals and arsenic on the east coast of the Middle Adriatic Sea using *Mytilus galloprovincialis*. Nucl Instrum Methods Phys Res B. 245(2):495–500.

Salvo A, Cicero N, Vadalà R, Mottese AF, Bua D, Mallamace D, Giannetto C, Dugo G. 2016. Toxic and essential metals determination in commercial seafood: *Paracentrotus lividus* by ICP-MS. Nat Prod Res. 30(6):657–664.

UNI EN 13805. 2002. Foodstuff. Determination of Trace Elements, Pressure Digestion.