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HEAVY METALS BASELINE CONCENTRATIONS IN SOFT TISSUES OF *Patella* sp. FROM THE STRATONI COASTAL ENVIRONMENT, NE GREECE

BAZOWY POZIOM ZANIECZYSZCZEŃ METALAMI CIĘŻKIMI W TKANKACH MIĘKKICH *Patella* sp. WYSTĘPUJĄCYCH W PRZYBRZEŻNYCH OBSZARACH STRATONI, GRECJA

Abstract: Species of the limpet *Patella* sp. were collected from a typical control coastal site (Artemida) and a metal contaminated marine environment (Stratoni), situated in Greece. The soft tissues were analyzed for their heavy metal (Pb, Zn, Mn, Cu, Ni, Cr) content by flame atomic absorption spectroscopy. High Pb, Zn and Cu concentrations were determined in limpet samples from Stratoni, which can be attributed to geological-mineralogical factors due to the natural heavy metal elevated substrate of the broader mineralized area. In contrast, concentrations of Mn, Ni and Cr in both areas are comparable to those reported for other non-polluted geographical regions of the Mediterranean area. Considering the refurbishment of the Stratoni port that will accompany the new large scale mining operations, these results provide a preliminary baseline metal level in these organisms, by which future local changes could be assessed. It is suggested that the species of *Pattella* sp. are appropriate to be used for biomonitoring research and should be included in routine monitoring programs regarding the Stratoni marine ecosystem. Further studies of metal levels in limpets of *Patella* sp. from the Stratoni area including the investigation of the possible effects of seasonal changes on metal concentrations and distribution are required.

Keywords: heavy metals, limpets, marine environment, *Patella* sp, baseline, Stratoni

Introduction

During the last decades, the state of marine environment, in terms of environmental contamination, has become a matter of growing international concern. Heavy metals in coastal areas can originate either from natural sources, as in the case of feeder river rich in trace elements, nor from anthropogenic sources, such as discharges from industries and naval activities. These chemical constituents are essential elements required to support biological activities, but they can also become toxic to living organisms when subject to high concentrations.

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It is now well accepted that the bioavailabilities of heavy metals in the marine environment, *ie* the biologically available levels of contaminants in the ecosystem, can better be evaluated with the use of specific organisms, named as biomonitors [1]. These organisms should fulfill several criteria that have been summarized by Phillips [2] and Rainbow [1]. Gastropod molluscs are commonly used as bioindicators because of their ability to take up metals from the dissolved phase and through ingestion from inorganic particulate material, presenting concentrations considerably higher than in other constituents of the marine environment. In particular, the limpet *Patella* sp. is among the commonest inhabitants of rocky shores in Greek coasts, with a well known capacity to accumulate metals, mainly in their soft tissues [3-7], but also in their shells [8]. Furthermore, these herbivorous gastropods are sedentary, available all year long, easy to collect, resistant to transportation and conservation to a laboratory, while their size can provide abundant tissue available for analysis. They also represent a potential route of entrance of contaminants into the highest trophic levels, since they are largely harvested by humans.

In general, when examining heavy metal bioaccumulation, a contaminated marine environment is designated and compared with a clear - control monitoring site. In the present study, the accumulation of potentially toxic metals (Pb, Zn, Mn, Cu, Ni, Cr) in the soft tissues of *Patella* sp., collected from two contrasting coastal environments of Greece (Fig. 1), was evaluated. Artemida site (South Evian Gulf) is situated near the capital of Greece, Athens, and therefore it is not completely uncontaminated. However, it is lacking of any industrial operations and is quite far away from the Rafina Port, so it can be considered virtually uninfluenced by hardly anthropogenic activities. On the other hand, the coastal area of Stratoni (Ierissos Gulf, NE Greece), located at the north east part of Chalkidiki peninsula is subject to potential high metal load due to the historical exploitation of the adjacent Pb-Zn (±Ag) sulphide deposits. The so-called Cassandra mines started to be exploited around 600 BC and continue in production today, producing galena (PbS) and sphalerite (ZnS) concentrates. The ongoing mine development plans include the exploitation of the Skouries porphyry copper-gold deposit, located some 35 km by road west of Stratoni village. According to the plans of the owner company [9], the existing port storage facilities at Stratoni will be extended to provide a new capacity covered shed for the Cu-Au concentrates. In addition, a new loading hopper and feeder will be provided while the present transfer conveyor and the ship loader conveyor will be refurbished.

In the recent past and up to 1983, the mine tailings were dispersed directly into the nearshore marine environment in the vicinity of Stratoni. As a result, surface sediment samples from the area near the load-out facility of the mining operations were found to be polluted by Pb, Zn and Cu [10]. Geochemical partitioning studies of sediments [11] demonstrated that Pb, Zn and Mn are associated with the reducible fraction that consists of Fe and Mn oxides, while Cu, Ni and Cr are mainly bound to the crystal structure of insoluble mineral particles. The latter author also attributed the elevated levels of Pb, Zn and Cu in the studied sediments to the past mainland mining activities. Nevertheless, there is no study up to now that examines the fate of the above potentially harmful elements in this heavy metal contaminated marine system. It is in this context that the present study is presented; the main objective is to determine the levels of Pb, Zn, Mn, Cu, Ni and Cr in the soft parts of individuals of the common limpet *Patella* sp., collected from the Stratoni coastal environment, and compare their concentrations with data from a typical control marine population in Greece (Artemida) and other coastal environments in order to evaluate

the relative significance of contamination. Considering the scheduled new mining operations in the region and the refurbishment of the Stratoni port, such a survey will allow to establish a preliminary metal baseline level in these organisms that can be used in future comparisons.

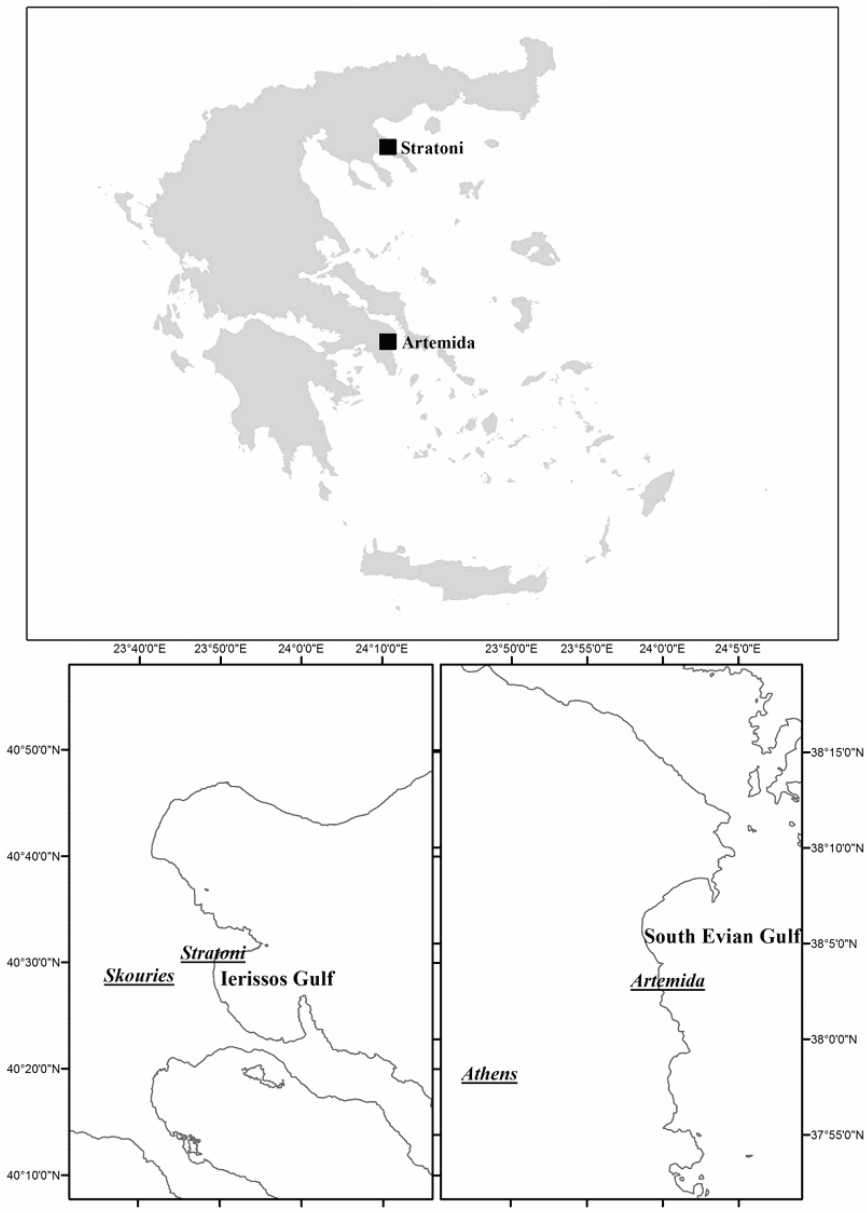

Fig. 1. Map of Greece showing the sampling sites

Materials and methods

Sampling procedure and chemical analyses

Specimens of limpet were collected along the Stratoni and Artemida coastline (Fig. 1) during July 2009. The organisms were removed from the substratum with a plastic knife, washed with clean seawater, placed in clean plastic bags, transferred to the laboratory under cooling conditions and then frozen until analysis. The limpets were handpicked from the top surfaces of the terraces, always at the same depth and distance from the shoreline, so that limpets have experienced similar periods of emersion and immersion. Special attention was paid to select individuals of quite similar dimensions, since it has been shown that the accumulation of heavy metals in limpets is dependent on body size [5, 12], meaning that the largest individuals contain higher levels of metals; hence, all the animals were collected at shell length of 35 ± 5 mm.

A total of 42 individuals from each sampling site were processed. The soft tissues were separated from the shells with a plastic knife, dried to constant weight at 80ºC and homogenized. Afterwards, they were classified into seven groups, 6 samples each, and then 0.3 g aliquot of dry tissue of these 6 subsamples from each group was digested with a mixture of concentrated HNO_3/H_2O_2 (6 + 1 cm^3) in a microwave digester. The completely digested samples were allowed cool to room temperature, filtered and diluted to 25 cm^3 with deionized water. Concentrations of Pb, Zn, Mn, Cu, Ni and Cr in the aqueous solutions were measured by air/acetylene *flame atomic absorption spectroscopy* (Perkin Elmer 1100B) with dublicate determination of each sample at the Laboratories of Department of Geology and Geoenvironment, University of Athens. The instrument was calibrated with metal standard solutions prepared by dilution from stock standard solutions of 1000 mg/dm^3. The resulting concentrations are expressed in mg/kg on dry weight basis. A number of analytical blanks were run in the same way as the samples. Detection limits were calculated on the basis of determination of the blanks as 3 times the standard deviation of the blank measurements. These values [mg/kg d.m.] were: 3.3 for Pb, 1.7 for Cu, Mn and Ni and 2.5 for Cr and Zn.

Statistical analyses

Mean metal concentrations were calculated together with standard deviations; the standard deviations refer to the variability within different replicates. Variation in metal concentrations was tested by using non-parametric statistical methods. In particular, the Mann-Whitney U test was conducted to test the significance of the differences in the metal content between the two sampling sites. The level of significance was set at $p < 0.01$. All statistical calculations were performed with Minitab 15.1 for Windows.

Results and discussion

The mean concentrations of six metals examined in *Patella* sp. collected in this study are tabulated in Table 1. It can be stated that the area of Stratoni is subjected to more intense heavy metal loading than Artemida. The highest levels of Pb, Zn, Mn and Cu are observed in organisms from Stratoni marine environment, decreasing according to the sequence of Zn > Pb > Mn, Cu. On the contrary, similar Ni and Cr concentrations were determined in the tissue of limpets from the two sites of investigation.

Table 1

Mean ± standard deviation values [mg/kg d.m.] of heavy metal concentrations in the soft tissues of *Patella* sp. from Stratoni and Artemida coastal areas

Heavy metal	Artemida	Stratoni
Pb	8 ± 5	96 ± 19
Zn	48 ± 4	196 ± 31
Mn	10 ± 5	36 ± 17
Cu	6 ± 1	28 ± 4
Ni	9 ± 3	9 ± 4
Cr	7 ± 3	8 ± 3

The Mann-Whitney test detected significant differences among the two sites. The results of comparison are shown in Table 2. The results demonstrated that Pb, Zn, Mn and Cu values of limpets from Stratoni were significantly higher ($p < 0.01$) than those from Artemida. However, it should be stated that Mn concentration in limpets from Stratoni has a very high standard deviation compared with the other chemical elements (Table 1), meaning that the values fluctuated too much. No significant difference was observed between the concentrations of Ni and Cr in soft tissues of *Patella* sp. from Stratoni and the other site of comparison.

Table 2

Comparison between the mean metal content of limpets from Stratoni with those sampled at Artemida

Site compared	Pb	Zn	Mn	Cu	Ni	Cr
Artemida	**	**	**	**	–	–

- indicates no difference between mean metal content, ** significantly higher at $p < 0.01$

Comparison of metal concentrations with corresponding values measured in species of *Patella* sp. from various geographical sites, including data from clean areas that can be considered as background levels, is shown in Table 3. Such a comparison will additionally provide more useful information on the contamination degree of Stratoni marine ecosystem. From a general point of view, the concentrations of Pb, Zn and Cu in limpets from Stratoni are not comparable to those reported by other investigators from clean coastal areas. In particular, the magnitude of these metals in *Patella* sp. population from Stratoni can be considered significantly higher in comparison with the published reference background levels for unpolluted sites in the Mediterranean area [13, 14]. The Mn concentrations from Stratoni are quite similar to those obtained by Catsiki et al [13], from a typical unpolluted area, located at Milos island (central Aegean Sea, Greece). Nickel and chromium fall within the lowest available data values in the literature.

The presence of high Pb, Zn and Cu concentrations in limpet samples from Stratoni should be attributed to geological-mineralogical factors, due to the natural enriched heavy metal substrate of the broader mineralized region. Another point of view is that the significantly higher levels of Pb, Zn and Cu in species from Stratoni may be directly related to contamination of the marine environment due to the past mining activities. Such results are in accordance with the heavy metal contamination of the sediment samples in the same area [10, 11], indicating that a substantial portion of these metals is readily available to limpets. It should be mentioned however that the uptake mechanisms and assimilation of

metals by limpets are largely dependent on the dissolved form of metals and the metal portion accumulated in the algae on which they graze [15].

Table 3
Selected references of metal concentrations [mg/kg d. m.] of limpets from different geographical areas (mean values are presented)

Location	Pb	Zn	Mn	Cu	Ni	Cr	Authors
Stratoni, Ierissos Gulf	96	196	36	28	9	8	Present study
Saronic Gulf, Greece, including areas affected by naval and industrial operations, and a naturally enriched heavy metal site		43-367		5.0-77.4	6.0-31.6	0.4-9.4	Kontopoulos et al [18]
Central Aegean Sea, Milos island, Greece 3 clean stations		31.9-56.5	11.3-32.1	7.6-12.0	19.1-28.6	5.1-6.2	Catsiki et al [13]
Central Aegean Sea, Milos island, Greece semi-enclosed gulf including the port		43.2	23	7.9	16	5.2	Catsiki et al [13]
North Evoikos Gulf, Greece, ferro-nickel smelting plant influence		40.2-71.9		5.7-12.6		5.0-32.7	Kozanoglou and Katsiki [19]
Canary Islands, Spain	0.3-10.2	1.6-24.1		0.6-5.01			Bergasa et al [15]
Goury, La Hague, NW France, slightly influence by industrial activities	0.9-3.7	40-91		3.0-6.6	0.6-2.5	0.2-2.4	Miramand and Bentley [20]
Turkish eastern Mediterranean coastline, sewage influence	0.3-3.2	44-96		3.5-13.7	2.1-83.7	1.5-10.6	Ramelow [21]
Ras Beirut, Lebanon	6.8-95.6			11.3-38.0	n.d-75.9		Shiber and Shatila [16]
Gulf of Gaeta, Tyrrhenian Sea, central Italy, small scale anthropogenic activities	0.5-1.5	87.4-117.1		10.2-19.2		0.7-1.0	Conti and Cecchetti [12]
Favignana Island (Silicy, Italy), stations not affected by human activities	0.14-0.27	3.5-5.8		1.21-2.35		0.19-0.46	Campanella et al [14]
western coast of the Gulf of Suez, land based and naval activities	6.2-70.9	56.5-191.4	24.9-76.3	1.6-12.2	3.1-9.9	2.3-8.0	Hamed and Emara [17]
İskenderun Bay, NE Mediterranean Sea, contaminated	4.3-14.5	23.1-46.6	1.4-4.3	1.6-4.0	3.6-12.2	4.8-8.3	Türkmen et al [23]
South coast of Portugal, estuarine contaminated site		73.4-172.0	3.9-86.3	4.2-15.2	2.9-11.3		Cravo and Bebianno [23]

It is of interest to compare the Pb, Zn and Cu concentrations in limpet samples from Stratoni with the maximum published concentrations found in organisms at other locations. Our mean Pb concentration was similar to the highest value determined in samples from a coast from Lebanon [16], which was considered to be contaminated by a highway adjacent to the coast and by industrial and domestic wastes. Slightly lower than ours were the Pb

values found in the Gulf of Suez, Red Sea [17], where the land based and intense naval activities are the main sources of metal pollution. Higher Zn and Cu content was manifested in species collected from some areas along Saronic Gulf, Greece, ascribed to industrial and naval activities, but also to metal rich natural substrate [18]. Zinc values were similar to those determined in organisms collected from the Gulf of Suez [17], whereas the Cu concentrations were lower than those in the coast from Lebanon [16]. Overall, the measured Pb, Zn and Cu concentrations are among the highest ever reported in species of *Patella* sp., highlighting the Stratoni area to be a contaminated marine site.

Conclusions

The refurbishment of the Stratoni port, as a result of the mine development plans of the owner company, brings up the urgent need to provide useful baseline data of metal content in individuals of *Patella* sp., by which future local changes could be assessed. Results of the present study demonstrated that heavy metal (Pb, Zn, Cu) loadings in Stratoni marine ecosystem are high in comparison with a typical control marine site in Greece and other non-polluted geographical regions of the Mediterranean area. As a result, it can be stated that bioaccumulation is at similar high levels with other contaminated marine systems. Furthermore, the very high Pb content, a non-essential metal in relation to Zn and Cu that can produce toxic effects on biological systems, is of great concern with regard to environmental health. This research also denotes that the geochemical conditions and particularly the presence of a natural substrate rich in heavy metals, contributes to elevated metal levels in the soft tissues of *Patella* sp, presenting a trace metal assemblage that can be interpreted in terms of environmental exposure. It is suggested that the species of *Pattella* sp. are appropriate to be used for biomonitoring research and should be included in routine monitoring programs regarding the Stratoni marine ecosystem. Further studies of metal levels in species of *Patella* sp. from the Stratoni area, including the investigation of the possible effects of seasonal changes on metal concentrations and distribution are required.

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BAZOWY POZIOM ZANIECZYSZCZEŃ METALAMI CIĘŻKIMI W TKANKACH MIĘKKICH *Patella* sp. WYSTĘPUJĄCYCH W PRZYBRZEŻNYCH OBSZARACH STRATONI, GRECJA

Abstrakt: W celu oceny zanieczyszczenia metalami ciężkimi przybrzeżnych wód morskich Grecji mięczaki z gatunku skałoczepów *Patella* sp. zostały zebrane na zanieczyszczonym obszarze (Stratoni) oraz w pobliżu typowego miejsca kontrolnego (Artemida). Metodą płomieniowej atomowej spektroskopii absorpcyjnej zbadano stężenia metali ciężkich (Pb, Zn, Mn, Cu, Ni i Cr) w tkankach miękkich. Wysokie stężenia Pb, Zn i Cu oznaczono w próbkach skałoczepów ze Stratoni, co może wynikać ze składu geologiczno-mineralogicznego podłoża. W przeciwieństwie do tego, stężenie Mn, Ni i Cr w próbkach zebranych na obu obszarach są porównywalne ze stężeniami opisanymi dla niezanieczyszczonych regionów śródziemnomorskich. Zważywszy na remont portu Stratoni, któremu będą towarzyszyły prace górnicze, wyniki te stanowią wstępną ocenę poziomu zanieczyszczenia metalami tych organizmów, co umożliwia ocenę przyszłych, lokalnych zmian. Przypuszczalnie, gatunki *Pattella* sp. są odpowiednie do wykorzystania w badaniach biomonitoringowych i powinny zostać uwzględnione w badaniach biomonitoringowych morskiego ekosystemu Stratoni. Wymagane jest prowadzenie dalszych badań zawartości metali w skałoczepach *Pattella* sp. z obszaru Stratoni oraz ocena sezonowych zmian stężeń i ich rozkładów.

Słowa kluczowe: metale ciężkie, skałoczepy, środowisko morskie, *Patella* sp, poziom bazowy, Stratoni