BIOCONCENTRATION FACTORS FOR METALS IN HUMIC WATERS AT DIFFERENT pH IN THE RÖNNSKÄR AREA (N. SWEDEN)

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Abstract. The metal content of bryophytes (*Fontinalis antipyretica*), invertebrates (*Asellus aquaticus*, *Sialis lutaria*, *Libellulidae*) and fish (*Perca fluviatilis*, *Esox lucius*) was measured along an aquatic concentration gradient of Zn, Cu, Cd, Pb, As, Hg, generated by air emissions from the Rönnskär smelters. The separation of pH effects on metal uptake, was facilitated by metal and pH vectors being mostly well separated from each others. The interpretation was facilitated by seasonal small variations in water metal content, which improved the calculation of bioconcentration factors (BCF) as a strict measure of bioavailability. It was found that BCF varied as function of element and taxon. When pH decreased, BCF also did so for Zn, Cd, Ni, Co in bryophytes, while BCF increased for Pb, Cu in in fish. We conclude and agree that one-sided focusing on total concentrations may overlook shifts in speciation due to pH in moderately acidified waters for elements like Cu and Pb.

1. Introduction

Metals are bioaccumulated in aquatic biota in relation to ambient concentrations and metal species (Bryan 1980, Luoma 1983). pH may exert effects on bioavailability by affecting speciation and properties of biological surfaces. The dominating mechanism is ionic exchange where hydrogen ions remove metals from organic complexes and biological surfaces and render them more (Pb) or less (Zn, Cd, Cu) accessible to biota (Campbell & Stokes 1985). These pH-relations may be modified by complexing organics and particles in situ (e.g., John et al. 1987, Welsh et al. 1993), and need verification in different water types. We tried this in humic lakes and brooks in gradients of metals which are generated by air-born pollutants from the Rönnskär smelters (Fig. 1). The discrimination of pH effects on bioavailability was facilitated by pH and metal vectors being rather independent of each others.

2. Methods

Surface water (0.2 m depth) for chemical analysis was sampled with a plastic Ruttner sampler in 12 lakes (Fig. 1). Samples were taken 4 times in 1981 and 3 times in 1982 (No.0:1984) from April/May to September/October. Unfiltered water was transferred to acid-rinsed plastic bottles and acidified with concentrated HNO_3 (p.a.)(1:100). The bryophytes *Fontinalis antipyretica and/or F. dalecarlica* were localized in 8 lake outlets and were sampled in July 1981/1983. As a complement, were top shoots of *Fontinalis antipyretica* transplanted in net boxes to 15 brooks in the Rönnskär area (Fig. 1), from a pristine brook, Rensjöån. The experiment was was carried out in July 1987 and lasted

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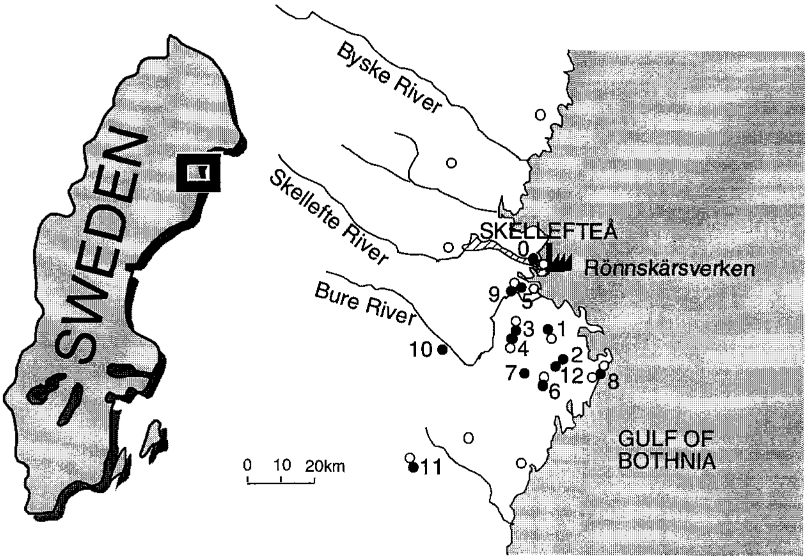

Figure 1. Studied lakes (●) and brooks (○).

for12-14 days. Water chemistry was checked at outset and uptake. The crustacean *Asellus aquaticus* was found in 9 of the lakes and was sampled in July 1981 and 1982/1984. Animals were kept alive in their water for 24 hours to permit gut clearance. Larvae of alderflies (*Sialis lutaria*) and dragon flies (*Libellulidae*) were found only in 6-7 lakes, and were sampled in July 1981. Euroasian Perch (*Perca fluviatilis,*) and Northern Pike (*Esox lucius*), occurred in 12 and 6 lakes, respectively. They were caught with nets in July/August 1981 except for No. 12 Lake Degerträsket which was investigated by local authorities in July 1982. Body-weight, length, sex and age was determined. The liver was picked out with plastic tweezers. A piece of dorsal muscle was dissected with a scalpel of stainless steel. Samples were deep-frozen in acid-washed polystyren capsules. They were then freeze-dried and homogenized in an agate mortar before digestion and analysis. Female fish were individually analyzed. Invertebrates were pooled with regard to size. Metals zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb), and aluminium (Al) were analyzed by flame and flameless AAS after digestion in concentrated $HNO_3+H_2O_2$ (Borg et al 1981). Arsenic (As) was determined after dry-ashing with $MgNO_3$ and MgO, mercury (Hg) by flameless AAS after digestion in conc. HNO_3. Water was analyzed for Zn, Cu, Cd, Pb by flameless or flame AAS (+DBC), with and without preconcentration (freeze-drying), iron (Fe) and manganese (Mn) by flame AAS, As (as ASH_3) by flameless AAS after acidification and UV-irradiation, and Al spectrophotometrically with pyrocatecholviolet. Quality assurance included NBS reference materials Bovine Liver (NBS 1577) (Zn, Cu, Cd, Pb) and Orchard leaves (CRM 1571) (Mn, Zn, Cu, Cd, Pb, Ni, Cr). Freeze-dried homogenates of Pike liver were analyzed for As and compared to NAA. The agreement with certified values was mostly good, except for Cr which was 30-40 % too low. The spread (S.D.) around the mean value was mostly <5-10 %. The statistical analysis included linear regression and Student's T-test, or Mann Whitney's U-test when unequal variance was indicated by Scheffe's test. Logarithmic transformation was used to increase the symmetry of data and compensate for extremes in linear regression.

3. Results

3.1 Water chemistry

Seasonal variations were mostly small and the amplitude of metals less than 2:1 in lake water. Inter-lake variation were 6:1 (Zn, Pb) to 10:1 (Cu, Cd, As), and inter-brook variations 50:1 to 100:1 (except for No. 0). Mean pH was 5.0-7.4 in lake water and 4.1-7.2 in brook water. Lake water was oligohumic to polyhumic and soft to ultrasoft (8-45) mg $CaCO_3$ L^{-1}) (Table 1). Concentrations of Zn, Cu, Cd, Pb, increased with the proximity to the Rönnskär smelters (brooks $R^2=0.55$ to 0.78). Concentrations of Fe, Cr, Co, Al increased with water colour (brooks $R^2= 0.81$, 0.73 0.67, 0.63, respectively). Al was slightly weaker correlated with pH (neg., $R^2=0.57$) than with water colour (pos.).

TABLE I
Water chemistry. Arithmetic mean±S.D. (N)

lakes	pH	Col[a]	Hard[b]	Fe[c]	Al[c]	Zn[d]	Cu[d]	Cd[d]	Pb[d]	As[d]
Circum-neutral (9)	6.5± 0.4	84± 36	19± 10	1.1± 0.5	0.18± 0.10	15.2± 14.1	4.6± 5.3	0.14± 0.19	1.8± 1.4	7.0± 11.4
Acidified (4)	5.6± 0.4	125± 8	14± 5	1.2± 0.2	0.38± 0.15	14.4± 3.4	2.4± 0.3	0.10± 0.02	1.4± 0.4	2.6± 0.4

brooks	pH	Col[a]	Hard[b]	Fe[c]	Al[c]	Zn[d]	Cu[d]	Cd[d]	Pb[d]	As[d]
Circum- neutral (8)	6.3± 0.4	117± 42	17± 8	0.7± 0.4	0.30± 0.14	19.9± 17.4	5.4± 6.0	0.12± 0.14	2.2± 3.1	9.1± 20.3
Acidified (7)	5.3± 0.6	195± 89	17± 13	1.7± 1.2	1.3± 1.4	32.4± 31.3	8.9± 13.5	0.29± 0.54	3.2± 6.5	6.8± 13.4

Brooks: 0.2-8.6 µg Ni L^{-1}. 0.07-6.6 µg Co L^{-1}. 0.13-2.17 µg Cr L^{-1}, 21-330 µg Mn L^{-1}

[a] water colour mg Pt L^{-1} [b] Hardness mg $CaCO_3$ L^{-1} [c] mg L^{-1} [d] µg L^{-1}

3.2 Biota in lakes

BCF had maximum for Cu, Cd in *Asellus*. It had minimum for Pb, As in predators and Al in fish liver. BCF-Cu in fish liver exhibited large interlake-variations (14-22:1) with highest concentrations in acidified respectively most polluted lakes (Cu>4 µg L^{-1}) ($p<0.01$)(Fig. 2a). BCF-Cu in Perch liver was inversely related to pH ($R^2=0.67$, $p<0.01$) (Fig. 2c) and did not correlate significantly with water colour. At circumneutral pH, Cu in Perch liver increased exponentially with the water Cu content (Fig. 2a; $R^2=0.979$, $p<0.001$). In *Asellus* was the Cu content linearly and positively correlated with the water Cu content ($R^2=0.97$, $p<0.001$). BCF-Pb of fish liver had maximum in acidified lakes and was inversely correlated with pH ($R^2=0.54$, $p<0.01$) (Fig. 2d). The correlation was stronger than corresponding positive correlation with water colour ($R^2=0.40$, $p<0.05$). BCF-Al of Perch liver was 2 times higher in acidified (N=2, pH 5.0-5.5) than in other lakes. At circumneutral pH, Pb in Perch liver was positively correlated with the water Pb content

Table II

BCF (x10^{-3}) (μg g^{-1} dry substance/μg L^{-1}). Arithmetic mean±S.D.(N)

	Al	Zn	Cu	Cd	Pb	As
Fontinalis (7)	6.6±3.2(6)	15±5	6.1±2	24±24.	17±9	8.7±9.5
Asellus(10) [2]	2.4±3.4(7)	8.2±5	75±31	65±35	6.9±11	2.2±0.9
Libellula(6)[2]	-	14±8	17±18	41±24	5.7±5.2(5)	1.9±0.6(5)
Silais(7)[2]	-	20±12	7.2±3	27±23	0.2±0.07	0.7±0.2
Perca liver(12)[3]	0.1±0.1(8)	11±4	12±13	164±60(11)	0.2±0.2(11)	0.2±0.1[1](10)
Esox liver(6)[4]	0.06±0.04(3)	16±11	31±50	27±23(5)	0.2±0.2(3)	0.8±0.3[1]

- =missing data. [1] Muscle [2] Mean of 1-4 pooled samples No. of ind.: 13-170 *Asellus*, 1-2 *Libellulidae*, 3-31 *Sialis*. Mean weight per individual: 0.6-2.1, 14-63 and 3-16 mg DS. [3] Mean of 12-22 Perches (No. 7: 6), mean weight: 64-152g (No. 8: 279 g), mean age 5-9 years Cv: Zn 4-8, Cu 8-38 and other elements 5-26 % [4] Mean of 4-8 Pikes, mean weight: 990-1460 g (No. 8: 200 g), Cv: Zn 9-22, Cu 14-40, and other elements 6-36 %.

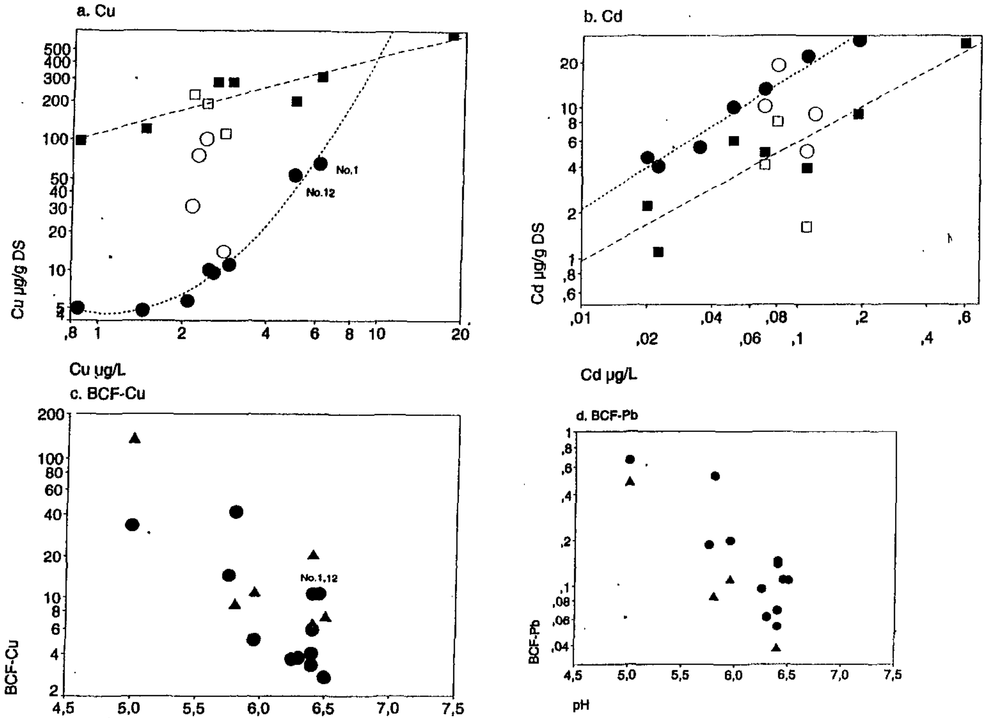

Figure 2. Metals in biota (μg g^{-1} dry substance) vs. metals in water (μg L^{-1})(top). BCF (×10^{-3}) vs. pH. (bottom) Logarithmic scales. *Asellus aquaticus* (■ □ ------), Perch liver (●O....), Pike liver (△▲) Filled symbols pH>6, open symbols pH<6.

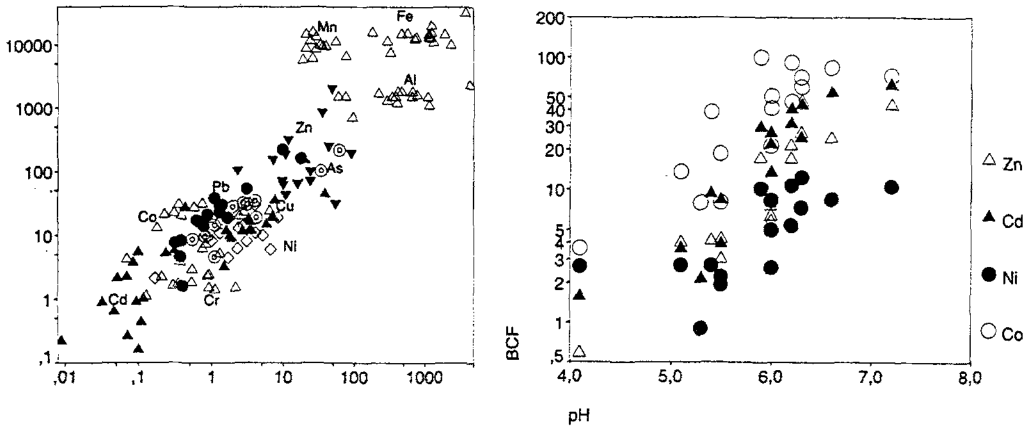

Figure 3. Metals in transplantated top shoots of Fontinalis antipyretica. (μg g^{-1} dry substance) vs. metals in water (μg L^{-1})(left). BCF (•10^{-3}) vs. pH (right). Logarithmic scales. In the left diagram are following relationships statistically significant: Pb (R^2=0.82), As (R^2=0.84), Cu (R^2=0.72), Ni (R^2=0.37), Cd(R^2=0.35), Co(R^2=0..31)

(R^2=0.746, $p<0.01$). Cd in Asellus was positively correlated with Cd in water (R^2=0.852) as was Cd in Perch liver at circumneutral pH (R^2=0.979, $p<0.001$) (Fig. 2b). In Perch liver BCF-Cd exhibited minimum in two of the acidified lakes, No. 4 Svarttjärn and No. 8 Svartsjön. In Pike liver, acidified lakes (N=3) exhibited lower BCF-Cd than remaining lakes (N=3). No significant pH relationships were found in invertebrates.

3. Transplanted *Fontinalis antipyretica.*

The pristine brook Rensjöån had circumneutral pH (pH≈7) and mesohumic water (c. 50 mg Pt L^{-1}). Concentrations of metals were mostly lower than in the Rönnskär area. After terminated exposure, top shoots contained metals roughly in proportion to ambient levels (Fig. 3a) (BCF≈ 10^4). The correlation was strongest in Pb, As, Cu (R^2=0.72-0.84). The weaker correlation in Ni, Co, Cd, (R^2=0.31-0.37) and Zn (n. s) was accompanied by a strong positive covariation between BCF and pH (R^2=0.48, 0.69, 0.82, 0.84, respectively) (Fig. 3b). At circumneutral pH, BCF decreased in the order Mn (30•10^4), Co (6•10^4), Cd (3•10^4), Zn,Fe,Pb (2•10^4), As (1•10^4), Ni (8•10^3), Cu,Cr,Al (4•10^3),

4. Discussion

Total water metal contents were fairly good predictors of Cd, Cu, Pb, As in biota at circumneutral pH. At pH<6, BCF did either increase (Cu, Pb, (Al) in fish) or decrease (Zn, Cd, Ni, Co in Fontinalis). Campbell & Stokes (1985) suggested on chemical grounds that the latter elements (plus Mn, Ag) are more likely than other elements to participate in competition between H^+ and Me^{n+} for binding sites on biological surfaces. They presented evidence for this in Zn, Cd, Mn at environmentally relevant pH values (e.g. pH>5). We confirm it for Zn, Cd, Ni, Co, but not for Mn, where precipitation of Mn-oxides is probably an important mechanism (as indicated by the high BCF-Mn of Fontinalis). The tendency towards lower BCF-Cd in fish liver in acidified lakes, might indicate that similar mechanisms were operating at higher trophic levels. Campbell & Stokes (1985) presented evidence for an anticipated increase of bioavailability in Pb, but not in Cu where they assumed H^+ competition at biological surfaces to be more important. We found strong covariation of Cu, Pb between Fontinalis and water, indicating that H^+ competition at biological surfaces was of minor importance or was balanced out by other mechanisms. The increase of BCF-Cu, Pb in fish liver at pH<6, rather indicated that their bioavailability increased as pH decreased. Since inorganic complexation is marginal in very soft waters, a likely explanation is that metal ions are liberated by being exchanged for H^+ in metal organic complexes (see, e.g., Saar & Weber 1982). Welsh et al. (1993) presented experimental support for this hypothesis in the laboratory. Thus, in soft water and the presence of organics (DOC=3.4 mg L^{-1}), they found Cu toxicity in fish to increase by a factor 2 from pH 6.5 to 5.5. At corresponding pH's we found a 5-10 fold increase of BCF-Cu in Perch liver which after correction for the nonlinear dose-response will be 3-4. The nonlinear dose response of Cu is supported by observations in the laboratory in species Bluegill (*Lepomis macrochirus*) and Brown Bullhead (*Ictaulsrus nebulosus*). Thus, Brungs et al. (1975) and Benoit (1975) reported a "threshold" for Cu uptake in liver at 20-40 μg L^{-1}, to be compared with 4 μg L^{-1} in this case. The discrepancey might reflect different species belonging, water quality (e.g. alcalinity) and/or exposure routes (additional exposure in Perch to high concentrations of Cu in food, e.g. *Asellus*). The utility of fish liver as an indicator organ and monitor of bioavailable metals, is supported by laboratory and pond experiments (e.g., op. cit., Gilderhus 1966, Olson et al. 1975, Benoit et al. 1976, Holcombe et al. 1976). Another important prerequisite for field studies to be successful is that samples were synoptically sampled during a limited period of time when temperature conditions were also favourable for metal uptake to occur in biota.

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