## TRACE METALS IN FLATFISH AROUND OUTFALLS

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During the past year, we conducted an intensive study of the extent of metals enhancement in flatfish collected from contaminated and control sediments. This study was a continuation of our initial investigation into metals concentrations in liver tissue from Dover sole. We had concluded earlier that specimens known to have lived in highly contaminated sediments showed no evidence of contamination by eleven trace elements. However, that study had involved only liver tissue and did not cover chromium, nickel, or lead three of the dozen or more trace metals of greatest interest. Also, the control specimens available at that time were considerably smaller than the outfall specimens, raising the possibility that our results had been biased by this size difference. Therefore,

we continued our studies on the Dover sole in an attempt to fill these gaps, using a different method of laboratory analysis, which provided independent data, and new

collections whenever possible.

Over 10,000 measurements of approximately 25 elements were made on a special emission spectrometer developed by George Alexander at University of California, Los Angeles. In this article, we will discuss seven trace metals that are wastewater constituents of great concern at present: These are silver, cadmium, chromium, copper, nickel, lead, and zinc. Four of the more volatile elements of interest (arsenic, mercury, antimony, and selenium) were not measurable by this method, but in our previous study, they were not found to be concentrated above background in livers taken from outfall specimens. Analysis of intercalibration samples provided by the National Bureau of Standards generally were within +10 percent of reference values for those elements detected (chromium, copper, lead and zinc). Detailed descriptions will be reported later in a technical memorandum.

The Dover sole is a particularly appropriate organism for this study because it is widely distributed along the southern California coast and can usually be obtained by trawl in relatively large numbers from the bottom sediments around submarine outfalls. In addition, this flatfish is affected by fin erosion disease at a high frequency around the largest discharges, and appears to exhibit the detrimental effects of the disease to a greater degree than any other species from these discharge sites that we have examined.

Because we have found that Dover sole from uncontaminated control regions are often of a different size than specimens collected around the outfalls, we first conducted an investigation into the importance of this parameter in comparing tissue concentrations. We looked at three size classes, selected on the basis of preliminary information relating age and length: 280 mm ("adults"). To obtain a sufficiently large number of specimens in each of these classifications, we used data on all Dover sole collected during 1971 73 from areas of negligible or only minor contamination (Santa Barbara Channel, Port Hueneme, Zuma Beach, San Pedro Canyon, Newport Beach, and Dana Point).

Application of the Wilcoxon rank sum test revealed no statistically significant differences at the 95 percent confidence level between the metal concentrations in prejuveniles and those in either the juveniles or the adults.

This lack of any detectable effect of size on metal concentration permitted us to obtain larger sample sizes for other comparisons. As discussed above, one question of considerable importance is whether or not flatfish living in contaminated sediments around major outfalls concentrate these metals above normal tissue levels. To investigate this matter, we compared concentrations of the seven metals in flesh, gonads, and livers of Dover sole collected from the Palos Verdes shelf and from the Santa Barbara Channel. The Palos Verdes samples consisted of tissues from up to sixteen individuals taken in a single bottom trawl at one highly contaminated station near the outfall (Station T4 450) in March 1973; the channel samples were obtained from up to ten individuals collected during November 1972 from several trawls in this control region. Estimated metal enrichment factors in the surface sediments of the outfall station (relative to control sediments), and median concentrations of the seven metals in the three tissues of outfall and control specimens, are presented in Table 1.

Of the metals for which we have quantified median concentrations in tissues in specimens from both areas, we found no large enrichments in the outfall samples. Although the median silver concentration in the flesh of the outfall specimens is twice that of the controls, the difference is not statistically significant at the 95 percent confidence level. However, the difference is significant at the 90 percent confidence level. This is also true for the nickel concentrations in liver tissue. Only for chromium in liver tissue is the higher median value for the outfall specimens statistically significant. There is a corresponding apparent enrichment of chromium in the gonad tissue of these specimens. This enrichment is not significant at the 95 percent confidence level, although it is at the 90 percent level. Because of the limits in our ability to detect chromium in the control liver tissues (median

In contrast to this single case where the outfall specimens exhibited a statistically significant enrichment, there are two cases where these specimens exhibited statistically significant depressions of metals, relative to the controls. These are cadmium in liver tissue and silver in gonadal tissue; the depression factors are somewhat less than two, and greater than three, respectively. In our previous study, we observed such depressions for several trace metals in outfall specimen livers. We do not yet understand the reasons for these apparent depressions in concentrations or whether or not they have any ecological significance.

In light of the unusually high incidence of fin erosion disease in Dover sole trawled from contaminated sediments around the Palos Verdes outfalls, we conducted preliminary tests to determine if metallic enhancements were associated with this disease. From the single trawl at outfall Station T4 450 in March, 1973, eight "obviously diseased" specimens were selected along with eight "apparently undiseased" specimens. We dissected seven tissues from each fish flesh, gonads, liver, kidney, heart, brain, and gill arches (the tissues

selected were those for which we could obtain a minimum sample without interference from the highly contaminated sediments from which the fish were taken).

Metal concentrations were determined using the same techniques as for the other samples. We then applied the Wilcoxon rank sum test to the values for the "diseased" and "undiseased" specimens, for all seven metals and all seven tissues. The significant differences are listed in Table 2.

All of the differences between medians for diseased and undiseased specimens are significant at the 95 percent confidence level, except for chromium in liver tissue; for that comparison, the difference is significant at the 90 percent confidence level. Because we conducted almost fifty statistical tests, at these confidence levels, several "significant" differences could be expected to occur purely by chance. Thus, for example, one could conclude that the observed significant difference in the case of lead in kidney tissue might be a random result of multiple testing. However, it seems unlikely that the remaining four significant differences should all occur for chromium purely by chance: It is quite possible that there is in fact an association between fin erosion disease and abnormal concentrations of this metal in gonad, heart, liver, and kidney tissue. The gonad and heart tissues of diseased specimens had depressed chromium levels relative to the controls, while the liver and kidney tissues had enriched chromium concentrations.

Both chromium and lead are known to have an affinity for epithelial tissue. Indeed, in the tuna, lead has been shown to exist principally in and on the skin. Also, it appears that Dover sole from the Palos Verdes outfall region are less "slimey" than those from control regions, indicating a possible epithelial involvement. In the next phase of this continuing program, we will prepare skin samples from diseased and apparently healthy outfall specimens to minimize sediment contamination before trace metal analysis.

The fact that there is a correlation between abnormal chromium levels in four tissues and fin erosion disease of course does not imply a cause and effect relationship. To establish or disprove that, definitive laboratory experiments would have to be performed. The Project hopes to begin such experiments during the coming year and also to replicate and expand critical sections of the field program completed to date.

Details of this investigation will be reported during the year in a technical memorandum and subsequent scientific publications.

## **TABLES**

**Table 1.** Estimated sediment enrichments and median concentrations (mg/dry kg) of seven metals in tissues from Dover sole trawled near the Palos Verdes outfalls (Station  $T_4$ -450) and from a control region in the Santa Barbara Channel.

|                |                                    |         | Flesh |         |         | Gonads |         |         | Liver |         |
|----------------|------------------------------------|---------|-------|---------|---------|--------|---------|---------|-------|---------|
| Trace<br>Metal | Sediments<br>Outfall to<br>Control | Outfall |       | Control | Outfall |        | Control | Outfall |       | Control |
| Silver         | 3.2                                | 0.2     |       | 0.1     |         |        | 0.3     | 0.4     |       | 0.5     |
| Cadmium        | 150                                |         |       |         |         |        |         | 3.8     |       | 6.4     |
| Chromium       | 16                                 |         |       |         | 0.4     |        |         | 0.4     |       |         |
| Copper         | 20                                 | 0.6     |       | 0.5     | 10.7    |        | 12.7    | 7.4     |       | 9.5     |
| Nickel         | 2.4                                |         |       |         |         |        |         | 2.6     |       | 1.4     |
| Lead           | 52                                 | 1.3     |       |         |         |        |         | 5       |       | 4.6     |
| Zinc           | 12                                 | 39.4    |       | 38.0    | 214     |        | 233     | 91.8    |       | 108     |

**Table 2.**Chromium and lead (mg/dry kg) in the tissues of diseased and apparently healthy Dover sole from the Palos Verdes shelf.

|          | Chromium | Lead |
|----------|----------|------|
| Gonads   |          |      |
| Diseased |          |      |
| Healthy  | 0.9      |      |
| Heart    |          |      |
| Diseased |          |      |
| Healthy  | 0.4      |      |
| Liver    |          |      |
| Diseased | 0.6      |      |

| Healthy  | 0.2 |     |  |
|----------|-----|-----|--|
| Kidney   |     |     |  |
| Diseased | 0.5 | 6.0 |  |
| Healthy  | 0.2 | 3.1 |  |