

In situ Measurement of Bioavailable Metal Concentrations at the Downstream on the Morava River using Transplanted Aquatic mosses and DGT Technique

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ABSTRACT: This work summarized the results of a long term monitoring programme performed downstream on the Morava river (Czech Republic). During this programme the total dissolved concentrations and bioavailable fraction of selected metals (Zn, Pb, Ni, Cu) were monitored. For the determination of bioavailable metals species, diffusive gradients in thin films technique (DGT) together with moss bags technique utilizing *Fontinalis antipyretica* moss species were used. All of the measured metal concentrations were compared with an amount of accumulated mass by *Fontinalis antipyretica*, represented as a concentration factor, CF. Obtained results shown that further investigation of DGT technique and metal accumulation processes by *Fontinalis antipyretica* is needed to conclude if the DGT technique is a good alternative for the biomonitoring technique using *Fontinalis antipyretica* moss bags as a means to measure (bio)available metal concentrations in natural water. Significant correlation with CF was found in the case of DGT_{Zn} and DGT_{Pb} concentrations. On the other site, accumulated mass of Cu by *Fontinalis antipyretica* correlated significantly with total dissolved concentration of Cu. In the case of Ni no correlation was found between total dissolved Ni concentrations, DGT_{Ni} concentration and CF of *Fontinalis antipyretica*.

Key words: Diffusive gradient, *Fontinalis antipyretica*, Water analysis, Passive sampling, Metals

INTRODUCTION

Metals water pollution can be measured by physical or chemical methods; however many of them have some limitations. The sampling schedule does not match the discharge events, elements of interest are presented in very low concentrations under the detection limits of analytical methods and the changes in metal speciation can occur during sampling and storage. The representativeness of obtained data is then questionable. Moreover, total and total dissolved metal concentration may not correspond to bioavailable fraction of metals (Tessier and Turner, 1995). These problems can be bypassed using *in situ* techniques and/or biomonitoring techniques. Biomonitoring techniques include the use of different kind of fish, invertebrates organisms or bivalves (Ji *et al.*, 2010; Rybak and Uminska-Wasiluk, 2007; Gerhardt *et al.*, 2007; Rhea *et al.*, 2006; Fialkowski *et al.*, 2003; Schilderman *et al.*, 1999; Romeo *et al.*, 2003; Smolders *et al.*, 2002; Rainbow *et al.*, 2000; Carru *et al.*, 1996; Camusso *et al.*,

1994). However, thanks to its suitable properties and wide availability, aquatic mosses have become used in recent years for biomonitoring of inorganic and organic pollutants in aquatic environment (Cesa *et al.*, 2006; Yurokova and Gecheva, 2004; Roy *et al.*, 1996; Siebert *et al.*, 1996; Mouvet *et al.*, 1993; Say *et al.*, 1981). The most commonly used *in situ* techniques in recent years include the use of Chemcatcher sampler and supported liquid membranes or permeation liquid membranes samplers (Vrana *et al.*, 2005). In 1994 diffusive gradients in thin films technique has been introduced (Davison and Zhang, 1994) and later this technique has become widely used for *in situ* assessment of thermodynamically and kinetically labile metal species in aquatic systems. The DGT technique employs two layers of hydrogel, a diffusive layer and a binding phase. Diffusive layer is placed in the DGT unit on the top of the binding phase and covered with a membrane (usually 0.45 μm). These three layers are sealed in the DGT unit so that only the diffusive layer

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covered with membrane is exposed to the solution to be analyzed (Zhang and Davison, 1995). Dissolved metal species smaller than membrane pore size diffuse through a hydrated polyacrylamide gel, of thickness Δg and area A , and are accumulated by a layer of binding agent. Most frequently an iminodiacetate chelating resin Chelex-100, is used and has been applied to a large number of divalent and trivalent metal ions (Garmo *et al.*, 2003), including heavy metals and other elements of environmental interest. After exposition of the DGT unit for a time t in a solution, the amount of metal ions absorbed by the resin is analyzed and the mass M of captured metals determined. The time-average concentration of metal in the bulk solution, c_{DGT} can be calculated with the help of Fick's 1st law of diffusion as:

$$c_{DGT} = (M \cdot \Delta g) / t \cdot A \cdot D \quad (1)$$

Where D is the diffusion coefficient of the metal in the gel, A an exposure surface area and Δg the thickness of the gel layer. As numerous experiments show that the biological effects of trace metals are related to the free metal ion activity (Buffle and Horvai, 2000), it has been tempting for researchers to understand a DGT measured metal fraction as the bioavailable fraction.

This study summarized the results from the monitoring programme performed on the Morava river during June 2007 and June 2008. The purpose of this

program was to monitor inorganic pollution by selected metals (Pb, Ni, Zn, Cd and Cu) at the down part of Morava river by the help of classical chemical analysis, passive sampling methods using DGT technique and aquatic moss *Fontinalis antipyretica* as a bioindicator. The aim of this study was to compare the results obtained by *Fontinalis antipyretica* with DGT results and total dissolved concentrations of studied metals to find the best concept for assessment of bioavailable metals forms.

MATERIALS & METHODS

The river Morava is the major Moravian river and it flows from the north to the south of Moravia, one of the three historical parts of the Czech Republic. The total length of the Morava river is 354 km and the drainage area is about 27 000 km². The lower reaches of the Morava river flows through agriculture areas and industrial cities with their textile, machinery and chemical industries. Location of sampling sites is shown on fig. 1. Temperature and pH in the river water were determined on-site. Filtered water samples were collected in glass bottles containing nitric acid as preservation agent using a plastic syringe and 0.45 μ m membrane filter placed in a plastic holder. Electrothermal atomic absorption spectrometr (AAS Zenit 60, Analytic Jena, Germany) was used for metal determination. The reference material (SLRS-4, river water, National Research Council of Canada, Canada) was used for method validation.

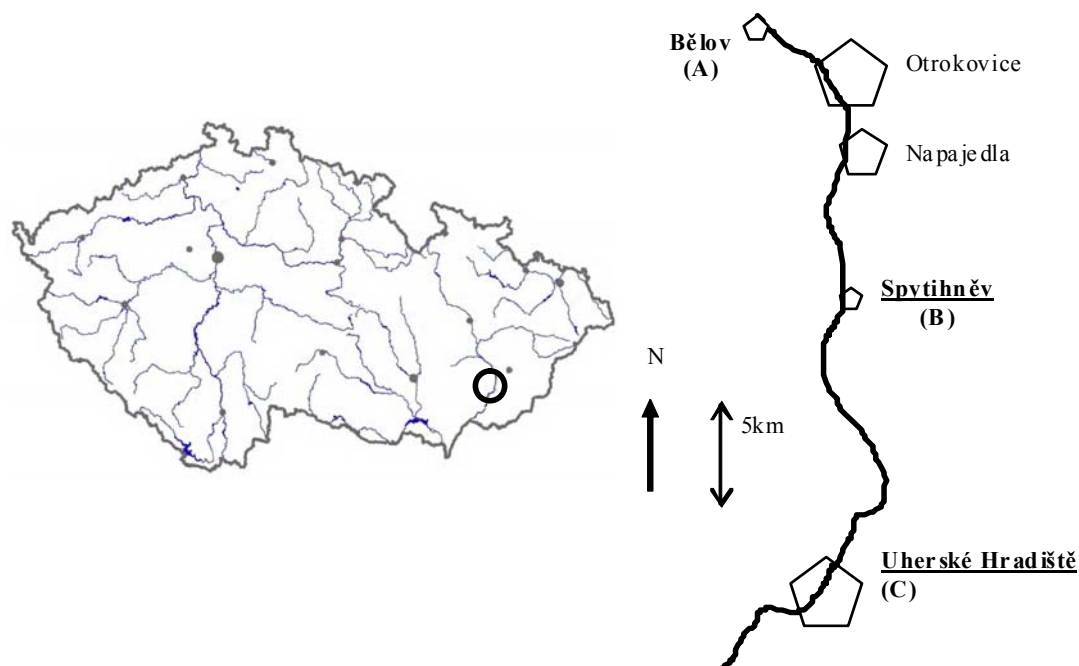


Fig. 1. Location of sampling sites

The DGT sampling units with Chelex 100 resin gel and 0.8 mm thick polyacrylamide diffusive gel (Diviš *et al.*, 2005) were tested in the test solution of the metals studied according to the regulations of DGT Research Ltd (DGT research Ltd., 1998). At the sampling site three DGT sampling units (DGT Research, United Kingdom) were anchored to the sampling station using nylon string. Similarly, two moss bags containing approximately 20g tufts of *Fontinalis antipyretica* (collected at Mlýnský potok, in natural park Litovelské pomoraví) in perforated, 0.5 cm mesh size plastic bag were anchored to the sampling station. After 28 days of deployment, DGT sampling units and the moss bags were removed, washed and transported into the laboratory. The resin gels of known volume (V_g) were extracted and metal concentrations (c_e) were determined after elution in 1 mL of nitric acid (V_e). The DGT metals concentrations were calculated using equation (2) and (1) respectively. The elution factor (f_e) was 0.85.

$$M = \frac{c_e \cdot (V_g - V_e)}{f_e} \quad (2)$$

The *Fontinalis antipyretica* samples were mineralized in a microwave furnace (MLS 1200, Milestone, Italy) using nitric acid and hydrogen peroxide (Analpure, Analytika) mixture. Flame AAS was used for metal determinations in the mineralized moss samples (Varian SpectraAA 30, Varian, Australia). To check the accuracy of analysis, quality control material (Metranal 8, green algae, Analytika) was used. The concentration factor CF was calculated as the ratio of accumulated mass of metal in *Fontinalis antipyretica* c_{FA} ($\mu\text{g/g}_{\text{dw}}$) after exposition in the river water and the mass of metal in *Fontinalis antipyretica* c_0 ($\mu\text{g/g}_{\text{dw}}$) before exposition: c_{FA}/c_0 (Cesa *et al.*, 2009).

RESULTS & DISCUSSION

All analytical methods used during this study passed the quality control tests. The recovery of Cu, Ni, Pb and Zn in material SLRS-4 (natural river water) measured by ET AAS were in the range 95-103 % and detection limits were sufficient for the metal analysis of selected samples. All prepared DGT sampling units meet the requirements of DGT Research Ltd. (DGT research Ltd., 1998).

From the calculated CF (Table 1) according to the Mouvet scale for the aquatic mosses (Cesa *et al.*, 2009), it can be say that at Bělov, there was no contamination by Zn and suspected contamination by Cu, Ni and Pb. At the Spytihněv station, there was no contamination by Cu but suspected contamination by Zn, Ni and Pb. Similar situation was found at the Uherské Hradiště station. Although some contamination was found

during the long term monitoring program, the average concentrations of total dissolved metals (Table 1) did not exceed the maximal long-term concentration limits warranting no negative impact upon aquatic ecosystem (USEPA, 1998).

There were no large differences in the DGT measured concentrations of Zn, Cu and Ni throughout the monitoring programme. Average DGT_{Zn} concentration was $0.74 \pm 0.27 \mu\text{g/L}$, DGT_{Ni} concentration $0.45 \pm 0.14 \mu\text{g/L}$ and DGT_{Cu} concentration $0.30 \pm 0.12 \mu\text{g/L}$. Lower DGT concentration was measured in the case of Pb (average value $0.026 \pm 0.009 \mu\text{g/L}$), but it corresponds to the lower total dissolved concentration of Pb in river water in comparison with Zn, Cu and Ni (Table 1). All DGT measured concentrations were approximately 10 times lower than total dissolved concentrations measured directly in the river water by atomic absorption spectrometry (Table 1). This indicated that some parts of dissolved metals in the Morava river was strongly complexed by dissolved organic carbon or by other strong natural ligands.

The amount of accumulated metals in *Fontinalis antipyretica*, expressed as concentration factor (CF), was correlated with DGT metal concentrations and total dissolved metal concentrations (Fig.2). Good correlations were found between measured DGT_{Zn} and DGT_{Pb} concentrations and CF. In the case of Cu measured values of CF correlated well with total dissolved Cu concentration, whereas no correlation was found to exist between all measured Ni concentrations and CF (Fig. 2). Similar results were also found in a previous study performed in the River Svitava (Diviš *et al.*, 2007).

The ability of aquatic mosses to accumulate not only inorganic copper, but also some copper bound in organic complexes has been reported by Ferreira *et al.* (Ferreira *et al.*, 2008). There are no other data in the literature which compares the metal concentration obtained by simultaneous application of moss bag technique and DGT technique. However, comparison with DGT measured concentrations and concentrations recorded by other bioindicators can be found. Jordan *et al.* (Jordan *et al.*, 2008) found good correlation of DGT measured concentrations with copper accumulated in *Saccostrea glomerata*. In contrast to these results, experiments with *Mytilus galloprovincialis* showed a significant correlation between Cd and Pb concentrations measured in the mussel tissues and bioavailable metal levels in water and proved that transplanted mussels did not accumulate Cu and Ni, although DGT showed significant concentrations of bioavailable forms of these metals in water (Schintu *et al.*, 2008). In the case

In situ measurement of bioavailable metal concentrations

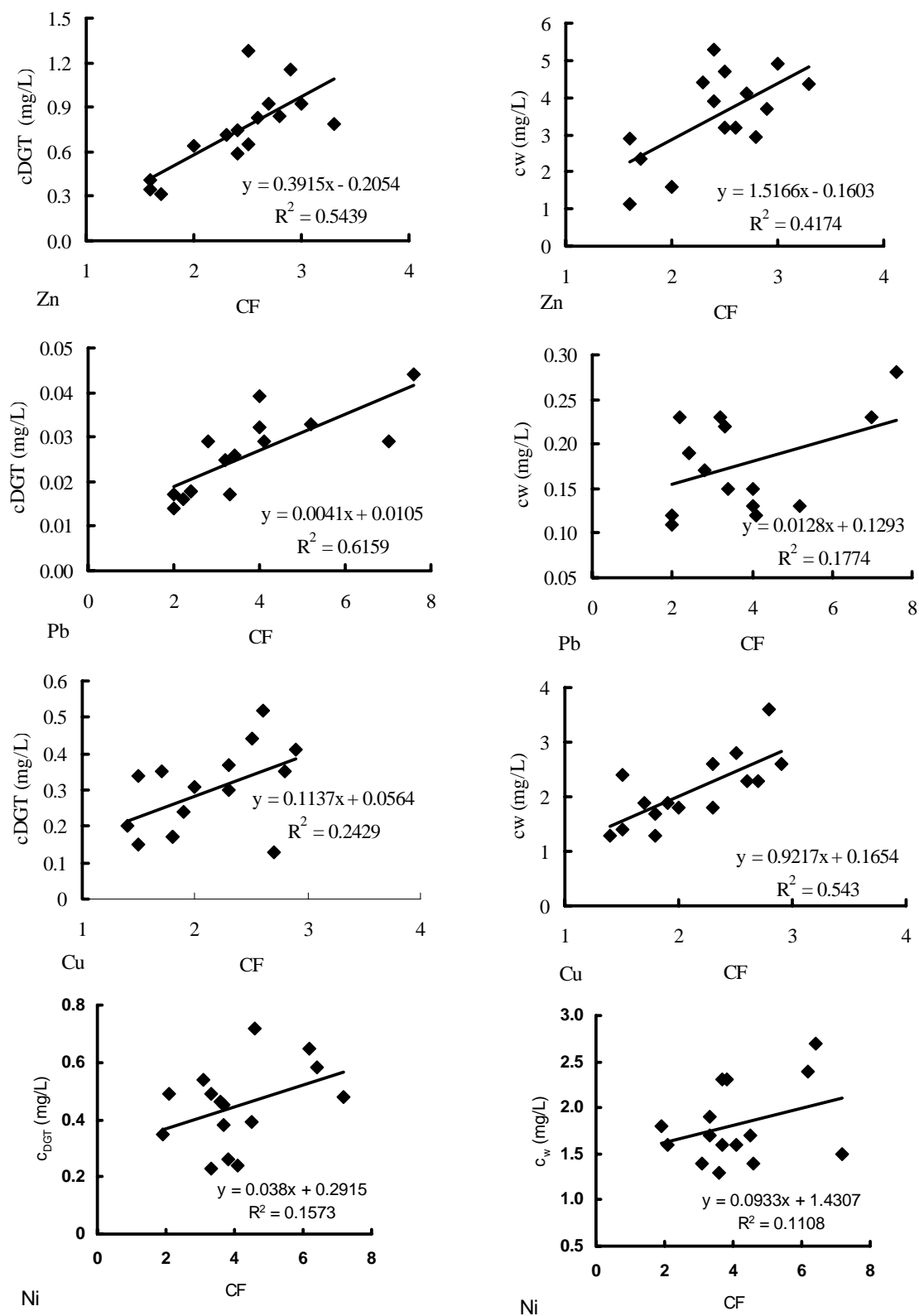


Fig. 2. Correlation of DGT measured concentrations and total dissolved concentrations with concentration factor of metals in *Fontinalis antipyretica*

Table 1. Measured concentrations in Morava river using conventional water sampling, DGT technique and moss bag technique (Continues)

station	A-06-2007	B-06-2007	C-06-2007	A-09-2007	B-09-2007	C-09-2007	A-12-2007	B-12-2007
Zn								
c_w	1.12 ± 0.09	2.94 ± 0.06	1.61 ± 0.13	2.33 ± 0.08	4.35 ± 0.16	4.7 ± 0.3	2.9 ± 0.2	3.9 ± 0.2
c_{DGT}	0.41 ± 0.08	0.84 ± 0.12	0.64 ± 0.15	0.31 ± 0.09	0.79 ± 0.18	0.65 ± 0.04	0.35 ± 0.13	0.74 ± 0.15
c_{FA}	392 ± 65	621 ± 118	539 ± 74	425 ± 52	782 ± 94	751 ± 83	452 ± 69	796 ± 109
c_0	245 ± 21	228 ± 15	263 ± 13	251 ± 8	237 ± 17	301 ± 35	287 ± 28	327 ± 42
CF	1.6	2.8	2.0	1.7	3.3	2.5	1.6	2.4
Cu								
c_w	2.61 ± 0.07	2.73 ± 0.15	1.85 ± 0.03	2.33 ± 0.09	1.39 ± 0.05	1.42 ± 0.09	2.63 ± 0.12	1.78 ± 0.07
c_{DGT}	0.37 ± 0.17	0.13 ± 0.04	0.24 ± 0.09	0.52 ± 0.16	0.17 ± 0.07	0.15 ± 0.06	0.41 ± 0.12	0.28 ± 0.08
c_{FA}	57 ± 16	49 ± 6	29 ± 5	68 ± 12	39 ± 9	43 ± 14	53 ± 8	33 ± 7
c_0	24 ± 3	18 ± 2	15 ± 3	26 ± 5	22 ± 2	29 ± 6	18 ± 4	14 ± 3
CF	2.3	2.7	1.9	2.6	1.8	1.5	2.9	2.3
Ni								
c_w	1.72 ± 0.05	1.63 ± 0.12	2.32 ± 0.08	1.57 ± 0.09	2.31 ± 0.16	2.68 ± 0.13	1.46 ± 0.09	1.41 ± 0.06
c_{DGT}	0.23 ± 0.05	0.24 ± 0.13	0.26 ± 0.07	0.49 ± 0.11	0.38 ± 0.16	0.58 ± 0.12	0.48 ± 0.09	0.54 ± 0.14
c_{FA}	21 ± 5	33 ± 7	35 ± 4	15 ± 3	23 ± 5	38 ± 7	35 ± 4	25 ± 6
c_0	6.5 ± 0.9	8.14 ± 1.26	9.3 ± 0.7	7.2 ± 1.3	6.3 ± 0.6	5.9 ± 0.8	4.8 ± 0.7	8.1 ± 0.9
CF	3.3	4.1	3.8	2.1	3.7	6.4	7.2	3.1
Pb								
c_w	0.12 ± 0.03	0.23 ± 0.06	0.17 ± 0.04	0.117 ± 0.009	0.19 ± 0.04	0.13 ± 0.03	0.224 ± 0.009	0.15 ± 0.03
c_{DGT}	0.014 ± 0.006	0.025 ± 0.004	0.038 ± 0.013	0.007 ± 0.003	0.018 ± 0.007	0.032 ± 0.005	0.017 ± 0.004	0.026 ± 0.009
c_{FA}	4.55 ± 1.27	7.9 ± 1.9	10.4 ± 3.9	3.8 ± 0.8	5.9 ± 0.6	9.3 ± 1.4	6.2 ± 0.7	8.4 ± 0.5
c_0	2.25 ± 0.19	2.53 ± 0.26	3.7 ± 0.3	1.92 ± 0.14	2.55 ± 0.21	2.3 ± 0.3	1.91 ± 0.15	2.5 ± 0.4
CF	2.0	3.2	2.8	2.0	2.4	4.0	3.3	3.4

c_w = total dissolved concentration ($\mu\text{g/L}$) $n=4$; c_{DGT} = DGT concentration ($\mu\text{g/L}$) $n=3$; c_{FA} = accumulated mass of metal in *Fontinalis antipyretica* after exposition ($\mu\text{g/g}_{dw}$) $n=3$; c_0 = mass of metal in *Fontinalis antipyretica* before exposition ($\mu\text{g/g}_{dw}$) $n=3$; CF = concentration factor

Table 1 (continuation): Measured concentrations in Morava river using conventional water sampling, DGT technique and moss bag technique

Table 1. Measured concentrations in Morava river using conventional water sampling, DGT technique and moss bag technique (Continuation)

station	C-12-2007	A-03-2008	B-03-2008	C-03-2008	A-06-2008	B-06-2008	C-06-2008
Zn							
c_w	4.1 ± 0.3	3.17 ± 0.05	4.92 ± 0.07	3.21 ± 0.14	5.39 ± 0.18	4.35 ± 0.16	3.7 ± 0.4
c_{DGT}	0.92 ± 0.18	1.28 ± 0.14	0.92 ± 0.16	0.83 ± 0.09	0.59 ± 0.17	0.71 ± 0.15	1.15 ± 0.23
c_{FA}	683 ± 71	528 ± 105	824 ± 138	617 ± 82	728 ± 97	548 ± 94	759 ± 123
c_0	249 ± 33	209 ± 31	278 ± 55	236 ± 27	301 ± 38	237 ± 17	258 ± 65
CF	2.7	2.5	3.0	2.6	2.4	2.3	2.9
Cu							
c_w	1.29 ± 0.04	3.55 ± 0.13	1.91 ± 0.05	2.75 ± 0.17	1.78 ± 0.15	1.69 ± 0.05	2.36 ± 0.08
c_{DGT}	0.19 ± 0.07	0.35 ± 0.09	0.35 ± 0.15	0.44 ± 0.07	0.31 ± 0.14	0.17 ± 0.07	0.34 ± 0.16
c_{FA}	27 ± 6	76 ± 19	37 ± 4	42 ± 8	63 ± 12	39 ± 9	29 ± 14
c_0	19 ± 5	28 ± 4	22 ± 5	17 ± 3	31 ± 6	22 ± 2	19 ± 7
CF	1.4	2.8	1.7	2.5	2.0	1.8	1.5
Ni							
c_w	1.88 ± 0.13	1.82 ± 0.05	1.43 ± 0.09	1.28 ± 0.08	1.66 ± 0.15	1.60 ± 0.07	2.42 ± 0.18
c_{DGT}	0.49 ± 0.15	0.35 ± 0.08	0.72 ± 0.19	0.46 ± 0.13	0.39 ± 0.16	0.45 ± 0.16	0.65 ± 0.24
c_{FA}	41 ± 9	18 ± 5	59 ± 8	27 ± 6	29 ± 3	23 ± 5	51 ± 7
c_0	12.5 ± 1.6	9.5 ± 1.3	12.7 ± 1.5	7.5 ± 0.9	6.4 ± 0.8	6.3 ± 0.6	8.3 ± 1.2
CF	3.3	1.9	4.6	3.6	4.5	3.7	6.2
Pb							
c_w	0.28 ± 0.09	0.23 ± 0.04	0.125 ± 0.018	0.23 ± 0.05	0.117 ± 0.013	0.19 ± 0.04	0.15 ± 0.06
c_{DGT}	0.044 ± 0.016	0.016 ± 0.005	0.033 ± 0.014	0.029 ± 0.012	0.019 ± 0.006	0.018 ± 0.007	0.022 ± 0.009
c_{FA}	10.6 ± 1.3	4.35 ± 0.96	8.9 ± 1.7	16.9 ± 2.7	6.3 ± 1.3	5.9 ± 0.6	9.3 ± 1.4
c_0	1.44 ± 0.26	1.95 ± 0.27	1.73 ± 0.19	2.4 ± 0.3	1.55 ± 0.27	2.55 ± 0.21	2.3 ± 0.3
CF	7.6	2.2	5.2	7.0	4.1	2.4	4.0

c_w = total dissolved concentration ($\mu\text{g/L}$) n=4; c_{DGT} = DGT concentration ($\mu\text{g/L}$) n=3; c_{FA} = accumulated mass of metal in *Foninalis antipyretica* after exposition ($\mu\text{g/g}_{dw}$) n=3; c_0 = mass of metal in *Foninalis antipyretica* before exposition ($\mu\text{g/g}_{dw}$) n=3; CF = concentration factor

of Ni, we suppose formation of strong complexes of Ni with dissolved organic matter. Metal species measured by DGT are limited by their volume and lability. Species larger than 5nm can not diffuse through diffusive gel and they are not detected by DGT technique as well as metal species which can not dissociate within the diffusion time-scale in the diffusive layer, or they are inert to binding phase (Li *et al.*, 2005). Therefore colloids, large metal complexes or very stable complexes are not detected by DGT technique. On the other hand, *Fontinalis antipyretica* may be able to accumulate these fractions of metals, which cannot be measured by DGT. For the interpretation of measured results different metabolism of Ni by *Fontinalis antipyretica* in comparison to other monitored metals should be taken also in to the consideration. As in the literature there is entire lack of information about Ni accumulation processes by *Fontinalis antipyretica* more studies on this issue are necessary.

CONCLUSION

The study demonstrated that the concentrations of Zn and Pb measured by DGT in river water were comparable with the concentration factor calculated from accumulated masses of metals in aquatic moss *Fontinalis antipyretica*. On the other hand, differences were observed between DGT_{Cu} and DGT_{Ni} concentrations and accumulated mass of metals in *Fontinalis antipyretica*. Significant correlation was found between the total dissolved concentration of Cu and mass of accumulated Cu in the aquatic moss. In the case of Ni, no correlation was found between the total dissolved and DGT measured Ni concentrations and concentration factor of *Fontinalis antipyretica*. Although we did not find any correlation between DGT_{Cu} , DGT_{Ni} concentrations and mass of metals accumulated by *Fontinalis antipyretica*, the diffusive gradients in thin films technique appeared to be a good alternative for the biomonitoring technique. However, further investigation of this promising *in situ* technique and metal accumulation processes by *Fontinalis antipyretica* is required.

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