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Pilot research on testing the reliability of studies on carabid heavy metals contamination

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Concentrations of heavy metals in the tissues of carabid beetles were assessed to evaluate a new rapid method of toxicity analysis. Beetles were collected from under stones in eight sampling sites in pristine and polluted environments across a pollution gradient. After transport to a laboratory the samples were frozen, dried, and digested with nitric acid prior to analysis by ICP-MS. Thirty trace elements were detected and significant concentration differences were found among beetles collected from different sites. Cluster analysis and Principal Component Analysis indicated correlations between element concentrations and pollution levels at particular sites as well as possible pollution from unexpected sources. The described rapid assessment method was found to be useful for preliminary screening, but this would be confirmed only with a considerably larger data set.

Keywords: bioindicators; Carabidae; heavy metals; multivariate analysis; pollution.

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INTRODUCTION

The accumulation of metals in insects has been clearly documented in the cells of several organs, e.g. in the genital and digestive systems, Malpigian tubules, and fat bodies, which provide the insects with highly resistant bioaccumulation systems against pollution damages (Ballan-Dufrançais 2002).

Metals tend to accumulate in the surface organic layers of soil, and herbivorous insects from this habitat apparently play a significant role in the transfer of toxic metals to higher trophic levels (Hunter et al. 1987). Data obtained from several sources in both laboratory studies (Braeckman et al. 1997, Augustyniak et al. 2006) and field studies (Hunter et al. 1987, Heikens et al. 2001, Migliorini et al. 2004, Gongalsky et al. 2007) have demonstrated metals accumulation in various arthropods, with many differences having been noted between different taxonomic groups.

The transfer of metals through trophic food webs may cause secondary effects in higher consumers, which can lead to the spreading of toxic met-

als through terrestrial food chains (Scheifler et al. 2002, Vickerman and Trumble 2003, Gongalsky 2006, Shipper et al. 2008), and recent studies have demonstrated biomagnification in plants and invertebrates (Vandecasteele et al. 2004, Mulder and Breure 2006, McConnell and Edwards 2008). Species living in metal-polluted areas for multiple generations may be exposed to sustained selection pressure. The energetic costs of living in such environments are high and may result in detrimental cytogenetic changes, or negative impacts on body mass and fecundity as well as sensitivity due to shortages of energy resources (Stone et al. 2001, Warchalowska-Sliwa et al. 2005, Augustyniak et al. 2006).

Previous studies demonstrate the role of invertebrates, such as insects, in facilitating the transfer of metals between different trophic levels within food chains, so that such organisms can be used as biological indicators of metal pollution, as well for assessing the potential biological consequences of metal pollution.

We have chosen Carabid beetles as the focal group, because they are reliable ecological indicators (i.e. (Landres et al. 1988) mirroring the state of environmental factors or habitat conditions) as found in a number of applied and theoretical ecological studies (den Boer 1977, Thiele 1977, Eyre et al. 1989, Hilty and Merenlender 2000, Szyszko 2002, Koivula 2011, Kotze et al. 2011), they are non randomly distributed in the environment (Sklodowski 2005), they are critically endangered by human activities (Brandmayr et al. 2009), even by tourism (Sklodowski 2011). Many carabids are predators, i.e. they occupy the trophic level above herbivores along potential metal pollution pathways in soils.

The storage of metals in carabids has been found as affected by laboratory (Kramarz and Laskowski 1997, Kramarz 1999, Maryanski et al. 2002, Scheifler et al. 2002, Bednarska and Laskowski 2008) or field conditions (Rabitsch 1995, Straalen et al. 2001, Heikens et al. 2001, Jelaska et al. 2007, Purchart and Kula 2007).

Since it is not clear if insects can be used by a rapid assessment approach for the detection of metal pollution in natural environments (cf. Hunter et al. 1987, and Gongalsky 2006), then, given that previous studies demonstrated that carabids are affected by the metal pollution of the environment, is it possible to use rapid sampling, based on a few specimens, for preliminary environmental screening? Such a method is expected to provide heterogeneous results that would mirror suspected pollution levels.

METHODS

Rationale behind

Our approach relies on previous studies, where relations between soil contamination and carabid metal bioaccumulation (Jelaska et al. 2007, Marianski et al. 2002, Straalen et al. 2001), and between human industrial activity and carabid physiology (Lagisz and Laskowski 2007) have been demonstrated. As a general rule, metal contamination affects carabids through an environment-to-carabids relationship, where the (falsified by previous studies) null hypothesis (Ho) says that environment contamination (ec) is congruent with no carabid contamination (n_cc).

Our approach is an operationalization of such a general rule, by assuming that the above relation has a symmetric value, so that it can be applied on the basis of a carabids-from-environment relation, where the Ho says cc H" n_ec (i.e., carabid contamination is congruent with no environment contamination). This means that if we find a contaminated carabid then there is a high probability that the environment is contaminated also, without need of control test because it is given by the previous studies. In other words, if we don't know the state of contamination (soc) of the environment, it will be possible to rise a reasonable hypothesis on the environment soc once known the carabid soc.

The study was implemented through three sequential steps, without information exchange, as follows. (i) sampling was performed by one researcher (who knew the environment soc on the

basis of previous studies), then (ii) samples (labeled from S1 to S8) were taken in charge by the chemical analysis team (who found the carabid soc), and finally (iii) results were analyzed by the data analysis team (who knew the carabid soc, but not the environment one). In such a way the monitoring protocol performed by three independent laboratories was tested.

Sampling Methods

Rapid sampling involved spending a few minutes looking for carabids under stones. One specimen was collected at each of eight sites, stored in a plastic tube, and within a few hours had been transported to the laboratory and frozen.

Carabids were collected over a large area in the region of Calabria, Italy. Three locations were selected many kilometres apart, based on stratified random sampling, with apparent levels of contamination as layers. The Sila mountain massif, 1400 metres above sea level, which is characterised by pine woods, pastures and fields was selected as the uncontaminated area, on the basis of Hernandez et al. (2003), Stefanowicz et al. (2010), Zhang et al. (2012); three sites (S1, S2, S3: Table 1) in pine woods were sampled. The "Università della Calabria" area (a university campus far from the city of Cosenza, but crossed by a street with intense traffic), was selected as the intermediate pollution area, as demonstrated by Akbar et al. (2006), Chen et al. (2010), Naima et al. (2012). Two sites were sampled (S4 and S5 in Table 1) at this location. An abandoned industrial area near the city of Crotone was selected as the severely polluted area, as demonstrated by Roberts and Johnson (1970), Rangoni (2008), Barone et al. (2010), Gowd et al. (2010), Pajak and Jasik (2011). This area had, in the recent past, been the subject of an inquiry into illegal trafficking of toxic wastes (including metals), and toxic material had been randomly dumped in various sites around the city. Three sites were sampled: S6, from just outside the boundary wall; S7, situated some distance from the main work zone, and S8, from inside the boundary, near the work zone. It should be underlined that the actual map of the toxic dumpsites is not available, so that homogeneity among these three sites is not to be expected.

Sample Preparation and analysis

Insects were dried in an oven at 110 °C for 24 hours after which drying was extended until a constant weight had been reached. The whole insect was then digested in a mixture of 8 mL of nitric acid and 2 mL of hydrogen peroxide (Merck Suprapure grade) in a closed PFA vessel, using a microwave digestion unit (Milestone Ethos 1, Bergamo, Italy). A 1 mL aliquot of the digest was then diluted to 10 mL in a polypropylene centrifuge tube. Analysis of the trace element content was carried out using an ICP-MS (Agilent 7500is) in semi-quantitative analysis mode.

Data analysis

The concentration range of the detected metals, as the ratio between (min–max)/max, was computed to highlight the extent of difference between minimum and maximum concentrations. The coefficient of variation (CV) was used to detect low variation (CV<1) vs. high variation (CV>1) distributions. Evenness (E, ranging from 0 to 1), computed as the ratio of metal Information (i.e., Shannon index) to maximum metal Information, gives an idea of how the result of a sum is partitioned among the addenda that gave that result, where E=1 indicates that the same concentration has been detected at every site, while E~0 indicates that the concentration may be very high at one site and low at others. Skewness and kurtosis were utilized to inspect heterogeneity of metal distribution.

Cluster analysis (R Development Core Team 2010) of the variables, based on the correlation coefficient and the average linkage clustering method, was used to highlight relationships among the elements, while cluster analysis of the sites, based on Euclidean distance and Ward's clustering method, was used to highlight the pollution similarity among sites (Pielou, 1984, Yongmin et al. 2006, Dragovic and Mihailovic 2009).

Principal components analysis (PCA) (R Development Core Team 2010) was used to identify new composite variates (i.e. the components, also known as dimensions or factors) for detecting variation in the distribution of the metals among the sites (Randerson993, Yongmin et al. 2006, Dragovic and Mihailovic 2009). PCA depicts components as orthogonal axes, and the relative position of metals on these axes was chosen as a tracer for each component.

RESULTS

Eight carabid specimens were sampled at a number of sites. The following species, which have predatory diet and similar geographical distributions, were collected: *Pterostichus melas* (from S1, S2 and S7), *Calathus montivagus* (from S3), *Calathus cinctus* (from S4 and S5), *Chlaeniellus vestitus* (from S6), and *Harpalus attenuatus* (from S8). All of these species are predators, except for *H. attenuatus*, which has a mixed diet (i.e. it is predatory, but also consumes seeds).

The results of the following computations should be read taking into account the limits of our study, i.e. small sample size and taxonomic heterogeneity, that could increase the variation range of the computed indexes.

Thirty trace elements (at a wide range of concentrations) were detected (Table 1). Only Cu, K and Mo had ranges of less than 80% of the maximum detected concentration. Such a variation is confirmed by the CV (Table 1), which showed values beneath the unity for Cu, K, Mg, Mo, Na and W, while for most cases CV values were above unity, and a value close to 3 (i.e. 2.78) was computed for Pb.

Evenness was low (<0.5) for Ag, As, Cd, Ge, Ni, Pb, Sb, Ti and Tl. This meant that 30% of the detected elements were more concentrated at one site and only five elements showed high Evenness (>0.9).

Distributions of all detected elements were positively skewed, i.e. relatively few had high values and distributions were concentrated towards low values. High values of kurtosis were computed for most of the elements, indicating that, relating to normal distribution, there is a higher probability of obtaining values above or below mean concentration levels.

Inspection of the dendrogram of Figure 1 provides an indication of the sources of the detected elements, that in our case cluster into one large group (A) and a smaller one (B). The relatively long vertical segment at the top of the dendrogram that links Group A with Group B indicates a low correlation between these two groups. Within the limits of our study, we found very high intra-group correlation for the most part of the elements of Group A (Fig.1, from Ag to Tl), where almost 80% of the elements have been clustered, while in Group B this holds true for K, Mg and Na over seven clustered elements (20%).

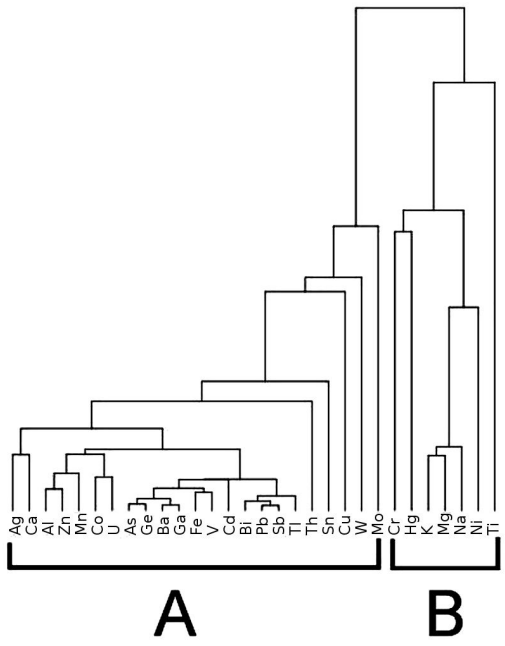

Fig. 1 Metal classification after cluster analysis based on correlation coefficient and average linkage clustering method. Two groups of elements are recognizable (A and B). There is high correlation among the elements of group A, while the same holds true for K, Mg and Na in group B.

Table 1. Concentration of metals (rows) in the sample sites (columns) ordered following an hypothesized pollution gradient S1-S8. Along each row the highest concentration is in bold, while the second maximum is underlined

	S1	S2	S3	S4	S5	S6	S7	S8	range%[a]	mean[a]	SD[a]	CV[a]	Equit[a]	median[a]	kurt[a]	skew[a]
Ag	0.053	0.037	0.000	0.000	0.000	**1.33**	0.53	0.003	*1.00*	*0.24*	*0.47*	*1.95*	*0.5*	*0.02*	*4.67*	*2.19*
Al	393.232	750.95	582.128	65.835	57.772	**2915.07**	365.738	206.970	*0.98*	*667.21*	*939.45*	*1.41*	*0.7*	*379.49*	*6.48*	*2.47*
As	0.120	0.845	0.410	0.000	0.000	**14.39**	1.65	0.109	*0.99*	*2.19*	*4.96*	*2.27*	*0.38*	*0.26*	*7.69*	*2.76*
Ba	3.077	5.163	3.018	6.491	1.468	**81.99**	13.61	1.322	*0.98*	*14.52*	*27.55*	*1.9*	*0.53*	*4.12*	*7.5*	*2.72*
Bi	0.099	0.099	0.15	0.139	0.142	**1.11**	0.116	0.115	*0.91*	*0.25*	*0.35*	*1.42*	*0.74*	*0.13*	*7.92*	*2.81*
Ca	256.455	469.343	528.227	194.724	84.316	**2186.3**	1402	201.221	*0.96*	*665.32*	*742.09*	*1.12*	*0.78*	*362.9*	*1.75*	*1.6*
Cd	0.142	0.164	0.000	0.000	0.000	**47.37**	8.74	3.737	*1.00*	*7.52*	*16.4*	*2.18*	*0.42*	*0.15*	*7.14*	*2.64*
Co	0.058	0.300	0.129	0.009	0.048	**1.71**	0.57	0.075	*0.99*	*0.36*	*0.58*	*1.59*	*0.61*	*0.1*	*5.58*	*2.32*
Cr	0.116	7.979	2.264	0.000	**17.18**	9.11	0.996	0.155	*0.99*	*4.72*	*6.2*	*1.31*	*0.69*	*1.63*	*1.16*	*1.34*
Cu	17.097	37.078	33.418	32.454	35.912	74.7	**81.28**	34.495	*0.79*	*43.3*	*22.36*	*0.52*	*0.95*	*35.2*	*-0.03*	*1.09*
Fe	239.358	469.343	355.745	76.962	312.281	**2368.49**	589.25	155.228	*0.97*	*570.83*	*744.68*	*1.3*	*0.75*	*334.01*	*6.86*	*2.56*
Ga	341.941	610.146	291.064	574.899	156.140	**7287.67**	1158.17	149.478	*0.98*	*1321.19*	*2433.06*	*1.84*	*0.55*	*458.42*	*7.56*	*2.73*
Ge	0.048	0.089	0.000	0.000	0.000	**1.15**	0.12	0.000	*0.96*	*0.18*	*0.4*	*2.26*	*0.48*	*0.02*	*7.68*	*2.76*
Hg	0.065	0.066	0.030	0.000	0.000	0.12	**0.35**	0.009	*0.97*	*0.08*	*0.12*	*1.45*	*0.74*	*0.05*	*5.02*	*2.16*
K	2906.496	**7040.14**	3773.050	2132.691	1717.544	4190.41	2844.632	2932.075	*0.76*	*3442.13*	*1656.5*	*0.48*	*0.96*	*2919.29*	*3.3*	*1.64*
Mg	1504.539	**3285.4**	1617.021	917.984	655.789	2004.11	1909.967	1379.800	*0.80*	*1659.33*	*800.57*	*0.48*	*0.95*	*1560.78*	*2.05*	*1.08*
Mn	47.872	84.48	54.979	9.273	6.870	**236.85**	42.669	20.122	*0.97*	*62.89*	*74.9*	*1.19*	*0.76*	*45.27*	*5.32*	*2.2*
Mo	0.855	1.643	2.264	0.733	1.046	3.1	1.280	**3.16**	*0.77*	*1.76*	*0.97*	*0.55*	*0.94*	*1.46*	*-1.38*	*0.61*
Na	4958.140	**11264.23**	4958.865	2039.965	1452.105	5465.75	4266.948	1437.292	*0.87*	*4480.41*	*3204.48*	*0.72*	*0.9*	*4612.54*	*2.66*	*1.41*
Ni	0.000	**1079.49**	0.000	0.000	0.000	0.000	162.55	0.000	*0.85*	*155.25*	*377.75*	*2.43*	*0.56*	*0*	*7.44*	*2.71*
Pb	0.188	0.390	0.000	0.000	0.000	**327.95**	3.45	0.535	*1.00*	*41.56*	*115.72*	*2.78*	*0.05*	*0.29*	*8*	*2.83*
Sb	0.011	0.084	0.172	0.195	0.128	**4.74**	0.2	0.195	*1.00*	*0.72*	*1.63*	*2.27*	*0.37*	*0.18*	*7.96*	*2.82*
Sn	0.154	0.113	0.464	0.399	2.65	**6.01**	0.173	0.024	*1.00*	*1.25*	*2.11*	*1.69*	*0.54*	*0.29*	*4.21*	*2.11*
Th	0.077	0.27	0.108	0.008	0.011	**0.55**	0.098	0.021	*0.99*	*0.14*	*0.18*	*1.3*	*0.7*	*0.09*	*3.47*	*1.88*
Ti	7.352	12.672	11.858	2.689	2.498	71.05	10.769	**2817.09**	*1.00*	*367*	*990.24*	*2.7*	*0.11*	*11.31*	*7.99*	*2.83*
Tl	0.019	0.033	0.036	0.000	0.011	**1.18**	0.08	0.010	*0.99*	*0.17*	*0.41*	*2.39*	*0.31*	*0.03*	*7.91*	*2.81*
U	0.024	0.070	0.029	0.004	0.000	**0.24**	0.09	0.025	*0.98*	*0.06*	*0.08*	*1.3*	*0.75*	*0.03*	*4.51*	*2.05*
V	0.291	0.469	0.399	0.019	0.067	**4.37**	0.996	0.155	*1.00*	*0.85*	*1.46*	*1.72*	*0.58*	*0.34*	*6.93*	*2.59*
W	0.031	0.2	0.108	0.039	0.048	**0.22**	0.045	0.000	*0.86*	*0.09*	*0.08*	*0.95*	*0.86*	*0.05*	*-0.69*	*0.96*
Zn	162.422	244.06	172.482	82.526	56.211	**1020.27**	144.263	155.228	*0.94*	*254.68*	*314.56*	*1.24*	*0.78*	*158.82*	*7.23*	*2.64*

[a] range%. is the ratio between (min - max)/max. SD. standard deviation. CV. Coeficient of Variation. ratio between SD/mean. E. Evenness. ratio of measured row Information (Shannon index) to maximum row Information. kurt. kurtosis. skew. skewness.

The dendrogram illustrated in Figure 2 gives an idea of how the detected elements concentrate at the sites. Two groups are clearly recognizable: Group 1 is structured into two subgroups with high intra-group similarity, but only the sites S4 and S5 have the same environmental features. Group 2 clusters S2 and S6, shows low intra-group similarity, and this is mirrored by the environmental features of these sites.

The link between the classification of elements (Fig. 1) and sites (Fig. 2) is shown in Table 1, where the bold-taped values highlight the most relevant weight of the co-occurence of element vs. site.

The PCA (Figure 3, Table 2) indicates that the first axis was responsible for 69% of the total variance, and that this variance is explained by 21 elements, i.e. the first factor is responsible for the high cosine square values of 70% of the detected elements. The second axis was responsible for 14.5% of the total variance, accounted for by four elements (13%). The elements tracing for factors 1 and 2 lie on the positive side of the axes: the firsts have very heterogeneous sources, while the seconds mainly originate from fertilizers. Only Ti has been clearly separated in the lower left quadrant of the PCA space. The ordination of sample sites (Figure 4, Table 3) indicates that S6 has high cosine square value on axis 1 (0.99), while the value for S2 on axis 2 was 0.97. It is worth noticing that among the other sites S7 has high osine square on axis 3 (0.97) as Hg (0.85), and that S8 has high cosine square on axis 4 (0.49) as Ti (0.73).

DISCUSSION

Our research suggests that previous studies can be used as control references because rapid assessment based on rapid sampling of carabid beetles at least partly mirrored the suspected state

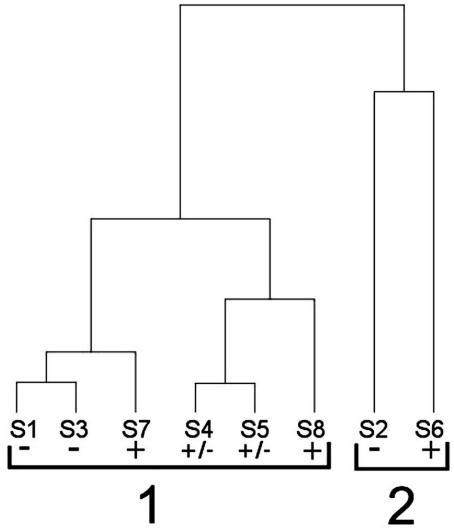

Fig. 2 Sample-site classification based on Euclidean distance and Ward's clustering method. Sample sites have been clusterd into groups 1 and 2, characterized by high and low intragroup similarity respectively. Sample sites with very different ecological characteristics have grouped in the same clusters, suggesting that the environmental availability of metals is the main factor determining the similarities and differences among sampled sites. The symbols -, +/- and +, refer to the uncontaminated, intermediate and polluted suspected state of the sample sites.

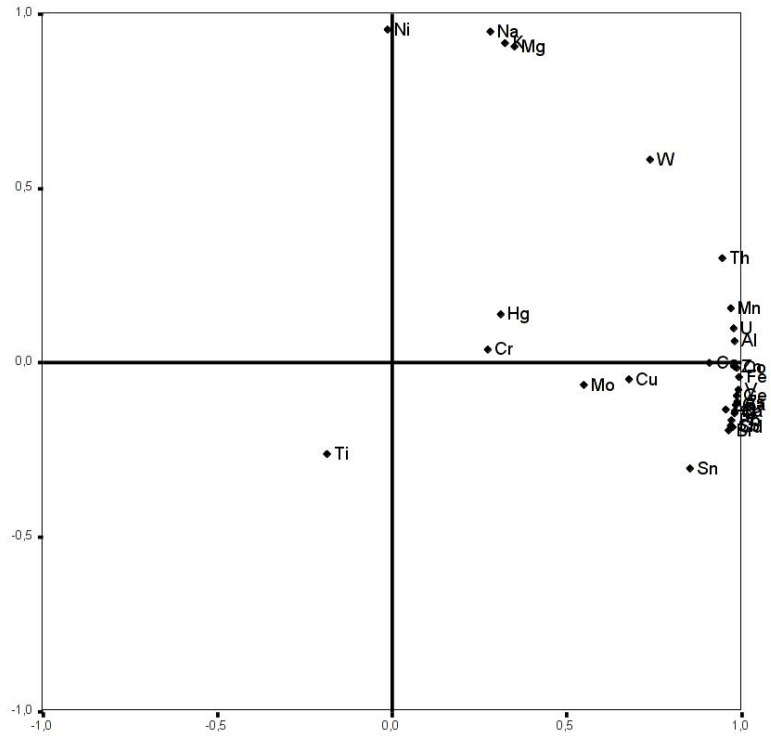

Fig. 3 Metal ordination after Principal Component Analysis. 1st and 2nd Components explain 68.97% and 14,46% of the total variance respectively. The ordination highlights two sources of pollution, one in the right part of the first dimension is given by most of the detected metals, and the other in the upper part of the second dimension is given by few metals that are recognizable as regular components of chemical fertilizers.

of environmental pollution. At the same time our results showed that the analysis of few specimens may be critical for tracing conclusions, while it can be helpful in case of preliminary screening. Environmental pollution is a source of contamination for carabids (Rabitsch 1995, Straalen et al. 2001, Heikens et al. 2001, Jelaska et al. 2007, Purchart and Kula 2007), a priori (see sampling methods paragraph) we knew that the sample sites were characterized by different levels of pollution. Then by simulating a condition where we knew the actual contamination of the carabids, while not the one of the environment, we demonstrated that, within the limits of our study, we are able to rise a reliable hypothesis on the actual state of contamination of the environment, which drives our attention to the need of a more complete data gathering. Furthermore our results gave some unexpected outcomes with regard to our starting hypothesis.

The hypothesis proposed that the metal content of the carabid tissues should mirror the variation of environmental metal pollution rather than the different ecosystem types, and we found that where the metal pollution was demonstrated by previous studies then there was a similar response in metal concentrations in carabid tissues. On the basis of descriptive statistics (CV), the metals separated into two groups: one in which metal concentrations moderately fluctuated around the mean (CV<1), and one with highly variable concentrations (CV>1). Since the first group was formed by six of the thirty detected metals, this indicated that the majority of metals did not have similar pollution sources in the sampled sites. Metal concentrations were also positively skewed, showed high values of kurtosis (i.e., leptokurtic), and a wide range of variation, i.e. they were unevenly distributed around the mean, suggesting that the source of the detected metals was not the same among these samples (Yongmin et al. 2006). A critical point in the interpretation of such differences could be that a sort of masking effect is possibly due to Carabid taxonomic differences, even if they have similar predatory diet, which is why rapid sampling should be carefully applied in preliminary studies only.

Since metal classification (Fig. 1) was based on tissue metal concentrations, results indicated that in groups A and B (Fig. 1) the manner in which bioaccumulation occurred in carabid tissues was similar for different elements. The strongest correlations in intra-group elements was found in

Table 2. Principal Component Analysis (PCA) main results for the metals after table 1. First and second dimension (Dim1 and Dim2) account for the 68.97% and 14.46% of the total data variation respectively. Relationship between dimensions and metals is depicted by axes 1 and 2 in figure 3

	cos2[a]		contribution[a]	
	Dim.1	Dim.2	Dim.1	Dim.2
Fe	**0.9929**	0.0021	4.7986	0.0477
V	**0.9897**	0.0067	4.7829	0.1543
As	**0.9826**	0.0136	4.7488	0.3127
Co	**0.9799**	0.0004	4.7356	0.0086
Ge	**0.9795**	0.0098	4.7341	0.2265
Ga	**0.9738**	0.0158	4.7062	0.3643
Ba	**0.9697**	0.0204	4.6865	0.4708
Zn	**0.9683**	0.0003	4.6798	0.0058
Al	**0.9679**	0.0033	4.6779	0.0761
Tl	**0.9673**	0.0223	4.6747	0.5151
U	**0.9625**	0.0089	4.6519	0.2051
Cd	**0.9567**	0.0357	4.6238	0.8221
Pb	**0.9500**	0.0287	4.5912	0.6618
Mn	**0.9471**	0.0231	4.5771	0.5321
Sb	**0.9460**	0.0342	4.5720	0.7886
Bi	**0.9346**	0.0394	4.5169	0.9071
Ag	**0.9190**	0.0192	4.4417	0.4430
Th	**0.8996**	0.0873	4.3477	2.0118
Ca	**0.8306**	0.0000	4.0141	0.0005
Sn	**0.7330**	0.0946	3.5423	2.1794
W	**0.5501**	0.3340	2.6586	7.6989
Ni	0.0001	**0.9038**	0.0005	20.8305
Na	0.0809	**0.8928**	0.3912	20.5763
K	0.1064	**0.8315**	0.5143	19.1628
Mg	0.1249	**0.8138**	0.6036	18.7556
Cu	0.4644	0.0027	2.2443	0.0623
Mo	0.3049	0.0046	1.4734	0.1066
Hg	0.0987	0.0180	0.4770	0.4158
Cr	0.0767	0.0011	0.3709	0.0256
Ti	0.0336	0.0708	0.1625	1.6324

[a]cos2, square cosine (also known as communality), gives a measure of the metal variance explained by each dimension. Contribution, is the relative contribution of each metal to the variance explained by each dimension

Group A with the maximum concentration being mainly at site S6 (bold-tape in Table 1), while in Group B the maximum concentration was observed at site S2.

Such differences are affected by the different environmental sources of the metals, as outlined in the classification of sites (Fig. 2), where sample sites with different ecological characteristics grouped in the same clusters, suggesting that the bio-availability of metals was the main factor in determining similarities among the sites. Figure 2 suggests that the Ho is not true thus providing support to our alternative hypothesis (i.e., carabid contamination is congruent with environment contamination). It should, however, be noted that the expected similarity between S2 and S3 was not confirmed. One possible explanation can be that the specimen collected in S2 had been contaminated with non-organic fertilizers. Accurate field inspections after having analyzed the specimens indicated that the site was close to a conventionally-fertilized oats field from where the tested carabid could have originated. As an al-

Table 3. Principal Component Analysis (PCA) main results for the sites after table 1. First and second dimension (Dim1 and Dim2) account for the 68.97% and 14.46% of the total data variation respectively. Relationship between dimensions and metals is depicted by axes 1 and 2 in figure 4

	cos2[a]		contribution[a]	
	Dim.1	Dim.2	Dim.1	Dim.2
S6	**0.99**	0.01	82.87	2.53
S2	0.001	**0.97**	0.01	77.11
S4	0.69	0.14	5.04	4.94
S1	0.67	0.003	3.26	0.06
S3	0.52	0.03	1.30	0.35
S5	0.36	0.18	3.84	8.88
S8	0.33	0.12	3.69	6.11
S7	0.0005	0.0005	0	0.02

[a] cos2, square cosine (also known as communality), gives a measure of the metal variance explained by each dimension. Contribution, is the relative contribution of each metal to the variance explained by each dimension

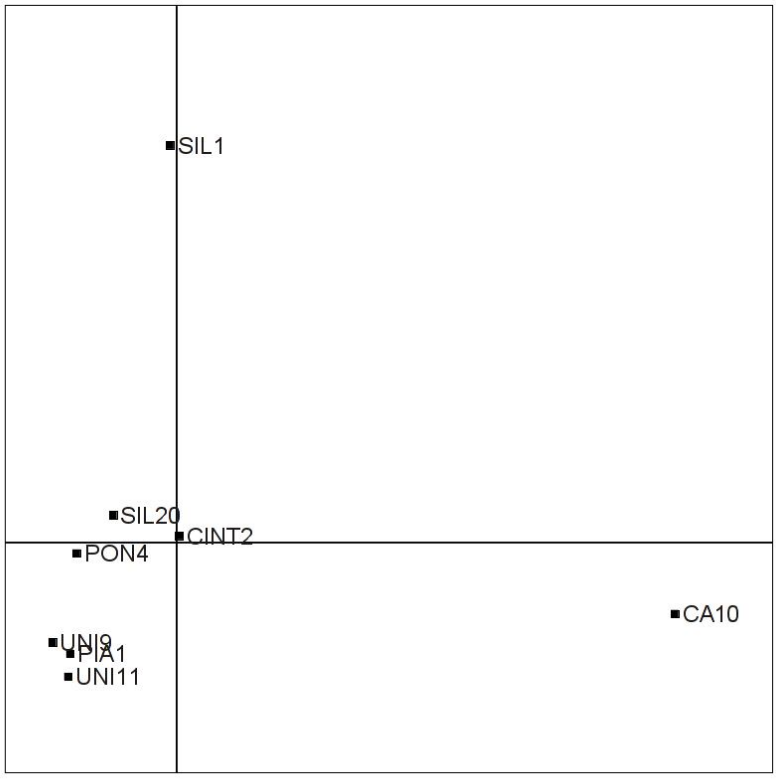

Fig. 4 Sample sites ordination after Principal Component Analysis. 1st and 2nd Components explain 68.97% and 14,46% of the total variance respectively. The ordination shows that there are two sites as different sources of pollution, i.e. S6 in the right part of the first dimension and S2 in the upper part of the second dimension.

ternative explanation farmers may have buried empty fertilizer bags at this site, which may have "contaminated" our measurements. The random distribution of S7 and S8 seems to be due to the random dumping linked to the illegal trafficking of toxic wastes, so that non-homogeneity among the three industrial sites follows the expected sampling conditions (see Methods).

PCA ordination of metals and sample sites along the first axis confirmed (as expected) that the main source of the pollution was the abandoned industrial site (i.e. high cosine square value of the most part of the metals and of S6 site on the same side of the first axis (Figures 3 - 4, Tables 2 - 3). The PCA also indicated that small components of the detected metals (regular components of chemical fertilizers) were linked to an apparently non-polluted forestry site (S2).

Fig. 4 shows the actual pollution gradient, which is more complex than expected because of the random pollution of the industrial area (S6, S7, S8) and of the unexpected contamination, probably due to farming, in S2. Furthermore, Fig. 4 (see also Tab. 3) with Fig. 2 shows that when industrial sites are sampled there is a strong pollution gradient driven by one main factor, i.e. the uncontrolled dispersal of industrial waste, which causes peaks of pollutant concentration in the area. Then, Fig. 4 shows that if the uncontrolled dispersal is excluded (i.e., second axis of PCA, see also Fig. 3), the second factor ordering the state of contamination of carabids is the use of chemicals in human activities, in our case farming. The lack of a clear gradient may result from the five species accumulating metals in different rates also, or the presence of the extremely polluted site S6, which cannot be treated as an outlier in our case.

Low metal concentrations at S8 could be a confirmation of results obtained by Purchart and Kula (2007) who found that the concentration of metals was affected by the feeding mode of the individual species. In our tests we only sampled an omnivorous species (*H. attenuatus)* at S8, even though this species is linked to Ti (as found by the PCA). This partially confirms our pollution hypothesis with respect to S8.

Worth of notice is that on the basis of the analysis of carabid state of contamination it is to be highlighted that humans, deliberately or not, may be the source of unpredictable deviation from expected outcomes (e.g. the similarity between S2 and S6, while not between S7 and S8). This gives value to our rapid sampling approach if, and only if, it is used in a preliminary screening, while a more rigorous approach must be followed in a decision-making inspection.

CONCLUSION

On the basis of our results we suggest to use the rapid sampling approach for detecting at least the gross differences among sites polluted by heavy metals in preliminary environmental screening. It could be applied for early warning detection, because it gives sharp spotted signals that mirror Carabid physiology, i.e. very high body metal concentration in the short period after contamination and high concentration when contamination remains time-constant (Laskowski et al. 2010).

Even if both the hypothesized pollutant source and an unexpected (site S2) source were detected, it should be emphasized that rapid sampling may be cautiously useful for preliminary studies aimed to highlight critical sites. As recommended by Hendrickx et al. (2003), hand collection (or *in vivo* trapping) is probably the best method for such studies.

The following recommendations should also be noted in case of further investigation needs for critical sites: on the basis of rapid sampling results, careful consideration should be given to the choice of new sampling site locations; sample sizes should be larger than was the case for the present rapid sampling (in which single samples were collected); and seasonal monitoring, as recommended by Hunter et al. (1987), should be undertaken, rather than the 'single visit' approach that was adopted in rapid sampling.

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