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Influence of a Ferronickel Smelting Plant Activity on the Coastal Zone through Investigation of Metal Bioaccumulation on Two Gastropod Species (*Patella caerulea* and *Phorcus turbinatus*)

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Abstract

The impact of a ferronickel smelting plant on the coastal zone has been investigated through metal bioconcentration by two common Mediterranean gastropod species (*Patella caerulea* and *Phorcus turbinatus*) collected seasonally together with seawater. *P. caerulea* presented higher Zn and Fe levels, while *Ph. turbinatus* higher Mn and Cu ones. The highest concentration of metals was measured in the stations close to the smelting plant in both species. *P. caerulea* displayed higher seasonal metal levels during autumn and winter as seawater did, while *Phorcus* displayed higher metal levels in spring. Statistically significant positive correlation was found between the dissolved concentration of Fe and Mn in seawater and the soft tissues in *Ph. turbinatus*, whereas the same was detected for Zn in *P. caerulea*. The Cluster analysis based of metals uptake was similar for both species except for Mn and Cu, in *P. caerulea* and *Ph. turbinatus*, respectively. The bioaccumulation metals in the two gastropods indicated that the ferronickel smelting plant has a heavily impact on the coastal zone, contaminating the coastal biocenoses with Fe, Mn and Zn. Finally, it seems that *Ph. turbinatus* reflects better the environmental conditions and could be considered as more efficient bioindicator.

Keywords: Smelting plant; Heavy metals; Bioaccumulation; Gastropods

Introduction

The rapidly expanding industrial and agricultural activities have led to extended contamination of the coastal marine environment. Among the polluting activities, mining and processing of metal ores is a significant source of heavy metals [1,2]. The availability of metals in seawater and suspended particles results to their uptake by the aquatic organisms via the dietary exposure, their accumulation and biomagnification into the biota tissues and later their remobilization through the food chain [3,4]. Therefore, it became an urgent need to monitor and investigate the impact of polluting activities such as mining and metal processing [5]. Such a case is a ferronickel smelting plant located at Larymna bay in the Northern Evoikos Gulf (Greece). The plant extracts iron and nickel from laterite ores and is among the biggest in Europe. The by-products of the smelting plant (namely cooling water from the furnaces, dust and slag) enter the marine environment directly and through spillages [6]. The slag is deposited into the sea at a rate of about 600 tons per day, in an area of about 30 km^2 which is located approximately 8 km far from the shore [7,8]. The slag is a brittle metal like material which contains 39.78 and 0.10% (wt %) of Fe_2O_3 and NiO, respectively [9,10].

The offshore deposition area is regularly monitored and there is enough information on the impact of the slag on the marine environment [4,7,11-16]. On the other hand very little is known on the smelting plant effect on the coastal zone. Moreover the existing research dates already two decades [6,17-32] although the industry continues its activity, there is lack of recent data and especially there is no comprehensive study. Additionally there is not any information on the level of metals in seawater near the shore and studies on metal levels of the bio-concentrated metals by the coastal biocoenosis and especially mollusks are few.

Mollusks are ecologically and commercially important on a global scale as food and as non-food resources. Most studies that provide comparisons among taxonomic groups indicate that bioaccumulation of pollutants in mollusks are in general, much greater than that in fish [27]. Mollusk shell and tissues are also good indicators of metal pollution, as these organisms are sessile and sedentary and reflect heavy metal availability [26]. The aim of the present study is a) to investigate the potential impact of the smelting plant on the coastal environment by studying the geographical distribution of bioaccumulated metals in two common gastropod species (*Patella caerulea* and *Phorcus turbinatus*) along the coastline of the smelting plant area, b) to provide information on metal bioaccumulation parameters in the two gastropod species such as their levels, their metal bioconcentration factor and their interrelation and, c) specify which gastropod is more suited as bioindicator species for detecting contamination from smelting plants.

Materials and Methods

In order to determine the metallic profile of the smelting plant area, seawater and gastropod samples were collected seasonally from autumn 2009 to autumn 2010 from seven stations along the coastline of Larymna bay and once from two reference stations far from the smelting plant (Figure 1). One litre of surface sea water was sampled manually near the shore and about 120 individuals from each gastropod species (*Patella caerulea* and *Phorcus turbinatus*) were handpicked from the tidal zone of each sampling station.

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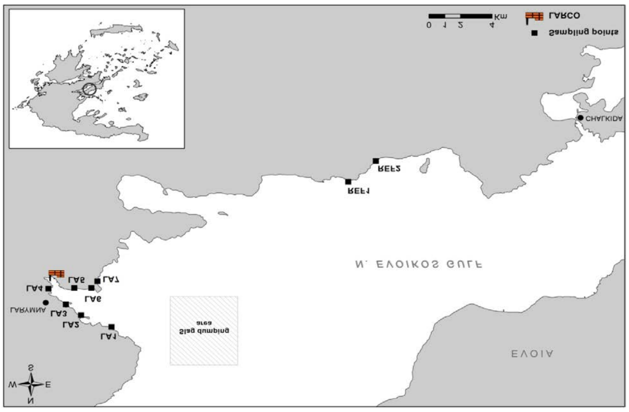

Figure 1: Map indicating the location of the sampling stations in N. Evoikos Gulf.

Both seawater and gastropod samples were stored in polyethylene bottles and polyethylene bags and brought to the lab in isotherm boxes. Heavy metals in dissolved phase were pre-concentrated on Chelex -100 resin columns [22]. The soft tissues of gastropods were separated from the shells and during each sampling occasion 8 to 12 pooled samples were prepared for each species containing 10 individual tissues of *P. Caerulea* (range size of 22-35 mm*)* and 15 of *Ph. turbinatus* (range size of 25-37 mm) respectively. Samples were freeze dried and homogenized. About one gram of dried tissue was digested with 6 ml of concentrated HNO_3 (65%) (Merck) and 2 ml of concentrated H_2O_2 (30%) (Merck) in microwave digestion system (CEM -MDS 2100) and consequently diluted up to 20 ml with Milli-Q water. Blanks were carried out in the same way.

Two groups of heavy metals were measured by Flame atomic absorption: Fe and Mn related to the smelting plant activity and Cu and Zn related to urban activities. The accuracy of the analytical procedure was checked by certified references materials. For water samples the certified reference material CASS-4 and spiked seawater and for biota samples CRM NO 279 (Ulva) and NIST 2976 (Mussel tissue) were used. The test revealed more than 90% recoveries for all metals including the water and biota samples. The statistical treatment of the data comprised control for normality and homogeneity with Levene's test, check for statistically significant differences among median concentrations of the various data sets with the Kruskal Wallis test and finally Cluster analysis (CA) in order to illustrate the similarities among sampling stations.

Results

In total 28 samples of seawater, 181 samples of *Ph. turbinatus* corresponding to 2715 individuals and 254 samples of *P. caerulea* corresponding to 2550 individuals were measured for Fe, Mn, Zn and Cu. Summary statistics including average concentrations and range of metals in biota and seawater are presented in Table 1, expressed in μg/g dry weight and μg/l respectively. It is easily observed that the two gastropod species bioaccumulated metals in different degree: *P. caerulea* had higher Zn and Fe concentrations, whilst *Ph. turbinatus* had higher Mn and Cu levels. The observed differences are notable; indeed Mn and Cu in *Ph. turbinatus* are respectively two and five times higher than those in *P. caerulea* and the concentrations of Zn and Fe in *P. caerulea* are twice higher than that in *Ph. turbinatus*. As expected, metal levels in water were significantly lower than those in the gastropods.

In order to investigate the effect of the smelting plant, the distribution of heavy metals along the coastline is presented as seasonal line plots (Figure 2). It can be clearly seen that these plots have a similar pattern in *Ph. turbinatus* for Fe and Mn; both metals related to the smelting plant activity. The same was observed for *P. caerulea* with the exception of the very high values in LA2 during winter 2010. The geographical distribution of metals apparently indicates that during the whole duration of the study the sampling stations located closer to the factory (station LA4 and during some seasons station LA5 too) are the most contaminated by Fe and Mn. Zn also showed high concentrations in both species collected at the vicinity of the industry (station LA4). On the other hand Cu presented quite completely different pattern: the higher concentrations were detected in both species in stations LA6 and LA7. In addition *Ph. turbinatus* collected at station LA3 was heavily impacted by Cu presenting concentrations even higher than those at stations LA6 and LA7. Kruskal Wallis test revealed that all the observed differences were statistically significant ($p<0.05$).

Concerning the seasonality, it has been observed that the two studied gastropods reached their maximum values during different seasons: *P. caerulea* followed the seasonal pattern of seawater presenting the highest levels during autumn and winter (2009-2010). On the contrary the highest metal concentrations in *Ph. turbinatus* were measured in spring. For both species the seasonal differences were statistically significant according to the Kruskal Wallis test ($p<0.05$).

Note that data from the reference stations, because of their limited number, due to technical reasons excluded from the statistical tests. In order to find out if the elevated metal concentrations in seawater and especially the dissolved ones related to the metallurgy industry which resulted in higher bioaccumulated levels in gastropods, correlation analysis was performed (Table 2). Additionally, correlation analysis was also applied to bioaccumulated metals in order to find out if any relation exists among them.

The two species of gastropods seem to present different sensitivity to seawater quality; for *Ph. turbinatus* a significant positive correlation ($p<0.01$) was found between concentrations of the dissolved Fe and Mn in seawater and the accumulated amount in its soft tissues, as for *P. caerulea* a significant positive correlation ($p<0.01$) was found between concentrations of the dissolved Zn in seawater and the accumulated amount in the soft tissues (Table 2). Concerning the interrelation of the bioaccumulated metals the positive significant correlation can be mentioned for both species between Fe and Mn with Zn and Mn with Fe (Table 2). It is also interesting to cite the order of magnitude of the bioaccumulated metals: in *P. caerulea* metal concentrations decrease in the following order: Fe>Zn>Cu>Mn while in *Ph. turbinatus* the metals are following a quite different order of magnitude: Fe>Cu>Zn>Mn. The Bioconcentration Factor (BCF) may be used to evaluate the bioaccumulation ability of a species in an ecosystem [18]. The formula used for the calculation is BCF=Co/Csw, where Co is the mean concentration of metals in the organism in μg/g dw and Csw is the equivalent concentration in seawater in μg/l [20].

The calculated Bioconcentration Factor (BCF) for the two studied gastropods indicates that *Ph. turbinatus* presented higher bioaccumulation capacity for Mn and Cu while *P. caerulea* got better ability in accumulating Fe (Table 3).

Finally in order to establish a better relationship between the different parameters in this study, a cluster analysis was run. This is a unique method which groups the cases that share the same variables. Ward's method and Euclidean distance were applied for this analysis.

Dataset		Zn	Mn	Fe	Cu
P. caerulea	Mean ± SD	65.1 ± 17.3	8.4 ± 5.3	4552 ± 3530	12.4 ± 5.3
	Range	38.1-126.0	1.9-33.6	843-16232	7.2-95.8
Ph. turbinatus	Mean ± SD	57.9 ± 20.9	20.9 ± 13.3	1684 ± 1036	68.1 ± 12.7
	Range	29.8-212.2	2.5-87.8	71.1-12492	29.6-117
Water	Mean ± SD	4.8 ± 1.9	1.9 ± 1.5	20.6 ± 31.1	0.6 ± 0.4
	Range	3.17-5.1	4.2-6.7	5.3-6.4	4.4-5.9

Table 1: Mean ± SD concentrations and range of heavy metals in *P. caerulea* and *Ph. turbinatus* (in µg/g dry weight) and in superficial seawater (in µg/l).

a) *Phorcus turbinatus*				
	Fe-M	Cu-M	Zn-M	Mn-M
Fe-M	1			
Cu-M	-.031	1		
Zn-M	.564**	-.136	1	
Mn-M	.895**	.079	.524**	1
Fe-w	.719**	-.133	.105	.521**
Mn-w	.773**	-.030	.191	.740**
Cu-w	-.176	.354	-.119	-.128
Zn-w	.354	.160	.348	.232

b) *Patella caerulea*				
	Fe-P	Cu-P	Zn-P	Mn-P
Fe-P	1			
Cu-P	.085	1		
Zn-P	.726**	.035	1	
Mn-P	.906**	.031	.632**	1
Fe-w	.074	.674**	.036	-.043
Mn-w	-.080	.351	-.048	-.156
Cu-w	-.182	-.100	-.057	-.187
Zn-w	.389*	-.190	.612**	.376

Table 2: Correlations among: a) metal levels in seawater and in gastropod tissues, b) among bioconcentrated metals (M: indicates *Ph. turbinatus*, P: indicates *P. caerulea*, W: indicates Seawater, **= significant correlation at the 0.01, *= significant Correlation at the 0.05 level).

Species	Bioconcentration Factor			
	Fe	Mn	Zn	Cu
Ph. turbinatus	180	13.0	12.5	312
P. caerulea	570	5.9	13.5	20.5

Table 3: Bio concentration factor (BCF) calculated for the gastropods *Ph. Turbinatus* & *P. caerulea*.

The cluster analysis was performed both for sampling stations and metals (Figure 3). It is observed that the contamination along the coastline of the smelting plant, based on *Ph. turbinatus* content is classified into three groups. The first group contained the two closer stations to the smelting plant and considered as contaminated (LA4 and LA5), the second included the two references areas and the third grouped the stations that are far from the smelting plant (LA1, LA2, LA3, LA6 and LA7). Although similar to *Ph. turbinatus,* based on *P. caerulea* content, the coastline stations are also grouped in three clusters; however the order is different. The two reference areas again formed a group, but in this case were grouped together with a station far from the metallurgic industry (station LA2), the second group contained the remained northern stations (LA1 and LA3), while the third was subdivided in one cluster containing the close to the smelting plant stations (LA4 and LA5) and in one containing the southern stations (LA6 and LA7).

The clusters based on metals presented some similarities for the two species; for instance Fe and Zn were grouped together. Meanwhile, in *Ph. turbinatus* Cu made an individual cluster and in *P. caerulea* so does Mn.

Discussion

Dumping activities, apart the enrichment of the marine environment with metals and other contaminants, have several side effects as the increase in turbidity and the consequently decrease in illumination [6]. Furthermore, the effects of tailings are comparable to those of organic and inorganic pollution since they cause ecological deterioration as reduction of the biodiversity and the total number of individuals [6].

The enrichment of the marine environment with metals results to elevated bioaccumulation in marine life that becomes potentially toxic if the concentrations were above a certain level [33]. Marine gastropods are recognized to accumulate high metal concentrations [3] due to their sedentary life cycle [6,34] and they are used as efficient bioindicators. It is known that several factors influence metal bioaccumulation by marine organisms such as seasonal dietary and food chain [6,34], climate conditions [14] physiology of species [21], body tissues [35] and of course the closeness to a pollution source, that is probably the most important one. On the latter are based both the concept of bioindicator species and the mussel watch programs [29,30].

Hence, the two common and more accessible marine gastropods of the Mediterranean (*Phorcus turbinatus* and *Patella caerulea*) have been chosen to study the impact of the smelting plant on the coastal zone of Larymna bay (Greece). The measured metal bioaccumulation values in the studied gastropods are high although the known pollution source -the slag dumping area- is located about 8 Km far away from the coast [7]. Moreover the sampling stations of the northern part of the Larymna bay (stations LA1, LA2) that are closer to the dumping site did not display any metal enrichment; instead LA2 at the cluster analysis was grouped together with the reference areas. On the contrary both *Ph. turbinatus* and *P. caerulea* collected from the two nearest stations to the smelting plant (LA4 and LA5) were heavily affected by Fe and Mn, metals abundant in the ores used by the smelting plant to produce steel. The phenomenon could probably be due to cooling and untreated waters released directly from the smelting plant, to metalliferous dust [6] escaping during transport and other activities or the smoke from the chimney of the metallurgy. Although it is confirmed by the plant authorities that metals in the smoke are trapped by special filters in the chimney, there is always possibility of metals to escape to the open air, agglomerate to particles and due to gravity and rain luckily fallout close to the smelting plant. Zn also was found in high concentrations in both species collected at the vicinity of the industry. Although this metal is not abundant in the used ores, it is usually measured near the plant in elevated concentrations probably due to side activities [36]. Zn with Cu is both considered as metals related to urbanization. We have however

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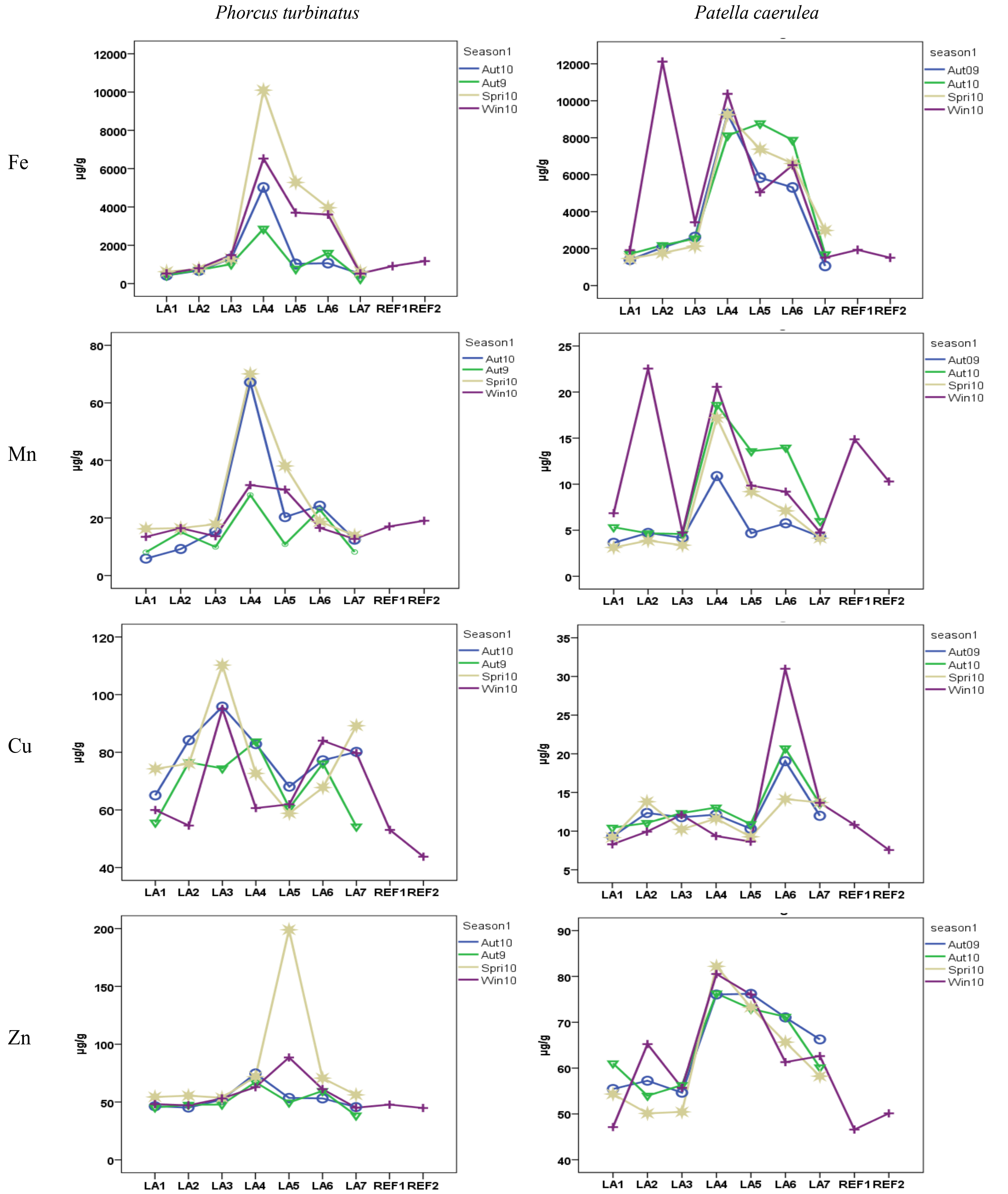

Figure 2: Heavy metals distribution in *Ph. turbinatus* & *P. caerulea* (µg/g dry weight) per sampling station and season. REF1 & REF2 indicate the Reference areas.

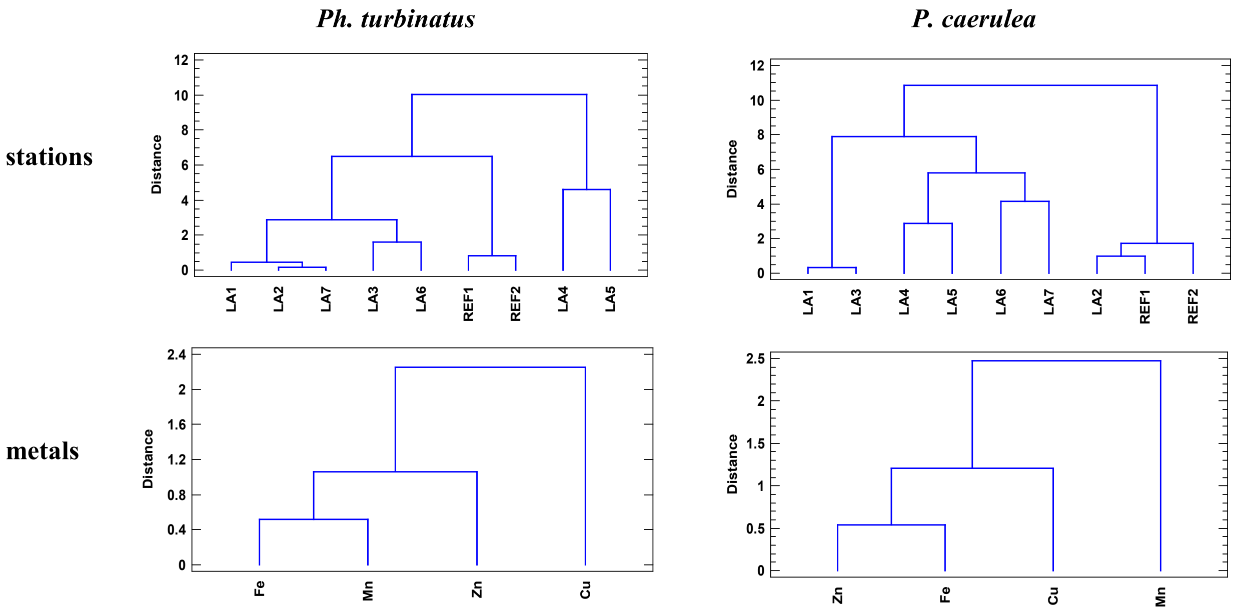

Figure 3: Cluster analysis in gastropods based a) on sampling stations, b) on metals (REF indicate the two reference areas).

to mention that the smelting plant is currently located at the border of the Larymna village and the worker's residential area. It is assumed that Cu, having different origin, was expected to present quite completely different pattern: the higher concentrations in both gastropods were displayed near aquacultures (stations LA3, LA6 and LA7). The cluster analysis for both gastropod species grouped together the closer to the smelting plant stations. In addition it grouped together the reference stations as well.

From the above is therefore clear that the metallurgy do impact the coastal zone at a distance of at least 1 km, contaminating the coastal biocenoses with Fe, Mn and Zn.

Concerning the seasonality of metal bioaccumulation, *P. caerulea* followed the seasonal pattern of seawater presenting the highest levels during autumn and winter. On the contrary, the highest metal concentrations in *Ph. turbinatus* were measured in spring, short after the wet seasons. Although it appears that the two studied gastropods reached their maximum metal values during different seasons, the season of the observed metal maxima are related to rain and runoff. The increased runoff and the concomitant introduction of suspended particulate materials to the water could augment metal uptake. Our findings are supported by Fowler and Oregioni [27] who found that the higher levels of trace metals in mussels living in the northwestern Mediterranean Sea occurred in the late winter during the time of heavy rain. Popham et al. [14] also discovered that trace metal concentrations increased in seawater and mussels after a rainfall. The essential metals such as Cu, Fe, Mn, and Zn in the whole body of *Onchidium struma* increased in autumn [35]. Moreover, *Patella vulgata* and Fucus collected from a contaminated by radionuclides area, presented high concentrations of Cd, Co, Cr, Cu, Fe, Ni, Pb and Zn in winter [37].

Besides to some factors such as the availability of metals in the environment and the different diets, the physiology of animals may play an important role in the bioaccumulation of specific metals. The comparison of bioaccumulated metal levels in the selected gastropods with those of other species from previous studies at the area [6,32,38] show that different body compartments exhibit specific metal bioconcentration pathway and mechanism [39]. Thus, it is detected that, with the exception of Fe, the concentration of metals is higher in viscera than the muscles and the whole body (Table 4). The higher metal concentrations in viscera are probably due to the large digestive gland with numerous granules that gastropods got. These granules provide electrostatic bindings sites for intermediate metals, including Mn, Zn, Fe, Co and Ni and act as site of metal localization and accumulation [6,40].

An important assumption underlying the use of bioindicators is that metal concentration in their tissues reflects the metal bioavailable level in the ambient environment [3]. However, BCF could also be influenced by the passage of contaminant through the trophic chain [19,20]. High BCF indicates the high potential of an organism to concentrate heavy metals in its tissues. Thus it is useful to express results in terms of Bioconcentration factor (BCF) to investigate the magnitude of metal uptake [25].

The calculation of the BCF indicated that *P. caerulea* accumulated Fe three times more than *Ph. turbinatus* and the latter concentrated Mn and Cu two and five times respectively more than *P. caerulea*. Similar results were reported by Conti [20], who found that *Phorcus* accumulated Cu three times more than *Patella*. Even when metals in the ambient seawater are in low levels, the relevant concentrations in the bioindicator tissues may be significant. Furthermore the uptake of some metals by marine organisms may induce that of other metals. For example Cd and Zn uptake in marine invertebrates are often coupled. The opposite, a competition between different metals, becomes probable in heavily polluted areas [20], as is the case of the present study. The strong positive correlations between the metals in the seawater samples and that in the soft tissues of both *Patella* and *Phorcus* may reflect the high metals level in the ambient water or in the trophy chain [15,33]. Among the bioaccumulated metals in both gastropod soft tissues, good correlation was found between Zn, Mn and

Species	tissue	Cu	Fe	Mn	Zn	References
Cerithium vulgatum	Viscera	110.3 ± 35.8	2674.9 ± 788	3645 ± 1234	3837 ± 1625	[39]
	Muscle	16.9 ± 6.7	355.6 ± 202.8	19.7 ± 10.07	57.9 ± 4.4	
Monodonta turbinata	Viscera	194 ± 24.7	1235 ± 398	58.2 ± 15.7	110 ± 20.5	[39]
	Muscle	39.9 ± 11.4	123.4 ± 12.3	10.08 ± 4.3	66.4 ± 9.4	
Monodonta turbinata	Whole body	68.2 ± 7.0	2307 ± 865		62.7 ± 2.9	[32]
Patella aspera	Whole body	12.6 ± 8.11	9421 ± 1153		71.0 ± 5.3	[32]
Monodonta turbinata	Whole body	20.2 ± 10.8			55.4 ± 4.9	[21]
P.caerulea	Whole body	5.9 ± 2.7			43.2 ± 4.9	[21]
P.caerulea	Whole body	2.3 ± 0.82	190.8 ± 3.2		11.8 ± 2.8	[42]

Table 4: Average metal concentrations (μg/g d w) in different gastropod species (the species Monodonta is renamed to Phorcus).

Fe which might be due to their chemical similarities [18,41]. Besides, these metals are abundant in the ores used by the metallurgy, are systematically released into the marine environment and they are found into the soft tissues of the studied gastropods in high amounts. There are very few studies dealing with the interaction of metals in marine biota [3]. Particularly in marine gastropods [42] it is difficult to discern any pattern due to the chemical properties of the metals, probably because these animals may regulate Zn and Cu to maintain a relatively constant concentration through seasons [34]. Any differences in metal chemistry can be confounded during transfer through the food chain and as demonstrated by Wang [3] trophic transfer is an important mechanism of overall metal bioaccumulation in marine invertebrates. However, for some metals regulated by biota, metal concentrations in the predatory may not necessarily reflect the bioavailable metal levels in the prey [3,24]. According cluster analysis metals presented some similarities for the two species; for instance Fe and Zn were grouped together.

The above foundlings suggest that the two gastropod species presented several similarities, as well dissimilarities in the uptake of metals:

Ph. turbinatus a) presented high concentrations in the stations closer to the smelting plant and displayed significantly high levels of Cu at a station close to an aquaculture, b) in cluster analysis based on stations the stations close to the smelting plant grouped together, as well as the reference stations, c) presented significant correlation for Fe and Mn between their concentrations in seawater and tissues, d) in cluster analysis Fe and Zn grouped together while Cu formed an individual part, e) has higher bioaccumulation capacity for Mn and Cu than *P. caerulea* and f) do not followed the same seasonal variations of metal in seawater and presenting high voncentration in spring.

P. caerulea a) presented high concentrations in the stations closer to the smelting plant, b) in cluster analysis based on stations although grouped together the stations closer to the smelting plant, as well as the reference stations which did not present a very clear picture of the area, c) presented significant correlation for Zn between concentrations in seawater and in the tissues, d) in cluster analysis based on metals, Fe and Zn grouped together while Mn formed a group apart, e) had higher bioaccumulation capacity for Fe and f) followed similar seasonal metal variations of seawater and presenting high concentration in autumn and in winter.

The reason for the observed differences could be related to the fact that although both species are herbivores (eating from microscopic plants) and found together on rocky sea beds of the intertidal and tidal bed of the medio-littoral zone [21], they are not similar species according to Gause [29]. He suggests that there are no similar species living in the same habitat and feeding on the same food, because, if they did, they would be in competition and, over time they would exclude each other. Hence, it is probable that these two species do not feed in exactly the same habitat or in the same manner on exactly the same food. Besides the depth of grazing on the rock surface of these species is different as well [29].

Based on the above findings, it seems that *Ph. turbinatus* reflects quite better the environmental conditions near a smelting plant and could be considered as more efficient bioindicator for this kind of investigation. However it is riskiness to propose only a single species for monitoring metal pollution since each species offers supplementary information.

Conclusion

In conclusion, the results of the bioaccumulation study on the coastal gastropods *Patella caerulea* and *Phorcus turbinatus* clearly indicate the impact of the ferronickel smelting plant on the coastal zone at a distance of at least 1 km, contaminating the coastal biocoenosis with Fe, Mn and Zn. The two gastropod species presented several similarities, as well dissimilarities in the uptake of metals.

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