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Polychlorinated biphenyls, organochlorine pesticides and trace metals in cultured and harvested bivalves from the eastern Adriatic coast (Croatia)

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HIGHLIGHTS

- PCBs, organochlorine pesticides, and trace metals were determined in bivalves.
- Spatial and seasonal distribution was dependent on the origin of contaminants.
- No significant differences in organic contaminant levels were found among species.
- Significant differences in Cu and Zn levels were found among species.
- Contaminant levels pose no harmful risk to bivalve consumers.

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ABSTRACT

Polychlorinated biphenyls, organochlorine pesticides and trace metals were determined in tissues of bivalve molluscs (*Mytilus galloprovincialis*, *Ostrea edulis*, *Venus verrucosa*, *Arca noae* and *Callista chione*), collected from 11 harvesting and 2 cultured locations along the eastern Adriatic coast, in May and November 2012. Concentrations (ng g^{-1} dry weight) of organochlorines ranged from 1.53 to 21.1 for PCBs and 0.68 to 5.21 for *p,p*′-DDTs. HCB, lindane, heptachlor and aldrin-like compounds were found in lower levels or were not detected. Metal concentrations (mg kg^{-1} dry weight) ranged from 0.23 to 4.03 for Cd, 0.87–3.43 for Cr, 3.69–202.3 for Cu, 0.06–0.26 for Hg_T, 0.62–9.42 for Ni, 0.95–4.64 for Pb, and 55.76–4010.3 for Zn. Established organochlorine and trace metal levels were lower than the maximum allowable levels in seafood set by the European Commission.

1. Introduction

Croatia is located in the eastern part of the Adriatic Sea with its coastline extending over 5835 km. Bivalve mollusc farming has been one of the most important economic activities in the Croatian coastal regions. The most commercially important species are Mediterranean mussel (*Mytilus galloprovincialis*) and European flat oyster (*Ostrea edulis*), which are cultured as well as harvested from natural populations. Other commercially important species are Mediterranean scallop (*Pecten jacobeus*), clam (*Venus verrucosa* and *Ruditapes decussatus*), and Noah's ark (*Arca noae*), which are harvested from natural populations (Župan et al., 2012). Until now, smooth clam (*Callista chione*) hasn't been exploited commercially in Croatian coastal areas, therefore, there is as strong interest and need for potential cultivation of this species (Ezgeta-Balić et al., 2011). To control and ensure the quality of shellfish growing in farming and harvesting areas, the Croatian authorities have established monitoring programmes, pursuant to the EU requirements that were transposed into Croatian legislation (CP, 2009; MAFWMRC, 2004). The marine environment is the ultimate repository for a considerable range of natural and anthropogenic contaminants that have the ability to accumulate in seafood for human consumption. Along with the unquestionable health benefits of fish and shellfish, there are also potential risks that can be associated with such contaminants.

Among organochlorine compounds (OCs), the most environmentally widespread are polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). PCBs were commonly used as

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dielectrics, heat resistant liquids, lubricating oils, and as plasticizers in plastics, paints, flame-retardants and adhesives (WHO-IPCS, 2003). Dichlorodiphenyltrichloroethane (DDT) was produced and used extensively in the past to control insects on agricultural crops and insect-borne diseases (ATSDR, 2002). Dichlorodiphenyldichloroethane (DDD), metabolite of DDT, was also used as an insecticide, albeit to a far lesser extent than DDT. Dichlorodiphenyldichloroethene (DDE) is the main metabolite of DDT and has no commercial use. Other pesticides, i.e. hexachlorobenzene (HCB), hexachlorocyclohexane (γ-HCH), heptachlor, aldrin, dieldrin, and endrin were mostly used as insecticides in agriculture. PCBs and OCPs released in the environment are highly resistant to biological and chemical degradation. Due to their hydrophobic nature (high K_{ow}) they become associated with lipid-rich tissues of marine organisms, particulate matter in seawater, and sediments. Organic contaminants accumulate in marine organisms and their concentration increases up the food chain (Gray, 2002). Due to their toxic properties, exposure to organochlorine compounds can lead to deleterious effects on human health and the environment (Jones and de Voogt, 1999). Therefore, the production and use of PCBs and OCPs has been restricted by Stockholm Convention (UNEP, 2009).

Trace metals accumulate in bivalve organisms in concentrations that are several orders higher than those of surrounding marine water (Casas et al., 2008). Certain trace metals and its compounds, such as mercury and methylmercury as well as arsenic, cadmium, lead, and organotin compounds, are considered to be highly toxic, whereas others, such as Cu, Cr, Zn, Mn and Fe, are essential for organism growth and are only toxic above a certain threshold level (Hoffman et al., 2003). Environmental conditions (Luoma, 1983; Phillips and Rainbow, 1993), metal speciation and bioavailability, as well as biological properties of bivalves, i.e. water filtering speed, growth, biochemical content, stadium of reproductive cycle, and metabolic processes (Fisher and Reinfelder, 1995) are factors affecting the level of trace metal accumulation in bivalve tissue. Due to trace metal ecotoxicity and their bioaccumulation properties, these contaminants pose a threat to marine life and a health risk to seafood consumers.

As filter feeding organisms, bivalve molluscs accumulate bioavailable contaminants in the marine environment. Therefore, they have been widely used as bioindicators for monitoring marine environmental pollution (Carro et al., 2014; Milun and Zvonarić, 2008; Kljaković-Gašpić et al., 2010). Presence of various chemical contaminants has been reported in several studies investigating wild (Zhang et al., 2012; Bellas et al., 2014; Fernández et al., 2013) and cultured bivalves (Sáenz et al., 2010; Suárez et al., 2013). However, studies have examined the presence of OCPs (Kožul et al., 2009; Herceg-Romanić et al., 2014) and trace metals (Bogdanović et al., 2014; Jović and Stanković, 2014; Kljaković-Gašpić et al., 2007) in cultured bivalves in the Croatian part of the Adriatic Sea.

The objective of the present study was to perform half-yearly monitoring of the quality of shellfish waters in new shellfish growing locations. The preliminary monitoring of organochlorines and trace metals at 13 locations should provide valuable baseline data necessary for registration and validation of new production areas in Croatian coastal waters. Moreover, findings regarding the levels of organic contaminants and trace metals in bivalves can be used to estimate a potential health risk for shellfish consumers.

2. Materials and methods

2.1. Reagents and standards

Dichloromethane and *n*-hexane, for organic trace analysis were supplied by J.T. Baker (USA), and sulphuric acid (96%) and anhydrous sodium sulfate by Merck (Germany). Florisil® (0.15–0.25 mm) for column chromatography was purchased from Acros Organics (USA) and extraction thimbles from LLG GmbH (Germany). Solution reference standards for organic residue analysis (HCB, lindane, heptachlor, aldrin, dieldrin, endrin, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, ε-HCH and deuterium labelled endosulfan Id4, and PCB congeners (IUPAC No. 28, 29, 52, 101, 105, 118, 138, 153, 156, 180, 194, 198) were supplied by Dr Ehrenstorfer GmbH (Germany). Reference material IAEA-432, mussel homogenate, was supplied by the International Atomic Energy Agency.

Aqueous trace metal standard solutions (Cd, Cu, Cr, Hg_T, Ni, Pb and Zn) were prepared from commercially available stock standard solutions of ultrapure grade, supplied by Merck (Darmstadt, Germany). The precision and accuracy of the applied analytical method were assessed by analysis of the standard reference material of marine biota sample (SRM 2976, freeze-dried mussel tissue, National Institute of Standards and Technology, Gaithersburg, MD, USA). Sample digestion procedure involved a mixture of acids: HNO_3 (68%, Trace analysis grade, Fisher Scientific UK, Loughborough, UK), $HClO_4$ (65%, Trace analysis grade, Fisher Scientific UK), and H_2O_2 (30%, Merck, Darmstadt, Germany). Water deionized by the Milli-Q Water Purification System (Merck Millipore, Molsheim, France) was used for cleaning glass, plastic and teflonware, preparation of stock solutions and dilution of samples.

2.2. Study area and sample collection

Bivalve samples were collected in May and November 2012, at 2 farming and 11 harvesting locations along the Croatian Adriatic coast (Fig. 1). Species with different ecological preferences were chosen for analysis. *Mytilus galloprovincialis*, *Ostrea edulis* and *Arca noae,* are three common sessile benthic species in the eastern Adriatic coast, where they can be found attached on different substrata. *Mytilus galloprovincialis* lives attached with its byssus filaments on natural substrata, from rocky coasts to gravel and sandy bottoms, as well as on different artificial surfaces. *Ostrea edulis* can also be found permanently attached to different substrata, natural or artificial, down to 50 m deep. It prefers hard rocky bottoms and concrete walls. *Arca noae* lives attached with its solid byssus on hard substrata, on all types of rocky bottoms. It is a common species in the Adriatic Sea, occurring down to depths of 40 m. Other two species, *Callista chione* and *Venus verrucosa*, burrow into different types of sandy sediments, and are among common species in the Adriatic Sea, found down to 25 m or deeper.

Mediterranean mussels (*Mytilus galloprovincialis*) were collected at seven locations, European flat oyster (*Ostrea edulis*), smooth clam (*Callista chione*) and warty venus (*Venus verrucosa*) at 3 locations, and Noah's ark (*Arca noae*) at 1 location. Specimens were transported to the laboratory in a portable refrigerator. Detailed information on the sampling locations, dates, species and its origin (cultured/harvested) are shown in Table S1 (Supplementary material).

Bivalves, separated for OC and metal analysis, were washed and scrubbed with a stainless steel knife to remove adhering detritus. The gut content of bivalves in present study was not depurated prior to analysis. Depuration procedure is commonly applied to ensure that contaminated particles in the bivalve's gut do not lead to overestimation of contaminant bioavailability (Solaun et al., 2015; Olenycz et al., 2015). However, according to Gunther et al. (1999), with the exception of lead and selenium, comparison of trace metal levels in depurated and non-depurated mussels revealed no significant differences. Moreover, according to Pruell et al. (1986), depuration of mussels' gut content has a negligible influence on the measured PAH and PCB levels.

The shell length and dissected soft tissue weight of each individual was measured. Pooled samples were weighed, before and