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Autor: Paoletto, Maurizio G. / Celi, Monica / Cipolat, Chiara

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Cave dwelling invertebrates: possible bio-indicators of cave pollution – an Italian case

Maurizio G. Paoletti, Monica Celi, Chiara Cipolat, Laura Tisat, Annamaria Faccio, Attilio A. M. Del Re & Raffaella Boccelli

ABSTRACT Contrib. Nat. Hist. 12: 1029–1047.

Cave and underground environments are dwelling places for an endemic and rich but fragile invertebrate fauna, which is mainly dependent on outside autotrophic food-webs. Organic matter and pollutants enter the cave and are ingested by living organisms. In a field study of floor mud, guano deposits, cave water, and some cave dwelling invertebrates found in eight natural carsick caves in the Veneto region of Italy, the concentration of some heavy metals (HM), e.g. Cd, Cr, Cu, Pb, Zn, were measured. In caves with heavy bat guano deposits, Cu and Zn pollution was very high (copper 132–2179 mg/kg and zinc 590–2090 mg/kg). Other HM were detected in various and not so high amounts in the cave mud. No accumulation along the cave foodchain was detected, as predators did not have higher HM contents than detritivores. In most cases the troglobitic specimens showed higher HM concentrations than topsoil invertebrates. Cr content was high both in mud and in detritivorous cave dwelling invertebrates such as *Typhloiulus tobias* (millipede). Some troglobitic species accumulated Cu, Zn, and Cd. Therefore the millipedes *T. tobias* and *Serradium* in addition to other invertebrates such as terrestrial isopods (*Androniscus, Spelaeonethes*) can be proposed as bioindicators to monitor the underground environments.

Keywords: cave dwelling invertebrates, heavy metals, caves, pollution, bat guano, bioindicators, terrestrial Isopoda, *Androniscus, Spelaeonethes, Thyphloiulus, Eupolybothrus,* millipedes, *Cansiliella.*

Introduction

An endemic invertebrate fauna of environmental and biological interest dwells in caves and in underground environments (Vandel 1965, Paoletti 1978) and a number of physiological and ecological adaptations have been observed in cave dwelling organisms (Ginet & Decou 1977; Juberthie & Decu 1994). The underground fauna of Southern Alps caves is rich in rare species and needs to be guarded from human contamination and landscape transformation (Paoletti 1977; Sauro 1989; Cobolli-Sbordoni & al. 1994).

The cave environment depends on trophic contributions from the outside, since photosynthesis in the absence of light is impossible (Gers 1987). Cave bacteria and other microorganisms that are part of the invertebrate diet are in general believed to be heterotrophic. The mud, the bat guano, and the organic matter that are accumulated in some caves support the trophic structure of some hypogeal communities (Wilkens & al. 2000, Vandel 1965), though recently an isolated cave fauna was found, apparently depending on sulfobacteria (http://www.geocities.com/rainforest/vines/5771/4research.htm: Movile cave, Rumania; Sarbu & Kinkle 1996).

Pollutants and water can also flow into most caves: their effect on aquatic biota was studied in Arkansas (Graening & Brown 2000) and significant consequences were observed. Although the impact of outside pollution on hypogeal environments is complex and little understood, it is likely to cause damage to the whole underground ecosystem (Boccelli & al. 1992; Miko & al. 2003).

Pollutants can also be carried outside by the living organisms migrating from the cave or from the underground (Turquin 1975) and the quality of drinking water can be affected. Invertebrates have been suggested as bioindicators of soil pollution or of poor soil management in agricultural areas (Paoletti 1977, 1999; Paoletti & al. 1991). Most heavy metals (HM) are toxic to invertebrates and can severely modify both structure and diversity of animal communities (Van Straalen & Wemsen 1986, Hopkin 1989, Paoletti & al. 1991, 1998, Van Straalen & al. 1997). For instance, copper and zinc concentrations in orchard soils can severely damage earthworm populations, making them collapse when copper content is over 200 mg/kg (Paoletti & al. 1998).

In this paper we tested the underground fauna as an indicator of HM (Cd, Cr, Cu, Pb and Zn) pollution in the cave environment, perhaps due to outside contamination sources.

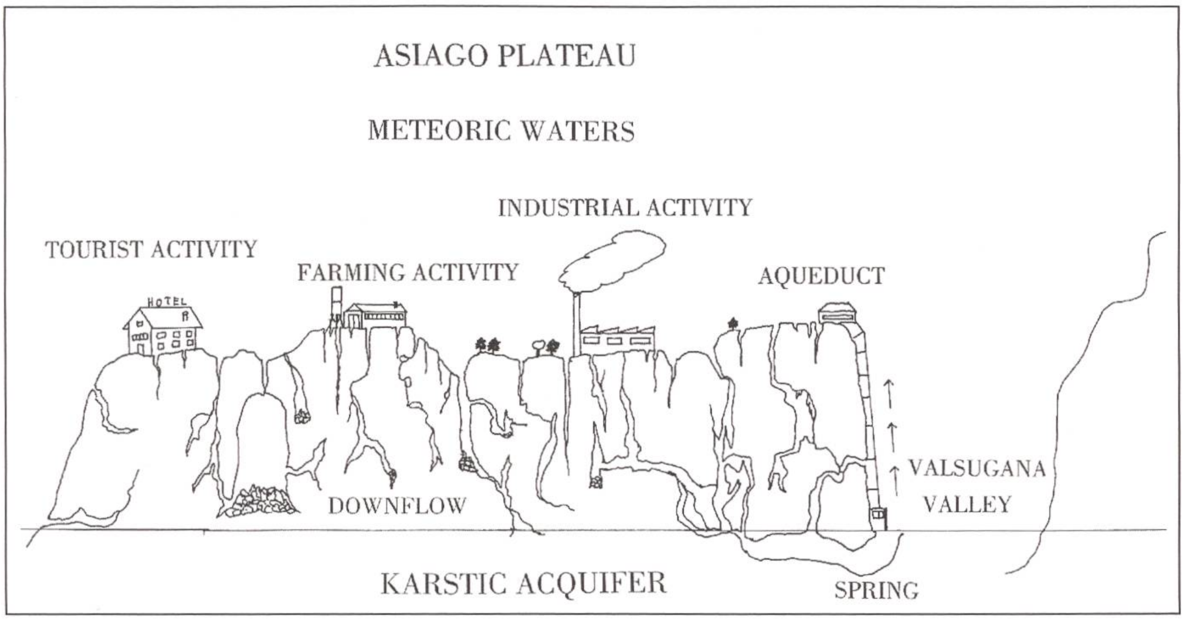

Fig. 1. Asiago Plateau karstic system: possible sources of pollutants in carsick waters.

Material and Methods

Area of study

The research was done in caves of the Valsugana valley, located in a prealpine area of the Veneto region, Northeastern Italy (Figs. 1, 2). Many caves are found in this limestone rich area, often including large underground springs. Relationships of caves with human activities are sketched in Fig. 1 together with likely water sources.

Three caves were selected in the Val Sugana area: Buso delle Anguane, Cogol dei Siori and Grotta di Ponte Subiolo; two near the Piave-Cismon Valley: Grottina Faller and Fogola de Toni Mondin; three others were analyzed for two metals (Zn and Cu) because of their heavy bat guano deposits (Tab. 1).

Sampling

Mud, water and troglobitic invertebrates were collected from the same sub-plot sites. Sampling times were unevenly spaced between November 1988 to December 1992. In some periods, caves were inaccessible and sampling impossible. All samples were kept in sterile jars at -8°C, for a maximum of three days before analysis. The mud was collected from three sites (400 cm^2 undisturbed area) in each cave by removing the upper 5 cm of surface mater-

Fig. 2. Location of the caves examined in the Veneto Region:
1 Grotta del Subiolo
2 Cogol dei Siori
3 Buso delle Anguane
4 Buso della Torta
5 Grotta di Veia
6 Grotta della Guerra
7 Fogola di Toni Mondin
8 Grotticella Faller

ial. Three kinds of water were sampled: 1) spring water, 2) seepage, 3) pools on the cave ground. Cave dwelling meso-invertebrates were captured with an aspirator after placing apples as bait and thereafter washed with distilled water.

Heavy metal (HM) measurement

Samples were weighed, oven-dried for 24–36 hours at 105° C, weighed and then ground to a fine powder. Two subsamples of 2 g each were taken for duplicate analysis and oven-concentrated for 24 hours at 95°C. Invertebrates were oven-dried for 24–36 hours at 105 °C.

Large organisms (0.1–0.4 g, e.g. *Troglophilus cavicola*, *Meta menardi*, *Typhloiulus tobias*) were individually analyzed. Smaller organisms, such as *Neobathyscia antrorum*, *Spelaeonethes nodulosus*, etc., were pooled in groups of 20–40 individuals. All samples were digested with nitric acid 65% and atomized in a graphite furnace Perkin-Elmer Mod. HGA 500. A pre-treatment with hydrochloric acid was used to destroy the excess of $CaCO_3$ in the mud

Cave	N	Cadmium		Chromium		Copper		Lead		Zinc	
		Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
Cogol dei Siori	27	0.82	0.34	22.25	13.13	12.48	0.34	18.02	8.86	75.53	32.02
Grotta del Subiolo	18	0.38	0.15	11.15	6.03	12.7	0.15	22.0	6.69	67.78	24.4
Buso delle Anguane	15	0.78	0.27	48.91	15.36	20.3	0.27	17.61	4.68	101.08	31.54
Fogola de Toni Mondin	32	0.55	0.36	129.0	23.0	54.2	0.36	30.4	5.5	121.8	23.2
Grottina Faller	32	0.0036	0.003	116.1	11.4	66.7	0.003	31.1	3.9	119.3	21.6
Buso della Torta*	30					2179.2	1324.1			2089.8	899.4
Grotta della Guerra*	10					132.6	83.5			589.5	233.6
Grotta di Veia*	8					144.3	74.2			679.2	246.2

Tab. 1. Mean concentrations of heavy metals (mg/kg) in cave mud. *: cave with consistent deposits of bat guano. S. D.: Standard Deviation. N: number of samples.

samples. HM were analysed by means of atomic absorption spectrophotometry (Perkin-Elmer Mod. HGA 460). For additional informations see Cipolat (1992) and Tisat (1992).

Results and Discussion

The number of more frequent species is variable: in the caves of Brenta river valley, 10–12 species are prevailing; in the two caves of the Piave-Cismon Valley, 19–32 species were detected.

Mud

The HM concentration in the cave mud samples varied seasonally; values were lower in March, April, and May than in other months (Fig. 3). Statistically significant differences (test *t* not shown) were found among Cr, Zn, Cu, and Cd contents in the mud of the various caves (Fig. 4, Tab. 1). Precipitation was inversely correlated with concentrations of copper, lead, and zinc (Fig. 5), while the correlations with Cd and Cr concentrations were statistically not significant (not shown). The Buso delle Anguane cave had the highest Cr content: this may be due to the bedrock type or to pollution from outside (Fig. 1).

Cave	N	Cadmium		Chromium		Copper		Lead		Zinc	
		Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
Cogol dei Siori	21	0,00007	0,0001	0,0031	0,0016	0,0226	0,0185	0,0096	0,0194	0,1381	0,0921
Grotta Subiolo	21	0,00006	0,00005	0,00007	0,00009	0,0092	0,0055	0,00008	0,0001	0,0691	0,0645
Max. conc.		0,001		0,05		0,05		0,05		3	

Tab. 2: Heavy metals concentrations (ppm) in cave water. Max. conc. = Maximum concentration in potable water according to Italian law; N: Number of samples.

Bat guano

Three caves were selected for their extensive bat guano deposits; the measured Cu and Zn concentrations are shown in Tab. 1. In the cave that had been used during the First World War as firearm storage (Buso della Torta, Celi 1991), the Cu and Zn concentrations were very high. In the other two caves they were also high, yet somewhat lower. In all cases, the Cu and Zn levels were so high that they must be considered a threat to cave foodwebs.

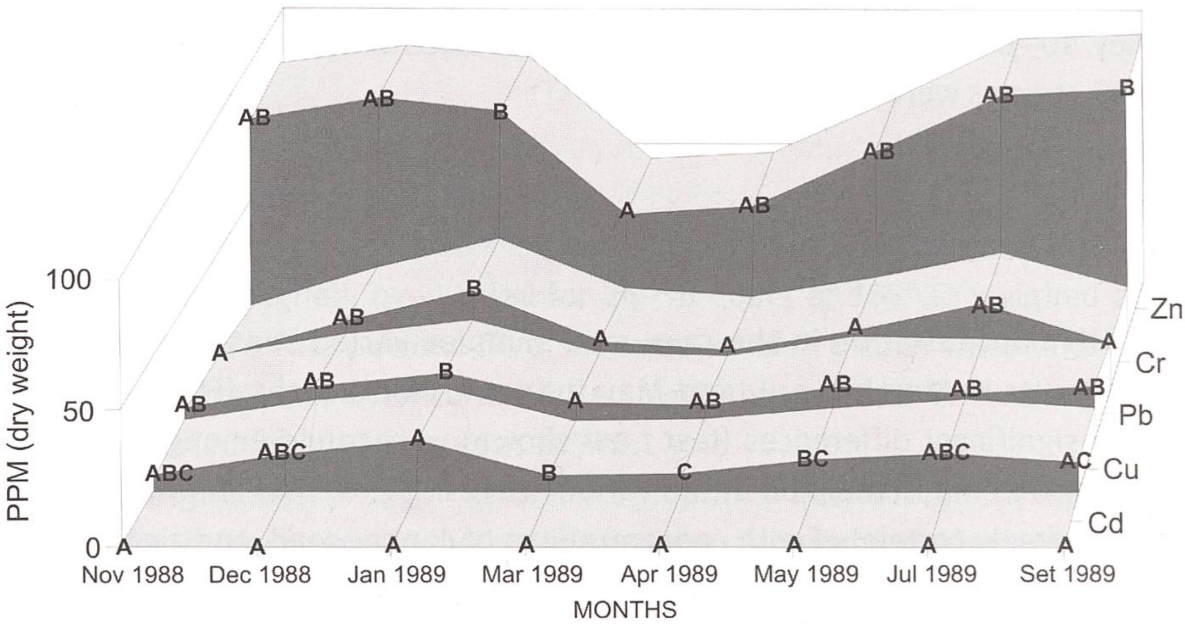

Fig. 3. Cogol dei Siori: heavy metals concentrations in mud. Different letters (A, B, C) indicate statistically significant differences (p<0.05) in the metal concentrations for different samples.

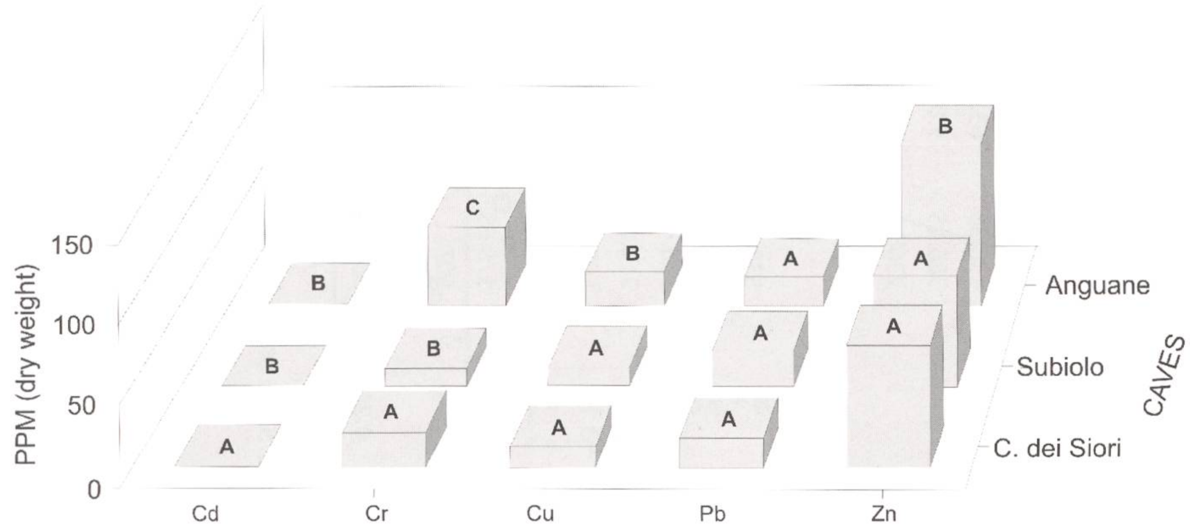

Fig. 4. Mean metal concentrations in the three caves studied: Cogol dei Siori, Ponte Subiolo and Buso delle Anguane. Different letters (A, B, C) indicate statistically significant differences ($p<0.05$).

Water

Metal concentrations in water were very low (Tab. 2), perhaps because of continuous leaching. They were under the Italian limits for drinking water, in agreement with data already published (Tisat 1992; Cipolat 1992).

Invertebrates

Different caves are inhabited by different invertebrates and comparison between cave faunas is not easy. Not all invertebrates were analyzed to detect HM, since sometimes sample size was too small. For instance, troglobitic beetles such as *Orotrechus* and *Neobathyscia* were scantily found and the sample size did not reach the minimum amount. Perhaps HM levels were too high to allow survival of some species: the heavy guano deposits in some caves seem to be harmful to invertebrates. Where the Zn and Cu levels in bat guano were highest (Buso della Torta, Tab. 1), no troglobitic beetles (like *Neobathyscia* or *Orotrechus*) or any other troglobitic invertebrates were found in appreciable numbers.

Some of the macroinvertebrates seem to bio-concentrate some of the HM (Tabs. 3–7), in some cases at a higher rate than in surface environments. The myriapods, *Typhloiulus tobias* and *Eupolybothrus tridentinus*, concentrated Cu to maximum levels of 2115.9 mg/kg and 1380.7 mg/kg. The isopods, *Androniscus brentanus* and *Spelaeonethes nodulosus*, did so with Cd to maximum

Species	N	Cadmium		Chromium		Copper		Lead		Zinc	
		Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
Cogol dei Siori cave											
Troglophilus cavicola	3	0.267	0.089	0.247	0.197	17.603	10.622	n.d.		616.81	567.674
Antisphodrus schreibersi	5	0.093	0.007	n.d.		49.075	37.945	n.d.		130.35	63.05
Neobathyscia antrorum	209	0.235	0.035	1.165	0.305	7.38	5.16	n.d.		386.45	5.05
Eupolybothrus tridentinus	3	1.111	0.002	n.d		1380.67	206.3	n.d.		2368	489.7
Androniscus brentanus	144	12.337	2.567	5.18	1.03	54.55	15.46	n.d.		252.5	44.89
Grotta del Subiolo cave											
Triphleba antricola	46	0.32	0.089	n.d.		15.8	6.89	n.d.		533.7	36.9
Orotrechus targionii	17	1.042	0.345	n.d.		56.67	13.9	n.d.		655.2	57.345
Androniscus brentanus	92	11.376	4.676	0.69	0.099	57.21	10.234	n.d.		156.4	30.678
Spelaeonethes nodulosus	21	8.262	3.45	1.124	0.546	70.08	9.238	n.d.		214.8	84.98
Typhloiulus tobias	29	0.3	0.09	0.884	0.458	1328.5	430.6	1.5	2.13	102.12	15.24
Buso delle Anguane cave											
Troglophilus cavicola	3	0.524	0.384	0.563	0.796	23.873	8.833	n.d.		178.63	22.016
Meta menardi	5	1.272	0.232	n.d.		117.285	12.785	n.d.		507	83.95
Neobathyscia antrorum	176	0.209	0.089	1.9	0.3	47.93	9.67	n.d.		302.4	77.698
Typhloiulus tobias	23	0.397	0.35	5.05	4.27	898.874	607.05	0.67	0.25	83.85	41.224

Tab. 3. Heavy metal concentrations (ppm) in cave dwelling invertebrates. N: Number of invertebrates analysed. n.d.: not determined. S. D.: Standard Deviation.

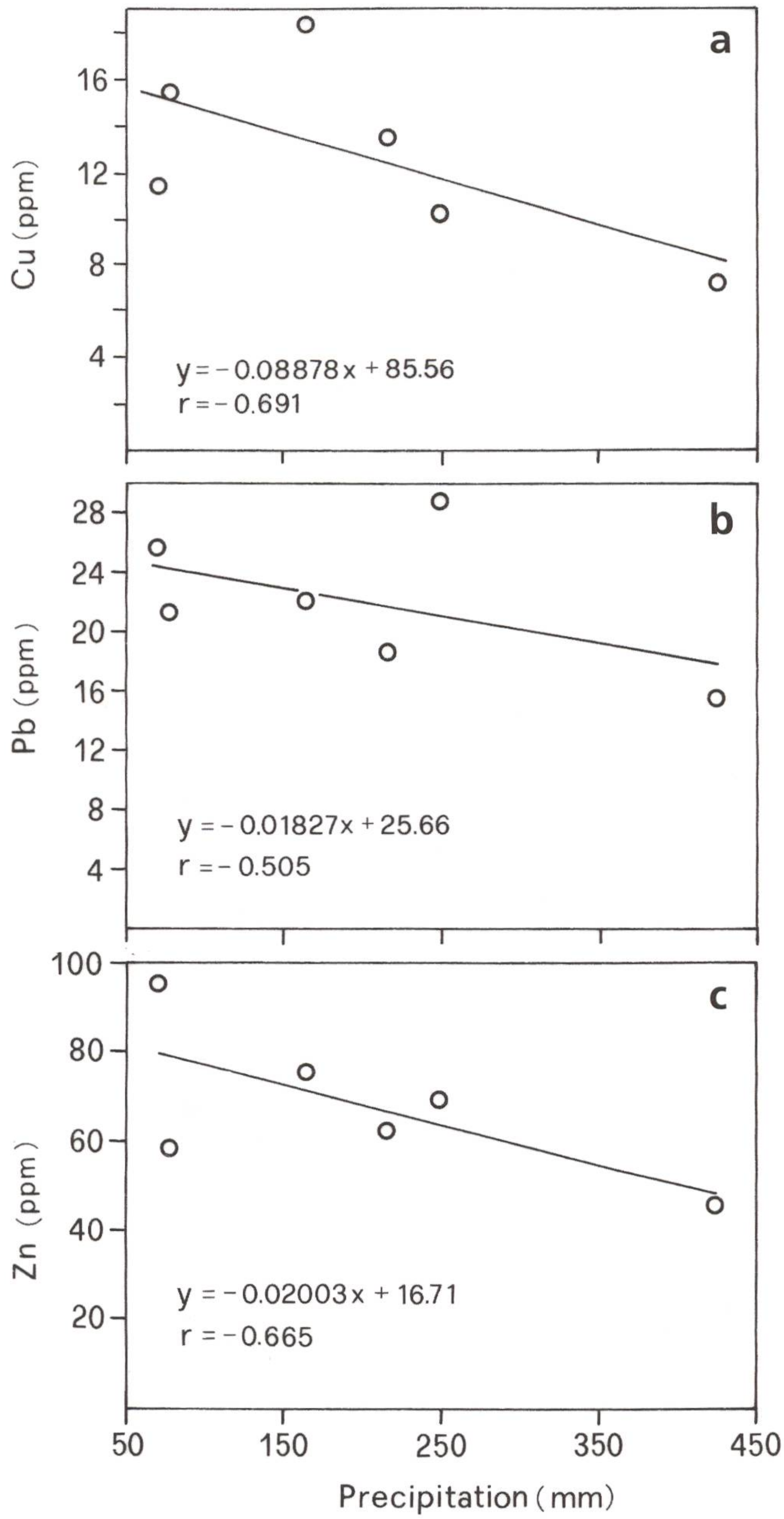

Fig. 5a–c. Regression lines obtained by fitting the metal (Cu, Pb, or Zn respectively) concentration of mud in the Cogol dei Siori cave and the average precipitation during thirty days before sampling.

Species	Site description	Zn (mg/kg)	Reference
Isopoda			
Porcellio scaber	Not contaminated	0-350	1
Porcellio scaber	Low contamination	350-450	1
Porcellio scaber	Modest contamination	450-550	1
Porcellio scaber	High contamination	550-1000	1
Porcellio scaber	Very high contamination	>1000	1
Oniscus asellus	Not contaminated	0-150	1
Oniscus asellus	Low contamination	150-250	1
Oniscus asellus	Modest contamination	250-350	1
Oniscus asellus	High contamination	350-500	1
Oniscus asellus	Very high contamination	>500	1
Trachelipus rathkei	Cultivated field	252.32	2
Chaetophiloscia sicula	Cultivated field	370	2
Androniscus noduliger	Cave	176.89	3
Androniscus brentanus	Cave	204.45	4
Spelaeonethes nodulosus	Cave	150.5	3
Spelaeonethes nodulosus	Cave	257.2	3
Spelaeonethes nodulosus	Cave	204.45	4
Diplopoda			
Several species	Alfalfa fields	321	5
Several species	Cultivated fields	335.2	2
Serradium hirsutipes	Cave	575.99	3
Typhloiulus tobias	Cave	96.9	4
Orthoptera			
Several species		200	6
Troglophilus cavicola	Cave	119.2	3
Troglophilus cavicola	Cave	148.37	3
Troglophilus cavicola	Cave	193.55	4

Tab. 4. Zn (µg/g dry weight) in macroinvertebrates from different sites.
References: 1: Hopkin & al. (1986); 2: Paoletti & al. (1988); 3: Cipolat (1992); 4: Faccio (1989); 5: Carter (1983); 6: Hopkin & Martin (1984).

levels of 12.3 mg/kg and 18.3 mg/kg. Some beetles such *Neobathyscia* and *Orotrechus* also concentrated zinc.

HM concentrations were apparently not related to the foodchain level that theses organisms belong to (Fig. 6). There was no accumulation along the actual food chain which we tentatively construe: detritivores and saprophages: *Typhloiulus tobias, Troglophilus cavicola, Triphleba antricola, Androniscus brentanus, Spelaeonethes nodulosus,* and *Neobathyscia antrorum*; predators: *Orotrechus targionii, Antisphodrus schreibersi, Meta menardi,* and *Eupolybothrus tridentinus.*

Species	Site	Cd (mg/kg)	Reference
Isopoda			
Porcellio scaber	Not contaminated	1.7	1
Porcellio scaber	Not contaminated	1.1	2
Porcellio scaber	Mean contamination	70	1
Porcellio scaber	Mean contamination	26	2
Porcellio scaber	contaminated	80.09	3
Porcellio scaber	contaminated	146	2
Armadillidium sp.	non contaminated	1.4	1
Ligidium sp.	non contaminated forest	5.5	2
Several species	non contaminated	1.1	4
Several species	contaminated	232	4
Several species	1 Km by a Cu factory	130	5
Severalspecies	Cu factory	231	5
Androniscus noduliger	cave	15.61	6
Androniscus brentanus	cave	11.86	7
Spelaeonethes nodulosus	cave	14.93	6
Spelaeonethes nodulosus	cave	22.73	6
Spelaeonethes nodulosus	cave	8.29	7
Diplopoda			
Several species	red clover field	0.2	8
Several species	1 Km by a Cu factory	14.2	5
Several species	Cu factory	18.9	5
Serradium hirsutipes	cave	6.59	6
Typhloiulus tobias	cave	0.33	7
Orthoptera			
Several species	1 Km by a Cu factory	0,32	5
Several species	Cu factory	1,96	5
Troglophilus cavicola	cave	0,69	6
Troglophilus cavicola	cave	1,36	6
Troglophilus cavicola	cave	0,39	7

Tab. 5. Cd (μg/g dry weight) in macroinvertebrates from different sites.
References: 1: Hopkin & al. (1986); 2: Dallinger & Berger (1992); 3: Dallinger & Prosi (1988); 4: Martin & Coughtrey (1982); 5: Hopkin (1989); 6: this paper; 7: Celi (1989); 8: Carter (1983).

Our data show that HM levels in cave dwelling invertebrates are linked mainly to the environment in which they live. For instance, we found a significant difference (t-test) between the Cr levels of *T. tobias* collected either in the Grotta del Subiolo or in the Buso delle Anguane. In the latter cave, *T. tobias* had higher Cr level, perhaps in relation with the mud Cr level, as pointed out previously (Tab. 3).

Species	Site situation	HM concentrations (mg/kg) Cu	Cr	Pb	Reference
Isopoda					
Porcellio scaber	non contaminated			11.9	1
Porcellio scaber	contaminated			25.9	1
Porcellio scaber	non contaminated			0.7	2
Porcellio scaber	non contaminated			4.6	2
Porcellio scaber	moderate contamination			142	2
Porcellio scaber	highly contaminated			106	2
Porcellio scaber	highly contaminated			328	2
Porcellio scaber	non contaminated	198		7	3
Porcellio scaber	highly contaminated	1205.6		1523.7	4
Trachelipus rathkei	contaminated (cultivated field)	533.7		4.1	5
Chaetophiloscia sicula	contaminated (cultivated field)	453.9		5.1	5
Several species	non contaminated	77.8		1.5	15
Several species	moderate contamination	838			15
Several species	highly contaminated	2390		1190	15
Oniscus asellus	non contaminated	115		4.15	6
Oniscus asellus	contaminated	567		413	6
Oniscus asellus	contaminated	196		37.8	6
Porcellio scaber	contaminated	243		18.8	6
Androniscus noduliger	Faller cave	87.8	70.13	2.53	8
Androniscus brentanus	non contaminated cave	56.03	2.94		8
Spelaeonethes nodulosus	non contaminated cave	70.08	1.12		9
Spelaeonethes nodulosus	Faller cave	91.53	31.29	4.48	9
Spelaeonethes nodulosus	Quero cave	86.51	20.19	3.09	9
Diplopoda					
Several species	non contaminated	221			7
Brachyiulus lusitanus	contaminated cultivated field	853.58		3.1	5
Brachydesmus proximus	contaminated cultivated field	442.5		4.8	5
Several species	non contaminated	138			6
Several species	moderately contaminated	511			6
Several species	highly contaminated	780			6
Typhloiulus tobias	non contaminated cave	1221.1	1.9	1.49	8
Serradium hirsutipes	Quero cave	3939.2	52.11	4.24	9
Orthoptera					
Several species	non contaminated	37.5			6
Several species	moderately contaminated	56.4			6
Several species	highly contaminated	333			6
Troglophilus cavicola	non contaminated	20.74	0.84		8
Troglophilus cavicola	Faller cave	22.02	2.85	0.43	9
Troglophilus cavicola	Quero cave	26.72	7.75	1.18	9
Concentration in soils:	mean soil concentrations	4–150		<50	10, 11
	mean cultivated soils	56.36		19.46	5
	non contaminated	13.3		4.6	6
	rather contaminated	494			6
	highly contaminated	9270			6
	highly contaminated by Pb	20		1314	12
	rather contaminated	252		629	12
	copper contaminated	335		42	12
	non contaminated	15.16	27.62		8
	non contaminated			19.21	16
	1 Km by the pollutant source	4160			13
		1200			13
		525			13
	non contaminated	3.1		41	14
	rather disturbed area	30		361	14
	Faller cave	56.7	116.12	31.06	9
	Fogola di Toni Mondin cave	54.19	129.01	30.4	9

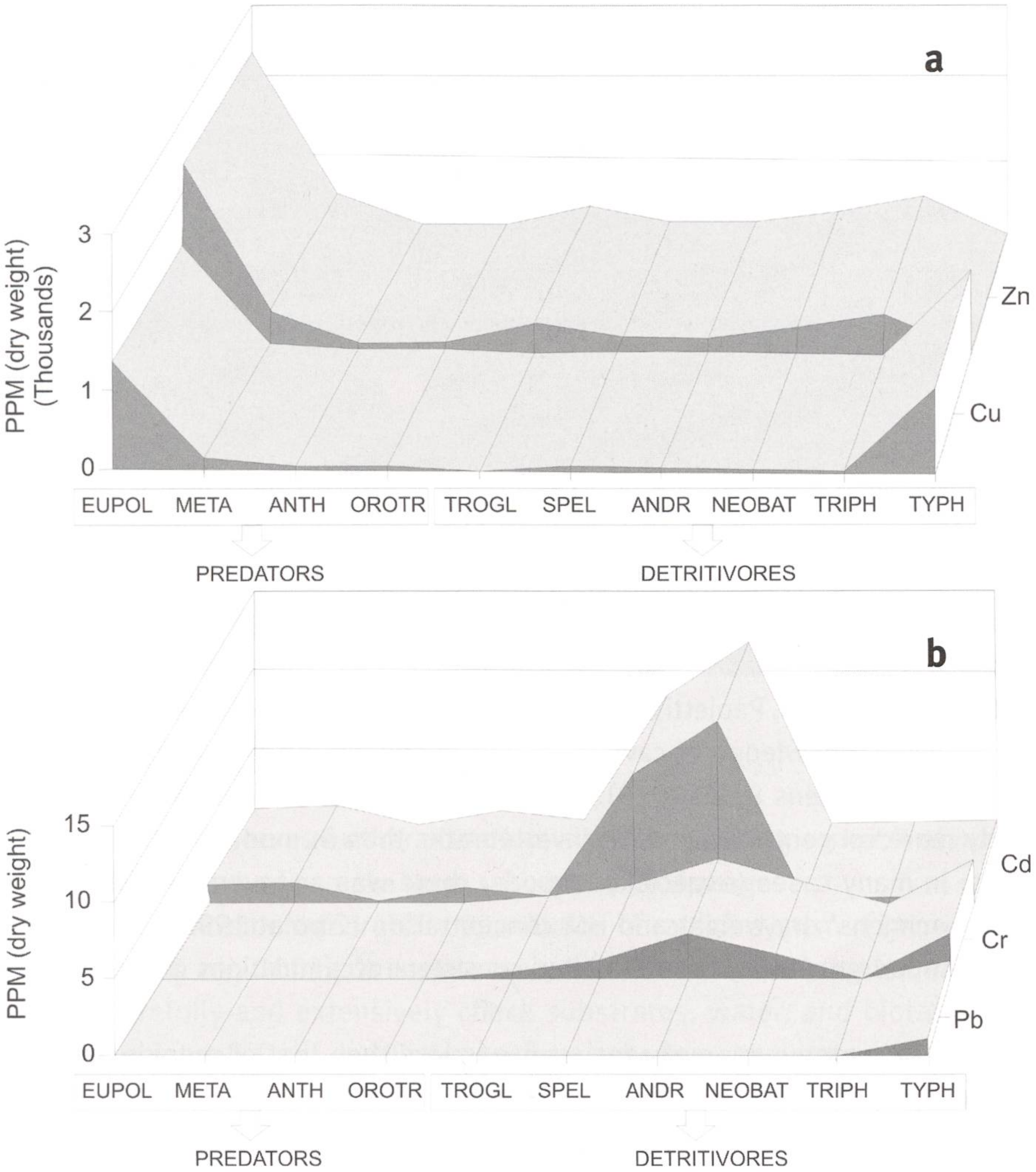

Fig. 6. Concentration of heavy metals (– a: Zn, Cu; – b: Cd, Cr, Pb) in some troglobitic macroinvertebrates (predators and detritivores). Typh.: *Typhloiulus tobias* (Diplopoda); Trogl.: *Troglophilus cavicola* (Orthoptera); Triph.: *Triphleba antricola* (Diptera), Andr.: *Androniscus brentanus* (Isopoda); Spel.: *Spelaeonethes nodulosus* (Isopoda); Orotr.: *Orotrechus targionii* (Coleoptera); Neobat.: *Neobathyscia antrorum* (Diptera); Meta: *Meta menardi* (Araneae); Anth.: *Antisphodrus schreibersi* (Coleoptera); Eupol.: *Eupolybothrus tridentinus* (Chilopoda)

Tab. 6. Heavy metal concentrations (Cu, Cr, Pb) in macroinvertebrates and in soils.
References: 1: Dallinger & al. (1992); 2: Dallinger & Berger (1992); 3: Prosi & Dallinger (1988); 4: Dallinger & Prosi (1988); 5: Paoletti & al. (1988); 6: Hopkin (1989); 7: Carter (1983); 8: Celi (1989); 9: this paper; 10: Williamson & Evans (1973); 11: Streit & Jaggy (1982); 12: Ireland (1979); 13: Hopkin & al. (1986); 14: Joosse & Verhoef (1987); 15: Martin & Coughtrey (1982); 16: Faccio (1989).

Species	Fogola de Toni Mondin	Grottina Faller	Fogola de Toni Mondin	Grottina Faller
	Zn	Zn	Cd	Cd
Serradium hirsutipes	4.727	-	11.9	-
Androniscus noduliger	-	1.481	-	2497
Spelaeonethes nodulosus	1.235	2.155	27	3637
Troglophilus cavicola	1.218	0.989	2.5	111

Tab. 7. Zn and Cd concentration factor in some troglobitic invertebrates (dry weight of invertebrates as compared to soil/mud).

Conclusion

HM bio-accumulation in cave dwelling invertebrates did not depend on their place in the foodweb (detritivores, saprophages, and predators), in agreement with previous studies on terrestrial invertebrates (Van Straalen & Van Wensen 1986, Janssen 1988, Paoletti & al. 1988, Van Straalen & al. 1989, Hopkin 1989).

However, knowledge of cave foodwebs is still limited or rudimentary (Turquin 1980; Wilkens & al., 2000). We have shown that several HM are consistently more concentrated in cave invertebrates than in mud or in the outside soils; in many cases (especially isopods) there was an inverse relation with the specimens' dry weight and HM concentration (Cipolat 1992, Tisat 1992). Both isopods and millipedes exhibited consistent accumulations of several HM (Tab. 6).

The Pb contamination of cave mud was less than that of outside soils, in agreement with findings in some Croatian caves (Miko & al., 2002, 2003). Other metals such as Cd seem to be at a rather similar level outside and inside caves and in some cases it can be concentrated in the bodies of macroinvertebrates (Tab. 7).

The high HM concentrations in myriapods (*Typhloiulus tobias*, *Serradium* sp.), and isopods (*Androniscus brentanus,* and *Spelaeonethes nodulosus*) collected in the Brenta caves, and in *S. nodulosus* and *Androniscus noduliger* in the two caves in Piave-Cismon valleys (Fogola Toni Mondin and Grotticella Faller), suggests that these macro-invertebrates can be proposed as cave system bioindicators.

Concentrations of Cr in *Typhloiulus tobias* in two different caves is related to the level of cave pollution, this species may therefore be used as a HM bio-indicator in the cave system. Troglobitic coleoptera (*Neobathyscia* and *Orotrechus*) seem to be useful as Zn bio-indicatiors, even if not fully examined in all caves. Bat guano is apparently a sink of HM and other pollutants (Boccelli &

Fig. 7. Large bat guano deposits in Grotta della Guerra cave.

al., 1992) and deserves further attention, since the scavenging habit of many troglobitic invertebrates make them susceptible to damage.

Given the extreme vulnerability to pollution of carsick systems (Sauro 1987, 1989; Miko & al., 2003), it is necessary to develop surveying techniques able to carefully and extensively check substrates, water, and biota of the cave environment. Cave dwelling macroinvertebrates can be used as bioindicators and supply new tools to monitor cave pollution. For example, for the high sodium and chlorium contents in the Bus della Genziana cave (Cansiglio), percolating water can be addressed as the potential pollution effect of salt applied on roads during winter to control ice formation. The absence of *Cansiliella* in this cave, a classical place of collection, can be associated to this pollution source (Paoletti & al., 2009)

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Addresses of the authors:

Maurizio G. Paoletti, Monica Celi, Chiara Cipolat, Laura Tisat, Annamaria Faccio
Department of Biology
University of Padova
I–35100 Padova, Italy

Attilio A. M. Del Re, Raffaella Boccelli
Faculty of Agriculture
Università Cattolica
I–29100 Piacenza, Italy