

## THE DISTRIBUTION OF Cd AND Cu IN SEDIMENTS COVERED WITH ALGAL MAT

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### ABSTRACT

In coastal marine environments, with shallow, warm and calm waters, it is sometimes possible to observe the presence of organosedimentary structures that are commonly flat and laminar in shape. These structures are called algal mats or recent stromatolites due to their remarkable similarities to fossil stromatolites. The aim of this work was the study of the distribution of the various forms of Cu and Cd in the main layers of coastal algal mats in a closed bay at Anavissos, on the coast of Saronikos Gulf.

Small sediment cores were collected from one point inside the cove on a seasonal basis and the trace metals extracted according to the BCR sequential extraction method. The main results of the study were:

Most of the labile Cd was associated with small inorganic grains or lime particles. The contribution of the fraction associated with Mn and Fe oxides, was similar to that adsorbed to organic compounds and sulfides. The high contribution of the labile Cd to the total element (over 85%) was a strong indication of anthropogenic origin.

On the other hand, most of the Cu in the sediment was associated with the crystal lattice (74%). Cu showed increased seasonal fluctuation; most of its labile forms were associated with organic compounds and sulfides whereas the less abundant form was that associated with Mn and Fe oxides.

On the whole, the sediments were slightly enriched in Cd, while the concentrations of Cu were normal for the region.

**KEYWORDS:** Anavissos, cadmium, copper, organosedimentary, cyanobacterial mat, recent stromatolites, Saronikos Gulf.

### INTRODUCTION

In coastal marine environments, with shallow, warm and calm waters, it is sometimes possible to observe the presence of organosedimentary structures, which are produced by sediment trapping, binding and/or precipitation as a result of the growth and metabolic activity of microorganisms, principally cyanobacteria. These structures are called microbial, cyanobacterial or algal mats and one of their typical properties is their laminated structure in which different functional groups of microorganisms occur in vertically stratified layers. This type of lamination can be attributed to different growth periods, seasonal events, periodical events (e.g. tides), and episodic or erratic events (e.g. storms) (Brock, 1976; Golubic, 1976; 1983; Stal, 2000).

Calcification is the general process of consolidation and preservation of microbial mats. Laminated rocks dating from the Precambrian or later eras are known as stromatolites (Krumbein, 1983). Still living well-laminated and consolidated stromatolites can be found in the Bahamas (Exuma Cays), as well as in Australia (Sharks' Bay) (Reid and Brown, 1991). Nowadays algal mats have remarkable similarities with fossil stromatolites and are often characterised as recent stromatolites. However, the most important difference between recent and fossil stromatolites is that the great majority of recent stromatolites do not form consolidated rocks.

These structures have been observed in various Greek coastal areas. The most important of these are mainly located in the peninsula of Attika (Anavissos and Rafina), in eastern Crete and on the island of Lesvos (east Aegean Sea).

The aim of this work was the study of the distribution of various forms of Cd and Cu in the main layers of coastal algal mats in a characteristic Mediterranean area. The area of Anavissos was selected for this study due to its ideal conditions for the development of such formations. Cd and Cu are environmentally significant metals, widely affected by anthropogenic inputs (Dassenakis *et al.*, 2003). Cd is classified as a dangerous pollutant and a monitoring priority in the Mediterranean region. On the other hand, Cu is a biologically important metal but is also used in antifouling and pesticide products. High concentrations of this metal in the marine environment may be dangerous to ecosystems.

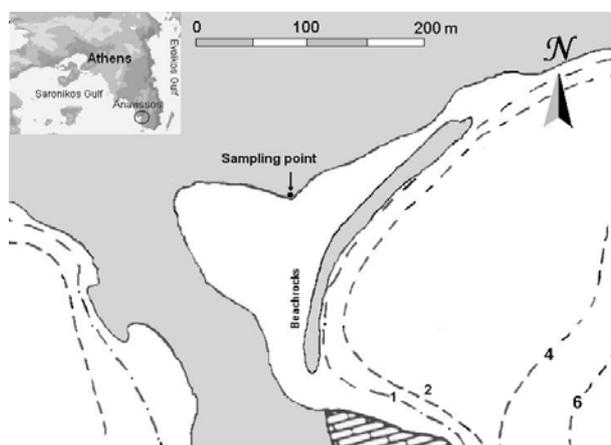


Figure 1. Area of study

The area under research is a closed bay at Anavissos on the coast of Saronikos Gulf, 50 Km away from Athens, covering an area of about 20,000 square meters (figure 1). A line of beach rocks shuts the bay's entrance, obstructing water renewal. A remarkable reciprocation of the cove's waters due to the wind is also recorded. Throughout the shallow bottom of the cove (max. depth of about 0.6 meters), a layer of green algae with a thickness of 0.2–2 mm is observed (Krumbein, 1983; Reid and Brown, 1991).

There is an occasional high inflow of fresh water into the cove, usually after heavy rainfalls. No significant pollutant point sources exist in the area but diffused pollution is possible because: (a) during the last few years, many holiday homes have been built in the nearby hills that surround the area. The use of septic tanks (of the cesspool type), in combination with the fact that the groundwater level is very close to sea level, create conditions that favour the contamination of the nearby marine area with urban sewage; (b) refuse is observed all around the area and sometimes in the sea; (c) a number of small recreation and fishing boats use the cove as a safe and free anchorage and their wastes may seriously affect the ecosystem.

## MATERIALS AND METHODS

### Sampling and sample treatment

Sediment cores of about 10 cm long were collected from one point inside the bay that is partly covered with seawater. Samplings were carried out seasonally from July 2000 to June 2001. The cores were separated into three layers according to their structure (Figure 2):

(a) The upper layer is 0.5 – 1.2 mm wide and includes the cyanobacterial mat;

- (b) The middle layer is up to 5 cm wide, dark in colour, anoxic and has the characteristic odour of hydrogen sulphide;
- (c) The lower layer is about 5 cm wide, brown in colour and represents the background sediment.

The separated layers were freeze-dried, sieved through 0.25 mm sieves and stored in plastic bottles.

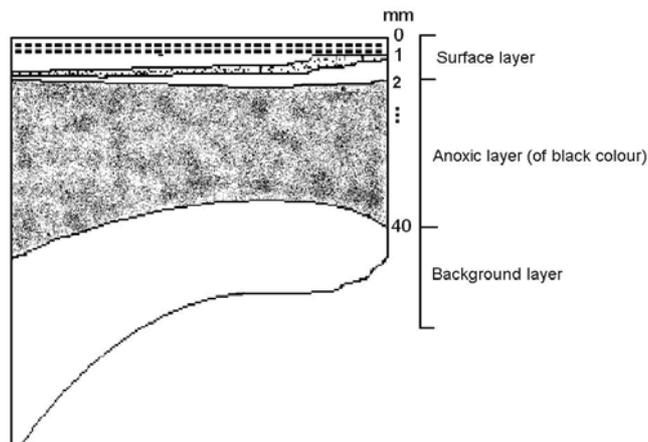


Figure 2. Structure of the algal mat

### Sequential extraction procedure

A slight modification of the BCR sequential extraction procedure was performed in sediment samples (Quevauviller *et al.*, 1997; Rauguet *et al.*, 2000). It was chosen because the mobility of trace metals, as well as their bioavailability and related eco-toxicity, depends strongly on their specific chemical forms or ways of binding. Consequently, these have to be determined rather than the total element content in order to assess the toxic effects and study geochemical pathways. According to this procedure, the trace metals are separated into four fractions:

- Fraction A: Metals connected to small inorganic particles and carbonates;
- Fraction B: Metals connected to oxides/hydroxides of Mn and Fe;
- Fraction C: Metals connected to organic compounds and sulphides;
- Fraction D: Metals strongly bound in the alumino-silicate lattice.

Briefly, the sequential extraction procedure applied was as follows:

- Step 1 - Fraction A: About 1g of the sediment sample was shaken with 40 ml  $\text{CH}_3\text{COOH}$  0.11M for 16 hours at room temperature.
- Step 2 – Fraction B: The residual of step 1 was shaken for 16 hours with 40 ml  $\text{NH}_2\text{OH}\cdot\text{HCl}$  0.5M at pH 2 at room temperature.
- Step 3 - Fraction C: The residual of step 2 was treated with 10 ml  $\text{H}_2\text{O}_2$  30% at 85 °C until the solution volume was reduced to 1 ml. Then 50 ml  $\text{CH}_3\text{COONH}_4$  1M (at pH 2) were added and the solution was shaken for 16 hours at room temperature.
- Step 4 – Fraction D: The residual of step 3 was treated with a mixture of strong acids ( $\text{HF}$ ,  $\text{HNO}_3$ ,  $\text{HClO}_4$ ) at 120 °C to total dissolution (Thompson and Walsh, 1983; UNEP, 1985).

A reference sediment (CRM-601) was treated by this method. The results are presented in Table 1.

### Apparatus

A Flame Atomic Absorption apparatus (Varian Spectra AA-200) equipped with Sample Introduction Pump System and  $\text{D}_2$  lamp for the background signal correction was used for the determination of Cu. A Graphite Furnace Atomic Absorption apparatus (Varian Spectra AA-640Z GTA 100) equipped with Zeeman background correction system was used for the determination of Cd.

Table 1. Recovery of the metals in the CRM-601 reference sediment using the BCR sequential extraction method (values in mg kg<sup>-1</sup>)

Metal	Fraction A		Fraction B	
	Certified value	Value found	Certified value	Value found
Cd	4.14	3.88	3.08	2.65
Cu	8.32	10.2	5.69	5.25
Metal	Fraction C		Fraction D	
	Certified value	Value found	Certified value	Value found
Cd	1.83	1.37	1.30	1.06
Cu	116	117	60.4	48.5

## RESULTS AND DISCUSSION

The mean percentages of the various fractions of Cd and Cu for all samplings are presented in Table 2.

The annual mean percentages of the various fractions of Cd and Cu in the surface, anoxic and background layer of the sediment are presented in Figure 3.

The labile forms of Cd are about 85% of the total metal content indicating that this metal is mostly originating from pollution sources.

Fraction A (i.e. the Cd associated with small inorganic grains and carbonates) contains most of the labile Cd (defined as the sum of fractions A, B and C). The content of the sediment in fraction A is similar within layers, as the annual mean values for the surface, anoxic and background layer are 0.600 mg kg<sup>-1</sup>, 0.624 mg kg<sup>-1</sup> and 0.593 mg kg<sup>-1</sup> respectively (Figure 4). This behaviour is attributed to the elevated lability of Cd and to the fact that this metal is not involved in biochemical procedures and therefore can easily be dispersed via the pore water to the various layers of the sediment in a considerable depth.

Table 2. Percentages (over total) of Cd and Cu fractions in the various sediment layers

Cd						
Layer	Season	Fraction A (%)	Fraction B (%)	Fraction C (%)	Fraction D (%)	Total (mg kg <sup>-1</sup> )
Surface	Sum 2000	8	38	34	20	1.230
	Aut 2000	38	18	28	16	0.430
	Win 2001	45	19	17	19	0.961
	Spr 2001	79	5	10	6	2.170
Anoxic	Sum 2000	45	14	22	19	0.266
	Aut 2000	39	25	20	15	0.335
	Win 2001	51	22	10	17	0.463
	Spr 2001	87	4	4	6	2.310
Background	Sum 2000	51	11	19	18	0.691
	Win 2001	60	14	17	10	1.390
Cu						
Layer	Season	Fraction A (%)	Fraction B (%)	Fraction C (%)	Fraction D (%)	Total (mg kg <sup>-1</sup> )
Surface	Sum 2000	3	1	13	84	9.14
	Aut 2000	5	2	35	58	8.26
	Win 2001	5	1	22	72	6.07
	Spr 2001	4	< 0.5	19	77	7.66
Anoxic	Sum 2000	3	1	37	59	6.52
	Aut 2000	5	1	40	54	6.35
	Win 2001	6	1	21	73	6.08
	Spr 2001	6	1	24	69	3.41
Background	Sum 2000	4	1	29	67	6.03
	Win 2001	1	0.5	2	97	9.05

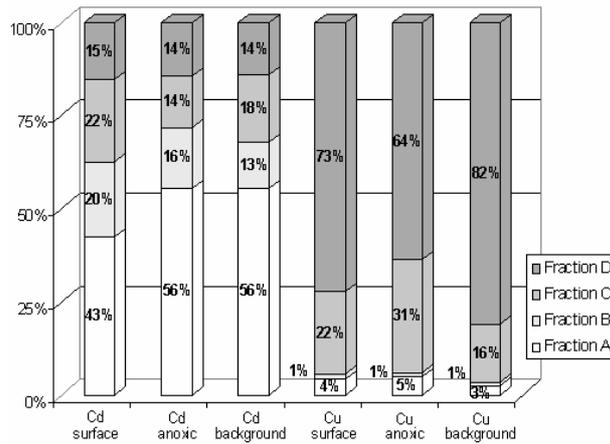


Figure 3. The mean annual percentage distribution of Cd and Cu fractions in the various sediment layers

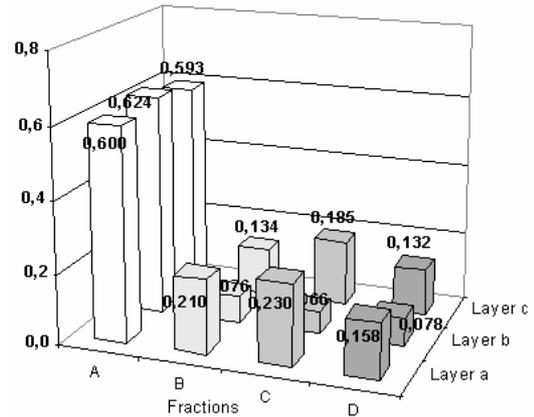


Figure 4. The distribution of Cd within the sediment's layers (values in mg kg<sup>-1</sup>)

The content of the labile forms of Cd in the sediment's surface layer has a similar seasonal fluctuation with the concentration of Cd in the water column as presented in Figure 5 (Ladakis et al., 2007). This indicates the influence of the water column on the surface sediment for the specific metal.

There are also no remarkable differences in the % contribution of Cd's B and C fractions in each layer (see table 2). The seasonal fluctuation of the sediment's content in Cd shows a remarkable increase of fraction A during spring and a slight increase of fraction B during winter in both surface and anoxic layers. The seasonal fluctuation of fraction C is relatively random.

With the exception of fraction A, the anoxic layer of the sediment contains less Cd than the surface and background layers.

No correlation was detected between the seasonal fluctuation of Cd and organic matter (Total Organic Carbon - TOC or carbohydrates - TCHO) in the sediment.

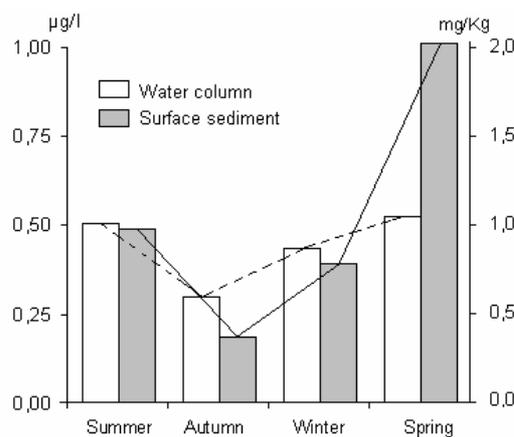


Figure 5. The seasonal fluctuation of Cd in the water column (values in µg l<sup>-1</sup>) and the surface sediment (values in mg kg<sup>-1</sup> - labile forms only)

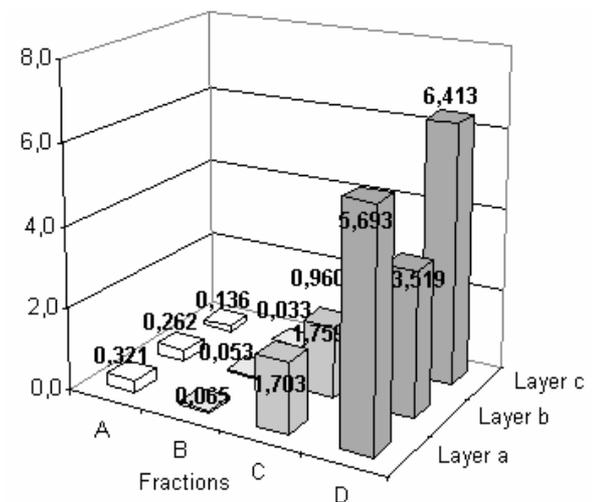


Figure 6. The distribution of Cu within the sediment's layers (values in mg kg<sup>-1</sup>)

Most of the Cu in the sediment is associated with the crystal lattice (fraction D) (Table 2 and Figure 3). Considering the biological importance of Cu, we conclude that the elevated

contribution of Cu's fraction D to the background layer, is attributed to the absence of biological activities in this layer, a fact that does not encourage its enrichment with the labile forms of this metal.

The most abundant labile form of Cu is the fraction associated with organic compounds and sulphides (fraction C), as is apparent from Figures 3 and 6. This distribution is in accordance with the well-known tendency of Cu to be bound to organic matter (Breault *et al.*, 1996; Salomons and Forstner, 1984). On the other hand, the lowest values were recorded in fraction B (Cu adsorbed to oxides of Mn and Fe). The vertical distribution of the labile forms of Cu in the sediment shows a decrease with depth. Specifically, the concentrations of the labile forms of Cu in the surface and anoxic layers of the sediment are about the same, whilst twice as much is contained in the background layer (Figure 6).

This distribution explains the low values of Cu's fraction B. Most of the labile forms of Cu accumulate in the surface and anoxic layers of the sediment that are rich in organic compounds and sulphides; most of the Cu is adsorbed on those compounds resulting in the depletion of Cu in fraction B.

With regard to the seasonal fluctuation of the labile forms of Cu, the highest values were recorded during autumn both in the surface and anoxic layer of the sediment (Figure 7). The increased values of Total Organic Carbon (3.7% w/w) and Carbohydrates in the sediment during the same period, is the probable reason for this fluctuation (Ladakis *et al.*, 2006). On the other hand, no correlation was detected between the concentration of Cu in the water column and the content of the labile forms of the same metal in the surface layer of the sediment.

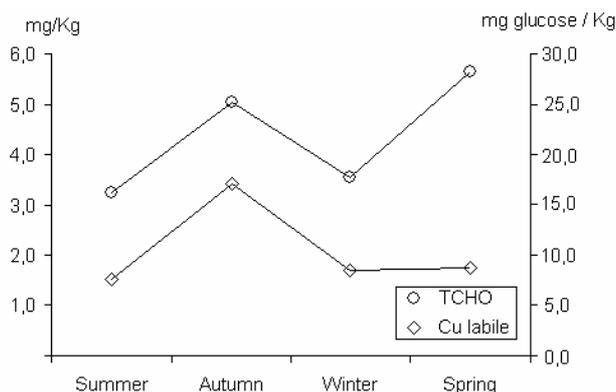


Figure 7. The seasonal distribution of the labile forms of Cu (left axis) and total carbohydrates (right axis) in the surface layer (values in  $\text{mg kg}^{-1}$ )

The mean concentrations of the total Cd and Cu in sediments of some areas of Saronikos gulf are presented in Table 3. According to this table, the content of the sediments under study in Cu is lower compared to the sediments of the open Saronikos gulf. On the other hand, Cd is present in higher levels in comparison to sediments of polluted areas such as Elefsis bay and the island of Psitalia. These two areas are heavily affected by human activities and are rich in organic mater and metals.

Table 3. Total Cd and Cu in sediments of some areas of Saronikos gulf (values in  $\text{mg kg}^{-1}$ )

AREA	Cd	Cu
Anavissos bay (Zotiadis, 2004)		18.0
Offshore Anavissos (Zotiadis, 2004)	<0.4	10.0
East Saronikos (Zotiadis, 2004)	0.4	19.0
Elefsis bay (Giannopoulou, 2005)	0.97	164
South Psitalia isl. (Giannopoulou, 2005)	0.81	85.0
Studied sediments (Surface)	1.20	7.78
Studied sediments (Anoxic)	0.84	5.59
Studied sediments (Background)	1.04	7.54

Moreover, the surface layer of the sediments originating from Elefsis bay (Sakelari, 2006) have a similar % distribution in the various fractions of Cd and Cu with that recorded in the surface layer of the area under study, as presented in Table 4:

Table 4. Comparison of the % distribution of Cd and Cu in sediments of Elefsis and Anavissos bay

Fractions	Cd (%)		Cu (%)	
	Elefsis Bay (2004-5)	Anavissos	Elefsis Bay (2004-5)	Anavissos
A	33	43	2	4
B	13	20	< 1	1
C	7	22	17	22
D	46	15	81	73

Therefore, this could indicate that in sediments rich in organic matter, the various forms of both Cd and Cu show a specific distribution pattern.

The TEL/PEL (Threshold Effect Level / Probable Effect Level, (Long *et al.*, 1998) criteria were applied in order to characterize the contamination level of each layer of the sediment. The results are presented in Table 5.

Table 5. Contamination level of the sediment according to the TEL/PEL criteria

Metal	Surface layer	Anoxic layer	Background layer
Cd	Low contaminated	Low contaminated	Low contaminated
Cu	No contaminated	No contaminated	No contaminated

## CONCLUSIONS

The existence of the cyanobacterial mat on the surface of the sediment influences the temporal, spatial and fractional distribution of Cd and Cu, although their distribution is also affected by their biological importance and geological background, as well as human influence. Thus, differences between the fluctuations of these two metals were recorded in all of the distributions mentioned above.

With regard to their seasonal distribution, the highest values for Cd were recorded during spring and the lowest during autumn, whereas the highest values for Cu were recorded during summer and the lowest during spring (Table 2).

As regards the vertical distribution of Cd, the anoxic layer is depleted in all fractions of Cd except fraction A (Figure 4). On the other hand no such behaviour was recorded for Cu.

Cd is mostly adsorbed in the sediment's small inorganic particles and carbonates (fraction A). Fraction D of this metal (crystal lattice) does not exceed 20% indicating that its origin is mostly human. This indication is in accordance with the fact that Cd is mainly associated with human activities. Also, the increased mobility of this metal is a possible danger to the ecosystem.

Cu, a metal with significant biological importance shows increased seasonal fluctuation, in the surface layer of the sediment. Its main fraction is D and most of its labile forms are associated with organic compounds and sulphides (fraction C). The abundance of extra cellular polymeric substances excreted by the cyanobacteria, as well as the high content of the sulphides in the anoxic layer (Stal, 2000), favour the agglomeration of the labile forms of Cu to this fraction.

The % fractional distributions of Cd and Cu are similar to those prevailing in Elefsis gulf, a marine area of Saronikos gulf, heavily affected by human activities and rich in organic matter and various metals.

Finally, according to the TEL/PEL criteria, we can conclude that the sediments of the studied area are partially contaminated with Cd but not with Cu.

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