AIR POLLUTION BIOMONITORING IN ARGENTINA, APPLICATION OF NEUTRON ACTIVATION ANALYSIS TO THE STUDY OF BIOMONITORS

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Abstract:

Due to low population density, total air pollutant emissions in Argentina are still low if compared with highly industrialised countries. Although a significant deterioration of air quality has been observed for a long time, air monitoring did not begin until the 90's and only in a few cities. The use of air pollution biomonitors represents an important contribution to Argentina, as measurements of air pollutants in large areas would require especial technical equipment not easily available and operated.
In this project, two lichen species (Ramalina ecklonii (Spreng) Mey & Flot and Usnea amblyoclada (Müll. Rg.) Zahlbr.) and a Bromeliaceae (Tillandsia capillaris) are used as biomonitors of air pollution at a 50,000 km^2 area in Córdoba province (central Argentina). AAS and INAA have been applied for the analysis of samples, determining As, Ba, Br, Ce, Co, Cr, Cs, Cu, Eu, Fe, Hf, Gd, K, La, Lu, Mn, Na, Ni, Pb, Rb, Sb, Sc, Sm, Ta, Tb, Th, U, Yb and Zn. The following physiological parameters were also determined: chlorophyll a, chloropyll b, phaeophytin a, phaeophytin b, hydroperoxy conjugated dienes, malonaldehide and sulphur. Some of these parameters were used for calculating a pollution index. These determinations were carried out on pools collected at the sampling sites.
AAS and physiological parameters were also applied to the analysis of five-replicate samples in order to study variability sources.
For data evaluation, different statistical and other evaluating tools were used: descriptive statistics and Spearman's correlation analysis were used on data from the three biomonitor species while factor analysis and mapping, only for R. ecklonii results.

1. INTRODUCTION

Due to the low population density, the total air pollutant emissions of Argentina are probably still low in comparison with highly industrialised countries. However, and like in some other South American countries, they are rapidly increasing and, even though the detailed emission inventories of urban and industrial centres are being compiled by state environmental agencies, they are scarce and inefficient [1].

Although a significant deterioration of ambient air quality, caused by the locally and regionally high air pollutant emissions from different kinds of sources, has been observed for a long time, the establishment of environmental agencies and the installation of air-monitoring networks did not take place until the 90's and only in a few cities. Major air pollution problems are occurring at urban and industrial centres, increasing pollution levels, however, they can also be observed at remote sites as a consequence of agricultural practices and mineral mining and processing [2].

Thus, the use of air pollution biomonitors represents an important contribution to Argentina where measurements of particulate matter or other types of pollutants in big areas would require expensive technical equipment, not available in the country at this moment.

In this project, we set to assess the behaviour of two lichen species (*Ramalina ecklonii* (Spreng.) Mey. & Flot. and *Usnea amblyoclada* (Müll. Arg.) Zahlbr.) and of one *Bromeliaceae* (*Tillandsia capillaris* f. *incana* (Mez.)) as biomonitors of air quality, considering both their accumulative properties and their physiological response.

Although several studies have shown that trace element concentrations in biomonitors show average concentration of particulate matter in air and both wet or dry depositions of pollutants over a certain time [3-4], this kind of research has not been carried out so far on a large scale in Argentina.

The possibility to systematically assess air quality from the lichens' physiological response was described about a decade ago [5] and has been studied for some species in Argentina [6]. Due to the specific response to different pollutants, some species transplanted to urban and industrial areas are excellent markers of different emission sources and good for biomonitoring atmospheric quality [7-8-9].

Besides lichens, other epiphytic plants have been used as biomonitors for metal and trace elements depositions [10-11]. *Tillandsia* genus, which is highly spread in South America, shows a large number of species, characterised because of their high tolerance to hydric stress. However, there are few works reporting on their physiological response to pollutants, and the species growing in Argentina have not been studied considering their biomonitoring ability.

2. METHODS

2.1. Study Area

The study area covering 50,000 km^2 in central Argentina, is a quadrilateral area whose four corners are at the following co-ordinates: to the West, 31° 25' 21" S, 65° 24' W; to the East, 31° 41' 15" S, 62° 38' 34" W; to the North, 30° 36' S, 64° 15' W; to the South, 32° 48' S, 64° 10' 12" W. Land morphology is highly variable, ranging from a mean altitude of about 250 meters in the south-east to more than 2,500 m to the mid-west. There are cities (high and medium sized) and many small villages in the area; industrial plants, mainly metallurgical, petrochemical, chemical, food, vegetable oil and cement, are mostly located in the centre and south where the highest population density is recorded.

For sampling purposes, the chosen area was divided according to a square pattern, each square of 25 by 25 km (80 sampling points in the area) collecting samples of selected species at each intersection point. At them, the collection sites were located at least 500 m from major routes and highly populated areas, and at least 300 m from streets with lower traffic density.

2.2. Sampling collection

Three bioindicators were selected, two lichen species: *Ramalina ecklonii* (Spreng.) Mey. & Flot and *Usnea amblyoclada* (Müll. Arg.) Zahlbr. and a Bromeliaceae, *Tillandsia capillaris* f. *incana* (Mez.). They are present in the study area but they are not evenly distributed as they represent different environments or phytogeographic provinces. *R. ecklonii* and *T. capillaris* are found in tree trunk substrates, whereas *U. amblyoclada* is found in rocks. The two lichens are fruticose species.

2.3. Experimental Design

Pools of the three different species were collected (when possible) at the sampling sites. Each pool consisted of 40 – 50 individuals, randomly taken along the four cardinal directions within an area of 100 x 100 m. Extraneous material was removed from each sample and they were put in paper bags. The collection was done using plastic gloves to avoid any risk of sample contamination [12].

In order to analyse intra-site variability, five-replicate samples were collected at 20 % of the sites [13]. At these sites, five pools were collected, from the same substrate if possible.

Sampling was conducted only if a five-days-without-rain condition was fulfilled. Once at the laboratory, the samples were let to dry on filter paper, in a clean enclosure at room temperature, for 24-48 hours.

2.4. Sample Pools

Each species pool was prepared mixing several individuals collected along the four cardinal directions, at each sampling site, no more than 100 m from the geographically referenced point. When possible, the samples were collected from the same kind of substrate (forophyte species). For *T. Capillaris*, similar diameter individuals were collected. Chemical determinations were done in triplicate, from independent sub-samples in each sample corresponding to each pool.

2.5. Replicates

The points for collecting the five-replicates were set using a random numbers table. Each replicate was formed by a pool of individuals collected from the same substrate, at the four cardinal directions and no more than 100 m from the point. The samples were put in paper bags and labelled.

Chemical determinations were done in triplicate, from three independent sub-samples in each sample corresponding to each replicate

2.6. Analytical methods

Multielemental analysis was carried out using Instrumental Neutron Activation Analysis (NAA) and Atomic Absorption Spectrometry (AAS) was used for some metal determination. For chemical-physiological parameter quantification, different methodologies were used [13].

2.7. Sample treatment previous to AAS

The samples (kept at room temperature) were washed with cold (4 °C) bi-distilled water in a relation 1:50 W/V. For washing purposes, each sample was put into a white nylon mesh bag (previously washed with bi-distilled water) and submerged in a glass of bi-distilled water for about 5 seconds while rotating it 180°. This procedure was repeated three times without changing the water.

Once washed, the samples were put in a Petri capsule and dried in oven at 50 ± 2 °C for 72 hours. Afterwards, *R. ecklonii* and *T. capillaris* were ground in a porcelain mortar, while *U. amblyoclada* was separated into small segments using Teflon covered tweezers. After

being ground and homogenised, the samples were dried in oven till constant weight. From this material, 0.5 g (dry weight) was taken for metal quantification by AAS.

2.8. Sample preparation for NAA

The samples were washed as described in point 2.7 and let dry at room temperature in a clean area. Plant material was ground in an agate mortar with the aid of liquid nitrogen addition and then freeze-dried for 24 hours.

2.9. AAS determinations

Masses of about 0.5 g of dry material were ground and reduced to ashes at 650°C for 4 hours. The ashes were digested with HCl (18%): HNO_3 (3:1) at mild heat and the solid residue was separated by centrifugation. Finally, the volume was adjusted to 50 ml with Milli Q water and analysed by AAS using a Buck Spectrophotometer Model 210-VGP in order to determine the concentration of Co, Cu, Fe, Mn, Ni, Pb and Zn. Likewise, blanks of the digest were prepared and analysed [14].

2.10. NAA determinations

Masses of about 200 mg of freeze-dried material were sealed in high purity quartz ampoules for their irradiation, together with two certified reference materials, NIST 1633b Coal Fly Ash and IAEA V-10 Hay Powder. The irradiations were done at the RA-3 reactor of the Ezeiza Atomic Centre (Argentine Atomic Energy Commission), of thermal flux 3.10^{13} $cm^{-2}.s^{-1}$ and 4,5 Mw, during 8 hours. Instrumental Neutron Activation Analysis was performed, measuring twice after 6 and 30 day decay, for medium and long-lived nuclides.

The measurements were carried out using GeHP detectors (30 % efficiency, 1,8 keV resolution for the 1332,5 keV 60Co peak) coupled to both a Canberra Series 85 multichannel analyser and to an Ortec 919 buffer multichannel module plus Gamma Vision software for data acquisition. Concentrations for the determined elements (As, Ba, Br, Ca, Ce, Co, Cr, Cs, Eu, Fe, Gd, Hf, K, La, Lu, Na, Rb, Sb, Sc, Sm, Ta, Tb, Th, U, Yb and Zn) were calculated using softwares developed at the NAA laboratory. The precision on 12 sets of three replicates was estimated. All the determined elements had a precision better than 12%, except for Ba (14%), Gd (17%) and U (14%). Control charts (z-values) [15] for inspection of the normalized concentrations of all elements in one control sample, for a series of measurements, were used. None of the values was outside the $|z| > 3$ range. As control samples, NIST SRM 1547 Peach Leaves, 679 Brick Clay and 2709 San Joaquín Soil were used.

2.11. Chemical-physiological parameter quantification

Lichen samples were dried and shredded to achieve homogeneity and then, freeze-dried. *T. capillaris* was kept and analysed without previous homogenisation of the initial material due to its high water content. Three sub-samples were run for all the determinations.

2.11.1. Dry weight / fresh weight ratio

The dry weight / fresh weight (DW/FW) ratio of the samples was determined by drying 1 g of fresh material at 60 ± 2 °C until constant weight. The results were expressed in g DW. g^{-1} FW.

2.11.2. Chlorophylls

A portion of 100 mg of plant material was homogenised in 10 ml EtOH 96 % v/v with an Ultra Turrax homogeniser and the supernatant separated. Afterwards, HCl 0.06 M was added to clear chlorophyll extract (1 ml HCl and 5 ml chlorophyll extract) in order to produce phaeophytin formation. Absorption of chlorophylls and phaeophytins, and phaeophytins alone (after addition of HCl) was measured with a spectrophotometer Beckman DU 7000. Concentrations of chlorophylls and phaeophytins were calculated on a dry weight basis [16]. The ratios chlorophyll b/ chlorophyll a (Chl b /Chl a) and phaeophytin a /chlorophyll a (Phaeoph a / Chl a) were also calculated.

2.11.3. Sulphur content

Five millilitres of Mg $(NO_3)_2$ saturated solution were added to 0.5 g of plant and dried in an electric heater. Subsequently, the sample was heated in an oven for 30 min at 500 °C. Ashes were then suspended in HCl 6 M, filtered and the resulting solution, boiled for 3 minutes. The solution was brought to 50 ml with distilled water.

The amount of SO_4^{2-} in the solution was determined by the acidic suspension method with $BaCl_2$ [17] which subsequently allowed for the calculation of sulphur contents in each sample. The concentration was expressed in mg of total sulphur g^{-1} DW.

2.11.4. Peroxidation product estimation

Malondialdehyde (MDA) was measured by a colorimetric method. The amount of MDA present was calculated from the extinction coefficient of 155 mM^{-1} cm^{-1} [18]. Results were expressed in μmol g^{-1} DW.

Hydroperoxy conjugated dienes (HPCD) were extracted by homogenisation of the plant material in 96 % v/v EtOH at a ratio of 1:50 FW/V. The absorption was measured in the supernatant at 234 nm and its concentration was calculated by means of $\varepsilon = 2.65 \times 10^4$ M^{-1} cm^{-1} [8]. Results were expressed as mmol g^{-1} DW.

2.12. Statistical Analysis and other data evaluation tools

The following statistical analysis and other data evaluation tools were applied:

1. Descriptive statistics, factor analysis and distribution maps for chemical elements and physiological parameters, using SPSS 8.0 and Surfer softwares.

2. Spearman's correlation coefficient for studying the relationships among chemical elements and physiological variables.

3. Hierarchical analysis of variance to evaluate the different sources of variability in the study. The GLM procedure of SAS was used to estimate the components of variance from the expected mean squares for Type III Square Sums.

3. RESULTS AND DISCUSSION

3.1. Ramalina ecklonii

For this species the determinations included:

i) Pools from 37 sites and five-replicates from five sites were analysed by AAS
ii) NAA was used for the analysis of pools from 26 sampling sites.
iii) Physiological parameters (chlorophyll a, chlorophyll b, phaeophytin a, phaeophytin b, HPCD, MDA and S were determined on pools from 33 sites and five-replicates from five sites .
A pollution index (PI) was calculated based on some of the above mentioned parameters [19]:

PI = ((Phaeoph a/Chl a)+ (S/ S mean)) (MDA/ MDA mean)

a) Descriptive statistics

Results are in Table I. The highest concentrations corresponded to Ca, K, Fe, Na, Mn, Zn, Ba, Cu, Rb, Ce, Cr, Ni, Nd and La. Physiological parameters and S values were found within the same range as reported for this species in previous works.

b) Spearman´s correlation analysis

b.1) Chemical element correlation analysis

High positive correlation coefficients were found among Na, K, Sc, Cr, Fe, Co, Se, Cs, Ba, La, Ce, Nd, Sm, Eu, Gd, Tb, Yb, Lu, Hf, Ta, Th and U. Zn showed positive correlation with Sc, Se, Ba, Ce, Sm, Eu, Gd, Tb, Yb, Lu and negative with Sb while Sb showed positive correlation with K, Co, As, La y U. Ni correlated with Na, K, Co, Ba, La, Nd and Eu and Cu with Na, Cr, As, La, Ta, and Pb, S showed positive correlation with Ni and Se, and a negative one with Pb, and Pb correlated positively with Cu, As and negatively with S, Zn and Gd.

b.2) Correlation analysis among physiological parameters and among physiological parameters and chemical elements

Significant positive correlations were observed for S with HPCD and for DW/FW with Sc, Cr, Fe, Co, Cs, La, Sm, Yb, Hf, although for this species, DW/FW has showed to be a poor physiological damage indicator caused by pollutants.

In general, pigments didn´t show correlation with chemical elements with the exception of Chl a which showed positive correlation with Mn, Co and Ba, Chl b/Chl a with Cs and Br and Phaeoph b, and HPDC which showed negative correlation with Pb. Pollution index PI presented significant positive correlations with Mn, Fe, Se and Nd and negative with Pb and Zn.

c) Factor analysis

d)

Chemical results from 25 sampling sites were analysed by factor analysis using SPSS software. By keeping those factors with eigenvalues higher than one, six factors were chosen (Table II). The first factor could be assigned to natural soil source and also probably, to fossil fuel combustion. Factor 2 was probably due to agricultural activities and refuse incineration

and factors 3, 4 and 5, to anthropogenic activities. Factor 7, although could be connected to a natural source, will require further investigation. A new analysis was tried including physiological results and 10 factors were obtained. Those factors with an important contribution of chemical elements were similar to the ones obtained previously. Factor 1, connected with soil and possibly fossil fuel combustion, was similar to factor 1 obtained using only chemical data analysis. Factors 3 and 6 reflected anthropogenic origin and factor 4 could be connected to an agricultural source. Factors 2, 5 and 9 included some physiological parameters. Factors 7 and 8 and also factor 10 (similar to factor 6 from the analysis using only chemical results) will require further investigation.

d) Distribution maps

Preliminary distribution maps for certain selected elements and physiological parameters were constructed based on correlation results and analysis of variability sources. Only some of these maps are presented here (see Figure 1, 2, 3 and 4).

e) Sources of Variability Analysis

Data from five sample points with five replicates were used to estimate components of variances of the physiological parameters, and data from eight sample points, for the analysis of variances of metals determined by AAS.

Table III (a) and IV (a) show the estimates of variance components obtained from a hierarchical analysis of variance, in which three random effects or sources of variability were considered:

- The variability among sample points (only sample points with five replicates), which will be called survey variance.
- The variability among localizations within a sample point (five replicates in each sample point), which is called "local variance".
- The variability among different sub-samples and chemical determinations, which will be named "experimental variance".

It must be pointed out that these estimates of variances are "components" of the total variance of each observation, and therefore, none of them includes any component of a lower level of variability.

If experimental variance were negligible compared to local variance, the signal-to-noise ratio proposed by Wolterbeek et al. [20], could be obtained as the ratio between the survey and local variance in Tables III (a) and VI (a). This was not the case as can be seen in Table III (a). Some variables were found in which experimental variance is at least as important as local variance, and others in which experimental variance is more important than local variance.

Tables III (b) and IV (b) show the estimates of variance with the following two sources of variabillity:

- The variability among sample points (survey variance)
- The variability among localizations within a sample point (local variance*) that in these cases includes a contribution due to the experimental variability.

Local variance* = Local variance + experimental variance / n

With n = number of sub-samples.

Survey variance1 = Survey variance for sample points with five replicates, using the mean value of the sub-samples determinations.

Survey variance2 = Survey variance for sample points with five replicates + Local variance / k + experimental variance / (k * n)

Survey variance3 = Survey variance of all the sample points + Local variance / k + experimental variance / (k * n)

With k = number of local replicates and n = number of sub-samples.

From Tables III (b) and IV (b) it can be seen that there are substantial changes in considering this new proposal for estimating the variance associated to "noise" in connection with the estimation obtained in Tables III (a) and IV (a) for local variances. As it has been proposed by Wolterbeek et al. [20], the denominator of the signal-to-noise ratio should contain all sources of variability others than the variability among sample points that are far enough to represent different environmental conditions.

Based on these estimates of survey and local variances, a signal-to-noise ratio was constructed and labelled 1. For those variables in which the experimental variance was negligible, the signal-to-noise ratio 1 was almost the same as the ratio obtained in the first table (see for example, Mn and Zn in Table IV). But in those variables with important experimental variances, the ratio could diminish noticeably (see for example, Chl b and Phaeoph b in Table III or Ni and Pb in Table IV).

Signal-to-noise ratios were calculated from these estimates of survey variances, using the local variance* estimate obtained from the analysis of variance of the data from those points with five replicates.

As can be observed in Table III (b) and IV (b), survey variance 2 was a little higher than the survey variance 1, as expected from the above expression. There were no important differences in calculating the signal-to-noise ratio using the estimate 1 or 2 of the survey variance. The only difference was that the survey variance 2, was not exactly a variance component but it contained a contribution of the lower components of variance, which would be less important with the increase in the number of replicates or sub-samples.

However, the estimates of survey variance based on all the data of the survey (survey variance 3) could be noticeably different from those based on sample points with five replicates (see for example Chl b, Phaeoph b or Chl b/Chl a ratio in Table III (b)). These results could be explained in connection with the fact that in using all the points a wider range of environmental conditions are included, giving more chances for extreme situations to appear. For metals (Table IV (b)) there were situations in which survey variance 3 (or signal-to-noise3) was lower than survey variance2 (or signal-to-noise2).

3.2. Tillandsia capillaris

For this species the determinations included:

i) Pools from 34 sites and five-replicates from five sites were analysed by AAS
ii) Physiological parameters (chlorophyll a, chlorophyll b, phaeophytin a, phaeophytin b, HPCD, MDA and S were determined on pools from 33 sites and five-replicates from eight sites

A pollution index (PI) was calculated based on some of the above mentioned parameters (unpublished data)

$$PI = [(Chl\ b/Chl\ a) + (S/S\ mean)]\ [(MDA/MDA\ mean) + (HPCD/HPCD\ mean)]\ (DW/FW)$$

a) Descriptive Statistics

Results of mean, standard deviation, median and range are in Table V. The highest concentrations corresponded to Zn, Cu and Ni.

Although Cu, Zn, Co and Ni mean values were similar to those found in *R. ecklonii,* the highest values were significantly lower. For Pb, values were one magnitude level lower than those for *R. ecklonii.*

b) Spearman's correlation analysis

Significant positive correlation coefficients were found between Ni and Co and between them and Pb. Cu showed positive correlation with Zn. S showed negative correlation with DW/FW and positive with Chl a, Phaeoph a, and Phaeoph b. HPCD correlated positively with Cu, and MDA negatively with the DW/FW ratio.

c) Distribution Maps

Preliminary distribution maps for metals and physiological parameters were drawn presenting here only those that according to the statistical analysis were considered as more representative (see Figures 5, 6, 7, 8).

d) Sources of Variability Analysis

Results for *T. capillaris* are shown in Tables VI and VII. Data from eight sample points with five replicates were used to estimate components of variances of the physiological parameters and data from five sample points with five replicates were used to estimate components of variance of metals determined by AAS.

As for *R. ecklonii*, Tables VI (a) and VII (a) show the estimates of variance components obtained from a hierarchical analysis of variance in which three random effects or source of variability were considered. Tables VI (b) and VII (b) show the estimates of variance obtained from this analysis under the names: Survey variance 1, Survey variance 2, Survey variance 3 and Local variance*.

It can be seen from Tables VI (b) and VII (b), that there were substantial changes in considering this new proposal for estimating the variance associated to "noise" compared to the estimation obtained in Tables VI (a) and VII (a) for local variances.

As can be observed in Tables VI (b) and VII (b), survey variance 1, was slightly higher than survey variance 2. Signal-to-noise ratio using the estimate survey variance 1 was higher than 2 for some parameters (Chl a, Chl b, Phaeoph a, Phaeoph b and sulphur), whereas for others it was lower (Chl b/Chl a ratio, Phaeoph a/Chl a ratio, MDA, and heavy metals).

For *Tillandsia*, the estimates of survey variance based on all the survey data (survey variance 3) and signal-to-noise 3 were not noticeably different from those based on sample points with five replicates.

3.3. *Usnea amblyoclada*

Chemical determinations in this species included:

i) Pools from 10 sites and five-replicates from three sites were analysed by AAS
ii) Physiological parameters (chlorophyll a, chlorophyll b, phaeophytin a, phaeophytin b, HPCD, MDA and S were determined on pools from 10 sites and five-replicates from two sites
A pollution index (PI) was calculated based on some of the above mentioned parameters [21].

PI = [(Chl b/Chl a)+ (Sulphur/Mean of Sulphur)] (MDA/Mean of MDA)

a) Descriptive Statistics

Results are in Table VIII. Highest concentration values corresponded to Zn, Cu y Ni. Mean values for Cu as well as for Zn, Co and Ni were similar to those found in *R. ecklonii* while the highest values were significantly lower. Pb values were in the same order that those obtained for *T. capillaris*, whereas the highest values were one order lower than those of *T. capillaris* and two orders than the ones for *R. ecklonii*.

b) Correlation Analysis

Due to the fact that only a few point were available for this species, the results for the correlation analysis should be confirmed in future studies using more data. Pb correlated positively with Co and PI, and negatively with Phaeoph a and Phaeoph b. Zn correlated positively with sulphur and Phaeoph a/Chl a ratio and negatively with Chl a and Chl b. Cu showed a significant positive correlation with Co, Chl b/Chl a ratio and HPCD. Ni correlated positively with Phaeoph a, Phaeoph b and negatively with MDA and PI. Co correlated positively with Pb, Cu, PI and negatively with Paeoph a and Phaeoph b. Sulphur correlated positively with Zn. PI showed a positive correlation with Co, Pb and sulphur and a negative one with DW/FW ratio, Phaeoph a, Phaeoph b and Ni. MDA correlated positively with Phaeoph a/Chl a ratio and negatively with Chl b, Chl a and Ni. HPCD correlated positively with Chl b/Chl a ratio and Cu.

c) Distribution maps

Due to the scarce number of points obtained for this species, no distribution maps are presented.

d) Sources of Variability Analysis

Results for *U. amblyoclada* are presented in Tables IX and X. Data from three sample points with five replicates were used to estimate components of variances of the physiological parameters and metals determined by AAS.

In the same way as for the other two species, Tables IX (a) and X (a) show the estimates of variance components obtained from a hierarchical analysis of variance in which three

random effects or sources of variability were considered. It must be pointed out that these estimates of variance are components of the total variance of each observation, and therefore, none of them includes any component of a lower level of variability.

Tables IX (b) and X (b) show the estimates of variance obtained from this analysis under the names: Survey variance 1, Survey variance 2, Survey variance 3 and Local variance* obtained in the same way as explained for *R. ecklonii* and *T. capillaris*.

From Tables IX (b) and X (b) it can be seen that there were substantial changes in considering this new proposal for estimating the variance associated to "noise" if compared with the estimation obtained in Tables IX (a) and X (a) for local variances.

As can be observed in Tables IX (b) and X (b), survey variance 2, was slightly higher than survey variance 1, as expected. For this species, except for HPCD, signal-to-noise ratio using the estimate survey variance 1 was lower than 2.

However, as for *R. ecklonii*, in this species the estimates of survey variance based on all the data of the survey, can be noticeably different from those based on sample points with five replicates (see for example Chl a, Chl b, b, Phaeoph a, Phaeoph b, Chl b/Cl a ratio, MDA and sulphur in Table IX (b) and Zn in Table X (b)). Signal-to-noise ratio 3 also was noticeably higher.

3.4. Interspecies comparisons

Spearman's correlation analysis was performed among all parameters taking pairs of species and considering data from the points where the two species were present. As for *U. amblyoclada* the number of common points with the other two species was low, only the results for the intercomparison between *R. ecklonii* and *T. capillaris* are reported. Chl b/Chl a, MDA and Cu showed high positive correlation coefficients between two species. However, due to the fact that data for trace elements were not available, these preliminary results should be considered as preliminary ones.

4. PLANS FOR FUTURE WORK

Chemical analysis of trace elements by NAA as well as the analysis and interpreting of results will be completed and new samples will be taken considering these results, in order to complete the study.

At those sites where high elemental concentrations were found for certain elements of anthropogenic origin, transplants will be tried. *R. ecklonii* and *T. capillaris* will be used in order to determine if the observed high-level results correspond to present high-value emissions or to accumulation over an extended period.

The results of this Project will be transferred to the Environmental Agency of the Government of the Córdoba province. This agency will set air quality standards and maximum emission levels for industry in the province of Córdoba. The data produced within this study are the first trace-element baseline level results for the area that will be used by organisms responsible for environmental legislation and control.

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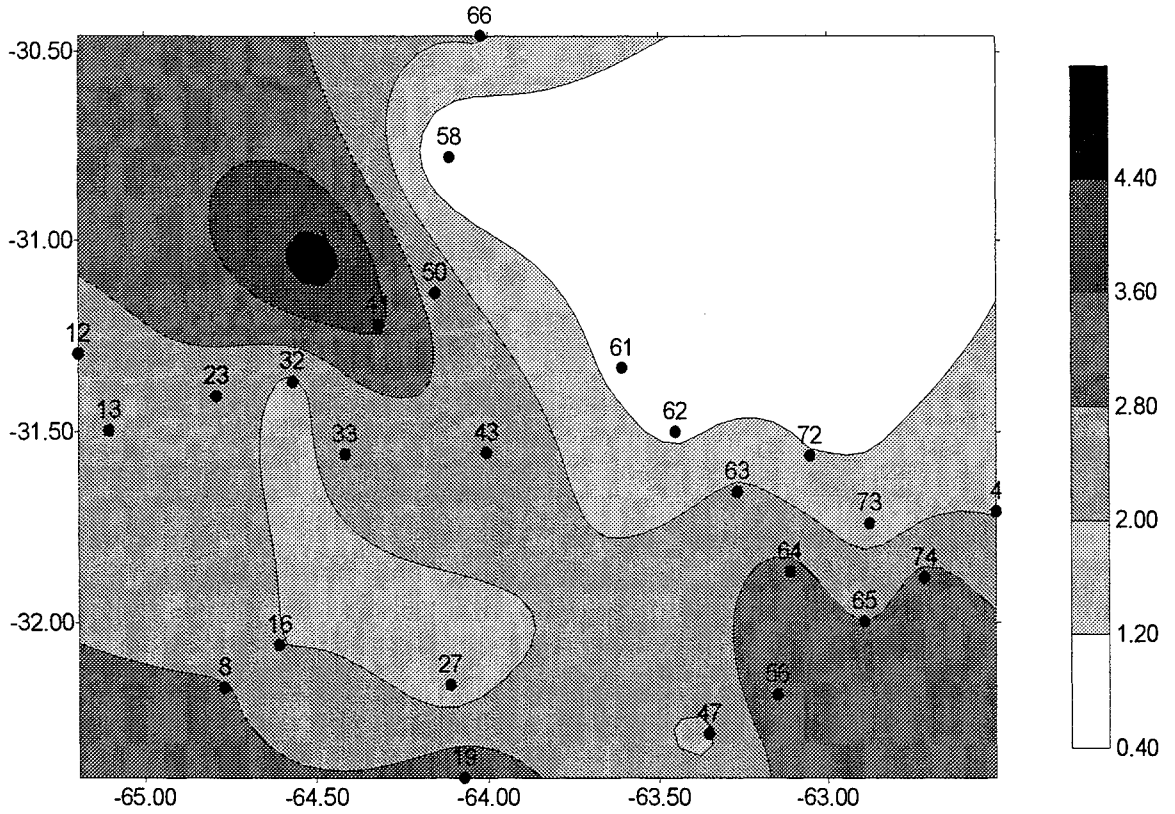

Figure 1. Distribution map of As (NAA) concentration in *R. ecklonii* (µg/g DW).

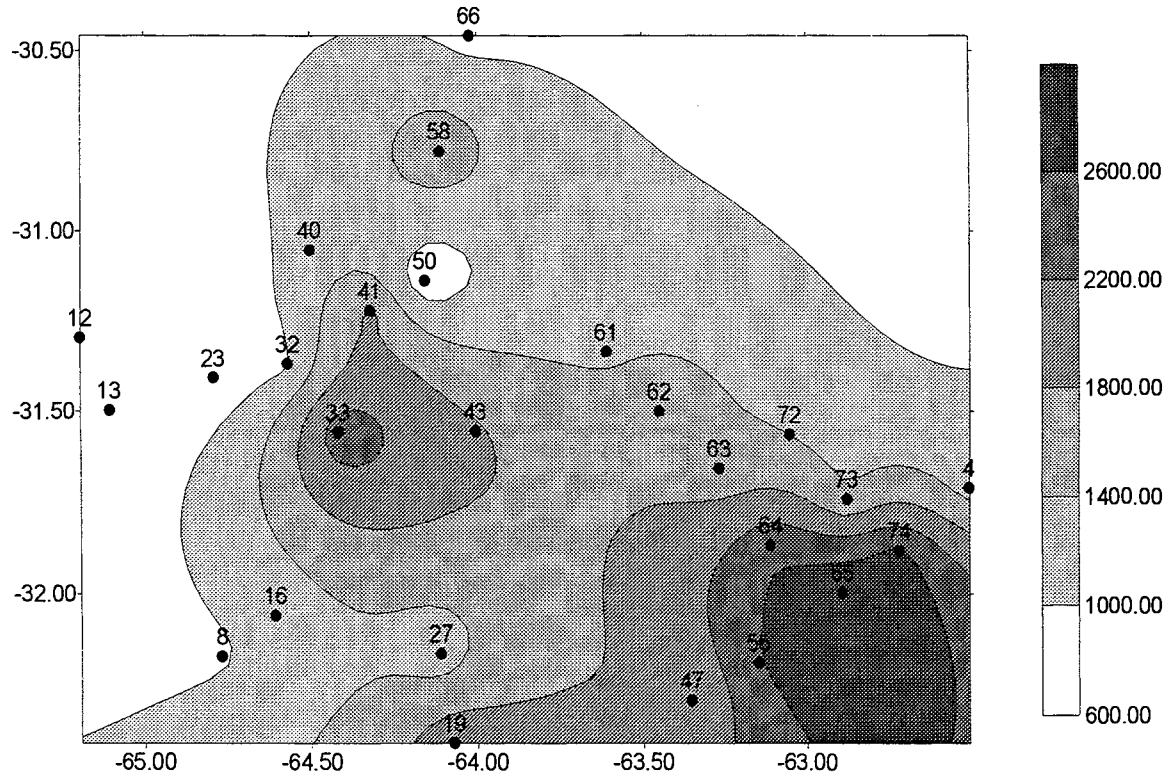

Figure 2. Distribution map of Fe (NAA) concentration in *R. ecklonii* (µg/g DW).

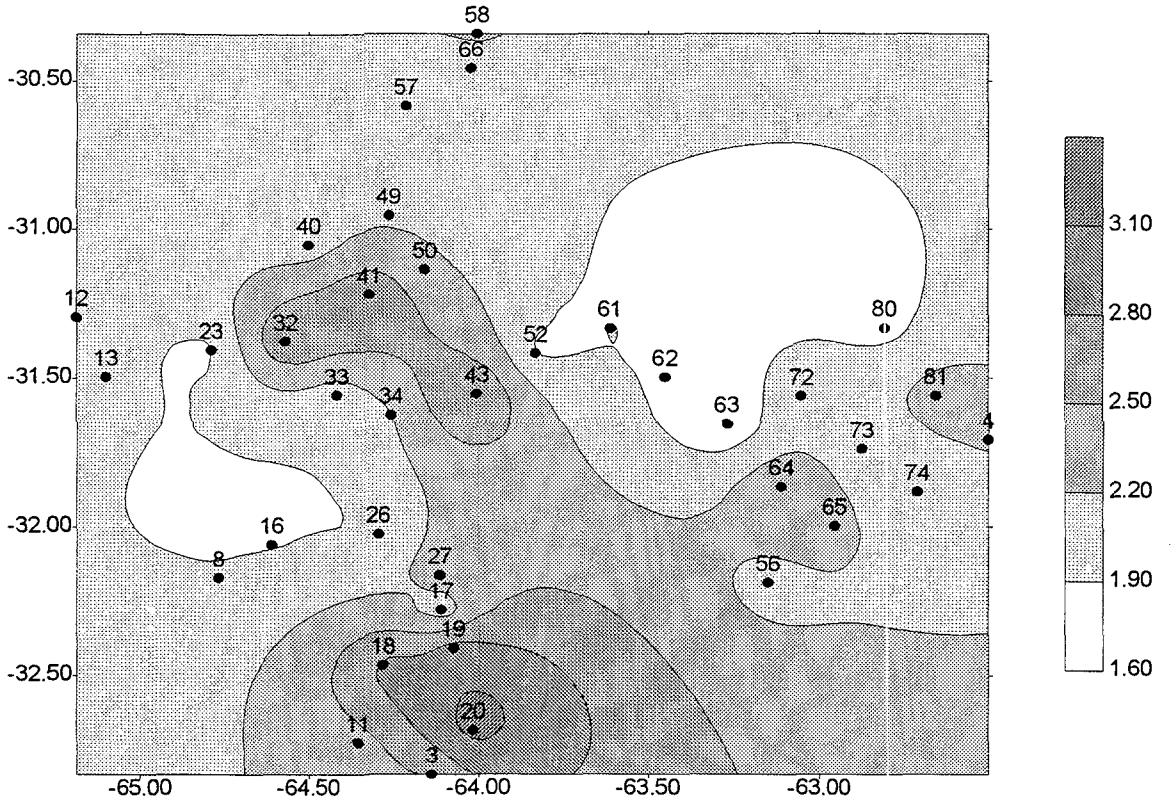

Figure 3. Distribution map of Ni (AAS) concentration in *R. ecklonii* (µg/g DW).

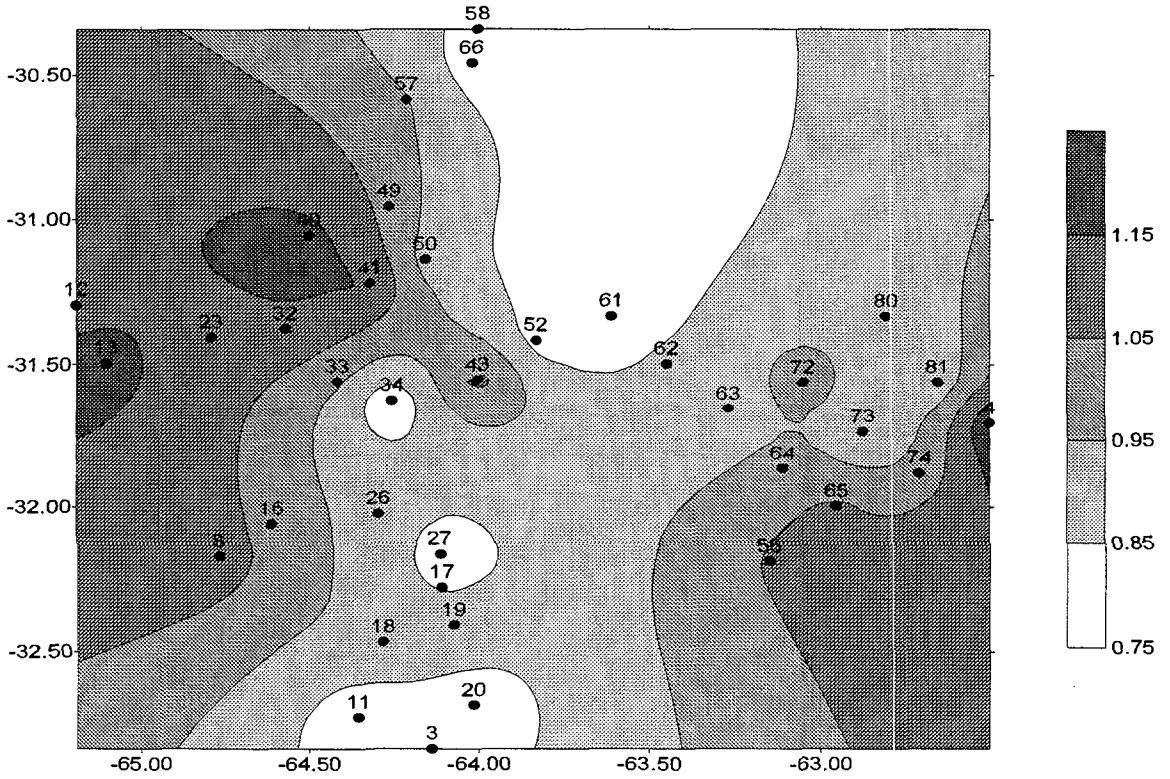

Figure 4. Distribution map of Pb (AAS) concentration in *R. ecklonii* (µg/g DW).

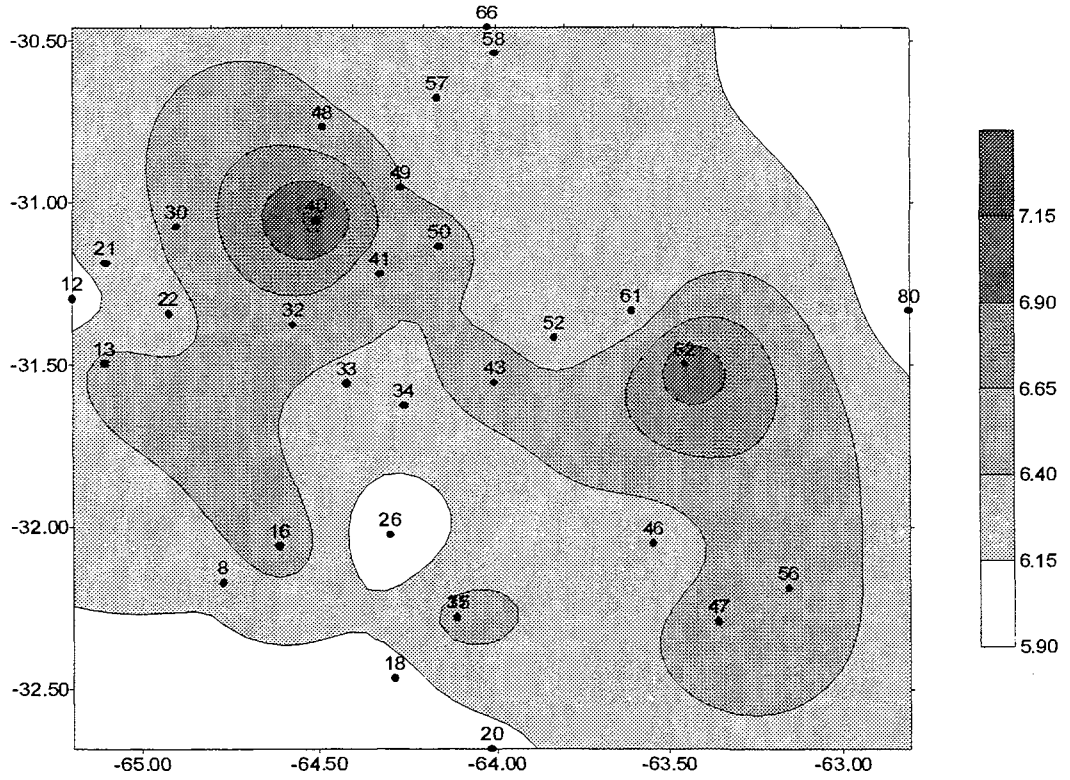

Figure 5. Distribution map of Cu (AAS) concentration in *T. capillaris* (µg/g DW).

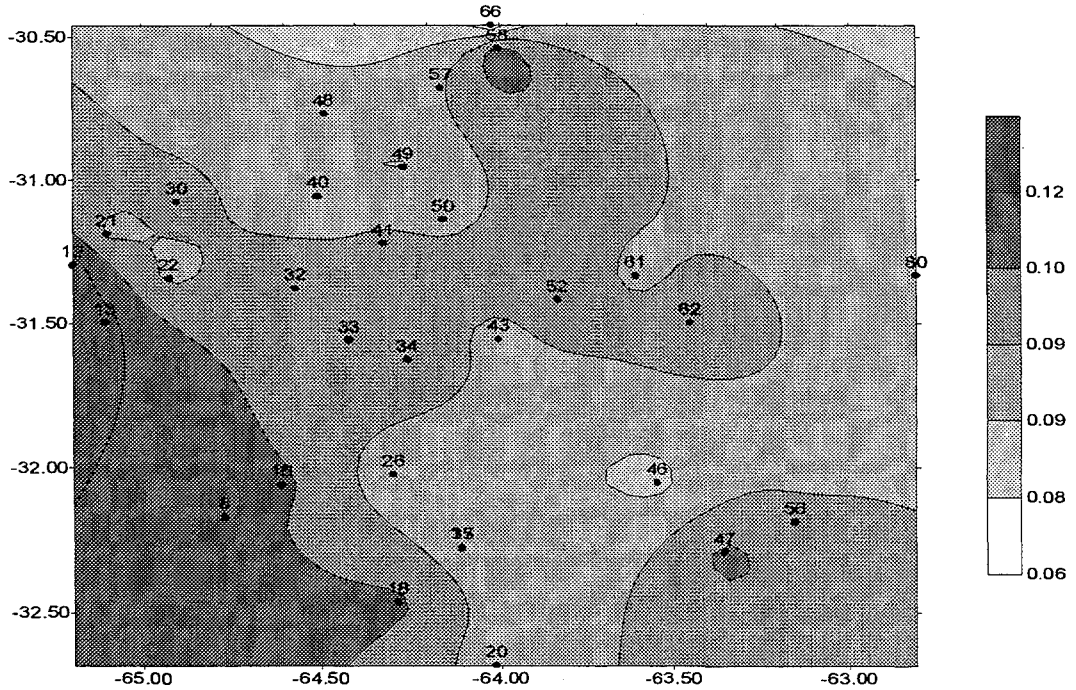

Figure 6. Distribution map of Pb (AAS) concentration in *T. capillaris* (µg/g DW).

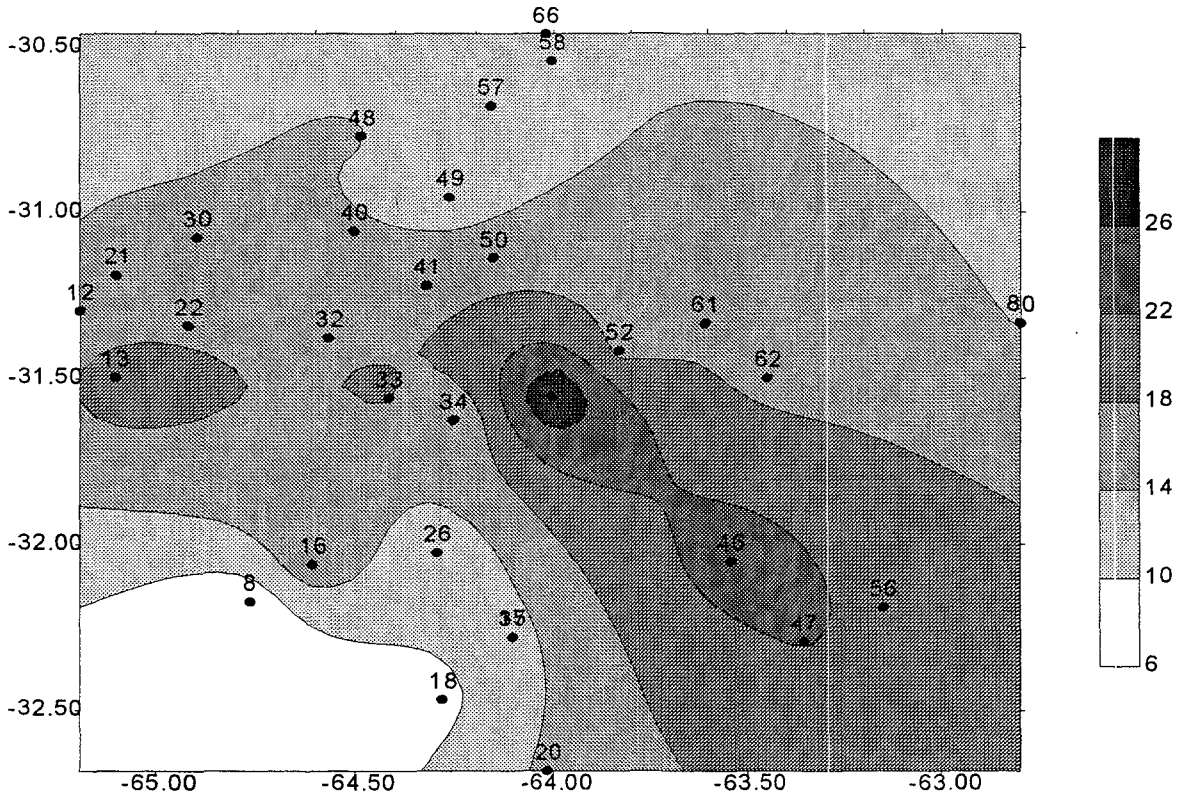

Figure 7. Distribution map of Zn (AAS) concentration in *T. capillaris* (µg/g DW).

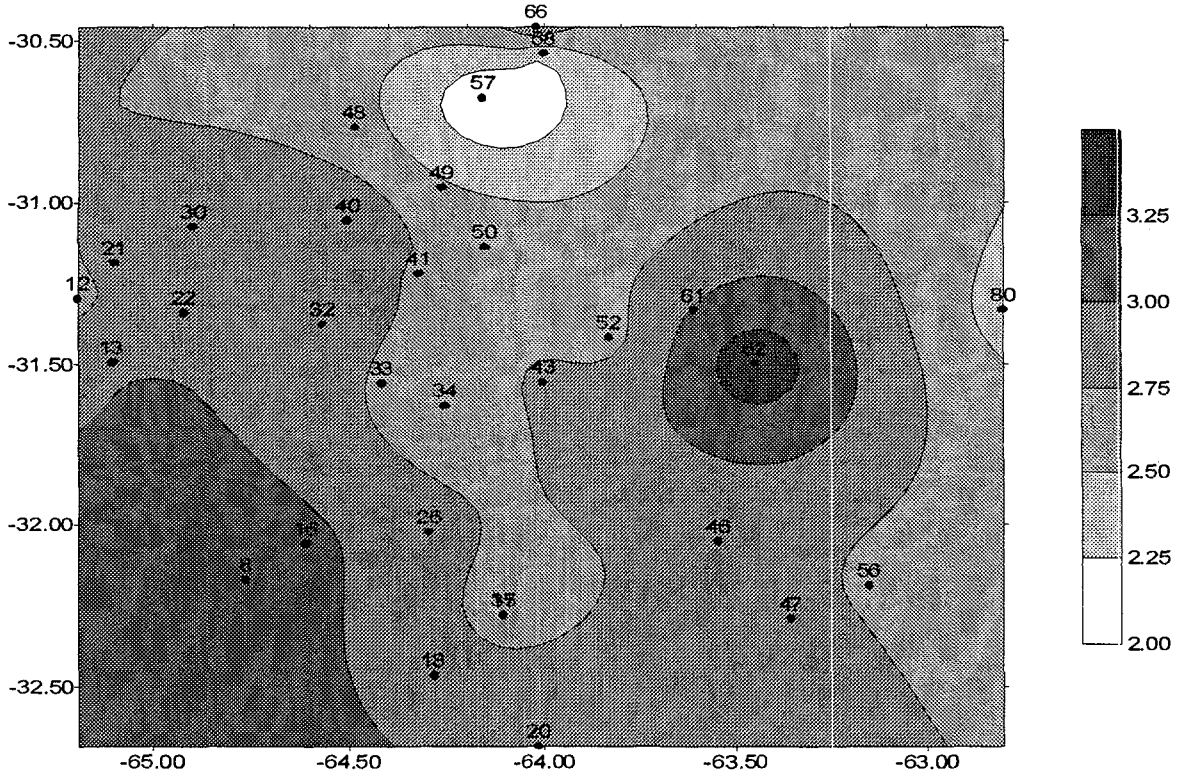

Figure 8. Distribution map of Ni (AAS) concentration in *T. capillaris* (µg/g DW).

TABLE I. SIMPLE STATISTICS FOR THE RESULTS OF *RAMALINA ECKLONII* (SPRENG.) MEY. & FLOT

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
Cu	37	6.76782	4.09012	5.14393	2.49931	16.89425
Zn(AAS)	37	20.60368	17.75915	12.92046	2.42585	75.40479
Pb	37	0.95756	0.13384	0.92762	0.77375	1.24090
Co(AAS)	37	0.06620	0.00997	0.06723	0.03815	0.08346
Ni	37	2.21406	0.37340	2.11883	1.63300	3.20637
Mn	37	53.99938	13.33885	52.41594	26.33140	80.75305
Fe(AAS)	37	171.50776	87.57934	145.09551	61.98080	513.14609
MDA	33	0.12946	0.03004	0.12754	0.06742	0.20363
HPCD	33	1.12489	0.56637	1.01963	0.43395	3.66428
Chl a	33	1.40365	0.40450	1.36106	0.59363	2.09438
Chl b	33	0.54812	0.20475	0.50857	0.28348	1.25758
Phaeoph a	33	1.68747	0.45312	1.68505	0.86275	2.59750
Phaeoph b	32	0.66151	0.34219	0.56930	0.32001	1.97857
Sulphur	33	1.92900	1.66718	1.79961	0.07186	6.30557
Chl b/Chl a	33	0.62131	1.08232	0.36305	0.22813	5.81522
Phaeoph a/Chl a	33	1.33828	0.73236	1.14878	0.96114	5.22704
PI	33	2.28517	1.00647	2.07966	1.04352	4.87969
As	26	2.33212	0.99164	2.30500	0.30000	4.95000
Ba	25	21.60840	10.04755	20.98500	9.62000	44.19000
Br	26	2.24904	1.38911	1.75750	0.68000	6.12000
Ca	23	3321	2160	2853	307.47000	8532
Ce	26	3.29135	1.31922	3.46500	1.33000	6.82000
Co(NAA)	26	0.64596	0.29683	0.64000	0.24000	1.38000
Cr	26	2.41981	2.20208	1.87000	0.80500	11.94000
Cs	26	0.35385	0.12521	0.35000	0.21000	0.68000
Eu	26	0.05982	0.02570	0.05880	0.02010	0.12000
Fe(NAA)	26	1555	667.92302	1424	695.87500	2959
Gd	26	0.25788	0.10598	0.23500	0.09500	0.48000
Hf	26	0.23250	0.10759	0.20000	0.10000	0.48500
K	23	2875	1570	2998	399.91000	6410
La	26	1.59606	0.84687	1.52750	0.01260	3.28000
Lu	26	0.02210	0.00990	0.02035	0.00900	0.04670
Na	26	466.53461	278.87335	401.11250	73.50000	1302
Nd	25	1.67680	0.85509	1.53000	0.58000	4.39000
Rb	26	5.93173	2.10817	5.76500	2.37500	12.51000
Sb	26	0.11465	0.07125	0.09585	0.04740	0.38500
Sc	26	0.54865	0.23874	0.54500	0.25000	1.13500
Se	24	0.26375	0.10743	0.23500	0.16000	0.53000
Sm	26	0.32596	0.14925	0.32500	0.12000	0.66500
Ta	26	0.04315	0.01519	0.03885	0.01850	0.08030
Tb	24	0.04026	0.01667	0.03750	0.01200	0.07590
Th	26	0.53692	0.20419	0.54500	0.21000	1.02500
U	26	0.12112	0.06771	0.09970	0.03470	0.34000
Yb	26	0.12940	0.06028	0.11740	0.04450	0.28000
Zn(NAA)	26	43.83769	40.48961	26.57500	5.07000	154.06000

TABLE II. *RAMALINA ECKLONII* (SPRENG.) MEY. & FLOT, FACTOR ANALYSIS

	Component					
	1	2	3	4	5	6
Tb	.931			-.126		.120
Ce	.927	.175		.152		
Co	.925	.281				.136
Eu	.925	.292		.142		
Lu	.905	.369		.117		
Yb	.890	.354	.145			
Th	.880	.286		.231		.150
Sc	.868	.354		.217		.184
Hf	.836	.368	.223	.226		.131
Fe	.835	.375	.153	.201		.210
Se	.802	.120	.144	.150	-.109	.178
Cs	.783	.308		.339	-.107	.247
Sm	.748	.473	.226	.157	.298	-.115
Ta	.687	.324	.174	.429		.307
Ba	.683	.504	.306	.239	-.157	
La	.674	.383	.325	.219	.358	-.158
U	.660	.308	.527		.106	
Mn	.637		.205		-.527	
Gd	.582	.425	.178	.113	-.435	.151
Cr	.335	.821				
K	.154	.755	.422	.278		
Br	.430	.718			-.108	.359
Na	.406	.707	.240	.442		.107
Zn	.367	.598	-.389	-.239		-.301
Ca	.335	.493	.301	-.154	.102	.409
Sb	.154		.890			
As	.102	.207	.601	.154	.589	-.153
Ni	.151			.803	.174	-.177
Nd	.483	.142		.620	-.354	.120
Cu	.226	.132	.387	.596	.380	.140
Pb	-.172		.196	.171	.817	.210
Rb	.350				.126	.764

TABLE III. PHYSIOLOGICAL PARAMETERS MEASURED IN *RAMALINA ECKLONII* (SPRENG.) MEY. & FLOT. SURVEY VARIANCE, LOCAL VARIANCE, EXPERIMENTAL VARIANCE AND SIGNAL-TO-NOISE RATIOS.

(a)

	Chl a	Chl b	Phaeo a	Phaeo b	Chl b / Chl a	Ph a / Chl a	MDA	HPCD	DW /FW	Sulphur
Survey variance	0.123	0.0088	0.165	0.015	0.0018	0.013	0.00046	0.038	0.00013	2.19
Local variance	0.022	0.00067	0.025	0.0002	0.00187	0.0017	0.00062	0.069	0.00014	3.84
Experimental variance	0.053	0.013	0.064	0.031	0.0076	0.033	0.0016	1.43	0.0016	2.14
Signal to Noise ratio	**5.44**	**13.16**	**6.49**	**75.85**	**0.94**	**7.52**	**0.77**	**0.55**	**0.93**	**0.57**

(b)

	Chl a	Chl b	Phaeo a	Phaeo b	Chl b / Chl a	Ph a / Chl a	MDA	HPCD	DW / FW	Sulphur
Survey variance 1	0.13	0.010	0.188	0.018	0.0015	0.0119	0.0004	0.00	0.0001	1.074
Survey variance 2	0.14	0.012	0.199	0.021	0.0025	0.0139	0.0007	0.00	0.0003	2.69
Survey variance 3	0.16	0.042	0.205	0.117	1.171	0.536	0.0009	0.321	0.0008	2.78
Local variance*	0.053	0.005	0.057	0.011	0.0047	0.010	0.0013	0.565	0.0007	8.08
Signal to Noise ratio 1	2.525	1.962	3.27	1.692	0.326	1.173	0.295	0.00	0.2024	0.133
Signal to Noise ratio 2	2.72	2.17	3.47	1.88	0.526	1.37	0.508	0.00	0.3937	0.333
Signal to Noise ratio 3	3.08	7.91	3.58	10.64	249.23	52.58	0.69	0.57	1.1581	0.34

TABLE IV. HEAVY METALS MEASURED IN *RAMALINA ECKLONII* (SPRENG.) MEY. & FLOT. SURVEY VARIANCE, LOCAL VARIANCE, EXPERIMENTAL VARIANCE AND SIGNAL-TO-NOISE RATIOS

(a)

	Co	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
Survey variance	0.00018	25.58	4228.49	288.39	0.079	0.0435	314.901
Local variance	0.00008	3.005	3173.30	41.60	0.0044	0.0031	259.523
Experimental variance	0.00034	7.872	1448.28	12.14	0.283	0.073	35.810
Signal to Noise ratio	**2.25**	**8.51**	**1.33**	**6.93**	**17.93**	**14.50**	**1.21**

(b)

	Co	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
Survey variance 1	0.00020	29.0033	4326.9911	277.0919	0.0552	0.0382	279.7265
Survey variance 2	0.00025	27.6403	5322.4320	278.2558	0.1279	0.0422	318.1942
Survey variance 3	0.00010	16.7291	7670.1410	177.9250	0.1394	0.0179	315.3874
Local variance*	0.00010	10.9315	3398.7271	44.4513	0.3392	0.0350	252.5633
Signal to Noise ratio 1	1.9557	2.6532	1.2731	6.2336	0.1628	1.0923	1.1076
Signal to Noise ratio 2	2.4649	2.5285	1.5660	6.2598	0.3772	1.2066	1.2599
Signal to Noise ratio 3	0.9932	1.5304	2.2568	4.0027	0.4110	0.5118	1.2487

TABLE V. SIMPLE STATISTICS FOR THE RESULTS OF *TILLANDSIA CAPILLARIS* F. *INCANA* (MEZ.).

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
Cu	31	6.35193	0.30838	6.33163	5.85035	7.28475
Pb	31	0.09621	0.01139	0.09383	0.06723	0.12020
Zn	31	15.86639	4.47128	14.86651	6.72220	30.60101
Co	31	0.07551	0.01406	0.07456	0.04563	0.10419
Ni	31	2.77213	0.27997	2.79166	2.00959	3.45611
DW/FW	26	0.38099	0.04468	0.37106	0.31096	0.46771
MDA	23	0.10394	0.01692	0.10196	0.06875	0.13616
HPCD	22	3.01767	1.24467	3.17057	1.23506	5.67427
Chl a	22	0.65691	0.26624	0.61919	0.28952	1.23786
Chl b	22	0.36592	0.12203	0.36018	0.20854	0.62182
Phaeoph a	22	0.87544	0.31743	0.83189	0.39748	1.55916
Phaeoph b	22	0.45049	0.22993	0.38832	0.21658	1.32828
Sulphur	26	0.05162	0.02198	0.05021	0.01319	0.10043
Chl b/Chl a	22	0.57930	0.07987	0.55167	0.48490	0.73866
Ph a/Chl a	22	1.36343	0.12440	1.33717	1.13333	1.67002
PI	19	1.23801	0.44509	0.99108	0.64973	2.22431

TABLE VI. PHYSIOLOGICAL PARAMETERS MEASURED IN *TILLANDSIA CAPILLARIS* F. *INCANA* (MEZ.). SURVEY VARIANCE, LOCAL VARIANCE, EXPERIMENTAL VARIANCE AND SIGNAL-TO-NOISE RATIOS

(a)

	Chl a	**Chl b**	**Phaeo a**	**Phaeo b**	**Chl b / Chl a**	**Ph a / Chl a**	**MDA**	**HPCD**	**DW /FW**	**Sulphur**
Survey variance	0.0027	0.00131	0.0033	0.0169	0.0017	0.0021	0.00005	0.656	0.00080	0.00008
Local variance	0.0222	0.00341	0.0421	0.0085	0.0025	0.0084	0.00023	0.502	0.00041	0.00017
Experimental variance	0.0107	0.0018	0.0081	0.0026	0.0043	0.0183	0.00059	0.218	0.00047	0.00013
Signal to Noise ratio	**0.12**	**0.38**	**0.079**	**1.98**	**0.68**	**0.23**	**0.22**	**1.31**	**2.0**	**0.47**

(b)

	Chl a	**Chl b**	**Phaeo a**	**Phaeo b**	**Chl b / Chl a**	**Ph a / Chl a**	**MDA**	**HPCD**	**DW /FW**	**Sulphur**
Survey variance 1	0.0448	0.0129	0.0676	0.0190	0.0021	0.0003	0.0001	0.5031	0.0008	0.0004
Survey variance 2	0.0097	0.0030	0.0201	0.0139	0.0029	0.0008	0.0001	6.1E-11	0.0009	0.0001
Survey variance 3	0.0709	0.0149	0.1008	0.0529	0.0064	0.0155	0.0003	1.5492	0.0020	0.0005
Local variance*	0.0162	0.0029	0.0257	0.0059	0.0036	0.0088	0.0002	0.5991	0.0004	0.0009
Signal to Noise ratio 1	2.7618	4.5051	2.6278	3.1901	0.5847	0.0293	0.2850	0.8397	2.1437	0.3923
Signal to Noise ratio 2	0.5965	1.0318	0.7835	2.3640	0.7921	0.0853	0.7321	0.0000	2.2801	0.1419
Signal to Noise ratio 3	4.3755	5.1347	3.9206	8.9605	1.7719	1.7585	1.4312	2.5859	4.9908	0.5368

TABLE VII. HEAVY METALS MEASURED IN *TILLANDSIA CAPILLARIS* F. *INCANA* (MEZ.). SURVEY VARIANCE, LOCAL VARIANCE, EXPERIMENTAL VARIANCE AND SIGNAL TO NOISE RATIOS

(a)

	Co	**Cu**	**Ni**	**Pb**	**Zn**
Survey variance	0.00007	0.0239	0.0582	0.00001	3.576
Local vaiance	0.00006	0.0208	0.0149	0.00002	7.718
Experimental variance	0.00060	0.213	0.0871	0.00042	5.727
Signal to Noise ratio	**1.17**	**1.15**	**3.90**	**0.5**	**0.46**

(b)

	Co	**Cu**	**Ni**	**Pb**	**Zn**
Survey variance 1	0.0001	0.0205	0.0556	0.00001	3.4497
Survey variance 2	0.0001	0.0403	0.0726	0.0001	5.5084
Survey variance 3	0.0002	0.0951	0.0784	0.0001	19.9923
Local variance*	0.0004	0.1012	0.0648	0.0002	11.1978
Signal to Noise ratio 1	0.1536	0.2021	0.8584	0.0082	0.3081
Signal to Noise ratio 2	0.3192	0.3980	1.1200	0.2249	0.4919
Signal to Noise ratio 3	0.4941	0.9397	1.2096	0.5522	1.7854

TABLE VIII. SIMPLE STATISTICS FOR THE RESULTS OF *USNEA AMBLYOCLADA* (MÜLL. ARG.) ZAHLBR.

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
Cu	9	5.84172	0.33953	6.00299	5.21211	6.25839
Pb	9	0.01064	0.00116	0.01066	0.00922	0.01245
Zn	9	24.50533	17.74014	15.42933	9.39576	54.98085
Co	9	0.04827	0.00720	0.04726	0.03713	0.06172
Ni	9	2.48339	0.18477	2.52414	2.14964	2.74553
DW/FW	10	0.92474	0.03021	0.92912	0.87205	0.96739
Sulphur	10	0.08235	0.02058	0.07423	0.06404	0.12802
Chl a	4	0.32734	0.29779	0.23523	0.08169	0.75719
Chl b	4	0.30016	0.21364	0.25808	0.10347	0.58099
Phaeoph a	4	0.41000	0.41617	0.21626	0.17399	1.03349
Phaeoph b	4	0.40182	0.19034	0.47303	0.12049	0.54073
HPCD	4	0.000696	0.000504	0.000691	0.000153	0.00125
MDA	9	0.16192	0.03502	0.17669	0.10614	0.20491
Chl b/Chl a	4	1.21971	0.69037	1.14986	0.45835	2.12077
Phaeoph a/Chl a	4	1.34814	0.58106	1.25791	0.74649	2.13027
PI	3	2.05128	0.53105	1.97317	1.56361	2.61707

TABLE IX. PHYSIOLOGICAL PARAMETERS MEASURED IN *USNEA AMBLYOCLADA* (MÜLL. ARG.) ZAHLBR. SURVEY VARIANCE, LOCAL VARIANCE, EXPERIMENTAL VARIANCE AND SIGNAL TO NOISE RATIOS

(a)

	Chl a	**Chl b**	**Phaeo a**	**Phaeo b**	**Chl b / Chl a**	**Ph a / Chl a**	**MDA**	**HPCD**	**DW /FW**	**Sulphur**
Survey variance	0.00038	0.00127	0.00169	0.00171	0.0527	0.176	0.000702	2.328	0.00020	0.000033
Local variance	0.0194	0.00559	0.00193	0.00661	0.00826	0.177	0.000127	0.347	0.000002	0.000076
Experimental variance	0.00083	0.0027	0.00211	0.00682	0.039	0.0842	0.000719	0.3	0.00167	0.000045
Signal to Noise ratio	**0.19**	**0.23**	**0.88**	**0.26**	**6.37**	**0.99**	**5.50**	**6.71**	**7.47**	**0.44**

(b)

	Chl a	**Chl b**	**Phaeo a**	**Phaeo b**	**Chl b / Chl a**	**Pha a / Chl a**	**MDA**	**HPCD**	**DW / FW**	**Sulphur**
Survey variance 1	0.0000	0.0009	0.0001	0.0001	0.0528	0.1770	0.0007	2.3528	0.0002	0.00003
Survey variance 2	0.0002	0.0018	0.0006	0.0013	0.0577	0.2221	0.0008	0.0000	0.0003	0.00005
Survey variance 3	0.0887	0.0456	0.1732	0.0362	0.4766	0.3376	0.0012	0.0000	0.0009	0.0004
Local variance*	0.0011	0.0037	0.0020	0.0052	0.0216	0.2007	0.0004	1.2546	0.0007	0.0001
Signal to Noise ratio 1	0.0000	0.2524	0.0653	0.0245	2.4496	0.8819	1.9110	1.8754	0.2445	0.3135
Signal to Noise ratio 2	0.1731	0.4745	0.2977	0.2492	2.6711	1.1067	1.9460	0.0000	0.4425	0.4803
Signal to Noise ratio 3	80.6167	12.3360	86.5994	6.9675	22.0650	1.6823	3.0665	0.0000	1.3041	4.2356

TABLE X. HEAVY METALS MEASURED IN *USNEA AMBLYOCLADA* (MÜLL. ARG.) ZAHLBR. SURVEY VARIANCE, LOCAL VARIANCE, EXPERIMENTAL VARIANCE AND SIGNAL TO NOISE RATIOS

(a)

	Co	**Cu**	**Ni**	**Pb**	**Zn**
Survey variance	0.000155	0.0574	0.0112	0.00026	9.832
Local variance	0.000114	0.0151	0.0394	0.00031	57.892
Experimental variance	0.000070	0.194	0.0691	0.00056	20.278
Signal to Noise ratio	**1.36**	**3.83**	**0.28**	**0.83**	**0.17**

(b)

	Co	**Cu**	**Ni**	**Pb**	**Zn**
Survey variance 1	0.0002	0.0572	0.0143	0.0002	0.0000
Survey variance 2	0.0002	0.0748	0.0281	0.0003	3.1350
Survey variance 3	0.0001	0.1153	0.0341	0.0000	314.7127
Local variance*	0.0001	0.0879	0.0686	0.0006	54.5363
Signal to Noise ratio 1	1.2789	0.6511	0.2090	0.3741	0.0000
Signal to Noise ratio 2	1.8769	0.8510	0.4090	0.5400	0.0575
Signal to Noise ratio 3	0.5190	1.3115	0.4977	0.0023	5.7707