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TECHNICAL NOTE

Elemental analysis of pitfall-trapped insect samples: effects of ethylene glycol grades

Mihály Braun[1], Edina Simon[2]*, István Fábián[1] & Béla Tóthmérész[2]

[1]*Department of Inorganic and Analytical Chemistry, University of Debrecen, Egyetem tér 1, Debrecen H-4032, Hungary, and* [2]*Department of Ecology, University of Debrecen, PO Box 71, Debrecen H-4032, Hungary*

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Introduction

Insects are abundant in terrestrial environments and play an important role in metal-transport chains among trophic levels due to their large biomass and diversity (Lindqvist & Block, 1997). As toxic inorganic and organic pollutants are often found in soil, they can be easily accumulated by soil-dwelling insects (Nakamura et al., 2005). Pitfall trapping using ethylene glycol as a killing and preserving fluid is the most widespread method for sampling soil insects (Southwood & Henderson, 2000; Magura et al., 2010; Purchart et al., 2010). In an earlier study, Jud & Schmidt-Entling (2008) reported that the vapour pressure of ethylene glycol was lower (0.0053 hPa) than other trapping fluids such as formalin and propylene glycol, making this liquid resistant to evaporation (Jud & Schmidt-Entling, 2008). There is no difference in capture efficiency between traps filled with diluted or undiluted ethylene glycol (Topping & Luff, 1995). As water has the highest vapour pressure (23.4 hPa) (Jud & Schmidt-Entling, 2008), evaporation of diluted ethylene glycol may result in differences in fluid concentrations. Therefore, undiluted ethylene glycol may be preferred as a trapping fluid under dry field conditions (Jud & Schmidt-Entling, 2008). The trapping fluid used may affect the capture efficiency, the condition of trapped insects, and the suitability of samples for further investigation (Sasakawa, 2007). However, the effect of trapping fluids on the concentration of elements in the insects has rarely been examined (Hendrickx et al., 2003; Braun et al., 2009).

In this study, the effects of four grades of ethylene glycol (analytical, puriss, technical grade, and common antifreeze) were compared. The aim of our work was to explore whether the condition of insect samples remains suitable for further elemental analysis after trapping with undiluted ethylene glycol. Trappings were modelled by soaking the insects in the trapping fluid for 2-week and 1-month periods, respectively.

*Correspondence: Edina Simon, Department of Ecology, University of Debrecen, PO Box 71, Debrecen H-4032, Hungary. E-mail: edina.simon@gmail.com

Materials and methods

Firebugs

Adult firebugs [*Pyrrhocoris apterus* (L.) (Hemiptera: Pyrrhocoridae)] were collected during August 2008 on the campus of the University of Debrecen, Hungary. Four hundred and fifty specimens were collected by hand. Live insects were put into polyethylene test tubes (10 specimens per tube). The firebugs were killed by freezing at −18 °C in the laboratory. Untreated wet body mass of the groups of 10 specimens was determined immediately after freezing, before treatment. Wet body mass was also measured after treatment. After drying, body mass was measured, whereas estimated dry body mass was determined based on the water content of control samples.

Five tubes were selected randomly for each treatment. Two factors were studied in this experiment: one was the quality of ethylene glycol and the other was the duration of trapping. Each test tube was filled with 10 ml glycol. Control samples were stored in a freezer for 2 weeks.

Elemental analysis

At the end of the treatment period, the contents of each sample tube were placed in a plastic sieve and flushed with 250 ml of double-deionised water obtained from a Millipore Milli-Q system (Millipore Corporation, Billerica, MA, USA). The firebugs were transferred into a 25 ml beaker and their wet body masses were determined immediately. The samples were then dried overnight at 105 °C and reweighed to determine their measured dry masses. The samples were then digested by adding 2 ml 65%

(wt/wt) nitric acid (Scharlau Chemie; Chem-Supply, Adelaide, Australia) to the container and keeping it at 80 °C for 4 h. Digested samples were diluted to 50 ml using 1% (wt/wt) nitric acid.

The elemental composition of the four types of ethylene glycol was analysed before and after the treatments. Five ml portions of analytical, puriss, and technical grade glycols and 1-ml portions of common anti-freeze were dried in containers at 80 °C for 4 h. After drying, glycols were digested with 2 ml 65% nitric acid at 80 °C for 4 h. The digested samples were diluted to 10 ml (analytical, puriss, and technical grades of glycol) or 50 ml (common anti-freeze) with 1% (wt/wt) nitric acid.

Analysis of the elements present was performed using an ICP-OES IRIS Intrepid II XSP (Thermo Electron Corporation, Franklin, MA, USA) equipped with a CETAC 45 000 AT$^+$ ultrasonic nebulizer (Cetac Technologies, Omaha, NE, USA). The optimised measurement procedure was applied for insect analyses (Braun et al., 2009). Two or three atomic or ionic lines were selected to avoid interferences. The following elements were determined: Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Sr, and Zn. Limits of quantification values were: Ba: 0.006, Ca: 0.008, Cd: 0.001, Co: 0.004, Cr: 0.002, Cu: 0.003, K: 0.003, Li: 0.002, Mg: 0.001, Mn: 0.001, Na: 0.002, Ni: 0.004, Pb: 0.013, Sr: 0.002, and Zn: 0.003 mg l^{-1}.

Statistical analysis

Two-way analysis of variance (ANOVA) was performed to detect the differences in the mean value of the body masses and the elemental composition of the specimens, as well as the sulphated ash content of glycol. Differences appearing among the treatments were analysed using Tukey's Honestly Significant Difference test (Everitt & Hothorn, 2009). Homogeneity of variances was examined by Levene's test. Data were ln(x + 1) transformed prior to analyses.

Results and discussion

Body mass

Wet and dry masses of insects are essential in studies related to biomass (Donald & Paterson, 1977; Wetzel et al., 2005). Before treatments, no significant difference was found in untreated wet body masses (Levene's test: $F_{8,36}$ = 2.027, P = 0.071; ANOVA: $F_{8,36}$ = 2.13, P = 0.061). Average untreated wet body mass was 42.9 ± 2 mg in the control samples.

After treatments, wet body masses did not differ significantly (Levene's test: $F_{7,32}$ = 1.083, P = 0.59) and the effect of glycol quality showed no significance either ($F_{7,32}$ = 0.673, P = 0.58). The duration of trapping had a significant effect on wet body masses ($F_{7,32}$ = 4.404, P = 0.044). Wet body masses were larger after 1 month than after 2 weeks of treatment. Interaction between the two factors (glycol quality and trapping duration) was not significant ($F_{7,32}$ = 0.385, P = 0.76). The glycol caused 43.8% increase in wet body mass after 2 weeks and 52.2% increase after 1 month of treatment compared with the control.

The measured dry mass of the control was 13.3 ± 1.7 mg (mean ± SE). Due to the effects of treatments, measured dry body mass increased significantly but the effect of trapping duration was not significant ($F_{8,36}$ = 1.153, P = 0.29). Ethylene glycol quality had a significant effect on measured dry body mass ($F_{8,36}$ = 3.805, P = 0.019). Interaction between the two factors was not significant ($F_{8,36}$ = 0.265, P = 0.85). Analytical grade (28.2 ± 3.7 mg), puriss (30.0 ± 3.1 mg), and technical grade (29.1 ± 3.1 mg) glycols had similar effects on measured dry body mass: their pooled average was 29.1 ± 1.7 mg. In the case of common anti-freeze, the effect on dry mass was lower (23.5 ± 3.1 mg) than with other ethylene glycols, the increase of dry mass being 39.1%. This may have been caused by the diffusion of glycol into the insect body, resulting in incomplete evaporation of the fluid. Heat-induced polymerisation of ethylene glycol may cause a significant increase in dry weight (Viciosa et al., 2008). This effect was detectable in all treatments, although to a lesser degree in the case of common anti-freeze.

Prior to treatments, the untreated wet body mass was measured for all collected specimens. After treatments, the dry body mass increased significantly. Then, the water content (60.5%) of control samples was determined. Unaffected dry body masses of treated samples were estimated based on the water content of control samples. Elemental concentrations were reported per mg untreated wet body masses, as well as per measured and estimated dry body masses.

In our earlier study, the increase in wet mass between the control and treated samples was 26 and 37% when 75% (vol/vol) diluted common anti-freeze was used (Braun et al., 2009). Donald & Paterson (1977) used 10% formalin and 70% ethanol, which caused a 32–47% decrease in wet mass. In another study, similar results were found with losses in wet and dry body masses varying between 13.1 and 36.7% (wet), and between 16.2 and 46.8% (dry) using ethanol (Leuven et al., 1985).

Dry weight is generally considered as a reference for elemental analyses (Elinder et al., 1994). Wet body masses can be measured directly only in the case of hand-collected animals. We found that wet body mass of trapped insects

Table 1 Summary statistics (mean ± SE) of element concentrations (mg kg^{-1}) in firebugs in the control, 2-week, and 1-month treatments

Ethylene glycol grade	Treatment	Reference base	Ba	Ca	Cu	Fe	K	Mg	Mn	Na	Sr	Zn
	Control	A	1.8 ± 0.1	746 ± 39	9.3 ± 0.2	98 ± 8	875 ± 72	508 ± 26	19.8 ± 1.2	266 ± 16	4.5 ± 0.2	70 ± 3
		B	4.6 ± 0.3	1879 ± 57	23.5 ± 0.5	246 ± 14	2207 ± 166	1282 ± 57	50.1 ± 3.2	671 ± 38	11.5 ± 0.4	177 ± 8
Analytical	2 weeks	A	1.3 ± 0.1	567 ± 46	7.4 ± 0.2	60 ± 7	684 ± 42	435 ± 16	15.2 ± 1.5	200 ± 9	3.9 ± 0.2	55 ± 3
		B	2.0 ± 0.2	887 ± 87	11.5 ± 0.7	93 ± 10	1070 ± 87	678 ± 40	23.8 ± 3.1	312 ± 20	6.0 ± 0.4	87 ± 8
		C	3.2 ± 0.3	1431 ± 116	18.6 ± 0.5	151 ± 17	1726 ± 107	1097 ± 40	38.2 ± 3.8	504 ± 23	9.7 ± 0.5	139 ± 8
	1 month	A	1.8 ± 0.4	746 ± 69	7.8 ± 0.3	88 ± 28	649 ± 33	461 ± 26	17.8 ± 2.0	180 ± 9	4.4 ± 0.3	62 ± 5
		B	2.6 ± 0.5	943 ± 108	11.7 ± 1.2	126 ± 36	964 ± 100	679 ± 52	26.1 ± 2.8	267 ± 26	6.5 ± 0.5	91 ± 10
		C	4.4 ± 0.9	1620 ± 173	19.8 ± 0.7	222 ± 70	1636 ± 84	1163 ± 67	44.9 ± 5.2	454 ± 23	11.1 ± 0.7	155 ± 13
Puriss	2 weeks	A	1.2 ± 0.1	530 ± 38	10.8 ± 2.7	51 ± 2	653 ± 35	421 ± 12	15.1 ± 0.4	190 ± 8	3.8 ± 0.2	54 ± 3
		B	1.9 ± 0.1	862 ± 87	17.2 ± 3.8	82 ± 4	1048 ± 48	679 ± 31	24.3 ± 1.2	305 ± 12	6.2 ± 0.5	87 ± 5
		C	3.0 ± 0.1	1338 ± 97	27.3 ± 6.8	128 ± 5	1646 ± 88	1062 ± 30	38.0 ± 1.0	479 ± 20	9.7 ± 0.4	136 ± 9
	1 month	A	1.4 ± 0.1	609 ± 26	7.2 ± 0.4	57 ± 5	574 ± 42	414 ± 20	15.8 ± 0.9	174 ± 8	4.0 ± 0.1	57 ± 5
		B	2.2 ± 0.2	923 ± 68	11.0 ± 0.8	86 ± 9	867 ± 71	626 ± 47	24.0 ± 2.1	263 ± 18	6.1 ± 0.4	85 ± 7
		C	3.6 ± 0.2	1537 ± 67	18.3 ± 1.0	143 ± 12	1449 ± 106	1043 ± 51	39.8 ± 2.4	439 ± 21	10.2 ± 0.2	143 ± 12
Technical	2 weeks	A	1.4 ± 0.1	541 ± 43	7.4 ± 0.2	69 ± 19	580 ± 38	388 ± 11	15.4 ± 0.6	186 ± 6	4.1 ± 0.3	49 ± 2
		B	2.4 ± 0.2	953 ± 86	13.0 ± 0.7	119 ± 29	1018 ± 68	682 ± 34	27.0 ± 1.4	327 ± 18	7.1 ± 0.4	86 ± 5
		C	3.4 ± 0.4	1364 ± 108	18.6 ± 0.6	174 ± 47	1462 ± 96	977 ± 27	38.8 ± 1.6	469 ± 16	10.3 ± 0.7	123 ± 6
	1 month	A	1.4 ± 0.1	605 ± 20	7.5 ± 0.3	56 ± 4	593 ± 41	416 ± 15	14.4 ± 0.5	181 ± 8	4.1 ± 0.2	52 ± 2
		B	2.0 ± 0.1	878 ± 47	10.9 ± 0.6	81 ± 8	858 ± 61	604 ± 32	21.0 ± 1.2	262 ± 14	5.9 ± 0.4	75 ± 4
		C	3.5 ± 0.2	1526 ± 51	19.0 ± 0.8	141 ± 11	1495 ± 102	1049 ± 38	36.4 ± 1.2	456 ± 20	10.3 ± 0.4	130 ± 5
Common anti-freeze	2 weeks	A	1.3 ± 0.1	626 ± 13	7.6 ± 0.1	60 ± 3	694 ± 57	463 ± 23	15.8 ± 1.2	149 ± 27	4.0 ± 0.1	58 ± 5
		B	2.4 ± 0.2	1163 ± 25	14.2 ± 0.2	111 ± 5	1284 ± 89	857 ± 33	29.2 ± 2.1	279 ± 54	7.4 ± 0.1	107 ± 9
		C	3.3 ± 0.3	1580 ± 33	19.2 ± 0.4	150 ± 8	1751 ± 143	1167 ± 59	39.8 ± 3.0	376 ± 67	10.0 ± 0.3	146 ± 14
	1 month	A	1.8 ± 0.3	718 ± 118	7.6 ± 0.6	82 ± 19	588 ± 66	481 ± 79	16.8 ± 1.7	168 ± 22	4.7 ± 0.5	61 ± 7
		B	2.8 ± 0.3	1128 ± 105	12.3 ± 1.1	127 ± 19	935 ± 56	756 ± 71	26.7 ± 1.3	275 ± 26	7.5 ± 0.3	97 ± 6
		C	4.6 ± 0.9	1811 ± 297	19.2 ± 1.5	207 ± 47	1482 ± 166	1214 ± 200	42.3 ± 4.3	441 ± 53	12.0 ± 1.3	154 ± 18

Reference base: element concentration reported (A) per mg untreated body mass before treatment, (B) per mg measured dry body mass, and (C) per mg estimated dry body mass.

were significantly different from untreated wet body mass. This was caused by the trapping fluid.

Effect of trapping fluid on elemental concentration

Elemental concentrations in biological samples are usually reported per mg wet or dry mass or in mg element per specimen (Elinder et al., 1994). We found remarkable changes in both wet and dry body masses during our experiment. This was caused by treatment with different grades of glycol. Effects on elemental concentrations were seen in mg kg^{-1} based on the untreated wet body mass as well as on measured and estimated dry body masses, as in the study by Hendrickx et al. (2003). Concentrations of cadmium (<0.4 mg kg^{-1}), cobalt (<1.5 mg kg^{-1}), lithium (<0.8 mg kg^{-1}), nickel (<1.5 mg kg^{-1}), and lead (<5.0 mg kg^{-1}) in the firebugs were below the quantification limit of the procedure (Braun et al., 2009). Descriptive statistics of the concentrations of the elements are given in Table 1.

Elemental concentrations of the specimens were compared using two-way ANOVA. Significant differences were found in the concentrations of potassium and sodium based on live body mass ($F_{8,36}$ = 3.25 and 3.86, respectively, both $P<0.01$) and estimated dry body masses; in the latter case, the concentration of potassium and sodium differed significantly ($F_{8,36}$ = 3.35 and 3.85, respectively, both $P<0.01$). Potassium concentration in the control samples differed significantly in the 2-week technical, 1-month puriss, 1-month technical, and 1-month common anti-freeze treated samples when the concentrations were calculated based on the live body mass and estimated dry body masses ($F_{8,36}$ = 3.25 and 3.35, respectively, both $P<0.05$). Concentrations of sodium based on untreated wet body mass and estimated dry body masses respectively, were significantly lower than the control in 2-week and 1-month common anti-freeze and 1-month puriss treatments ($F_{8,36}$ = 3.86 and 3.85, respectively, both $P<0.05$). When the elemental concentrations were based on measured dry body masses (which were increased due to the polymerisation of glycol), significant differences compared with control samples were found for each element (Table 2).

When the reference base was the untreated wet body mass or the estimated dry body mass, composition in the 2-week or in the 1-month treatments was not significantly different for any of the elements, except for K and Na (Table 2). Our results show that trapping with ethylene glycol caused differences between the control and treated samples. When glycols were used, a significant decrease in elemental concentration was observed in the insect material. This effect was caused by the increase in body mass. Therefore, body mass of live insects should be used in all calculations (Table 2). Comparing hand- and pitfall-collected isopods and carabids, a similar difference was reported by Zödl & Wittmann (2003).

Common anti-freeze contained relatively high levels of impurities (Ca, Fe, K, and Na). Consequently, using puriss or technical grade glycols is recommended as a trapping fluid for insect collection. Using puriss grade was cost-effective and its quality seemed to be appropriate. We found that potassium and sodium leached from insect tissues. Metabolites of ethylene glycol may have been responsible for the membrane damage (Guo & McMartin, 2005). They may also have contributed to the osmolarity gap in the plasma membrane (Leth & Gregersen, 2005) allowing some elements to flow into the cell, whereas others (for example potassium and sodium) may flow out. Our results also indicated that the undi-

Table 2 Outcome of two-way analysis of variance in elemental concentrations in *Pyrrhocoris apterus*, based on untreated wet body mass, measured dry body mass, and estimated dry body mass

	Untreated wet body mass		Measured dry body mass		Estimated dry body mass	
Element	$F_{8,36}$	P	$F_{8,36}$	P	$F_{8,36}$	P
Ba	1.89	0.093	8.27	<0.001	1.98	0.078
Ca	1.95	0.082	9.21	<0.001	2.02	0.072
Cu	2.04	0.082	8.17	<0.001	2.07	0.066
Fe	2.08	0.064	7.00	<0.001	2.13	0.058
K	3.25	0.007	14.81	<0.001	3.35	0.006
Mg	1.66	0.14	14.32	<0.001	1.72	0.13
Mn	1.69	0.14	9.99	<0.001	1.68	0.14
Na	3.86	0.002	10.41	<0.001	3.85	0.002
Sr	1.56	0.17	13.18	<0.001	1.67	0.14
Zn	2.10	0.061	11.55	<0.001	2.08	0.064

Table 3 Mean (± SE) concentrations (mg l^{-1}) of elements in different grades of ethylene glycol before and after 2-week and 1-month treatments

Trapping fluid	Duration	Ca	Fe	K	Mg	Mn	Na	Zn
Analytical	Initial	0.45 ± 0.09a	0.19 ± 0.06	0.12 ± 0.06a	0.06 ± 0.01a	0.003 ± 0.001	0.7 ± 0.1a	0.31 ± 0.03
	2 weeks	1.37 ± 0.29ab	0.15 ± 0.02	8.49 ± 1.32b	0.95 ± 0.28b	0.009 ± 0.001	2.9 ± 0.4b	0.31 ± 0.03
	1 month	2.36 ± 0.36b	0.28 ± 0.03	13.44 ± 1.33c	1.80 ± 0.21b	0.015 ± 0.002	4.0 ± 0.2b	0.37 ± 0.03
Puriss	Initial	0.32 ± 0.02a	0.06 ± 0.01	0.16 ± 0.03a	0.07 ± 0.01a	0.001 ± 0.001	0.5 ± 0.03a	0.05 ± 0.002
	2 weeks	1.06 ± 0.08ab	0.16 ± 0.04	9.01 ± 1.26b	0.75 ± 0.06b	0.008 ± 0.001	2.8 ± 0.4b	0.16 ± 0.05
	1 month	1.69 ± 0.23b	0.15 ± 0.03	12.93 ± 1.06b	1.36 ± 0.15b	0.009 ± 0.001	3.9 ± 0.2b	0.20 ± 0.02
Technical	Initial	0.59 ± 0.20	0.06 ± 0.01	0.24 ± 0.06a	0.17 ± 0.01	0.006 ± 0.003	0.09 ± 0.1a	0.04 ± 0.02
	2 weeks	1.15 ± 0.14	0.49 ± 0.20	10.25 ± 1.11b	0.93 ± 0.17	0.014 ± 0.002	3.6 ± 0.4b	0.10 ± 0.01
	1 month	1.12 ± 0.09	0.29 ± 0.04	11.28 ± 0.96b	1.16 ± 0.22	0.012 ± 0.001	3.7 ± 0.2b	0.13 ± 0.01
Common anti-freeze	Initial	15.81 ± 1.32	1.33 ± 0.17	197 ± 6	3.02 ± 0.09a	0.024 ± 0.002	717 ± 43	1.18 ± 0.18
	2 weeks	9.99 ± 0.94	1.90 ± 0.86	190 ± 14	1.43 ± 0.23b	0.027 ± 0.010	691 ± 35	1.05 ± 0.13
	1 month	16.92 ± 4.02	1.99 ± 0.60	232 ± 7	4.62 ± 1.67a	0.038 ± 0.006	740 ± 8	1.56 ± 0.35

Means within a trapping fluid followed by different letters are significantly different (ANOVA: $P<0.05$).

luted trapping fluids caused an increase in body mass (Braun et al., 2009). In contrast with formaldehyde, ethylene glycol increased the mass, and consequently caused a decrease in elemental concentration in treated insect samples (van Straalen et al., 2001).

Grades of glycol

We used the sulphate ash content to characterise trapping fluids. Total amount of inorganic materials leached out of insect bodies were estimated by means of sulphate ash content, and calculated based on the measured elemental contents (Table 3). In the case of analytical, puriss, and technical grade ethylene glycols, the sulphated ash content was not significantly different ($F_{11,40} = 1.881$, $P = 0.23$). Its pooled average was 5.6 ± 1.4 mg l^{-1}, which was consistent with the certificates (<50 mg l^{-1}). The sulphated ash content of common anti-freeze was high (2 730 ± 602 mg l^{-1}), but there was no difference between 2-week and 1-month treatments ($F_{11,40} = 2.638$, $P = 0.12$).

The effect of glycol grade was tested by two-way ANOVA. Factors compared were the quality of ethylene glycols and duration of treatments. Variances did not differ significantly according to Levene's test ($F_{8,30} = 2.431$, $P = 0.069$). The effect of glycol quality was not significant ($F_{11,40} = 0.462$, $P = 0.63$). Duration of treatments had a significant effect on sulphated ash content ($F_{2,49} = 84.885$, $P<0.001$). Sulphated ash in trapping fluids increased to 40.3 ± 6.1 mg l^{-1} during 2-week treatment, and to 54.9 ± 5.5 mg l^{-1} during 1-month treatment (Figure 1).

We studied the effects of four grades of undiluted ethylene glycol (analytical, puriss, technical, and common anti-freeze) on body mass and the elemental composition of firebugs as a model organism of surface dwelling

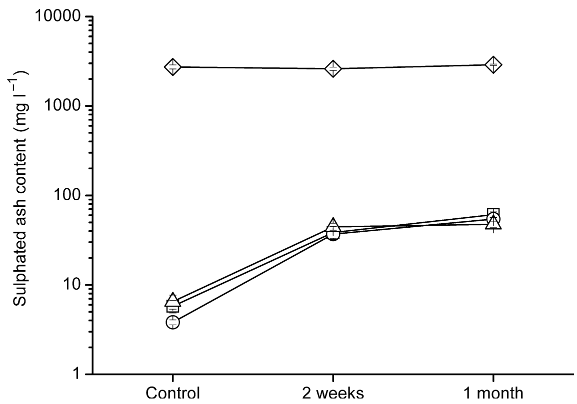

Figure 1 Sulphated ash content of the grades of ethylene glycol (mg l^{-1}; mean ± SE). Squares: analytical grade of ethylene glycol; circles: puriss grade of ethylene glycol; triangles: technical grade of ethylene glycol; diamonds: ethylene glycol or common anti-freeze.

insects often collected in pitfall traps. Our results indicated that both wet and dry masses of firebugs increased significantly in all glycol treatments. We found significant differences in the concentration of elements among the treatments when the elemental concentrations were reported per unit measured dry mass for all elements. There were no significant differences when the reference bases were live body mass and estimated dry body mass, except for potassium and sodium. Our results showed that the sulphated ash content was lowest in the analytical glycol. The sulphated ash content was also low in puriss and technical glycols. Based on our findings, the cost-effective technical grade is recommended as trapping fluid. Moreover, we found that undiluted trapping fluids resulted in an increase in body mass, biasing the results of elemental analysis of invertebrates; therefore, using diluted technical ethylene glycol is recommended.

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