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# Trace Metals in Limpets (*Patella* sp) from the Coast of Santa Cruz de Tenerife (Canary Islands)

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It is well known that plankton, molluscs and fish tend to accumulate metal pollutants as they generally are not well able to regulate these pollutants in their body. Thus, they concentrate these elements to the extent available in their environment. Body size as well as season, are factors which can influence tissue levels and must be taken into consideration (Boyden 1974, 1977). It has also been shown (Martin 1974; Galindo *et al.* 1986) that the concentration of heavy metals in some marine organisms are related to each other.

As a part of a monitoring program of the coast of Santa Cruz de Tenerife, Canary Islands (Díaz *et al.* 1990), limpets were chosen for analysis because they are almost sedentary, widely distributed and concentrate heavy metals (Boyden 1974, 1977). They are also important as food in these islands, being eaten raw, fresh or preserved in vinegar. For this reason, they were not subjected to purification prior to analysis nor were they taxonomically identified. However, limpets found in the Canary Islands belong to the *Patella candei candei*, *Patella candei crenata*, *Patella piperata* and *Patella ulisiponensis* species (Hernández Dorta 1986). Due to their almost sedentary character, it can be said that limpets sampled for this study live in similar habitats and were exposed to similar levels of heavy metals.

The present paper examines the relationships between trace metal contents and wet body weight of limpets, as well as the intermetallic relationships for a wide range of limpets collected at three locations along the coast of Santa Cruz de Tenerife. Principal component analysis and Ward's agglomerative methods of data analysis available in the pattern recognition packages ARTHUR (Harper *et al.* 1977) and CLUSTAN (Wishart 1982) were applied in order to show the overall structure of the data in multidimensional space.

## MATERIALS AND METHODS

570 limpets ranging from 0.5 to 3g soft tissue were collected at three locations, at mid-tide, in eight samplings from July 1984 to

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January 1987, and they were frozen at -20°C until analyzed. After thawing, they were removed from their shell and weighed in porcelain dishes, dried under IR irradiation and then ashed at 450 ±10°C in an electric furnace until white ash was obtained. This was then dissolved with 10mL hot HCl (1+1), filtered and made up to 25mL with deionized water in a volumetric flask. The resulting solutions were analyzed by flame atomic absorption spectrophotometry (Pye Unicam SP1900) using deuterium arc background correction (Perkin Elmer 3030B) for Cd, Pb and Zn.

Statistical analysis was carried out on Digital VAX/VMS 11/780 and IBM 3085 computers.

## RESULTS AND DISCUSSION

Since the different *Patella* species found in the Canary Islands are used as food under the generic name of "lapas" (limpets), we did not distinguish between them either analytically or statistically.

The mean concentrations of Pb, Cd, Fe, Ni, Cu and Zn are shown in Table 1. In general, of the metals for which the Spanish Food Directorate has set tolerances for molluscs (Pb, Cd and Cu: 3, 1 and 20ppm, respectively), there is no indication of any health hazard in the individuals analyzed. Only 4 (0.7%) and 6 (1%) individuals showed higher concentrations of cadmium and lead, respectively, than these tolerance levels.

Table 1. Mean concentration (X±SD, ppm) and range, wet weight basis, for Pb, Cd, Fe, Ni, Cu and Zn in the overall samples and in each sampling station.

| Element | Overall | Station 1 | Station 2 | Station 3 |
|---|---|---|---|---|
| Pb | 1.19±0.79 | 1.99±1.00 | 2.17±0.61 | 2.37±0.68 |
| | 0.50-6.36 | 0.50-6.36 | 0.74-4.14 | 0.88-4.56 |
| Cd | 0.31±0.37 | 0.39±0.63 | 0.26±0.11 | 0.28±0.10 |
| | 0.09-7.54 | 0.10-7.54 | 0.09-1.13 | 0.10-0.75 |
| Fe | 327±341 | 222±149 | 531±490 | 208±69 |
| | 1.20-4,083 | 1.20-1,300 | 98-4,084 | 53-474 |
| Ni | 1.84±0.04 | 1.96±0.07 | 2.06±0.07 | 1.47±0.04 |
| | 0.67-10.0 | 0.74-5.36 | 0.79-10.0 | 0.67-4.90 |
| Cu | 3.23±1.85 | 2.74±1.96 | 3.61±1.91 | 3.29±1.56 |
| | 0.10-15.2 | 0.10-11.6 | 1.17-15.2 | 1.05-11.0 |
| Zn | 10.2±3.20 | 0.98±5.00 | 11.4±3.20 | 10.2±3.80 |
| | 0.33-48.0 | 0.33-42.5 | 6.41-30.6 | 4.82-48.0 |

Concentration factors for these metals were calculated taking into account the corresponding concentrations of metals in the surrounding seawater previously reported (Díaz *et al.* 1990). The result was Fe(6,500) > Cd(1,600) > Zn(850) > Ni(620) > Cu(450). However, the calculated value for iron concentration factor varied significantly with sampling date, due perhaps to seasonal variations of iron in seawater caused by increased rainfall (Díaz *et al.* 1990).

The results of regression between metal content (μg/individual) and wet body weight (g) of limpets collected at each of the three sampling stations and the three stations considered together are given in Table 2. Considering the three stations together, the regression coefficients ranged from 0.723 for Cu to 0.882 for Pb, being significantly lower for Cd, Fe, Ni and Cu than for Pb and Zn. Regression coefficients calculated for each sampling station ranged from 0.677 to 1.061 for Fe at stations 2 and 1, respective-

Table 2. Parameters of the log-log relationship between trace metal (μg/animal) and wet body weight (g) of limpets collected at the three sampling stations.

| Metal | station | N* | Regression coefficient** logW±2σ(logW) | Intercept** logM±2σ(logM) | R*** |
|---|---|---|---|---|---|
| Pb | 1 | 184 | 0.874±0.060 | 0.229±0.019 | 0.764 |
| | 2 | 198 | 0.874±0.030 | 0.343±0.010 | 0.875 |
| | 3 | 183 | 0.886±0.037 | 0.383±0.032 | 0.873 |
| | 1,2,3 | 565 | 0.882±0.026 | 0.332±0.008 | 0.824 |
| Cd | 1 | 184 | 0.693±0.075 | -0.444±0.094 | 0.663 |
| | 2 | 199 | 0.919±0.044 | -0.598±0.043 | 0.830 |
| | 3 | 180 | 0.771±0.041 | -0.529±0.038 | 0.810 |
| | 1,2,3 | 563 | 0.814±0.032 | -0.531±0.010 | 0.728 |
| Fe | 1 | 185 | 1.061±0.201 | 2.122±0.059 | 0.363 |
| | 2 | 199 | 0.677±0.071 | 2.689±0.011 | 0.558 |
| | 3 | 182 | 0.819±0.045 | 2.335±0.019 | 0.803 |
| | 1,2,3 | 567 | 0.787±0.077 | 2.406±0.025 | 0.394 |
| Ni | 1 | 185 | 0.770±0.055 | 0.305±0.003 | 0.721 |
| | 2 | 198 | 0.854±0.048 | 0.304±0.003 | 0.787 |
| | 3 | 183 | 0.807±0.041 | 0.187±0.002 | 0.826 |
| | 1,2,3 | 566 | 0.803±0.029 | 0.268±0.009 | 0.758 |
| Cu | 1 | 161 | 0.839±0.128 | 0.342±0.090 | 0.435 |
| | 2 | 199 | 0.728±0.052 | 0.564±0.053 | 0.708 |
| | 3 | 183 | 0.712±0.047 | 0.548±0.041 | 0.746 |
| | 1,2,3 | 543 | 0.723±0.048 | 0.490±0.016 | 0.542 |
| Zn | 1 | 185 | 0.966±0.087 | 0.896±0.007 | 0.631 |
| | 2 | 198 | 0.822±0.029 | 1.075±0.043 | 0.898 |
| | 3 | 179 | 0.845±0.034 | 1.026±0.019 | 0.878 |
| | 1,2,3 | 562 | 0.861±0.033 | 1.005±0.010 | 0.741 |

*number of possitive samples; ** 95% confidence limits;*** significance of the correlation coefficient P≥0.001.

ly. Regression coefficients were higher at station 1 than those for stations 2 and 3. For Cd and Ni regression coefficients were higher at station 2 than at stations 1 and 3.

It has been suggested that variations of slope from one location to another could be caused by differences in gonadal development between animals from different sites (Cossa *et al.* 1979, 1980; Cossa and Rondeau 1985). Thus, we further analyzed these relationships as a function of season. Table 3 shows that, except for Fe, the other metals studied, Pb, Cd, Cu, Zn and Ni, show significant minimum in winter while for Cu and Zn the regression coefficient increased steadly from summer to winter. Fe displayed an "anomalous" behavior in that it showed a continuous decrease of regression coefficient from spring to winter, which can be associated with high Fe concentration of the surrounding seawater during rainy weather. This phenomenon may enhance individual variations by fine particulate matter remaining in the gut or lumen of the digestive tubule of the limpets analyzed as they were not depurated before analysis.

Table 3. Seasonal variation of the regression coefficients for the overall samples.

| Season | Equation | R** | N*** |
|---|---|---|---|
| Spring | log[Pb] = 0.932logW + 0.295 | 0.735 | 93 |
| | log[Cd] = 0.866logW - 0.557 | 0.835 | 93 |
| | log[Fe] = 0.977logW + 2.420 | 0.766 | 92 |
| | log[Ni] = 0.875logW + 0.207 | 0.682 | 93 |
| | log[Cu] = 0.722logW + 0.507 | 0.737 | 93 |
| | log[Zn] = 0.952logW + 0.995 | 0.891 | 93 |
| Summer | log[Pb] = 0.870logW + 0.335 | 0.864 | 193 |
| | log[Cd] = 0.799logW - 0.534 | 0.807 | 193 |
| | log[Fe] = 0.799logW + 2.509 | 0.560 | 193 |
| | log[Ni] = 0.811logW + 0.356 | 0.842 | 193 |
| | log[Cu] = 0.747logW + 0.564 | 0.704 | 193 |
| | log[Zn] = 0.765logW + 1.058 | 0.805 | 192 |
| Autumn | log[Pb] = 0.989logW + 0.334 | 0.804 | 117 |
| | log[Cd] = 0.888logW - 0.501 | 0.705 | 119 |
| | log[Fe] = 0.667logW + 2.466 | 0.667 | 99 |
| | log[Ni] = 0.892logW + 0.218 | 0.755 | 122 |
| | log[Cu] = 0.838logW + 0.587 | 0.726 | 99 |
| | log[Zn] = 0.826logW + 1.005 | 0.876 | 99 |
| Winter | log[Pb] = 0.860logW + 0.319 | 0.835 | 158 |
| | log[Cd] = 0.797logW - 0.542 | 0.592 | 159 |
| | log[Fe] = 0.616logW + 2.538 | 0.467 | 161 |
| | log[Ni] = 0.779logW + 0.219 | 0.783 | 158 |
| | log[Cu] = 0.860logW + 0.424 | 0.727 | 159 |
| | log[Zn] = 0.885logW + 1.026 | 0.834 | 160 |

*[] = µg/animal; W = wet weight, g; **for $P \le 0.0001$; ***number of positive samples.

The regression coefficients of the log-log relationships between a given metal and limpet wet weight obtained in this study are consistent with values given by Boyden (1974, 1977) for *Patella vulgata* and *Patella intermedia* for every metal stuied but for Cd. The value obtained for Cd (0.814) in our study is definitely lower than those previously reported (1.35-2.05). In addition, while Boyden observed almost uniform regression coefficients for this metal, we found significant differences for the different seasons. These differences can be tentatively explained in terms of the formation of Cd-thionein which depends on the concentration of Cd in the surrounding seawater (Noël-Lambot *et al.* 1980).

Our reults also show that Ni behaves similarly to Cd and Pb, with regression coefficient of 0.80; a behavior not previously established by Boyden for this mollusc.

However, no changes in slope was detected between smaller and larger individuals as found for *Mytilus edulis* (Cossa *et al.* 1980) The seasonal changes of slope observed are probably due to biochemical variations asociated with reproduction and seasonal adaptation (Orton *et al.* 1956; Dare and Edwards 1975), in addition to the influence which can be exerted by seaonal variations of the metal concentration in the surrounding seawater (Boyden 1977). However, no correlation could be established between metal content or concentration in limpets and in the surrounding seawater using the data previously reported by Díaz *et al.* (1990) for the same sampling years.

The similar behavior of some metals in limpets suggests the possibility of their inter-relation. Thus, we have carried out firstly a study of simple correlation between their concentrations in order to establish whether there were correlations significant enough within the population studied to establish positive metabolic or pollution relationships between these heavy metals.

Table 4. Equations for the metal-to-metal regressions

| Equation: $Y = (b \pm SD_b)X + (a \pm SD_a)$* | R |
|---|---|
| log[Ni] = (0.613 ± 0.077)log[Cd] + (0.907 ± 0.002) | 0.427 |
| log[Ni] = (0.340 ± 0.031)log[Zn] + (0.056 ± 0.001) | 0.462 |
| log[Ni] = (0.401 ± 0.038)log[Fe] - (0.295 ± 0.007) | 0.453 |
| log[Fe] = (0.478 ± 0.037)log[Cu] + (1.240 ± 0.033) | 0.511 |

* [] = ppm.

Table 4 summarizes the results of the logarithmic metal-to-metal regressions whose significance values indicate the intimate relationship of each other pair. Even though correlation coefficients are not very high, significances are high enough ($P \leq 0.0001$). Table 4 also indicates that the concentration of Ni is related to that of Cd, Zn and Fe, and the Fe concentration is related to those of Cu, Cd, Ni, etc. Based on these facts, analysis of multiple regression was carried out to determine if the concentration of one metal could be explained in terms of the concentration of the others. Results shown in Table 5 indicate that the concentrations of these heavy metals are inter-related from metabolic or pollution standpoints. Nevertheless, further studies are needed in order to explain such correlations, as correlation does not indicate causality.

Table 5. Equations resulting from the multiple regression analysis of the data

| Equation* | multiple R |
|---|---|
| 1/[Cu] = 6.298/[Fe] - 0.112/[Ni] - 0.016/[Cd] + + 3.037/[Zn] - 1/[Pb] + 0.068 | 0.793 |
| 1/[Fe] = 0.041/[Cu] - 0.003/[Ni] - 0.002/[Cd] + + 0.055/[Zn] - 0.009/[Pb] + 0.004 | 0.648 |
| 1/[Ni] = 0.033/[Cu] = 0.245/[Fe] + 0.058/[Cd] + + 0.189/[Zn] + 0.443/[Pb] + 0.120 | 0.516 |
| 1/[Cd] = 0.212/[Cu] - 4.608/[Fe] + 2.565/[Ni] + + 0.741/[Zn] + 3.079/[Pb] + 0.434 | 0.538 |
| 1/[Zn] = 0.137/[Cu] - 0.383/[Fe] + 0.029/[Ni] + + 0.031/[Cu] + 0.074/[Pb] - 0.013 | 0.741 |
| 1/[Pb] = - 0.024/[Cu] - 0.297/[Fe] + 0.308/[Ni] + + 0.051/[Cd] + 0.352/[Zn] + 0.068 | 0.540 |

*[] = ppm.

To obtain more information about these behaviors, data were statistically analyzed by applying Ward's agglomerative method (Wishart 1982) and principal component analysis (Harper *et al.* 1981) to show the multidimensional distribution of the metals studied. In a way similar to other studies undertaken to manage the quality of environmental systems (Favretto and Favretto, 1984a, 1984b, 1988).

On using the Ward's linkage agglomerative procedure of hierarchical clustering, no clear conclusion could be reached. However, when sampling date or sampling station is taken as the main variable some tendecy to cluster formation is observed, which can be tentatively explained in terms of the almost sedentary character of limpets.

The eigenvalues obtained from the principal component analysis of the data indicate that at least four principal components are needed to account for more than 80% of the total variance. From the factor loadings obtained after a Varimax rotation and its comparison with the corresponding values before rotation, it can be seen that the correlation between a principal component and a metal is ameliorated as a general rule. More than four principal components are still needed to explain about 80% of the total variance. This cluster configuration can be tentatively explained considering that the limpets analyzed are almost sedentary and grow in an almost homogeneous seawater (Díaz *et al.* 1990).

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