

The Impact of Possible Mercury Source-Point Contamination in the Coastal Area of Skiathos Island [†]

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Abstract: In Skiathos Island the water is not potable due to mercury contamination and salinization. The mercury's origin is natural due to the existence of cinnabar in the Skiathos aquifer as a mineral in the Earth's crust. The possibility of mercury contaminants ending up in the coastal area was investigated through a field experiment. Mussels (*Mytilus galloprovincialis*) were employed as mercury monitoring biomarkers at the outflow of the wastewater treatment of the island. Using the RNA:DNA ratio, it was revealed that the organisms were stressed after three months of exposure to Skiathos' coastal waters. The mercury concentration was directly measured at the bulk mussels' tissue showing differences between the station located at the outflow of the WWT and the reference station. Although the results may imply mercury contamination in the coastal area of the island, the precise origin of the mercury in mussels is difficult to define.

Keywords: mercury; biomarkers; Mediterranean; caged mussels; *Mytilus galloprovincialis*

1. Introduction

Mercury (Hg) is one of the most chronic and toxic pollutants released into aquatic ecosystems through natural and anthropogenic sources. Methylmercury (MeHg), mercury's most toxic form, bioconcentrates in organisms and biomagnifies along the aquatic food chains [1]. Total mercury (THg) is distributed by affinity to certain tissues, particularly in muscle and liver [2]. The animals that are at the top of the food web present higher concentrations than those in lower trophic levels, due to MeHg which is more bioavailable than other forms and is quickly absorbed and slowly excreted [3]. The presence of Hg and MeHg in the aquatic environment affects animal as well as human health [4].

In aquatic systems the effects of pollution and contamination can be observed at different levels of biological organization, from the molecular to the community level. Changes at the organism level are possible to lead to changes at the population and community levels. For the assessment of the good chemical and ecological status of water bodies, biomarkers can be used as an early warning signaling agent [5]. They constitute a useful index of the environmental disturbance having an especially good response to heavy metals, thus operating as a useful monitoring tool [6]. Mussels are of the most widely used biomarkers as sentinel organisms and applied to evaluate the toxic effects of chemical pollutants in marine organisms [7].

Humans are the top predators of the food chain, so it is easy for them to bioaccumulate mercury, especially for individuals living in the Mediterranean who consume larger quantities of seafood and fish. In addition, the Mediterranean basin has several deposits containing mercury minerals where mercury has been released by both natural and anthropogenic activities [8]. Compared to the world ocean, higher mercury concentrations are recorded in the Mediterranean Sea in the order of 2.5 pM [9]. The presence of cinnabar deposits and volcanoes (the so-called Mediterranean geochemical “anomaly”) results in Mediterranean species showing elevated concentrations of mercury compared to the same species in the Atlantic [10,11]. Taking into consideration the aforementioned mercury concentrations combined with increased seafood and fish consumption, the limits for the mercury tolerable weekly intake are stricter in Europe compared to the US.

Global health organizations have developed an index to determine the safe limit of weekly mercury intake, called the Provisional Tolerable Weekly Intake (PTWI), which is expressed on a weekly basis per kilogram of body weight (b.w). PTWI, last updated on 2018 [12], defines the quantity of mercury that can be consumed and bioaccumulated in the human body without presenting significant health risks. The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) has determined the limit of 1.6 µg/kg of body weight for MeHg and 4 µg/kg of body weight for inorganic mercury expressed as mercury [12]. This limit was institutionalized considering epidemiological studies for the effect of mercury on neonatal health when mothers were exposed to this metal [13,14]. For the European Food Safety Authority (EFSA) the limit was finally established as is 1.3 µg/kg body weight, for both forms of mercury. With respect to the established limits, the organization recommends the maximum consumption of 3–4 portions and 1–2 weekly portions of seafood for adults and children, respectively [15].

According to Commission Regulation (EC) No 1881/2006, MeHg is mercury’s chemical form of most concern and can make up more than 90% of the total mercury in fish and seafood. In many studies it is documented that total mercury is present at almost 100% in the form of MeHg in animal tissues [2,16–18]. Thus, in new studies for the safe limit of weekly mercury intake of seafood only the limits for MeHg are used even when THg is measured. The last update for THg limits was in 2001 well before the PTWI index was even established with the Commission’s regulation about fishery products and muscle meat of fish setting the maximum level at 0.5 mg/kg wet weight [19]. Since aquatic biota present a direct relationship with the environment, MeHg can be used as a bioindicator of the presence of mercury [4,18,20].

2. Materials and Methods

2.1. Case Study

Skiathos is a small island that belongs to the complex of the Sporades Islands at the North Aegean, Greece. The permanent residents are about 6000, while the island population grows rapidly during the tourist season lasting from April to early October [21]. The water demand presents high seasonal variability peaking in August when water pumping shows an increase often exceeding 130% of the winter minimum, indicating a link between touristic activity and the water demand [22]. The entire island water network is served by one drilled well, supported by a smaller well when required, which results in serious water supply issues for the island. The problem becomes more intensive by the sharp increase in water demand during the touristic period, while at the same time the potable water which comes only from groundwater is of low quality due to aquifer salinization and the presence of heavy metals [23]. The water utility of Skiathos (DEYAS) has announced that the water is not potable or safe for household use.

Based on data from DEYAS which is conducting monthly samplings for monitoring the water quality in the main well drilling of the island, mercury has been detected systematically above the permitted limit for more than a decade. The maximum permitted limit for mercury concentration in drinking water is determined by the WHO as 1 µg/L [24], while in Skiathos the measured value has

been found to be up to six times ($6 \mu\text{g/L}$) higher. The presence of mercury in Skiathos groundwater comes from natural sources, since the underlying carstic limestone contains cinnabar (HgS) deposits. The mechanism that mobilizes the mercury to leach from the rocks and arrive in the water network is described thoroughly in Spyropoulou et al. [23].

2.2. Field Experiment

In total, mercury may end up in the coastal environment of Skiathos Island through surface leaching after rainfall events (non-point source), or from point sources related to the water network or through the aquifer interaction with the sea. In order to address the question and to study the possible mercury contamination in the coastal waters, a three-month field experiment was carried out using caged mussels. Following the methodology introduced by Tsangaris et al. [5,7] mussels *Mytilus galloprovincialis* L. were employed as biomarkers which initially had approximately 60 mm shell length and were supplied from an aquaculture farm in Kitros Pierias, Central Macedonia, Greece. Three sampling sites near the coastline were chosen at areas possibly affected by the point source of wastewater treatment (WWT) of the island (Figure 1).



Figure 1. Map of Skiathos Island with locations of the three sampling stations (S_1 , S_2 , S_{Ref}). The waste water treatment (WWT) is marked at the left picture with the red cycle.

The first site was located at the outflow of the WWT pipeline, the second was situated about 100 m southwards in the direction of the reference site in order to investigate the possible mercury dispersion, and the third was located in the open sea to be used as a reference. The depth of the selected sites was between 15 to 30 m. During transplantation the mussel cages were kept on board in tanks provided with flowing seawater. The transplantation was performed by private speedboat in collaboration with the director of DEYAS, Mr. Ioannis Sarris. In each cage, 2 kg of mussels were stored. The cages were immersed under a buoy at a 3 m depth below the surface and were fastened by a rope to an anchor of about 20 kg at the seabed. The caged mussels were placed at the sites in early June and remained there until the end of September 2018. The mussels were recovered after three months by divers. From the three indicative sites only two were found and retrieved, the third one (S_2) was either removed by unauthorized actions or lost due to the cyclone Zorbas that swept the area at that time. Samples were conditioned immediately after collection on board and stored in ice until their arrival at the laboratory where they were deep-frozen at -20°C .

2.3. Laboratory Analysis

Before the mussels were placed into the cages, a proportion was stored at $-20\text{ }^{\circ}\text{C}$ in order to allow testing the changes in the samples before and after the three months stay in the sea. The dimensions of the mussels were measured with a caliper and the removed tissue was weighed. The application of appropriate solutions was followed by centrifugation to proceed with the analysis of the supernatant liquid. The first part of the analysis of the solid tissue samples took place at the laboratory of Microbial Communities and Habitats in Aquatic Environments (MiCHAE), at the Department of Ichthyology and Aquatic Environment, University of Thessaly. The method of simultaneous DNA and RNA extraction was applied to mussel's tissue according to Tsangaris et al. [5]. Comparative biochemistry and physiology C-toxicology and pharmacology (151:369-378) along with the corresponding protocol was applied using the Quick-RNA/DNATM Miniprep Kit, which provides a quick method for the isolation of high quality genomic DNA and total RNA from small amounts of cells and tissue. The DNA, RNA and the purity of each sample was measured. In order to investigate the possibility of mercury's existence in the mussels' tissues, further treatment was required. For the second part of the analysis, pooled mussel tissue samples were homogenized and the mercury concentration was measured with the cold vapor atomic absorption spectrometry method at the Laboratory of Environmental Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, in collaboration with Professor Manos Dassenakis.

3. Results and Discussion

3.1. Laboratory Results

Solid tissue samples were collected and the DNA, RNA contained were measured. The RNA:DNA ratio served as an indicator of protein synthesis and a biochemical biomarker of growth (Figure 2). The ratio reveals a general response to environmental stressors [25]. The first application of the RNA:DNA ratio as a biomarker of the effects of pollution in the genus *Mytilus* was performed by Tsangaris et al. [4].

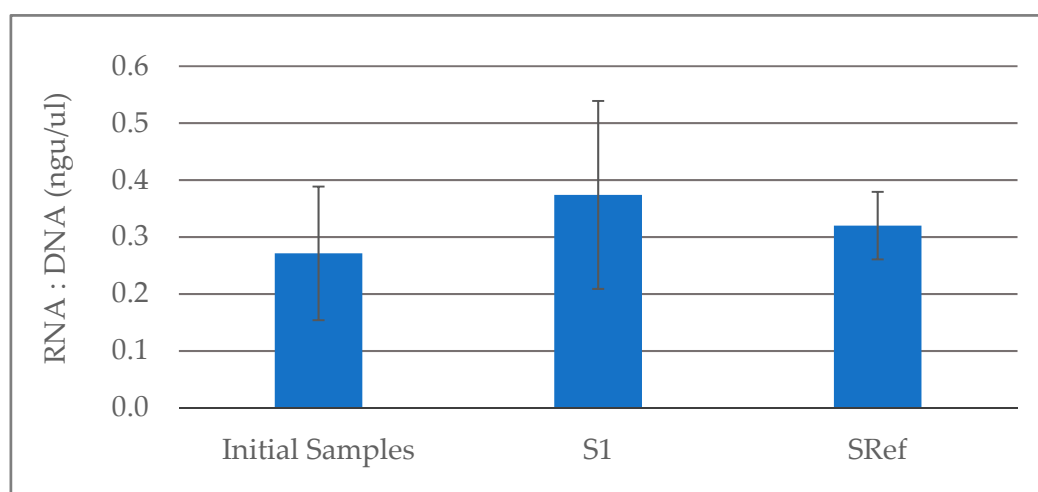


Figure 2. RNA:DNA ratio for the initial samples, and the samples at the sites S_1 , S_{Ref} .

In general, the amount of DNA remains constant, but when an organism is under stress conditions, growth is inhibited so RNA is decreased and the corresponding ratio is increased. As it is observed from Figure 2, the samples at both sites S_1 and S_{Ref} appeared to be under stress compared to the initial samples. In particular the S_1 site which was located at the outflow of the WWT pipeline seemed to be more influenced by a stressful condition. Nevertheless, it was not easy to identify the condition that stressed the samples. Although, this result is indicative, it cannot provide adequate evidence for mercury concentration in the mussel tissues. Many factors could have caused this

stressful condition; there could be environmental factors like strong currents, cyclone or leaching loads from the land.

In order to investigate these possibilities, further mercury measurements were carried out on the mussel tissue directly. To reduce variability, the whole soft tissue from 7 specimens was pooled from the initial samples [5]. The results showed mercury concentrations for sites S_1 and S_{Ref} of 0.11 and 0.02 $\mu\text{g/g}$, respectively. A difference between the two sites was observed although it was not high. At the S_{Ref} site the concentration was expected to be lower since it was located in the open sea (North Aegean) and the station was used as a reference. The difference between the two sites may imply that the mercury detection at S_1 site was linked with contaminated loads from the land or from the aquifer. Bioaccumulation and bio magnification processes were possibly affected by biotic as well as abiotic factors influencing mercury concentration [24]. In addition, climatic, ecological, and physical conditions including age, size, sex, growth rate, trophic position, food web size, population density, position in the water column, water pH, organic matter richness, oxygen saturation, seasonality, salinity and temperature play an important role in mercury accumulation in the mussel tissues [3,26,27]. In addition, since the Mediterranean basin is rich in mercury deposits, the corresponding concentration in seawater is higher [9]. Specifically, cinnabar deposits are abundant in the crustal rocks of Skiathos. Therefore, the effort to achieve a safe result of the mercury origin in the sea becomes more difficult.

3.2. Quantity of Mercury through Mussels that Can Be Consumed on A Weekly Basis from the Coastal Area of Skiathos Island

Based on the aforementioned information and in respect to the corresponding regulation and global health organizations, we calculated the permitted quantity to be consumed on a weekly basis that is safe for human health. The limits of PTWI are expressed per body weight, thus the calculations were carried out for four target groups (men, women, teenagers, children). In general, there is no acceptable mean body weight since it depends on the gender, the age and the height. Thus, the body mass index (BMI) established by the WHO was used, in order to calculate indirectly an indicative body weight for each group. BMI is defined as the weight in kg divided by the square of height in meters [28] and constitutes a measure for indicating nutritional status in adults.

Regarding adults, a different approach was applied compared to that for children and adolescents. The BMI for a healthy adult individual varies between 18.8 and 25 based on values defined by WHO. Evaluating the equation result against healthy BMI values, an indicative male height of 1.80 m and body weight of 80 kg were considered acceptable. The corresponding female values are 1.65 m height and 60 kg body weight. The PTWI was calculated for the mercury concentration of 0.11 $\mu\text{g/gr}$ (of site S_1) measured in Skiathos Island using the aforementioned values for male and female adults (Table 1). For children (5–11 years) and adolescents (12–18 years), growth curves were used which give the mean BMI as well as the mean height per age. These two groups were divided in gender sub-groups due to different growth curves [29,30]. From the results of the BMI equation, the body weight estimation for these two groups was used for the calculation of PTWI, taking into consideration the mercury measurements of S_1 site (Table 1).

Table 1. Mean indicative b.w per target group and quantity of mercury through mussels from the study area that is safe to consume weekly with respect to Provisional Tolerable Weekly Intake (PTWI).

	Mean Indicative Body Weight per Target Group (WHO)	Quantity of Mercury That Can Be Consumed Weekly	Provisional Tolerable Weekly Intake (PTWI) for MeHg ($\mu\text{g/gr}$)	Source
Adult (men)	80 kgr	945 gr		
Adult (women)	60 kgr	709 gr		
Adolescents boys	26.07–38.28 kgr	308–452 gr	0.0013 $\mu\text{g/gr}$ b.w	EFSA 2012, (last update 2018)
(12–18 years) girls	27.45–34.63 kgr	324–409 gr		
Children (5–11 years) boys	17.08–24.31 kgr	202–287 gr		
girls	17.54–25 kgr	207–296 gr		

From Table 1, it is revealed that the edible quantity in kg of consumed mussels that contain mercury is directly linked with the body weight. In respect to the PTWI index and the corresponding mercury limits of 0.0013 $\mu\text{g}/\text{gr}$ b.w, a man of 80 kg and 1.80 m height is safe to consume 945 g mussels of the coastal area of Skiathos per week without exceeding the intake weekly limit. Respectively an average woman of 65 kg and 1.65 m height can eat 709 g. For an adolescent boy the weekly intake varies depending on the mean b.w for the specific age of 12–18 years between 308–452 g, while for a girl of the same age it varies between 324–409 gr. For the children the safe edible weekly quantity is even less; for a boy of 5–11 years with 17.08–24.31 kg body weight it is safe to consume 202–287 g, and for a girl of the same age with 17.54–25 kg about 207–296 g. It must be noted that EFSA 2015 [15] recommends a maximum consumption of 3–4 portions and 1–2 weekly portions of seafood for adults and children respectively.

4. Conclusions

To investigate whether mercury contaminants may end up in the coastal area of Skiathos island a field experiment was conducted with the use of mussels as monitoring biomarkers, from June to September 2018. From the analysis of solid mussels' tissue, the RNA:DNA ratio was measured to represent an index of stressfulness of the organisms. The results show that the samples located at the sites near the WWT plant as well as at the open sea had undergone a stressful growth period. The S_1 site which was located at the outflow of the WWT pipeline seems to be subjected to more stressful factors compared to the S_{Ref} . However, it is not easy to come to a safe conclusion about the factors that may have stressed the samples. An additional analysis was carried out in order to measure directly the mercury concentration in the mussels' tissues, which was found to be 0.11 and 0.02 $\mu\text{g}/\text{g}$, for sites S_1 and S_{Ref} , respectively. Although the difference between the two sites was not high, it may imply that mercury detection at S_1 site is linked to mercury contaminated loads coming from the land or from the aquifer. Since many factors may affect bioaccumulation in organisms including biotic-abiotic processes, environmental and physical factors, the origin of mercury is difficult to be clearly determined. In respect to the limits of the PTWI for Hg and MeHg consumption and taking into account the measured concentration of mercury for the area of study, the quantity of mussels that is safe to consume from the study area without being dangerous for the human health was calculated. The calculations referred to four target groups (men, women, adolescents and children) since the permitted consumed quantity is strongly linked with the gender, body weight and height. Although it is safe to eat mussels from the coastal area of Skiathos Island, it may be not safe to consume unlimited quantities because there is the danger of exceeding the permitted limits. Overall, this is an important issue for the local community which hosts many visitors consuming large quantities of fish and seafood, and the economy, which is based on taverns, restaurants etc. (tourist industry), thus it is interesting to examine further probably through a new field experiment with a more dense station network in different coastal areas.

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