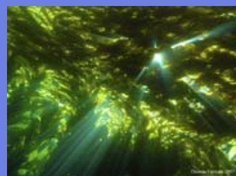
Identifying and Characterizing Polluted Surface Waters in the Southern California Bight Based on Metal Level in Kelp (*M. pyrifera*) Sieve Tube Sap #1145



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Abstract:

Metal concentrations in kelp sieve tube sap (STS) may be useful in determining the dispersal of coastal effluent plumes. Common trace metals average a 100-fold increase in concentration in STS as compared to their total concentration in seawater. In this study, concentrations of 19 different metals were measured in STS using ICP-MS. STS was collected from *Macrocystis pyrifera* fronds obtained from several coastal southern California locations, including a presumed "non-polluted" reference site on the seaward side of Catalina Island. Results indicate the presence of 8 new elements not formerly identified within *Macrocystis* STS including: Rb, Pd, Ag, Sn, and Cs. Several metals (*e.g.* Cr, Cu, Zn, Pb) were found at significantly higher levels within STS collected from populations inside the Port of Los Angeles/ Long Beach in comparison to Catalina Island. Also, significant differences in levels of other metals (*e.g.* Fe, As, Ag, Cd) were found between populations within and directly outside the harbor breakwall. These results indicate the potential usefulness of this method in spatially describing metal pollution arising from coastal sources.

Introduction

Kelps have a primitive translocation system that transports a mannitol-based fluid called sieve tube sap (STS) throughout the individual; STS contains metals at much higher levels than their average concentration in the surrounding seawater (Table 1). STS is primarily composed of mannitol (60% dry weight) and amino acids (15%), which are the major products of kelp photosynthesis, and inorganic ions (20%) (Manley, 1984), which include nutrient and non-nutrient trace metals such as As, Mn, Cd, Pb, Cr, Cu, and Zn. (Manley, 1983). Many of these trace metals are released into the Southern California Bight (SCB) through terrestrial runoff and treated waste water (Schiff et al., 2000). Since STS is a cellular fluid, the trace metal concentrations within it should reflect their bioavailability in the surrounding seawater although the response time is unknown. Information provided by this research will provide a new method for accessing levels of bioavailable metal pollution released into the nearshore ecosystem. It will also be used to find spatial relationships between metal concentrations in the sap and pollutant outfall into the coastal waters of the SCB.

This study uses STS to identify metal-contaminated surface waters in Los Angeles County and attempts to characterize the degree of their coastal influence by measuring elevated metal levels in STS. Little is known about the effective range of such plumes and their impact on coastal communities. However, this information is crucial in risk assessment for different biological populations. Although previous studies (not in California) have monitored amounts of metal accumulation in whole algal tissues, kelp sieve tube sap has never before been used as a pollution indicator. It is anticipated that results from this research will be used to assess and differentiate metal concentrations from each pollution source, allowing the comparison among input from urban rivers, sewage runoff, harbors and ports on a toxicity level.

Figure 1: Map of study area.

Methods:

Macrocystis STS has been extracted monthly from 10 sites in Los Angeles County waters (Fig. 1). Fronds were selected randomly and sap was extracted (300µL each) while in the field to ensure high flow. All samples were analyzed for chloride content by GranÕ method using a Cl^- electrode to ensure that no seawater contamination occurred during collection. 50 STS samples were extracted each month (weather allowing) from natural *M. pyrifera* populations and analyzed for natural elemental isotopes with masses ranging from 27-208 using the IIRMESÕ Perkin Elmer 6100 ICP-MS (Fig. 2). Over 250 STS samples have been analyzed to date. Continued sampling over 12 consecutive months is planned in anticipation of wet-weather runoff (precipitation events) causing increased metal levels in STS.

Sample preparation prior to ICP-MS analyses requires HNO_3 digestion followed by dilution. Ga, Y, and Tl are added to the dilutant as internal standards. At least 5 reagent blanks and a blank spike are prepared similarly during each run to ensure instrument detection limits for each metal of interest. A calibration curve prepared from a standard containing a suite of metals with known concentrations provides a comparison to find concentrations of relevant metals within the sap.

Results and Discussion:

•8 new elements have been identified within *Macrocystis* STS; including Rb, Pd, Sn, and Cs (Table 1, Fig. 5)

•All the trace metals identified are concentrated in the STS as compared to their seawater concentrations (Table 1). Although certain trace metals (i.e., Co, Cu, Zn, V, Mn) are known micronutrients for *M. pyrifera*, many of the STS metals have no known biological function (i.e. Pb, Rb, Sr, Ag, Ni, Cs) (Kuwabara & North, 1980, Pinto et al, 2003).

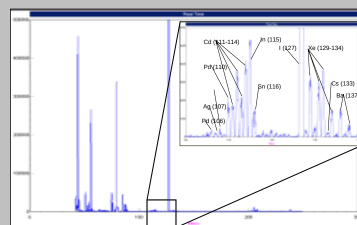

Figure 2: An STS sample from the LB Harbor analyzed by ICP-MS in the scanning mode through the periodic table to define the elements present in the sample. Expanded part of the mass spectrum from 100-140 showing elevated Cd, Ag, I, Sn, Cs, and Ba. The In is from the internal standard while the Xe is an impurity in the argon used in the plasma.

Metals	L.B. Harbor Inside BW	L.B. Harbor Outside BW	Bunker Pt., Palos Verdes	Escondido, Malibu	Kelp Pt., Catalina	Average Standard Deviation	Detection Limits on ICP-MS	Concentration in Seawater	Bio-concentration in STS: ([STS]/[seawater])
V	203	163	133	103	128	25.7	1.84	2.5	81.2
Cr	244	243	237	259	291	27.3	3.30	0.3	812
Mn	47.6	37.8	21.5	24.1	21.6	9.10	5.64	0.2	238
Fe	1.91×10^3	1.16×10^3	611	164	384	298	162	2	956
Ni	11.7	BDL	BDL	BDL	BDL	10.1	10.6	1.7	6.89
Co	7.82	3.48	1.97	1.51	2.30	1.50	1.55	0.05	156
Cu	322	160	148	188	201	51.3	90.6	0.5	644
Zn(64)	1.48×10^3	780	431	395	366	243	361	4.9	301
As	3.13×10^4	2.98×10^4	2.67×10^4	2.73×10^4	4.03×10^4	6610	7.22	3.7	8460
Se	338	139	159	144	220	56.6	3.02	0.2	1690
Rb	2.74×10^3	2.32×10^3	2.20×10^3	2.17×10^3	2.42×10^3	499	4.69	120	22.9
Sr	1.16×10^3	1.01×10^3	816	530	639	196	5.99	8×10^4	-
Ag	1.67	2.35	6.72	4.89	BDL	0.89	1.51	0.04	168
Cd(114)	586	474	661	664	634	133	3.19	0.1	6610
Ba(138)	26.4	36.6	BDL	11.3	28.4	14.2	9.90	2	18.3
Pb	241	888	136	170	153	108	61.6	3×10^{-2}	2.96×10^4

Table 1: Metal values found at 5 sites in September 2007 within *Macrocystis* STS, analyzed by ICP-MS. All values are in ppb (µg/L^{-1}) and represent a mean of 5 samples taken from the canopy. Column 8 shows detection limits for this analysis; calculated as 3x SD of the blanks. Last two columns indicate the average abundances of these elements in seawater, taken from Brewer, (1975), and the bio-concentration factor of these metals in STS (STS concentration over SW concentration). Metals highlighted in red exhibited highest values found in the Long Beach Harbor (either within or directly outside the breakwall). BDL indicates that values were below detection limits. Although the SD shown for each metal represent averages; it is important to note that the harbor samples regularly show higher variation.

- **Most trace metals exhibit higher levels in STS in the Long Beach Harbor/ Port of Los Angeles than in other coastal locales (Table 1).** The majority of metals measured have shown significance when comparing the Catalina reference sites with those in the harbor. Significant differences have also been found between sap populations separated physically only by the Long Beach breakwall for Mn, Zn, Fe, Cu (p=0.03, 0.03, 0.0004, 0.05 respectively).
- High levels of arsenic (As) present at all sites (Table 1) may be a result of increased arsenate uptake in the absence of phosphate, a process that has already been described in higher plants (Maugh, 1979; McSheehy, 2003).

- **Some STS metals (Type 1 in Fig. 3) levels decrease with increasing distance from the Port of Los Angeles.**

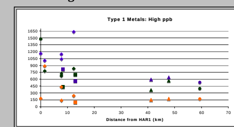

Figure 3: Type 1 metals (Zn, Sr, and Pb) indicate concentrations up to 1600 ppb, µg/L^{-1} in kelp STS at a site within the Long Beach Harbor (HAR1). These levels appear to decrease with increasing distance from the Port. All values characterized in these graphs represent a mean of 5 samples taken from a given site.

- **For others (Type 2, Fig. 4), there seems to be no correlation with distance from the Port;** however relating distance from another point source might produce different results.

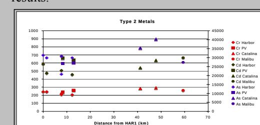

Figure 4: Type 2 metals (Cr, Cd, and As) do not indicate a gradient of metal concentration in STS with distance from the L.B. harbor. Values for As are on the right axis, values for Cr and Cd on the left.

- **Some metals (type 3) show higher concentrations at other sites than the Port.** This could be due to different runoff sources; Ag shows increased concentration especially in Malibu (Fig. 6), but also in adjacent sites along Palos Verdes.

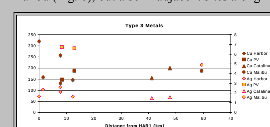

Figure 5: Type 3 metals (Cu, Ag) have shown a different pattern in STS metal concentration with distance from the Port.

- **Malibu samples exhibited low concentrations of most trace metals analyzed (Pb, Zn, Fe, Cu, etc.), but contained higher concentrations of Ag than at any other sampled area. High levels of Ag have been reported in both bivalves and fish sampled from Malibu Lagoon (Cohen et al., 2001); most likely a result of sewage runoff (Sanudo-Willhelmy et al., 1992).**

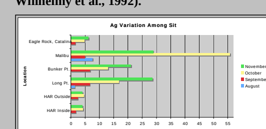

Figure 6: Comparing Ag concentrations among sites from months of August-November 2007. Higher Ag concentrations appear at sites within Santa Monica Bay, especially Malibu, when compared to others.

- **Collection sites within the harbor breakwall have shown consistently high levels of Zn from months of June through November. Temporal variation to date shows increased concentrations during fall months (compared to summer) for many metals.**

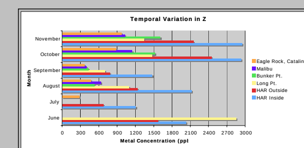

Figure 7: Concentrations of Zn vary from June-November 2007; it appears that many sites show increased concentration during fall months. Harbor sites demonstrate consistently higher values than all other sites.

- **Temporal variation is evident; precipitation events are most likely causing the increase in metal concentration seen in October and November samples. Since the months of December-January typically have more severe storm events, it is predicted that a correlated increase in metal concentration will be determined.**

Conclusions:

- **STS metals have been classified Type 1, 2, and 3 based on distance from the Long Beach Harbor/ Port of Los Angeles.** Further analysis of these values could indicate gradients from different outfall points (*e.g.* Ballona Creek, Malibu Lagoon, or the Hyperion treatment plant) along the studied coastline.
- **Micronutrient (*e.g.* Zn, Mn, Fe) and non-nutrient (*e.g.* Ni, Pb, Cs) STS metals levels are elevated in the harbor/port sites (Figs. 3 and 7).** Elevated STS metal levels appear to reflect exposure to elevated metal levels in polluted locations (*i.e.* port/harbor); despite the presence or absence of a metabolic function.
- **This STS technique may allow for the determination of the origin, range, and temporal variability of polluted seawater plumes**. Potentially a ÒSTS metal fingerprintÓ could identify a pollution source, such as Zn, Cu, and Ni for LB Harbor/ Port of L. A.
- **High levels of these metals, caused by exposure to polluted waters, are known to be toxic to marine organisms that live in or around kelp beds. This research strongly suggests that other members of the kelp forest ecosystem are also being exposed, especially those that consume *Macrocystis*.**

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