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HEAVY METALS IN RYEGRASS SPECIES VERSUS METAL CONCENTRATIONS IN ATMOSPHERIC PARTICULATE MEASURED IN AN INDUSTRIAL AREA OF SOUTHERN ITALY

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Abstract. The aim of this paper is to evaluate the reliability of ryegrass species as active biomonitors by assessing atmospheric metal concentrations. We show a procedure for measuring atmospheric concentrations of heavy metals by means of biomonitors and present the data collected between July 1997 and October 2000 in the industrial area of Tito Scalo (Basilicata region, Southern Italy). In particular, we discuss the reproducibility of the biomonitoring measures, the influence of plant age and the correlation between metal concentrations in plants and in atmospheric particulate. Statistical analysis of measured data suggests us that in the investigated site, Cd, Cr and Ni are suitable to be monitored by means of ryegrass species. For the other metals, their emission patterns in atmosphere make it difficult to identify the correlation structure between plants and particulate, and as a result the interpretation of the biomonitoring data is complex. On the basis of the results, we believe that for correct application of active biomonitoring procedure, a careful preliminary analysis of the monitoring site and integration of the biomonitoring and chemical–physical observation is necessary.

Keywords: atmospheric pollutants, biomonitoring, correlation analysis, *Festuca arundinacea*, *Lolium italicum*

1. Introduction

Biomonitoring methods, based on vegetal organisms, have been widely applied for investigating atmospheric concentrations of trace elements, in monitoring programs both on large-scale and on local-scale (Rossbach *et al*., 1999; Falla *et al*., 2000; Cuny *et al*., 2001; Wolterbeek, 2001). These methods are very advantageous in comparison with the physical–chemical techniques for the analysis of soil, water and air. In fact, biomonitors are less expensive than the conventional analysers; biomonitors are widespread and available also in remote areas; biomonitors may directly highlight the effects of pollutants on living organisms. The drawbacks are mainly related to the measurement quality, in terms of reproducibility and sensitivity, because of the high heterogeneity of the living conditions.

In order to monitor the pollution level of a site by means of biomonitors, the pollutant concentrations may be measured in different native species (lichens, fungi, mosses, grass, etc.) or in part of them (leaves, pods, seeds, stems) (Aksoy and Ozturk, 1997; Garcia and Millan, 1998; Aksoy *et al*., 1999; Conti and Cecchetti, 2001 and references therein; Cuny *et al*., 2001; Lau and Luk, 2001; Pacheco *et al*., 2001; Pyatt, 2001; van Dobben *et al*., 2001; Loppi and Corsini, 2003; Moreno *et al*., 2003). All these methods, based on the use of native species, may be classified as passive biomonitoring techniques. It is an efficient way for characterising the investigated area. Nevertheless, the collected data may be biased by the heterogeneity of the sampled organisms and may show low precision and limited reproducibility.

On the contrary, active biomonitoring methods are based on the use of specific vegetal biomonitors (such as transplanted or explanted lichens, moss bags, vascular plants) expressly selected and prepared to be exposed in the area that has to be monitored (Dietl *et al*., 1997; Reis *et al*., 1999; Bari *et al*., 2001). In these cases, many efforts have to be made for pointing out the biomonitoring capability to evaluate the atmospheric contamination and its effectiveness as an alternative tool to conventional devices for measuring the levels of pollutants. In fact it is necessary to achieve a higher degree of standardisation of experimental procedures, in order to improve the quality of the evaluation of contaminant level by means of biomonitors.

The aim of this paper is to evaluate the reliability of ryegrass species as active biomonitors by assessing the atmospheric metal concentrations. Ryegrass species show many advantages, for example, they may be able to monitor short-term changes in pollution level or they may allow differentiating between airborne and soil-borne heavy metals (Aksoy *et al*., 1999).

Starting from experimental protocols used for air quality control and for studying the effects of gaseous pollutants on vascular plants (Posthumus, 1982; Nussbaum *et al*., 1995; VDI 3957-2, 2001; EuroBioNet Project Report, 2001), we have developed an original biomonitoring procedure (Caggiano *et al*., 2001a). In particular, we compare the concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn measured in ryegrass and in atmospheric particulate, and we test the measure of reproducibility, the data independence from plant age and the plant–air data correlation. The examined data were collected between July 1997 and October 2000 from the industrial area of Tito Scalo (Basilicata region, Southern Italy).

2. The Study Area

The monitoring site is located in the industrial area of Tito Scalo, 10 km away from Potenza (Figure 1). The area is located about 750 m a.s.l. and it is surrounded by low hills.

Regarding the characterisation of local meteorological conditions, we show in Figure 2 the monthly mean values of ambient temperature, precipitation and wind speed calculated on the base of daily values recorded from 1997 to 2000.

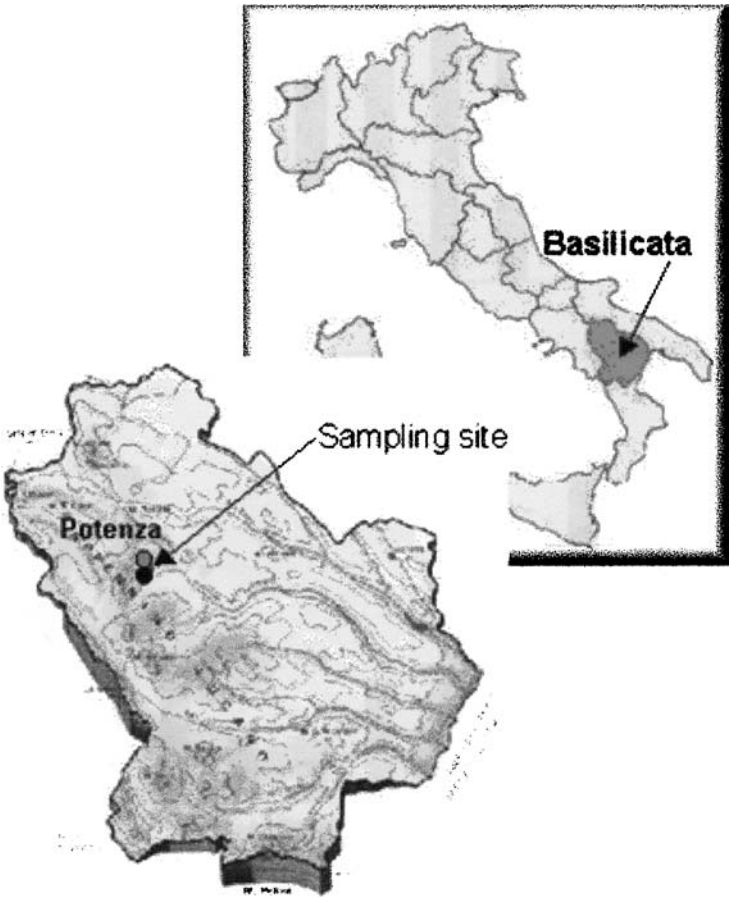

Figure 1. The study area.

The monthly mean temperature ranges from 3 to 22 °C; rains are more frequent during October–November; wind speed is low (1.9–3.1 m s^{-1}). The wind dominant directions are SSW–W and NNW–NNE. As an example, we show in Figure 3, the monthly wind roses of two sampling periods (May–July 1998 and September–November 1998).

Agriculture, pasture and woodlands are the existing uses of land. There are few commercial activities and no relevant residential units. The railway crosses this zone; the high-way SS. Basentana is about 1 km away from the sampling area, but the amount of the commercial traffic in this area is low. The industrial area is characterised by the presence of many small and medium scale industries. The main activities are building-material production, structural steel manufacture, industrial and agricultural vehicle construction, cement factory, railway sign production, metallurgical and mechanical industries.

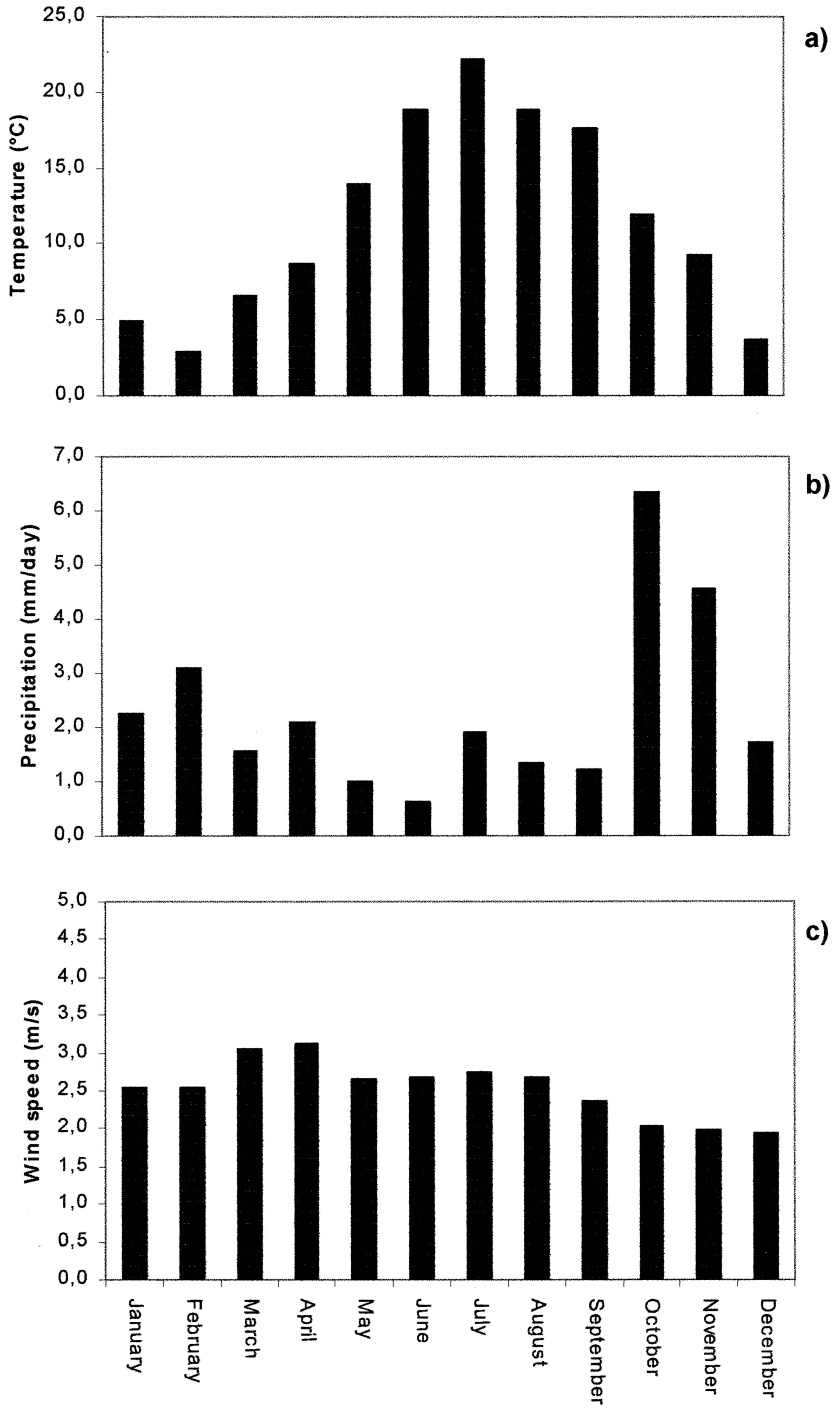

Figure 2. Monthly mean values of ambient temperature (°C), precipitation (mm day^{-1}), wind speed (m s^{-1}).

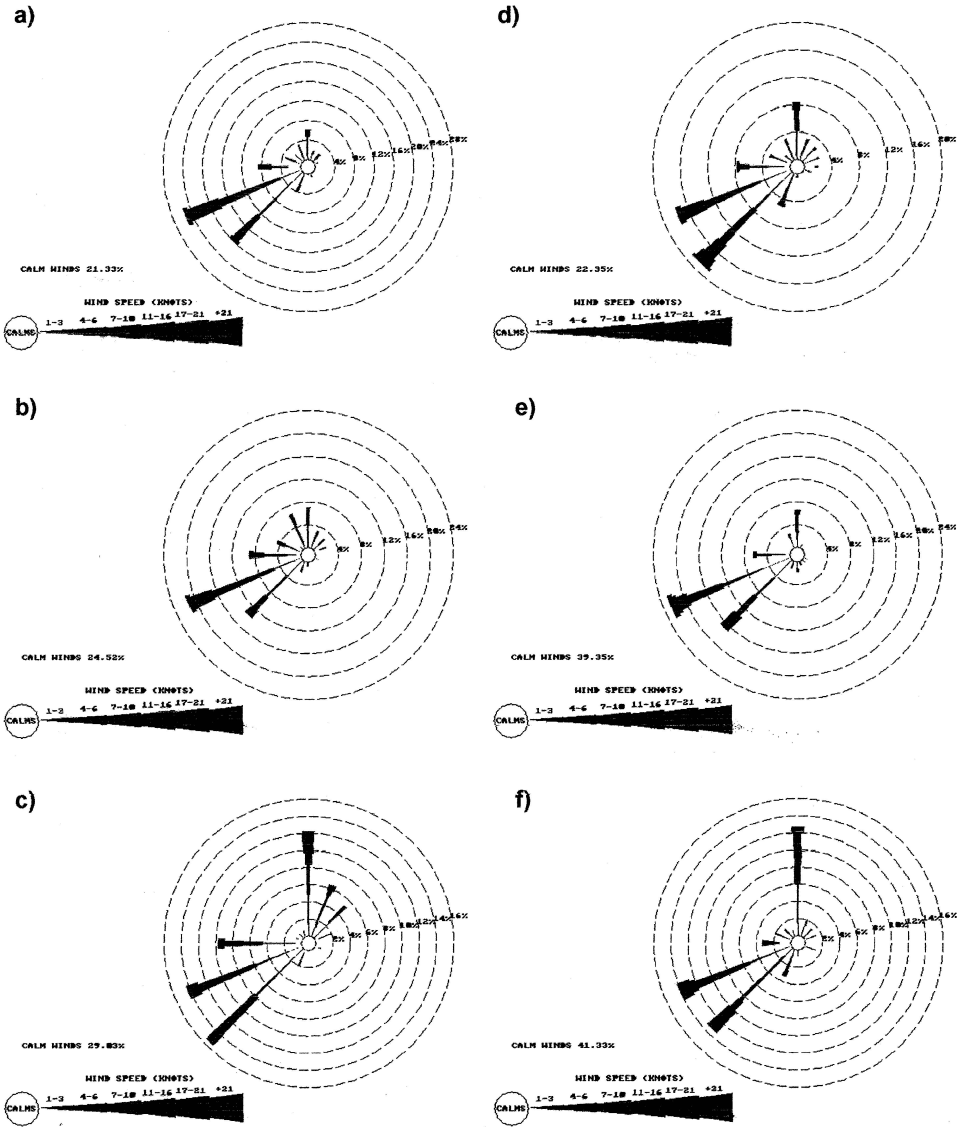

Figure 3. Monthly wind roses of (a) May 1998, (b) June 1998, (c) July 1998, (d) September 1998, (e) October 1998, (f) November 1998.

3. Experimental Methods

3.1. Ryegrass exposure and sampling protocol

For biological monitoring of heavy metal, we have used two ryegrass species, *Lolium italicum* and *Festuca arundinacea*. For each culture cycle, we used three equivalent cultivation pots (30 cm × 25 cm × 12 cm) prepared with 10 g of a 1:1

TABLE I
Scheme of biomonitoring experimental procedure

Week	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Sowing	*S*1	*S*2	*S*3	*S*4	*S*5	*S*6				
First cut			*S*1	*S*2	*S*3	*S*4	*S*5	*S*6		
										
Second cut					*S*1	*S*2	*S*3	*S*4	*S*5	*S*6
										
Exposed pots			3	3	6	6	6	6	3	3

mixture of *Lolium* and *Festuca*. The pots were filled with a soil substrate and provided with a half-automatic watering system. We replicated the same measures three times and evaluated the measure of reproducibility. A week after the sowing and the germination, the pots were brought to open air and kept over a stand, positioned 1.5 m above the ground. After 15 days of exposition (sampling period) the plants were cut with a ceramic scissor (first sampling in 3-week-old plants or first cut **Ic**) and exposed again for another 15 days. At the end, they were cut again (second sampling in 5-week-old plants or second cut **IIc**) and substituted with new pots (Caggiano *et al.*, 2001a). In Table I, we schematise the application of sampling procedure for 10 weeks. We have two sampling periods in which only 3-week-old plants are exposed and sampled (three cultivation pots), four sampling periods in which 3- and 5-week-old plants are exposed and sampled contemporaneously (six cultivation pots) and two sampling periods in which only 5-week-old plants are sampled. We carried out the measures only during spring–summer and autumn because in winter the local meteorological conditions (low temperature, frequent snow falls) are unfavourable for plant growth. In Table II, we give a complete list of the examined sampling periods ($N_S = 57$).

3.2. Particulate sampling protocol

The sampling and the mass measurements of particulate matter (TSP) are performed by means of a low volume β-gauging sampler, ADM9000 (Wedding and Wergand, 1993). The instrument is installed in an insulated box, next to the cultivation pots, in the Institute of Methodologies for Environmental Analysis of National Research Council. It has four sections: a selective inlet; a sequential unit for 12 filters; a β-gauge system for measuring mass of particulates deposited on the filters; a pumping system. Cellulose filters (Gelman filters) with a porosity of 0.8 μm and a diameter of 47 mm are used. The air-flow rate is 20 l min^{-1} and it is standardised (25 °C and 1013 mb). The total sampling time is 24 h. The precision of β-gauge measurement is 0.02 mg with β-time = 15 min and lower limit of detection is 1 μg N m^{-3}.

TABLE II
List of the start date of the 57 sampling periods

1997	1998	1999	2000
	21 May		
	28 May	27 May	25 May
	04 Jun		01 Jul
	11 Jun	10 Jun	08 Jun
	18 Jun	17 Jun	15 Jun
	25 Jun	24 Jun	22 Jun
02 Jul	02 Jul	01 Jul	29 Jun
16 Jul	09 Jul	08 Jul	06 Jul
	16 Jul	15 Jul	13 Jul
	23 Jul	22 Jul	20 Jul
31 Jul	30 Jul	29 Jul	27 Jul
10 Sept			
18 Sept	17 Sept	16 Sept	
24 Sept	24 Sept	23 Sept	
01 Oct	01 Oct	30 Sept	
08 Oct	08 Oct	07 Oct	
15 Oct	15 Oct	14 Oct	12 Oct
	22 Oct	21 Oct	19 Oct
	29 Oct	28 Oct	26 Oct
			02 Nov
9	18	16	14
		N_S	57

3.3. TRACE ELEMENTS ANALYSIS IN VEGETAL SAMPLES

The sampled grass is dried at 70 °C for 48 h in a forced stove. Dry sample weighing 0.6 g is added to 6 ml of HNO_3 and 0.5 ml of HF and put in a microwave system. The solution is filtered and stored in PTE bottles with the addition of deionised water til 50 ml. This solution is analysed for its content concentration of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn using AAS techniques (FAAS and GFAAS). For quality assurance of the analytic procedure, in the same experimental conditions and using the same protocol, we carried out the analysis of standard reference material CRM 281 from IRMM-JRC. The data reported in Table III show that all the measured values $\pm\ 3\sigma$ are enclosed in the range of certified values $\pm 3\sigma$.

3.4. TRACE ELEMENTS ANALYSIS IN PARTICULATE

For each sampling day, the filter is analysed for its content of eight heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn). Each filter is treated with 3 ml of HNO_3 and

TABLE III
Comparison between measured values and certified values of heavy metal concentrations in standard reference material (the table is published in Caggiano *et al*. (2001a)).

Metal	Measured values	Certified values
Cd	0.120 ± 0.003	0.15 ± 0.01
Cr.	2.6 ± 0.6	2.1 ± 0.4
Cu	9.5 ± 0.3	9.6 ± 0.6
Mn	74 ± 1	82 ± 4
Ni	3.8 ± 0.3	2.9 ± 0.3
Pb	3.4 ± 0.7	2.4 ± 0.2
Zn	42 ± 4	32 ± 2

a drop of HF by the method described in Caggiano *et al*. (2001b). The elemental analysis is performed by Varian AA200 atomic absorption spectrophometer (FAAS and GFAAS). The blank contribution from filters and reagents has been evaluated and taken into account (for all the measured elements, the blank values are less then 10%).

4. Data Analysis

This work aims at necessarily performing a careful statistical evaluation of data quality. In particular, we evaluate the reproducibility of data [see (1)], we verify the independence of data from plant characteristics [see (2)] and we analyse the correlation of air–plant data [see (3)].

1. A good index for estimating the precision of the data is the percentage of data reproducibility. For each metal and for each sampling period, let us indicate the metal concentration measured in 3-week-old plants (first cut) with $C_{1,I}, C_{2,I}, C_{3,I}$ and the metal concentration measured in 5-week-old plants (second cut) with $C_{1,II}, C_{2,II}, C_{3,II}$. We calculate, for each sampling period, the average concentrations on the three equivalent cultivation pots with their percentage errors ε_I and ε_{II} (Weiss and Hassett, 1987). The values of percentage errors, obtained by averaging for all the sampling periods, represent significant indices to estimate the data reproducibility in 3- and 5-week-old plants.
2. Metal concentrations in plants may depend on availability of pollutants in environment, on plant characteristics (species, age, state of health, etc.) and on other parameters (temperature, rain, moisture availability, substrate characteristics).In order to minimize these biases, we always use the same materials for plant cultivation and, from year to year, we expose the plants in the same periods.

Nevertheless, for evaluating the incidence of biological variability in our data, we carried out contemporaneous measures by means of plants with different ages. So we may compare metal concentrations measured in 3-week-old plants to concentrations measured in 5-week-old plants, exposed to open air in the same sampling period.

The following examined values of concentrations are mean values calculated on the three equivalent pots.

At first, for comparing the values, we apply a small-sample pooled over two-tailed t-test with $\alpha = 20\%$. For each metal and for each sampling period in which we have sampled 3- and 5-week-old plants, we test the null hypothesis $\mathbf{H}_0$:$C_I = C_{II}$ (with $C_I \neq C_{II}$ as an alternative hypothesis) (Weiss and Hassett, 1987). Then, to study the relationships between C_I and C_{II} on all the sampling periods, we calculate the Pearson's coefficient ρ_{I-II} for each metal.

Combining the two tests, we assume that for each metal the incidence of biological variability is low if the t-test is satisfied in more than 50% of the cases and if the ρ_{I-II}-coefficient is significant with a probability higher than 3%.

3. At the end, we examine the relationships between concentrations measured in plants (C_I and C_{II}) and data measured in air (C_{air}). In order to compare the data collected on different temporal scales (for plants the exposition period is 15 days, for filters it is 24 h), we calculated for each biomonitoring sampling period, the cumulative value of metal concentration measured in air for the 15 corresponding days. In this way, for each metal, we may calculate the non-parametric rank correlation coefficient ρ_{np} for $\{C_I - C_{\text{air}}\}$ and $\{C_{II} - C_{\text{air}}\}$ (Weiss and Hassett, 1987). Furthermore, for each value of ρ_{np}, we calculate the statistic $t(\rho_{\text{np}}) = \rho_{\text{np}}\sqrt{\frac{N_S-2}{1-\rho_{\text{np}}^2}}$ that is distributed as a t-variable with $(N_S - 2)$ d.f. and we apply the rank-correlation test with $\alpha = 5$–10%, for evaluating the statistical significance of ρ_{np} values (Barlow, 1989).

5. Results and Discussions

In Table IV, we present the mean values and the observed ranges of our measured data in particulate and values measured in other sites. Metal levels observed in atmospheric particulate sampled in Tito Scalo are similar to the values measured in other industrial or urban sites of Mediterranean area and they are higher than the values measured in the European remote sites chosen for the EMEP network (EMEP/CCC Report, 2/2000). In a previous paper (Ragosta *et al*., 2002), by means of a multivariate procedure, we characterized the source profiles of heavy metal atmospheric concentrations measured in Tito Scalo. We determined three profiles: the first one includes Zn, Mn, Cu and a fraction of Ni (**SP1**), the second one includes Cd, Cr, a fraction of Pb and a fraction of Ni (**SP2**), the third includes Fe, Mn, Zn, a fraction of Pb (**SP3**). **SP3** includes natural sources and contributions coming from

TABLE IV
Mean values and observed ranges of heavy metal concentrations in atmospheric particulate (daily values in ng/Nm^3) collected in Tito Scalo (PZ) from 1997 to 2000

	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Tito Scalo (PZ, Southern Italy)								
Mean	2.4	24	36	658	29	28	58	309
Range	0.2–18	0.4–120	0.3–5036	13–15164	1–1469	0.1–127	2–1006	9–7565
Remote sites of EMEP network[a]								
Svratouch (Czech Republic)	0.31						8,98	
Westerland (Germany)	0.15		1.31	99.92	3.74	1.06	6.68	
Keldsnor (Denmark)			1.69			1.77	8.01	14.48
Storhofdi (Iceland)	0.10	7.95	0.67	512.83	8.18	6.75	0.74	6.69
Preila (Lithuania)	0.32		2.32		3.34		8.41	20.49
Rucava (Latvia)	0.22		1.10				2.84	22.36
Kollumerwaard (Netherlands)	0.20						9.68	29.51
Spitzbergen (Norway)	0.03	0.16	0.36		0.34	0.12	0.71	1.38
Stara Lesna (Slovakia)	0.44	0.85	5.27		4.75	0.69	26.02	62.89
Industrial and urban sites in Mediterranean area								
Milano[b]		14	90	2440	45	10	310	285
Cartagena[c]	3		70				239	2000
Thessaloniki[d]	2.3		70		100	12.8	62	750

[a](EMEP/CCC-Report, 2/2000).
[b](Marcazzan *et al*., 2001).
[c](Moreno-Grau *et al*., 2000).
[d](Voutsa and Samara, 2002).

non-industrial anthropogenic sources that have a minor impact in the investigated area. Instead **SP1** and **SP2** include metals showing concentrations comparable to the level observed in other industrial or urban sites and they are both linked mainly to the industrial emissions.

In Table V, we present the mean values and the observed ranges of our measured data in plants and the threshold values determined in the EuroBioNet project (EuroBioNet Report, 2001). Regarding the comparison between our values in ryegrass and EuroBioNet threshold values, we highlight that the EuroBioNet protocol is based on an exposure period of 28 days, while our protocol is based on an

TABLE V
Mean values and observed ranges of heavy metals concentrations in ryegrass (values in ppm DW) collected in Tito Scalo (PZ) from 1997 to 2000

	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Tito Scalo (PZ, Southern Italy)								
Mean	0.29	1.5	14	136	215	4	4	184
Range	0.12–0.72	0.2–5.2	2–51	54–384	74–447	2–14	1–10	73–801
Eurobionet network threshold values[a]								
Very low	<0.04	<0.8	<7.1	<180		<5.2	<0.8	<31.7
Low	0.05–0.07	0.9–1.6	7.2–11.6	181–309		5.3–7.6	0.9–1.6	31.8–45.1
Elevated	0.08–0.10	1.7–2.4	11.7–16.0	310–438		7.7–9.9	1.7–2.4	45.2–58.6
Distinctly elevated	>0.10	>2.4	>16	>438		>9.9	>2.4	>58.6

[a](EuroBioNet report, 2001).

TABLE VI
Results of reproducibility test

Metals	ε_I^m (%)	ε_{II}^m (%)
Cd	20	19
Cr	29	31
Cu	12	15
Fe	10	11
Mn	12	18
Ni	30	32
Pb	15	16
Zn	14	12

exposure period of 15 days; this is the reason why we compare only the orders of magnitude. In this context, from Table V, we may observe that all the concentration ranges agree with the threshold values.

1. In Table VI we show the values of reproducibility index. We may distinguish three groups of metals: Cu, Fe and Zn with $\varepsilon_I^{\mathrm{m}}$ and $\varepsilon_{II}^{\mathrm{m}}$ lower than 15%; Cd, Mn and Pb, with $\varepsilon_I^{\mathrm{m}}$ and $\varepsilon_{II}^{\mathrm{m}}$ in the range 15–25%; Cr and Ni, with $\varepsilon_I^{\mathrm{m}}$ and $\varepsilon_{II}^{\mathrm{m}}$ in the range 25–32%. Taking into account the peculiarities of biomonitoring technique and particularly, the intrinsic variability of the living organisms, we may assume that the data have a good reproducibility. Furthermore, there are no significant differences between $\varepsilon_I^{\mathrm{m}}$ and $\varepsilon_{II}^{\mathrm{m}}$ for all the examined metals. This suggests that the data reproducibility is independent from plant parameters. Only for Mn, we note different values: $\varepsilon_I^{\mathrm{m}} = 12\%$ and $\varepsilon_{II}^{\mathrm{m}} = 18\%$, respectively.

TABLE VII
Results of statistical tests for the evaluation of the influence of plant age

	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
$P\%$ ($H_0 : C_I = C_{II}$)	52	73	31	43	47	73	63	33
$\rho_{I\text{-}II}$	0.54	0.84	0.61	0.83	0.45	0.46	0.89	0.65
(d.f.)	(41)	(43)	(42)	(43)	(43)	(43)	(43)	(43)
P_N	<3%	<3%	<3%	<3%	<3%	<3%	<3%	<3%

$P\%$ = percentage of cases in which $\mathbf{H}_0$ is satisfied; $\rho_{I\text{-}II}$ = correlation coefficient; d.f. = degrees of freedom; P_N = level of confidence for testing the significance of ρ-values

2. Regarding the tests on the independence of data from plant age, in Table VII we show the percentage of the examined cases in which we accept the null hypothesis of t-test and the values of correlation coefficients. All the correlation coefficients are significant with the level of confidence $\leq$3%. So we may assume that for all the metals the concentrations measured in 3-week-old plants (**Ic**) are linearly correlated with concentrations measured in 5-week-old plants (**IIc**), exposed in the same sampling period. Contemporaneously we note that the percentage of successes in the t-test is higher than 50% only for Cd, Cr, Ni and Pb.
3. The results of correlation analysis between air and plant data is shown in Table VIII. We may note that Cd, Cr and Ni measured in plants (**Ic** and **IIc**) are correlated with the values measured in particulate. In Figure 4, for each of these metals, we show the temporal pattern of observations, in plants and in air, using normalised values (zero mean and unit variance).

TABLE VIII
Results of test for the evaluation of the correlation between data measured in plants and in air. The cases in which the two variables are correlated are highlighted in bold

	Ic			**IIc**		
Metals	d.f.	ρ_{np}	$t(\rho_{np})$	d.f.	ρ_{np}	$t(\rho_{np})$
Cd	37	**0.36***	2.35	36	**0.58***	4.25
Cr	40	**0.29****	1.92	37	**0.24****	1.50
Cu	40	0.27	1.79	36	0.02	0.10
Fe	39	0.19	1.22	37	**0.33***	2.14
Mn	40	−**0.40***	−2.77	37	−0.02	−0.10
Ni	40	**0.48***	3.50	37	**0.75***	6.81
Pb	39	−**0.29****	−1.92	37	−**0.38***	−2.51
Zn	40	0.14	0.91	37	0.09	0.55

d.f., degrees of freedom; ρ_{np}, non-parametric correlation coefficient; $t(\rho_{np})$, $t-$variable.
*Significance level 5%.
**Significance level 10%.

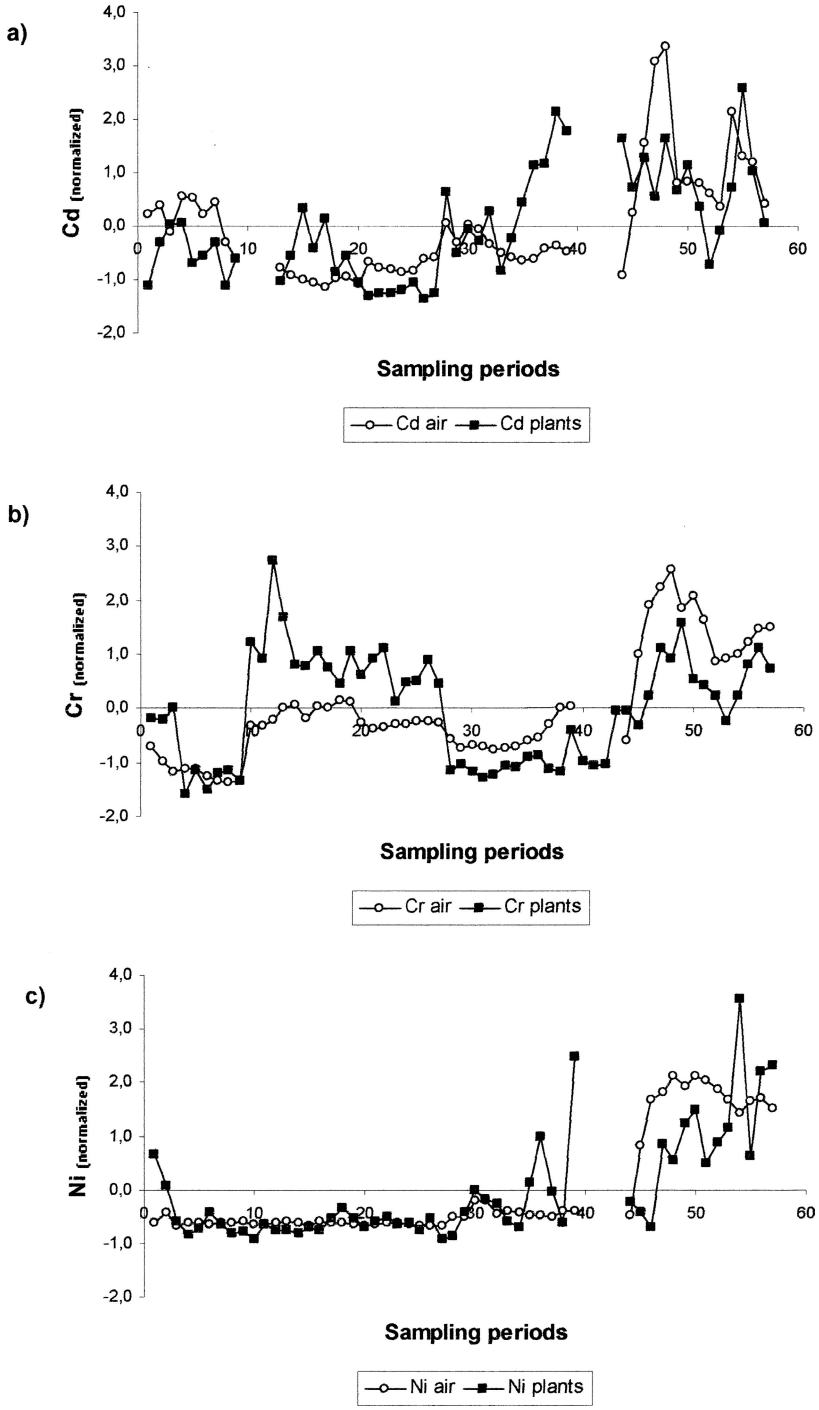

Figure 4. Temporal patterns of Cd (a), Cr (b) and Ni (c) measured in plants and in air (normalised values).

TABLE IX
Mean values of Cd and Ni concentrations measured in particulate and in biomonitors during the sampling periods (n) of each year

	Cd		Ni	
	Air (ng/Nm^3)	Plant (ppm DW)	Air (ng/Nm^3)	Plant (ppm DW)
1997				
N	9	9	9	9
Mean	2.8	0.22	3.0	3
1998				
N	18	18	18	18
Mean	1.4	0.18	2.0	3
1999				
N	16	16	16	16
Mean	2.1	0.38	9.8	5
2000				
N	14	14	14	14
Mean	4.3	0.36	68.0	7

It is important to note that for all the three metals the signal related to plant data and the signal related to particulate data show a similar behaviour although the three temporal patterns are different. In particular Cr seems to show a seasonal pattern probably due to a driving action of ambient temperature (Caggiano *et al.*, 2001b), while Ni and Cd show a positive trend (Table IX) related to an emission increase.

A good agreement may be evaluated by means of air–plant scatterplots also (Figure 5). We may determine three significant correlation lines

$$\begin{aligned}
\mathrm{Cd_{plant}} &= 34.9\mathrm{Cd_{air}} + 0.2 \quad (\rho_{\mathrm{Cd}} = 0.46 \quad N = 53,\ p < 0.05\%);\\
\mathrm{Cr_{plant}} &= 35.2\mathrm{Cr_{air}} + 1.1 \quad (\rho_{\mathrm{Cr}} = 0.53 \quad N = 53,\ p < 0.05\%);\\
\mathrm{Ni_{plant}} &= 61.3\mathrm{Ni_{air}} + 2.9 \quad (\rho_{\mathrm{Ni}} = 0.66 \quad N = 53,\ p < 0.05\%).
\end{aligned}$$

The plant concentration was calculated by averaging all the values (**Ic** and **IIc**) measured in each sampling period and the air concentration was calculated by averaging the data measured in the corresponding sampling period.

Regarding the other metals, Cu and Zn show low correlation coefficients (Table VIII), probably because we record frequent concentration peaks that may mask the correlation structure. Regarding Fe and Mn, we note a different behaviour between 3-week-old plant (**Ic**) and 5-week-old plants (**IIc**) in

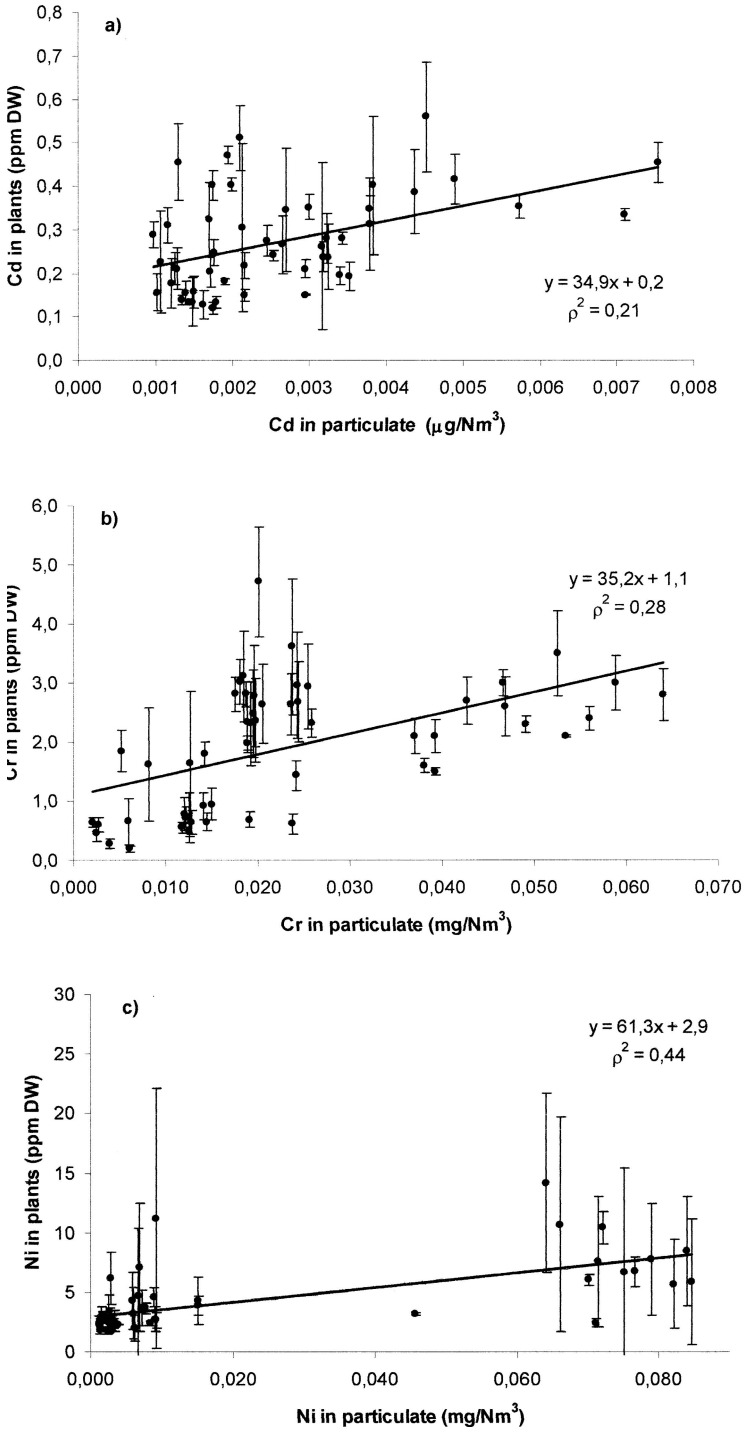

Figure 5. Air–plant scatterplots for Cd (a), Cr (b) and Ni (c).

the correlation structure (Table VIII): only Fe(**IIc**) is correlated with atmospheric values while only Mn(**Ic**) is negatively correlated with values measured in particulate. This particular behaviour of Mn was observed also in other biomonitoring field surveys (Bari *et al.*, 2001; Pacheco *et al.*, 2001). This is probably linked to the natural presence of these metals in plants that may affect the levels observed in biomonitors. At the end, we observe a significant negative correlation for Pb: the concentration increase recorded in particulate was not measured in plants. This is probably due to a different amount of increase in Pb fractions in the investigated area. As shown by source profiles, Pb emissions are linked to two different groups of sources: the dominant one coming from industrial activities (fraction of Pb in profile $\mathbf{SP}_2$), suffering a positive trend; and the second one due to traffic (fraction of Pb in profile $\mathbf{SP}_3$). So we need more information, such as particle size distribution, to put in evidence the correct relationships between Pb level in particulate and in plants.

6. Conclusions

In this paper we focused our attention on the measure of atmospheric heavy metals by means of ryegrass. To perform a reliable active biomonitoring procedure, it was necessary to standardise the experimental methods, to investigate the data in terms of sensitivity and reproducibility of measurements and finally to compare measures obtained by biomonitoring procedure to measures obtained by chemical–physical techniques.

From the data analysis, we may conclude that biomonitoring measures, performed by means of our experimental protocol, have a good reproducibility and that the data reproducibility is independent of plant parameters.

Regarding the metal monitoring, on the basis of the results of statistical tests, we may affirm that Cd, Cr, Ni seem to be well measured, by means of biomonitors in our test site. Particularly for these three metals, we may determine three significant correlation lines that may be used to define a calibration procedure among chemical–physical techniques and biomonitoring methods. For the other metals some statistical tests fail because their emission patterns make it difficult to identify the correlation structure between plants and particulate.

In conclusion, the results presented in this paper suggest us to promote the use of biomonitoring techniques, taking into account that it is necessary to test and adapt the protocols to the monitored area. We conclude that the use of bioindicators give much information about air quality and, even if bioindicators does not substitute completely the physical–chemical methods, they may be considered as a useful tool to integrate monitoring networks for air-quality survey. In fact, the integration of these two kinds of monitoring techniques may give an additional, useful and valid support for innovative monitoring programs.

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