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Analysis of Cu and Zn concentrations in shellfish from Korean coasts

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한국 연안에서 채취한 조개류의 구리와 아연 농도 분석

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Abstract The coastal environment is affected by Cu and Zn contamination through diverse routes, including antifouling agents, industrial wastewater, and domestic activities. In this study, *Crassostrea gigas* and *Littorina brevicula* were used to examine the distributions of Cu and Zn, which are related to the increase in the use of new antifouling agents that have been used for ships since the TBT ban in 2003. The average Cu and Zn concentrations in the samples were 231.8 μg/g and 492.6 μg/g, respectively. Both the Cu and Zn concentrations were highest in the shipyard samples, followed by the harbor sample for Cu and the industrial area sample for Zn. The results indicated that the Cu concentration was affected by the antifouling agents, and the Zn concentration was affected by both the antifouling agents and industrial wastewater. The areas that need intensive control to address the Cu and Zn contamination in major Korean coasts were identified.

요 약 해양 환경에서 구리와 아연의 오염은 방오제, 산업폐수, 가정하수와 같은 다양한 경로를 통해서 유입이 되고 있다. 이 중 2003년 유기주석화합물사용 금지 이후 선박에 사용되는 신방오제의 사용량증가와 관련된 중금속인 구리와 아연의 오염분포를 *Crassostrea gigas*와 *Littorina brevicula*을 통하여 확인하여 보았다. 이 결과 이들 샘플 중에 구리와 아연의 평균농도는 각각 231.8 μg/g, 492.6 μg/g으로 나타났다. 구리와 아연 두 물질 모두 수리조선소에서 채취한 샘플에서 가장 높은 농도로 측정되었으며, 다음으로 구리의 경우 항구에서 채취한 샘플에서 구리의 경우 산업단지샘플에서 고농도로 측정되었다. 이 결과를 참고 할 때 구리는 방오제에 기인된 영향을 받고 있는 것으로 아연은 방오제 이외에 산업단지폐수에 영향을 받고 있는 것으로 판단되었다. 본 연구를 통하여 한국 내 주요 연안에 대한 구리와 아연에 의한 오염 개선을 위하여 집중적으로 관리해야 되는 지역을 파악할 수 있었다.

Key Words : Cu Zn *Crassostrea gigas* *Littorina brevicula* Korean coast

1. Introduction

In many countries, marine life is used as food. Therefore, marine contamination is an important issue in many countries. Marine food resources include fish and shellfish, and are mostly collected from coastal waters rather than from mid-sea. Accordingly, contamination of coastal environments will directly affect humans who eat marine food, so such coastal environments must be properly controlled.

Coastal environments are contaminated by industrial activities, incoming and outgoing ships, and wastewater from residential areas. The heavy metal contamination caused by such industrial activities and incoming/outgoing ships imposes an especially significant burden on the ecosystem. Many countries are continuously studying the

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contamination of their coastal environments [1]

The contamination caused by incoming/outgoing ships is mostly due to the antifouling agents for ship surfaces, which has recently become a big issue. The damage wrought by compounds of tributyltin (TBT), which is the most toxic Sn antifouling agent, led to its complete ban by IMO [2]. The new antifouling agents are also basically toxic, however, and are constantly affecting coasts. They include the pesticides, Cu and Zn, which are used instead of Sn. They are reportedly contaminating the seas around ports [3].

Therefore, indicator organisms must be monitored to examine the effects of the aforementioned contaminants on marine life. *Bivalia* and *gastropoda* are frequently used for this purpose. In this study, *Crassostrea gigas*, which belong to the *bivalia* species, and *Littorina brevicula*, which belong to the *gastropoda* species, were analyzed. The contamination of Korean coasts by the two species since the TBT ban in 2003 was examined. The heavy metal contamination sources around the sampling area were classified in terms of four types, and the contamination in Korea was compared with that in other countries.

2. Materials and Methods

2.1 Reagents.

Standard solutions of Cu and Zn for AAS(1000 ppm) were purchased from Joensai. The standard materials were used without further purification. The working solution was prepared daily from the solutions. Nitric acid was purchased from Merck (78%). Deionized water was also used (18 MΩ).

2.2 Study area.

The sampling areas were classified into four types based on the contamination sources. A total of 39 samples were taken from 14 harbors, 3 industrial areas, 20 general tidal flats, 2 shipyard areas [Table 1]. Harbors were selected because they were directly affected by antifouling agents (Cu and Zn). The samples from areas adjacent to industrial areas were selected to examine Cu and Zn contamination of wastewater. General tidal flats were selected because many edible organisms exist there. Finally, shipyards were selected to examine the effects of wastewater from small ship repair yards that was discharged to coastal areas without treatment. The samples were taken from July 19 to 23, 2009. All the samples were protected by storing them in a deep freezer (-20°C).

[Table 1] Locations of the sampling points and classifications of the tidal flats

Site		Type	Kind
Incheon	I1	H	*Crassostrea gigas*
	I2	H	*Crassostrea gigas*
	I3	H	*Crassostrea gigas*
	I4	S	*Crassostrea gigas*
	I5	G	*Crassostrea gigas*
Jebu Island	J1	G	*Crassostrea gigas*
	J2	H	*Crassostrea gigas*
	J3	G	*Crassostrea gigas*
	J4	G	*Crassostrea gigas*
	J5	G	*Crassostrea gigas*
Asan	A1	H	*Crassostrea gigas*
	A2	H	*Littorina brevicula*
Taean	T1	G	*Crassostrea gigas*
	T2	H	*Crassostrea gigas*
	T3	H	*Crassostrea gigas*
Gunsan	G1	G	*Crassostrea gigas*
	G2	G	*Crassostrea gigas*
	G3	I	*Littorina brevicula*
	G4	I	*Crassostrea gigas*
	G5	H	*Crassostrea gigas*
Mokpo	M1	G	*Crassostrea gigas*
	M2	G	*Crassostrea gigas*
	M3	G	*Crassostrea gigas*
	M4	G	*Crassostrea gigas*
Suncheon	S1	G	*Crassostrea gigas*
	S2	G	*Crassostrea gigas*
	S3	G	*Littorina brevicula*
	S4	G	*Littorina brevicula*
Yosu	Y1	H	*Crassostrea gigas*
	Y2	H	*Crassostrea gigas*
	Y3	H	*Crassostrea gigas*
Gwangyang	GW1	I	*Crassostrea gigas*
	GW2	G	*Crassostrea gigas*
	GW3	G	*Crassostrea gigas*
	GW4	G	*Littorina brevicula*
	GW5	G	*Crassostrea gigas*
Pusan	B1	H	*Crassostrea gigas*
	B2	H	*Crassostrea gigas*
	B3	S	*Crassostrea gigas*
H (Harbor), General tidal flat (G), I (Industrial area), Shipyard (S)			

2.3 Procedure.

Oysters were extracted using the microwave extraction method. First, 0.5 g of oysters was put in a Teflon vessel for extracting, after which 20 ml of concentrated nitric acid for extraction was added to it and the vessel was placed in the microwave oven. The power of the microwave oven was set at 285 W, and the extraction was performed for 3 min. After the extraction, the entire solution was put in a test tube and centrifuged for 10 min at 4,000 rpm. After the centrifugation, 20 ml of the supernatant was collected and diluted in a 250 ml volumetric flask. The resulting solution was analyzed using graphite furnace atomic absorption spectroscopy (GF-AAS). The extraction method and time for Zn were the same as those for Cu, but only a 5 ml supernatant was collected and diluted in a 250 ml volumetric flask, and the resulting solution was analyzed using flame atomic absorption spectroscopy (FAAS).

2.4 Analytical measurements.

A Perkin-Elmer Analyst 700 Model Atomic absorption spectrometer equipped with Zeeman background correction and an AS-800 autosampler (Perkin-Elmer) was used throughout this study. To analyze the Cu, a 324.8 nm wavelength and a 0.7 nm slit width were used. The experiment parameters of GFAAS are listed in [Table 2]. A EUTECH cyberscan 500 pH meter equipped with a combined glass calomel electrode was used to adjust the pH.

The Zn was determined via FAAS using a Perkin Elmer Analyst 700 model atomic absorption spectrometer. The air-acetylene flame was adjusted according to the manufacturer's recommendations (wavelength: 213.9 nm, slit width: 0.7 nm, oxidant flow: 17.0 L/min, and acetylene flow: 2.0 L/min).

The 2100-mode microwave oven of CEM was used for the extraction.

[Table 2] Operating conditions for Cu via GF-AAS

	Temp. (℃)	Ramp time (min)	Holding time (min)	Internal flow (ml/min)
1	100	5	20	250
2	140	15	15	250
3	1,000	10	20	250
4	2,300	0	5	0
5	2,600	1	5	250

2.5 Recoveries and limits of detection.

To analyze the recovery, 20.0 μg/g and 120.0 μg/g of Cu was added to the standard sample and the real seawater sample, respectively, using a matrix. The replicate analyses of the spiked matrices (n = 3) demonstrated adequate precision with good recovery and repeatability. The mean recoveries (RSD) were calculated to be 98.2 ± 2.4% for Cu and 101.2 ± 3.6% for Zn. The Cu calibration curves were evaluated at 30, 60, 90 and 120 μg/g, and the Zn calibration curves were evaluated at 120, 240, 480 and 960 μg/g. All showed good linearity. The limit of detection was calculated via regression analysis, as suggested by the EPA. The detection limits for Cu and Zn were 0.001 and 0.08 μg/g, respectively.

3. Result and Discussion.

3.1 Summary of the concentration of Cu and Zn in Korean coast.

[Table 3] Summary of the concentrations of Cu and Zn in shellfish from Korean coastal areas in 2009.

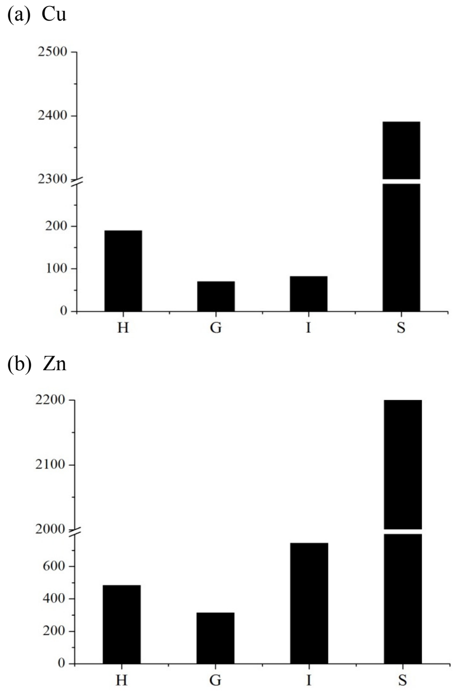
	Cu	Zn
Range (μg/g)	N.D.-3,986.6 (N.D.-571.8)	N.D.-3,587.0 (N.D.-1,432.0)
Mean (μg/g)	231.8 (115.1)	492.6 (397.0)
Frequency of detection (%)	95	100

[Table 3] summarizes the analysis results in terms of the range, mean, and frequency of detection. Because the concentrations of the shipyard samples were very high, the other results were separately indicated in parentheses. The results, including the shipyard sample concentrations, were N.D.-3,986.6 μg/g and N.D.-3,587.0 μg/g for Cu and Zn, respectively. Excluding the results for the shipyard samples, however, the Cu and Zn concentrations were N.D.-571.8 μg/g and N.D.-1,432.0 μg/g, respectively. The difference between the two results indicates that the ship repair yard significantly affected the Cu and Zn contamination in the adjacent sea. The results are very similar to those for the shipyard samples from other countries [1].

The mean Cu and Zn concentrations, including in the ship repair yard, were 231.8 μg/g and 492.6 μg/g, respectively; but without those in the ship repair yard, 115.1 μg/g and 397.0 μg/g, respectively. The Cu concentration was two times higher with the ship repair yard results than without them, and the Zn concentration differed less than the Cu concentration. This indicates that the Zn contamination was affected by the highly concentrated wastewater from industrial complexes, etc., as well as by antifouling agents. Kim et al. (2011) reported that wastewater with a high Zn concentration was being illegally discharged to Korean coastal areas through broken pipes [4].

As for the detection frequencies, Zn was detected in all the samples (100%), but Cu, in only 95%. The heavy metal concentrations were below the detection limits in Suncheon (S5) and Jebu (J4). Suncheon has a clean tidal flat that is registered with the Ramsar Convention, and Jebu is an island has around which there is no major contamination source. Accordingly, the sampling points and adjacent areas showed low Cu and Zn concentrations.

3.2 Cu and Zn levels of each sampling site



[Fig. 1] Average concentrations of (a) Cu and (b) Zn according to the area type: harbor (H), general tidal flat (G), industrial area (I), and shipyard (S). (Unit: μ g/g)

types. To further analyze the Cu and Zn concentrations, the sampling areas were classified into four types [Fig. 1]. The samples from the shipyard area had significantly higher Cu and Zn concentrations than those from the other area types, as mentioned. The high concentrations in the shellfish from the ship repair yard seemed to have been because the wastewater was directly discharged to the sea without proper treatment. In Korea, small ship repair yards discharge wastewater to the sea without proper treatment, as does the shipyard sampling area in this study. High concentrations in shipyard biosamples were also reported in other studies. According to the study of Chiu et al. (2006), the Cu and Zn concentrations in soil were 28-1,292 μg/g and 145-3,641 μg/g, respectively [5].

In the harbor, general tidal flat, and industrial area, the Cu and Zn concentration results differed. The Cu concentration was next highest in the harbor, and the Zn concentration was next highest in the industrial area. This seemed to have been due to the difference between the contamination sources for the two metals.

The high Cu concentration in the harbors was because the Cu concentration was affected by the antifouling agents used therein. The results of a study in another area also indicated that the Cu contamination was caused by antifouling agents from harbors [3].

The Zn concentration in the industrial area was the next highest to the shipyard, because the Zn contamination was affected by the wastewater from the industrial complex rather than by the antifouling agents in harbor. Many literature have reported that the wastewater discharged from industrial complexes directly affects the Zn concentration in coastal environments, and the results of this study seem to support such claim [6]. In addition, the Zn concentration was seemingly lower in the harbor than in the industrial area because Zn is a more minor component of antifouling agents than Cu [7]. Finally, the General tidal flat concentrations were the lowest of the four types.

3.3 Detailed analysis by region

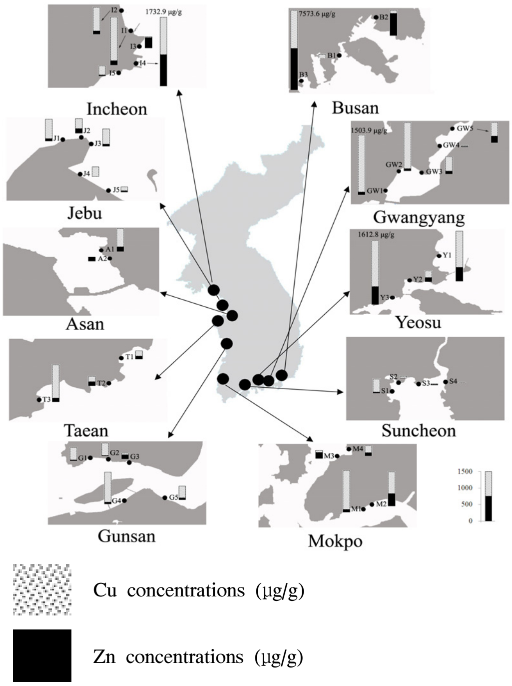
[Fig. 2] shows the data for each region. In Incheon, a ship repair yard was located in I4, where high Cu and Zn concentrations were measured. In area I5 that is a relatively clean general tidal flat, the Cu and Zn concentrations were 39.2 μg/g and 231.0 μg/g, respectively, which were lower than those in I1, I2, I3, and I4.

In the small Jebu island, three or four small fishing ships were anchored during the sampling. The average Cu concentration in the area was 56.3 μg/g, and the average Zn concentration, 294.8 μg/g, which were lower than those in the other areas.

Asan has large harbors for vehicle import and export. The average Cu and Zn concentrations in the sample harbor were 109.4 μg/g and 231.0 μg/g, respectively. The results were lower than the total averages (Cu: 231.8 μg/g and Zn: 492.6 μg/g, respectively), seemingly because the contamination was diluted in the harbor areas that were larger than the other areas.

Taean is a fishery harbor. During the sampling in T1, T2, and T3 there, about 10 ships were anchored. The Zn concentration in T3 was 848.0 μg/g, however, even though it is small. This high concentration requires detailed investigation.

In Gunsan, the Cu concentration in G3 was 117.7 μg/g, which is two times higher than in the other areas. The ship repair yard that was somewhat far from this area may have caused the high Cu concentration.



[Fig. 2] Area distributions of heavy metals (Cu and Zn) in major Korean ports

In Mokpo, the Zn concentration ranged from 58.0 to 982.0 μg/g, which was higher than in the other areas. The high concentrations in M1 and M2 were seemingly due to the large harbor 500 m to the right of M1 and the large industrial complex 3 km also to the right.

The Suncheon area has been managed as a wetland conservation area since January 2006, according to the Ramsar Convention. The average Cu concentration there was 18.2 μg/g, and the average Zn concentration, 101.3 μg/g, which are very low.

In Yeosu, the average Cu and Zn concentrations were 307.6 μg/g and 749.7 μg/g, respectively, which are very high. In the sampling site description in [Table 1], the area is an ordinary harbor, but there are military coast guard ships in Y1. Because any IMO-designated antifouling agent can be used for military coast guard ships, different antifouling agents and TBT may have been used. In Y3, about 300 fishery ships were anchored during the sampling. Y2 had a relatively low concentration, even though many ships were anchored there. This seemed to have been because the fast water flow from the narrow waterway formed by the island on the opposite side diluted the contaminated water. If many ships are anchored, they will continuously discharge blast water, and the antifouling agents that flow out from the ship surfaces reportedly contaminate the adjacent sea. The Yeosu area was seemingly also affected by the contamination [8].

In Gwangyang, the Zn concentrations in GW1 and GW2 were very high at 1,432.0 μg/g and 1,174.0 μg/g, respectively. This was seemingly due to the large steel industrial complex to the right of the area. The farther areas, such as GW3, GW4, and GW5, had Zn concentrations of 362.0 μg/g, 17.5 μg/g, and 350.0 μg/g, respectively. Teixeira et al. (1999) reported Zn contamination in the steel industry area from the higher Fe and Zn concentrations there than in ordinary areas [9].

In Busan, which had the first harbor in Korea, the Cu concentration was especially high at 571.8 μg/g in B2 and 3,986.6 μg/g in B3, where a ship repair yard is located. The Cu concentration in B1 was 5.5 μg/g, however,

3.4 Comparison of the test results with foreign study results

because the island and narrow waterway sped up the water flow, which lessened the contamination, as in Y2 in Yeosu

The test results were compared with those of other countries [Table 4]. Many studies have been conducted on *Crassostrea gigas*, but none on *Littorina brevicula*, even though it is a very common species in Korea. The results for a species that was considered similar to it were compared with the results of this study. Then the surveyed literature were classified by type and compared with the results of this study.

In an ordinary estuary in Australia, the Cu concentration ranged from 11.12 to 128.19 µg/g, and was 21.6 µg/g on the average; and the Zn concentration, from 351.63 to 531.06 µg/g, and 277 µg/g on the average [10]. In the study of Oliver et al. (2011) at a shipyard, the Cu concentration ranged from 63 to 2013 µg/g, and was 1,043 µg/g on the average; and the Zn concentration, 1,354 to 7,896 µg/g, and 5,783 µg/g on the average. Thus, the contamination by the shipyard was verified [1]. In the results of the study of Wang et al. (2011) for industrial areas with active economic development, the Cu concentration ranged from 692 to 14,380 µg/g, and was 4,412 µg/g on the average; and the Zn concentration, 4,692 to 21.050 µg/g, and 11,891 µg/g on the average [11]. The results showed that without proper control, contamination by industrial complexes could be worse than by shipyards. In the results of a study on edible oysters, the Cu concentration ranged from 1.08 to 60.18 µg/g, and was 29.05 µg/g on the average; and the Zn concentration, 192.47 to 862.04 µg/g, and 455.91 µg/g on the average [12]. The concentrations were much lower than those of the samples from the estuary, shipyard, and industrial areas. The high Zn concentration was seemingly due to the abundant Zn content of the oysters; but if it was due to contamination, the oyster farm area should be properly controlled.

In the study results for the other areas on *Littorina littorea*, a *Littoraria* species, the Cu concentration ranged

[Table 4] Levels of Cu and Zn concentration in shellfish as reported in the literature

	Species	Sampling year	Kind	Country	Description	Concentration (µg/g)	Mean (µg/g)	Type	Ref.
Cu	*Crassostrea gigas*	2006-2007	Estuary	Australia	All kinds	11.12-128.19	21.6	Dry	[10]
	Crassostrea gigas	1995	Bay	USA	Shipyard	63-2,013	1,043	Dry	[1]
	Crassostrea gigas	2010	Estuary	China	Industrial area	692-14,380	4,412	Dry	[11]
	Ostrea edulis (oyster)	2006-2007	Market	Turkey	Edible	1.08-60.18	29.05	Wet	[12]
	Crassostrea gigas	2009	Bay	Korea	All kinds	N.D.-3,986.6	258.3	Wet	(This study)
	Littorina Littorea	1992-1994	Estuary	United Kingdom	All kinds	0.34-1.34	0.61	Dry	[13]
	Littoraria Strigata	2009	Bay	Korea	All kinds	N.D.-117.7	50.3	Wet	(This study)
Zn	*Crassostrea gigas*	2006-2007	Estuary	Australia	All kinds	351.63-531.06	277	Dry	[10]
	Crassostrea gigas	1995	Bay	USA	Shipyard	1,354-7,896	5,783	Dry	[1]
	Crassostrea gigas	2010	Estuary	China	Industrial area	4,692-21,050	11,891	Dry	[11]
	Ostrea edulis (oyster)	2006-2007	Market	Turkey	Edible	192.47-862.04	455.91	Wet	[12]
	Crassostrea gigas	2009	Bay	Korea	All kinds	N.D.-3,587.0	577.7	Wet	(This study)
	Littorina littorea	1992-1994	Estuary	United Kingdom	All kinds	37.7-98.8	66.7	Dry	[13]
	Littoraria strigata	2009	Bay	Korea	All kinds	N.D.-45.0	20.1	Wet	(This study)

from 0.34 to 1.34 μg/g, and was 0.61 μg/g on the average; and the Zn concentration, 37.7 to 98.8 μg/g, and 66.7 μg/g on the average [13]. Compared with the measured concentration of *Littoraria strigata*, the Cu concentration was lower and the Zn concentration was higher.

Conclusion

In marine environments, heavy metal (Cu and Zn) contamination is caused by wastewater from industrial complexes, vehicles, and antifouling compounds. In this study, the Cu and Zn concentrations in *Crassostrea gigas* and *Littoraria strigata* shellfish were analyzed. The Cu and Zn concentrations ranged from N.D. to 3,986.6 μg/g and from N.D. to 3,587.0 μg/g, respectively. The sampling points were classified into four types, and the Cu concentration turned out high in the shipyard and harbor area samples, and the Zn concentration, in the shipyard and industrial area samples. Cu concentration of shipyard's samples is 13 times higher than harbor's samples; Zn concentration of shipyard's sample is 3 times higher than industrial area samples. And then Korea coast was affected by shipyard wastewater. The result seemed that the Cu pollution was directly influenced by the antifouling compounds from ships, but that the Zn pollution had many causes other than the antifouling agent, including industrial wastewater. From this study, our lab was able to identify key locations within Korea where focused pollution control of antifouling agents is necessary.

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