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Metal sources to the Baltic clam *Macoma balthica* (Mollusca: Bivalvia) in the southern Baltic Sea (the Gulf of Gdansk).

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1 **Abstract**

2

3 Metal concentrations of Cu, Fe, Mn, Ni, Pb and Zn in an infaunal facultative deposit-feeding
4 bivalve, the Baltic clam *Macoma balthica*, in the Gulf of Gdansk (southern Baltic Sea) were
5 assessed and compared to selected concentrations of metals in the environment. Between
6 October 1996 and September 1997, dissolved and easy extractable (by 1 M HCl) metal
7 fractions of total suspended particulate matter (TPM) in the overlying water and of surficial
8 sediments (< 63 μm) were measured monthly at five sublittoral sites in the Gulf of Gdansk,
9 and accumulated tissue metal concentrations in *M. balthica* were determined simultaneously.
10 The study highlights the importance of sediment geochemistry as a factor modifying ambient
11 trace metal bioavailabilities. Surficial sediments appeared to contribute most to the
12 accumulation of Cu and Pb in *Macoma balthica*, reflecting the high metal availability in the
13 Gulf. Assimilation of Cu from sediments is controlled by Mn components possibly through an
14 inhibitory effect of Mn oxyhydroxides, while Pb accumulation from sediments depends on the
15 organic content of the sediment. A dual metal uptake pathway, with a suspended particulate-
16 bound fraction and surficial sediments, was apparent for Mn and Zn. Partitioning of Mn in
17 sediments was related to the concentration of labile Fe, with increased levels of Fe tending to
18 inhibit the accumulation of Mn by the clam. Tissue accumulated Zn might have been altered
19 by the clam's internal regulation, making Zn tissue concentrations, to some degree,
20 independent of its environmental level. The principal source of Ni accumulated by the clams
21 exists in the soluble phase.

22

23 **Keywords:** metal source; *Macoma balthica*; surficial sediments; overlying water; Baltic Sea

24 **1. Introduction**

25 Marine animals can take up metals from solution and the diet which may, for example,
26 consist of particles in suspension or deposited in the sediment. The direct uptake of
27 dissolved metals takes place through passive facilitated diffusion of metal ions or via
28 channels (and pumps) for major ions (for example calcium channels in the case of cadmium)
29 over the permeable external body surface (Simkiss & Taylor, 1989). The soluble fraction is
30 commonly considered the most bioavailable form of trace metals in the marine environment
31 irrespective of feeding strategy, life history of an individual and geographical region, although
32 Wang (2002) has highlighted that the diet can represent the major route of trace metal
33 uptake. The uptake and subsequent accumulation of elements from particles involves the
34 ingestion of suspended material by suspension feeders such as bivalves, and deposited
35 organic material by sediment dwellers such as polychaetes (Wang & Fisher, 1999). For these
36 groups suspended and deposited material may thus constitute a considerable source of
37 metals, particularly at organically rich sites. The bioavailability of soluble and particulate-
38 bound metals is dependent upon a complex of geochemical factors including the strength of
39 metal-substrate binding, the composition of particles, the abundance of other elements and
40 compounds, potentially affecting metal speciation (Blackmore & Wang, 2002). A number of
41 studies has addressed the bioavailability of metals from particles by marine benthic
42 invertebrates. It has been shown that availability of Cd to the deposit feeding sipunculid
43 *Sipunculus nudus*, for instance, is partially controlled by the amount of organic carbon in
44 sediments (Wang, Yan, Fan, & Xu, 2002). The enhancing effect of an easily reducible Mn
45 phase was shown to be important in dietary inputs of Cu, Cd, Zn and Pb from surficial
46 sediments to *Macoma balthica* (Thomas & Bendell-Young, 1998), while the oxygen
47 conditions are well known to mediate relative mobility and bioavailability of Pb and Zn (Shine,
48 Ika, & Ford, 1998; Chong & Wang, 2000).

49 The efficacy of metal uptake and accumulation can vary also due to factors related to the
50 biology and ecology of animals studied such as feeding behaviour, nature of the diet, size,
51 age and physiology (Rainbow, 1995, 2002). One approach that throws light on the effects of

52 such factors on the kinetics of metal uptake and accumulation is a survey of seasonal
53 variations of the soft tissue body weight, providing information on the physiological condition
54 of an individual (Rainbow & Philips, 1993). Furthermore, it has been proposed that the
55 feeding and digestion strategy may change in feedback response to temporary fluctuations in
56 environmental metal concentrations. Decho and Luoma (1996) showed that two clam
57 species *Macoma balthica* and *Potamocorbula amurensis* are capable of modifying the
58 digestive processing of food to reduce exposure to high ambient biologically available Cr
59 concentrations.

60 Although the overall mechanisms for uptake of elements by various benthic molluscs with
61 respect to environmental conditions have been established in several regions (Rainbow,
62 1996; Maurer, Gerlinger, Robertson, & Nguyen, 1997), spatial heterogeneity with regard to
63 hydro-geochemical conditions and ecological factors precluded generalisation of metal
64 uptake and accumulation mechanisms. Patterns of metal uptake routes and subsequent
65 accumulation can vary interspecifically for the same metal and intraspecifically for different
66 metals (Rainbow, Philips, & Depledge, 1990). Site-specific studies are therefore required to
67 clarify unequivocally metal uptake sources in the area of interest, as it is inevitable that local
68 differences in habitat will affect tissue accumulated metal concentrations. This study has set
69 out to investigate metal uptake sources to the Baltic clam *M. balthica* in the Gulf of Gdansk
70 (southern Baltic Sea), distinguished from other marine basins by low salinity and
71 comparatively high pollution level (Sokolowski, Wolowicz, & Hummel, 2001; Szefer, 2002).

72

73

74 **2. Materials and Methods**

75 *2.1. Sampling*

76 Samples of overlying water, sediments and the Baltic clams *M. balthica* were collected at
77 monthly intervals from October 1996 to September 1997 at five sublittoral sites to depths of
78 10 m ("SW10"), 20 m ("SW20"), 30 m ("SW30"), 40 m ("SW40") and 60 m ("SW60") in the
79 Vistula River plume, the Gulf of Gdansk (Fig. 1).

80 Overlying water was sampled in triplicate with a Teflon GoFlo modified water sampler (5 l)
81 equipped with metal-free end stoppers and Teflon sealed valves. The sampler was fitted on
82 stainless-steel and polyethylene-coated rope with a polypropylene-coated counterweight at
83 the end, and was used always on the windward site of the vessel. Modification was designed
84 in order to sample water at a constant distance of 20 cm over the bottom surface and to
85 prevent entrance of particles that were resuspended from the sediment due to usage of the
86 sampler. The samples were pressure filtered with acid-scrubbed N₂ through 0.45 µm
87 precleaned cellulose-nitrate filters under an on-board clean box. Following filtration, water
88 samples were acidified with concentrated HCl to a final pH 1.8 and stored in dense
89 polyethylene bags at room temperature. The filters containing the retained particulate matter
90 were sucked dry and stored individually in acid-cleaned polystyrene Petri dishes at -20 °C. A
91 second series of water samples was collected at the same sites and on the same occasions
92 and filtered on a standard filtration system for the determination of total particulate matter
93 (TPM) content.

94 Samples of the top 5 cm of sediment were collected in triplicate with a polypropylene
95 sediment corer of 20 cm length and 2 cm diameter. This layer represents the fraction of the
96 sediments that *M. balthica* can penetrate and is the biological relevant portion of the
97 sediment. According to Zwartz and Wanink (1989) in a sandy and muddy substrate an
98 optimal burrowing depth of the clam, > 10 mm long, ranges from 20 mm in summer to 50 mm
99 in winter. After sampling, sediments were stored in dense polyethylene bags at -20 °C until
100 the time of analysis. Additional sediment samples were collected according to the same
101 procedure for the determination of the fine-grained fraction (< 63 µm) and its organic
102 component.

103 The clams were sampled by dredging and transported immediately to the laboratory where
104 they were kept in GF/F (glass microfibre filter; 1.6 µm) filtered sea water at a temperature
105 (3.5 to 19.6 °C) and salinity (7.1 to 8.6) corresponding to the ambient environmental situation
106 for 24 h to purge gut contents. Special care was taken to select individuals of a restricted
107 size range (11.4-15.0 mm that refers to individuals of 3-4 years old) as size has been

108 demonstrated to sometimes affect accumulated soft tissue metal concentrations in bivalves
109 (Wallace, Lee, & Luoma, 2003). Three pools of 20 individuals each were then prepared to
110 obtain mean metal concentration in each month, and animals were frozen at -20°C.

111

112 2.2. Analysis

113 Dissolved Cu, Pb and Zn concentrations were measured by differential pulse anodic stripping
114 voltametry (DPASV) and the dissolved Ni concentration was determined by differential pulse
115 cathodic stripping voltametry (DPCSV) after exposure to UV irradiance in the presence of
116 H₂O₂ (25 ml of sample+0.01 ml of H₂O₂) for 4 h to destroy dissolved organic ligands.
117 Measurements were carried out up to one month after collection in a laminar flow bench.
118 Manganese and Fe were measured directly on sea water samples with a graphite furnace
119 atomic absorption spectrometer (GF-AAS). Quality assurance and quality control was
120 maintained through inclusion of the estuarine water certified reference material (SLEW-2)
121 from NRC-INMS, Canada, and reagent blanks. Recovery and precision (calculated on the
122 basis of coefficients of variation) varied between metals and were 90-121 % and < 12 %,
123 respectively.

124 The extraction technique used to leach the particulate matter was that given by Luoma and
125 Bryan (1981). This method determines the fraction of elements in the particulates associated
126 with Fe- and Mn-oxides and by this estimates the relatively labile and potentially bioavailable
127 phase of the metal (Bryan & Langston, 1992). Filters with the retained suspended material
128 were extracted with 5 ml 1 M HCl for 2 h at room temperature, and shaken occasionally. The
129 final extracts were analysed by GF-AAS. Analysis of the estuarine sediment standard
130 reference material (BCR-277) from NRC-INMS was systematically performed using *Aqua*
131 *Regia* solution (HNO₃/HCl/H₂O=9:3:10 v/v/v) in a Microwave Digestion System (MDS-200,
132 CEM) to control the quality of the results. Recovery (81-103 %) and precision (< 16 %) were
133 considered satisfactory. A detailed description of the procedures used to clean field and
134 laboratory materials, analytical methods for dissolved and particulate metals, and the results
135 of the quality control tests is in Sokolowski, Wolowicz, & Hummel (2001). Total particulate

136 matter content (TPM) was determined according to standard methods recommended for the
137 Baltic Sea (Kramer, Brockman, & Warwick, 1994).

138 Prior to trace metal analyses, sediments were air-dried at 55°C to constant weight and dry
139 sieved through a 63 µm mesh to obtain the fine-grained fraction. Separation of the < 63 µm
140 fraction is a commonly used standardised procedure to compensate for grain size effects on
141 metal variability in sediments (Szefer, Glasby, Pempkowiak, & Kaliszan, 1995). The residue
142 retained after sieving was separated into two subsamples. One subsample was subjected to
143 extraction with 1 M HCl according to the same method as used for TPM, with a modification
144 in the final part of the procedure. After extraction, the extraction mixture (sediments+1 M HCl)
145 was centrifuged in glass acid-precleaned test tubes at 3000-3500 rpm and 10°C for 10 min.
146 to separate sediment particles from the solution, and the aliquot was pipetted off. Samples
147 were then made up to a volume of 10 ml and metal concentrations were determined by a
148 flame atomic absorption spectrometer (F-AAS). The second subsample of the < 63 µm
149 sediment fraction was subjected to ignition at 500°C for 8 h in order to determine organic
150 matter content.

151 When required for analysis, the clams were thawed, cleaned of debris and dissected using
152 Teflon or polypropylene instruments. The whole soft tissue was freeze-dried, homogenised
153 and 300 mg of aliquot was digested with 5 ml 14 N HNO₃ in a Microwave Digestion System
154 (MDS-200, CEM). The solution remaining after digestion was made up to 5 ml and measured
155 for metal concentrations in F-AAS. Quality control for sediments and clams was provided by
156 concurrent checks of the estuarine sediment (MESS-2) and lobster hepatopancreas
157 reference materials (TORT-1) from NRC-INMS, respectively and analytical blanks that were
158 analysed according to the same procedures. The results for recovery and precision met
159 acceptance criteria (Table 1).

160

161 2.3. Statistical analysis

162 Untransformed data were included in all statistical models followed by analysis of normality
163 (the Kolmogorov-Smirnov test for goodness of fit) and homogeneity of variances as

164 prerequisites to the parametric approach. The significance of the influence of month and site
165 on metal concentrations in the environment and clams was evaluated by analysis of variance
166 (two-way ANOVA) and functional relationships between pairs of variables (i.e. dissolved,
167 particulate-bound and sediment metal concentrations vs. metal concentrations in the clam)
168 were assessed with regression analysis. For particulate-bound metals, analyses were
169 performed separately for the concentration ($\mu\text{g dry wt}^{-1}$) and the content of elements
170 expressed per unit of water volume ($\mu\text{g l}^{-1}$) to calculate the concentration of suspended
171 fraction in the water column. The level of significance for all tests was set as $p < 0.05$ (Sokal
172 & Rohlf, 1995). Statistical analyses were carried out using STATISTICA for Windows
173 (StatSoft, Inc., 1997).

174

175

176 3. Results

177 3.1. Total Particulate Material (TPM) and sediment parameters

178 Spatial and temporal variations in TPM in the overlying water in the Vistula River plume are
179 described in Sokolowski, Wolowicz, & Hummel (2001). Briefly, high concentrations of TPM
180 were recorded near-shore due to riverine outflow, with a trend to decrease with depth and
181 distance from the river mouth. Elevated concentrations of TPM occurred in spring and late
182 summer/early autumn, particularly at the shallow sites SW10 and SW20, and were attributed
183 to intense primary production and algal blooms in the water column enhanced by increased
184 temperature in this season and eutrophication of the river. The percentage contribution of the
185 $<63 \mu\text{m}$ fraction in surficial sediments and the contribution of organic components in the
186 fraction showed strong spatial differences but no clear seasonal trends were apparent
187 (Table 2). The highest contribution (69.4%) of the $<63 \mu\text{m}$ fraction was at the deepest site
188 SW60 which lies below the thermo-halocline and furthest off-shore. This fraction was also
189 relatively rich in organic matter (average 7.1%). In the area adjacent to the river mouth, the
190 fine-grained fraction constituted on average 21.6-23.1% of the sediments and decreased to
191 6.1-6.7% in the mid-deep part of the plume (SW30 and SW40). In contrast, the contribution

192 of organic components in the fraction at SW10 and SW20 ranged from 9.4 to 9.7%, while at
193 SW30 and SW40 it varied between 6.1 and 6.7%. Such a distribution pattern reflects the
194 overall scheme of sorting and sedimentation processes of suspended matter and is typical
195 for the estuarine areas and the regions adjacent to the mouths of big rivers.

196

197 3.2. Concentrations of dissolved and easily extractable fractions of particulate- and sediment-
198 bound metals

199 Spatial and temporal variability of metal concentrations in different compartments of the
200 environment within the plume was high (Table 3 and Fig. 2-5). All dissolved metals, except
201 Mn, showed minima in the mid-deep zone of the plume (SW30) and maxima in shallow-
202 (SW10 and SW20) and deep-water parts (SW40 and SW60), most pronounced for Pb (Fig.
203 2). Dissolved Mn showed the highest concentrations near-shore with a tendency to decrease
204 gradually with depth and the distance from the Vistula River mouth. Particulate-bound Cu,
205 Fe, Mn and Ni demonstrated similar distribution profiles with lowered concentrations at SW30
206 (Cu) and SW40 (Fe, Mn, Ni) and an increase towards the coast and towards the more open
207 part of the Gulf (Fig. 3). In contrast, highest concentrations of particulate-bound Pb and Zn
208 were found in the deep zone (SW60) to decrease adjacent to the river mouth (SW10). For
209 most particulate metals lowest concentrations were recorded in May and highest
210 concentrations in June and July, except Pb whose lowest level occurred mainly in May (Fig.
211 3-4). A strong effect of site on metal concentrations was also apparent for surficial sediments
212 (Table 3) but no consistent seasonal trends were observed (Fig. 5). The concentrations of
213 Cu, Fe, Ni, Pb and Zn in the fine-grained fraction of sediments increased from the shallow
214 site SW10 up to a maximum at SW20 followed by a slight decrease towards SW40 (Cu, Fe,
215 Pb, Zn) and the deep zone (SW60, Ni). The distribution profile for Mn was distinct from other
216 metals, highest concentrations of Mn being found near-shore with a decrease towards an
217 open part of the Gulf.

218

219 3.3. Metal concentrations in clams

220 Accumulated soft tissue metal concentrations in the clam varied between sites and seasons
221 (Table 3). Copper, Fe and Zn exhibited parallel distribution profiles along the transect studied
222 with elevated concentrations in the mid-deep part of the plume and a marked tendency to
223 decrease seawards and landwards (Fig. 6), a reverse pattern being demonstrated for Ni. The
224 concentration of Fe increased slightly from the shallow-water to the deep-water zone and
225 towards an open part of the Gulf while concentrations of Mn showed maxima adjacent to the
226 river mouth and a decrease in the deep-water zone. The pattern for Pb was intermediate.

227 Accumulated metal concentrations of *M. balthica* varied significantly with season among
228 metals and in some cases also with sites, suggesting different accumulation patterns for
229 each element. Elevated levels of Cu, Pb and Zn were noticed during autumn and winter
230 (September through February) to diminish in spring and summer (March through August), the
231 pattern being most pronounced for Pb. The tissue concentration of Cu in the shallow-water
232 zone remained fairly stable throughout much of the year with only minor variations. High
233 concentrations of Mn were recorded in autumn (September and October) and spring (March
234 through May) and low concentrations occurred in winter, particularly at a depth of 10-30 m.
235 The concentration of Fe in *M. balthica* increased in spring (March and April) at all sites,
236 except SW60 where the maximum was observed already in February, followed by a sharp
237 decrease in summer and autumn (July through September). No distinct seasonal cycle was
238 evident for Ni.

239

240 3.4. Extractable metals vs. metals accumulated in *Macoma balthica*

241 Relationships between (extractable) metal concentrations in different components of the
242 environment and metal concentrations in the Baltic clam were determined, using the
243 correlation coefficient at the confidence level of 95 %, to assess potential metal sources to
244 the bivalve. Since the liability of metals and thus their potential bioavailability from sediments
245 is dependent on various sediment components, mathematical normalisation for the effects of
246 concentrations of labile forms of Fe and Mn, the percentage contribution of <63 μm fraction
247 and its organic component in surficial sediments was additionally employed to model the

248 bioavailability of sediment-bound metals. The normalisation procedure was performed
249 individually on each metal by dividing metal concentration by a respective factor. Surficial
250 sediments and TPM appear to be the main sources of metals to the facultative feeding *M.*
251 *balthica* (Table 4). Tissue Cu concentration was positively correlated to the Cu /Mn ratio in
252 sediments ($R^2=0.14$ $p<0.01$ $n=56$), suggesting that the bioavailability of Cu is controlled by
253 the amount of “reactive” Mn oxide, with increased levels of Mn tending to diminish the uptake
254 and accumulation of Cu from the substrate. Tissue concentrations of Zn were related to the
255 Zn/<63 μm fraction ratio in sediments ($R^2=0.13$ $p<0.01$ $n=56$), indicating that the fine-grained
256 fraction accounts for the bioavailability of sediment-bound Zn. Soft tissue Zn was also
257 significantly correlated with the Zn concentration in the particulate fraction in the overlying
258 water ($R^2=0.12$ $p<0.05$ $n=27$). The concentration of Mn in the clams was linearly related to
259 three different environmental metal concentrations: (1) particulate-bound fraction ($R^2=0.19$
260 $p<0.05$ $n=27$), (2) Mn in sediments ($R^2=0.42$ $p<0.001$ $n=56$) and (3) the ratio of Mn/Fe in
261 sediments ($R^2=0.38$ $p<0.001$ $n=56$) that may imply that Mn is taken up in the Baltic clam
262 through different pathways. The concentration of Pb in the bivalves was positively correlated
263 with the ratio of Pb/organic component of <63 μm fraction in sediments ($R^2=0.11$ $p<0.016$
264 $n=54$). The removal of the results for SW60 from the regression improved the R^2 value to
265 0.12 ($p<0.010$ $n=48$) so metal uptake in the deep-water part of the plume is presumably
266 distinct from that in the shallower parts and/or the bioavailability of Pb in this region can be
267 different. Nickel was the only element which exhibited a positive correlation between the soft
268 tissue metal concentration and its dissolved phase ($R^2=0.17$ $p<0.05$ $n=27$). No relation of Fe
269 in *M. balthica* with any of its environmental fractions measured was found.

270

271

272 4. Discussion

273 4.1. Bioavailability of metals

274 Bioavailable metals in the marine environment may correspond primarily to soluble phases
275 and the phases which are weakly associated with ingested particles (Rainbow & Phillips,

1993; Rainbow, 1995). These fractions represent metal forms, which can pass across permeable body epithelia or alimentary tract epithelia after digestion. The chemical nature of the metals in the environment depends greatly on external physiochemical factors and thus they are susceptible to high spatio-temporal variations. The recognition of the contamination profiles in different regions with the aim to define the local geographical variation of trace metal bioavailabilities and to assess the effects of the metals on the resident (indigenous) biota requires therefore site-specific studies. Literature addressing profiles of metal bioavailability in various geographical regions is growing as these data are a baseline for coastal monitoring programmes of metal pollution against which future changes in metal levels in the marine environment can be established (e.g. in the Hong Kong coastal waters (Blackmore, 1998), British coastal waters (Philips & Rainbow, 1993) and San Francisco Bay (Hornberger, Luoma, Cain, Parchaso, Brown, Bouse et al., 2000).

On the basis of direct measurements of three metal forms that might contribute to the total package of bioavailable metal, namely dissolved, labile particulate- and labile sediment-bound fractions, the profile of trace metal bioavailability in the Gulf of Gdansk has been established. The SW10 and SW20 were often the sites with the highest extractable trace metal concentrations (Fig. 2-5), highlighting the importance of the Vistula River as a source of metals into the Gulf. Since the Gulf receives a huge input of freshwater with the river, which drains ca 54 % of the area of Poland including the major industrial regions and cities of Katowice, Krakow and Warsaw, not surprisingly the Vistula is considered to be an important source of trace metals to the Gulf (Rainbow, Fialkowski, Sokolowski, Smith, & Wolowicz, 2004; Szefer, 2002). The concentrations of all three metal fractions at the two mid-deep sites (SW30 and SW40) were generally low with exception of particulate Pb and Zn, the levels of which were elevated. Physical processes of dilution and dispersion, that are intense in the mixing zone of fresh riverine and brackish waters, can directly explain such a distribution pattern. This is also consistent with the border of the low salinity zone and the extent of the riverine water impact based on horizontal and vertical reduced salinity to be at the distance of 3-4 km from the river mouth. At SW60 concentrations of dissolved and particulate metals

304 increased, most pronounced for Cu, Fe and Ni (Fig. 2-4), possibly due to benthic fluxes
305 through desorption from detrital and/or resuspended particles. Other mechanisms accounting
306 for enrichment of Fe and Ni include reductive dissolution of Fe oxyhydroxides resulting in a
307 release of dissolved Ni (Sokolowski, Wolowicz, & Hummel, 2001). Metal concentrations in
308 the surficial sediments at this site remained at a similar (low) level to the mid-deep sites.
309 However, regarding the high contribution of the <63 µm fraction in sediments at SW60 (on
310 average 69.4 %; Table 2), hypoxic/anoxic conditions and the presence (temporal) of
311 hydrogen sulphide, phenomena often observed in deeper parts of the Baltic Sea (Janas &
312 Szaniawska, 1996), the overall metal bioavailability at SW60 from the substratum can be
313 assessed high.

314 In addition to geographical differences in the extractable concentrations of the trace metals,
315 there were also changes in time over the period of sampling. Comparisons against future
316 data sets need therefore to allow for temporal effects (Rainbow, Fialkowski, Sokolowski,
317 Smith, & Wolowicz, 2004).

318

319 4.2. Metals in clams

320 Accumulated metal concentrations in bivalves are integrated records of the metals taken up
321 from all sources over a particular period. These concentrations are a measure of the recent
322 bioavailability of the metal and are the results of complex and interrelated hydrological,
323 physico-chemical and biological factors (Rainbow, 1996).

324 In the Gulf of Gdansk, a similar distribution pattern of tissue accumulated Cu and Zn in space
325 suggests that the accumulation mechanisms of these metals in *M. balthica* can be similar.

326 However, the highest concentrations of Cu and Zn in the clams at SW30 and SW40
327 corresponded with relatively low environmental extractable metal concentrations. Biologically
328 controlled processes likely account for the increased accumulated metal concentrations. One
329 explanation can be that they are essential elements and play an important physiological role.
330 Seasonal fluctuations of tissue metal concentrations provide further support to the regulation
331 of body concentrations of Zn but not Cu. Variations in Zn concentrations in the clams (higher

332 levels in winter and summer and lower during spring and autumn) were opposite to changes
333 in body weight and physiological state of the organism, suggesting a dilution/concentration
334 effect of body weight (Sokolowski, Bawazir, & Wolowicz, 2004). The tissue concentration of
335 Ni exhibited a reverse distribution pattern to Cu and Zn, but no clear temporal trends were
336 apparent. It is therefore possible, that Ni accumulation in the clams depends mostly on
337 environmental concentrations. The concentration of Fe in *M. balthica* increased gradually
338 along the transect studied with a maximum in the deeper parts of the plume. Iron behaviour
339 in marine bivalves is generally considered to reflect the influence of both biotic and abiotic
340 factors, a relative effect of a given factor being species-specific (Kumari, Kaisary, &
341 Rodrigues, 2006). Tissue concentrations of Mn tended to increase with depth, and from the
342 near-shore site to the deepest site, that generally agrees with a gradient of hydrological
343 parameters such as temperature, dissolved oxygen concentration and salinity in the Gulf of
344 Gdansk (Sokolowski, Wolowicz, & Hummel, 2001). Coincidentally, the tissue concentrations
345 of Mn corresponded also with its particulate-bound and sediment concentrations, indicating
346 that the metal accumulation is largely influenced by its external concentration and
347 environmental factors. The distribution profile for Pb was intermediate; increased
348 concentrations occurred at SW20 and SW30 to diminish gradually towards the deep-water
349 zone, suggesting the importance of external factors in controlling Pb concentrations in *M.*
350 *balthica*.

351

352 4.3. Routes of metal uptake

353 *Macoma balthica* lives preferentially burrowed in sediments and uses long extensible siphons
354 to vacuum up particles from the surface of the substrate or overlying water (Zwarts &
355 Wanink, 1989). The bivalve is generally considered a deposit-feeder, but is also able to filter
356 suspended material from the water column (Absil, Kroon, & Wolterbeek, 1994). Thus,
357 dissolved and particulate metals as well as metal associated with bottom sediments offer
358 potential sources of trace metals to this infaunal bivalve.

359 Copper in the bivalve tissue was correlated with the Cu/Mn ratio in sediments (Table 4).
360 Sediment Mn components are presumably modifying what Cu species is bioavailable for
361 uptake, possibly through an inhibitory effect of Mn oxyhydroxides (Bendell-Young,
362 Chouinard, & Pick, 1994). Low concentrations of Cu in *M. balthica* near-shore indeed
363 corresponded to high sediment concentrations of Mn, and elevated concentrations of Cu
364 were observed in a deep-water region of low extractable Mn in the sediments. In the deep-
365 water region, a high fine-grained fraction and organic content in sediments (Table 2)
366 apparently contributed to the increased metal accumulation in the clam (Hummel, Magni,
367 Amiard-Triquet, Rainglet, Modderman, van Duijn, et al., 1998). The importance of surficial
368 sediments as a source of Cu to *M. balthica* has also been highlighted in the North Sea
369 (Hummel, Modderman, Amiard-Triquet, Rainglet, van Duijn, Hersevoort, et al., 1997) and
370 San Francisco Bay (Cain & Luoma, 1990).

371 The accumulated tissue concentration of Zn was proportional to the Zn/<63 μ m fraction ratio
372 in sediments (Table 4), suggesting that the fine-grained fraction acts as a modifier of Zn
373 uptake, and higher tissue concentrations of Zn can be expected at sites rich in sediment-
374 bound Zn and/or poor in the <63 μ m fraction. However, an increased tissue Zn concentration
375 at SW30 and SW40 coincided with low concentrations of Zn in sediments and a decreased
376 percentage of the fine-grained fraction (correlation coefficients between tissue Zn and Zn in
377 sediments, and between tissue Zn and the percentage of <63 μ m fraction were $R^2=0.05$
378 $p<0.10$ $n=56$ and $R^2=0.02$ $p<0.27$ $n=57$, respectively). Possible explanations for these
379 contradictory findings are: (1) the bioavailability to benthic fauna of Zn from the sediments is
380 controlled by other environmental factors (e.g. by Fe oxyhydroxides) and/or (2) uptake of Zn
381 from the sediments and/or its subsequent accumulation is regulated by the organism. The
382 importance of sediment geochemistry on tissue Zn accumulation in marine bivalves is poorly
383 documented (e.g. Griscom, Fisher, & Luoma, 2000), while regulation of body Zn
384 concentrations has been shown in some but not all bivalve species (Mason & Jenkins, 1995;
385 Rainbow, 1996). Zinc in *M. balthica* from the Gulf of Gdansk was also positively correlated to
386 metal concentration in the suspended particulate matter (Table 4), providing an alternative

387 metal source (Ross, Bidwell, Williams, & Boland, 2003). The higher tissue Zn concentrations
388 indeed corresponded to higher particulate-bound Zn concentrations in the overlying water,
389 but extractable metal concentrations in the suspended particulate matter were not as high as
390 would be consistent with net Zn accumulation in the bivalves. Therefore, the clams likely
391 assimilate both particulate- and sediment-bound fractions of Zn in the southern Baltic Sea. A
392 dual dietary source of metals for Zn in the Gulf contrasts the results of Harvey and Luoma
393 (1985) who showed the relative importance of water as a dominant exposure pathway in *M.*
394 *balthica*. It is therefore concluded that partial biological regulation of the metal in the clam
395 can account for low Zn variations in the tissue, making metal soft tissue accumulated
396 concentration, to some degree, independent of its external level. This is in a good agreement
397 with the fact that Zn is an essential element and is involved in a number of metabolic
398 processes.

399 The concentration of Mn in the soft tissues of *M. balthica* correlated with extractable Mn
400 concentrations in sediments and the Mn/Fe ratio in sediments. Manganese associated with
401 surficial sediments represents a considerable source of this metal to the clams and reflects
402 the importance of Fe hydroxides in the partitioning of Mn in the sediments of the Gulf, as also
403 postulated by Szefer, Glasby, Pempkowiak, & Kaliszan (1995). An increase of Fe
404 oxyhydroxides inhibits the bioavailability of Mn from sediments by reducing its lability through
405 stronger binding to deposited particles (Gerringa, 1991). Accumulated tissue Mn
406 concentration was also correlated with the particulate-bound metal concentration, implying
407 that this phase can also contribute to Mn accumulation. Assimilation of Mn in marine bivalves
408 takes place primarily following ingestion and digestion in digestive glands where the
409 concentration of Mn can reach up to 35 $\mu\text{g dry wt}^{-1}$ (Irato, Santovito, Cassini, Piccinni, &
410 Albergoni, 2003).

411 Organic components of the sediments can be an important modifying factor for Pb
412 bioavailability and uptake by the clams, as the tissue concentration of Pb was correlated with
413 the ratio of Pb/organic component in sediments (Table 4). Thomas and Bendell-Young
414 (1998) found that the availability of Pb to two closely related clams *Scrobicularia plana* and

415 *M. balthica* was controlled mainly by metal concentration in sediments extracted with a weak
416 acid digestion. The distribution pattern of tissue Pb concentrations in the Gulf of Gdansk
417 corresponded well with the profile of extractable Pb concentrations in sediments ($R^2=0.11$,
418 $p<0.016$). Excluding the values for the deepest site SW60 from the analysis improved R^2 to
419 0.12 ($p<0.011$), indicating presumably different behaviour of Pb in sediments of the deep-
420 water region. In deeper stagnated waters of the Baltic, mineralisation of organic matter
421 through biological catalysed processes under low oxygen conditions results in hypoxia and
422 even anoxia, leading often to the production of hydrogen sulphide (Janas & Szaniawska,
423 1996). The presence of as low as 1.8% WW/WW of H_2S reduces the mobility of Pb from
424 surficial sediments and particulate matter by binding to non-reactive PbS (Bourgoin, Risk, &
425 Aitken, 1991). Reduced lability of Pb due to association with Fe oxyhydroxides in deeper
426 sediments provides another explanation as Fe oxyhydroxides inhibit Pb assimilation in some
427 sediment-dwelling bivalves such *Eliptio complanata* (Tessier, Campbel, Auclair, & Bisson,
428 1984).

429 Nickel was the only element whose concentration in *M. balthica* was positively correlated
430 with dissolved metal concentration, highlighting the importance of the dissolved fraction in
431 metal accumulation. A similar assimilation mechanism was also suggested for a typical
432 suspension feeder *Dreissena polymorpha* (Klerks & Fraleigh, 1997), although a second
433 metal uptake pathway from sediments cannot be excluded (Kumblad, Bradshaw, & Gilek,
434 2005). Uptake of Ni from solution can appear thus to be an important uptake pathway for
435 benthic bivalves. This agrees with a very low affinity of Ni for particulate matter in marine and
436 estuarine waters (Brügmann, Bernard, & van Grieken, 1992; Chiffoleau, Cossa, Auger, &
437 Truquet, 1994). Even in the presence of a high concentration of particulate matter Ni exists
438 preferentially in the dissolved phase which can contribute to the total load of the metal in the
439 water column (dissolved + 1 M HCl extractable particulate fractions) in the Vistula River
440 plume up to 96% (Sokolowski, Wolowicz, & Hummel, 2001).

441 Iron concentrations in the tissues did not correlate with any of the extractable metal
442 concentrations measured. Possible causes for a lack of any relationship would include the

443 metal origin and behaviour in the marine environment, and the functional role that iron plays
444 in organisms. In the southern Baltic, iron has a natural origin and is released to the sea by
445 natural erosion processes in the catchment area of the Vistula (Szefer, 2002; Szefer, Glasby,
446 Pempkowiak, & Kaliszan, 1995). Once in the sea, iron is very insoluble in water and exists
447 mainly in a form associated with lithogenic particles. High sediment bioavailability of Fe is
448 therefore found in regions of elevated contribution of lithogenic non-organic material. In
449 addition, Fe is an essential metal, present in bivalve's tissues primarily in a metabolically
450 available form required in cytochromes and haemoglobin of marine invertebrates (Rainbow,
451 1996) that plausibly affects its concentration in an organism. The conclusion stands out that
452 iron levels in southern Baltic are likely not in excess of metabolic requirements of the clams,
453 making its tissue metal concentrations (although variable with sites) in equilibrium with
454 environmental levels. It is therefore not surprising that separation of the routes of Fe uptake
455 in *M. balthica*, based on measurements of metal concentrations in various compartments of
456 the environment, appeared complex.

457

458

459 **5. Conclusions**

460 Measurements of dissolved metals and the easily extractable (by 1 M HCl) fraction of
461 particulate-bound metals in the overlying water and surficial sediments (< 63 µm) permitted
462 three main potentially bioavailable metal sources to be defined. Regression of metal data vs.
463 metal concentrations in the soft tissue of *Macoma balthica* enable different metal uptake
464 sources to the Baltic clam to be identified. Surficial sediments and particulate matter
465 appeared to contribute mostly in metal accumulation in *M. balthica*. Copper and Pb are
466 primarily assimilated from surficial sediments, Zn and Mn can be taken up from both
467 suspended and deposited particles, while solution is the principal source of Ni. The uptake of
468 Cu from sediments is controlled by Mn components possibly through an inhibitory effect of
469 Mn oxyhydroxides, and Pb depends on the sediment organic components. In deep-water
470 areas, the accessibility of Pb from sediments might be additionally inhibited by hydrogen

471 sulphide due to binding to non-reactive PbS. Tissue accumulation of Zn in the clams is
472 postulated to be internally regulated by the animal, making Zn tissue concentrations, to some
473 degree, independent of its environmental level. Partitioning of Mn in sediments was found to
474 be mediated by the concentrations of labile Fe, with increased levels of Fe tending to inhibit
475 the absorption of Mn by the bivalves.

476

477

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Figure Captions

- | <u>Figure</u> | <u>Caption</u> |
|----------------|--|
| Fig. 1. | Location of sampling sites (▲) |
| Fig. 2. | Concentrations of dissolved metals ($\mu\text{g l}^{-1}$) in the overlying water in the Vistula River plume, April-September 1997 (after Sokolowski, Wolowicz, & Hummel, 2001). Horizontal constant lines represent average values for all months at each site (n=5 or 6) and horizontal dotted lines represent average value after excluding the extreme data from computations (n=5). Data are presented as mean and standard error (n=3). |
| Fig. 3. | Concentrations of 1 M HCl extractable fractions of particulate-bound metals (mg dry wt ⁻¹ for Fe and $\mu\text{g dry wt}^{-1}$ for other metals) in the overlying water in the Vistula River plume, April-September 1997 (after Sokolowski, Wolowicz, & Hummel, 2001). Horizontal lines represent average values for all months at each site (n=6) and horizontal dotted lines represent average value after excluding the extreme data from computations (n=5). Data are presented as mean and standard error (n=3). |
| Fig. 4. | Contents of 1 M HCl extractable fractions of particulate-bound metals ($\mu\text{g l}^{-1}$) in the overlying water in the Vistula River plume, April-September 1997 (after Sokolowski, Wolowicz, & Hummel, 2001). Horizontal lines represent average values for all months at each site (n=6) and horizontal dotted lines represent average value after excluding the extreme data from computations (n=5). Data are presented as mean and standard error (n=3). |
| Fig. 5. | Concentrations of 1 M HCl extractable fractions of sediment-bound metals (mg dry wt ⁻¹ for Fe and $\mu\text{g dry wt}^{-1}$ for other metals) in the Vistula River plume, October 1996-September 1997. Horizontal lines represent average values for all months at each site (n=12). Data are presented as mean and standard error (n=3). |

Fig. 6. Metal concentrations in *Macoma balthica* ($\mu\text{g dry wt}^{-1}$) in the Vistula River plume, October 1996-September 1997. Horizontal lines represent average values for all months at each site ($10 \leq n \leq 12$). Data are presented as mean and standard error ($n=3$).

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Table 1. Comparison of mean (\pm 95% CL, n=3) measured in this study and certified (\pm 95% Tolerance Limits) trace metal concentrations Cu, Fe, Mn, Ni, Pb and Zn (mg dry wt⁻¹ for Fe and μ g dry wt⁻¹ for other metals) in certified reference materials (Estuarine Sediments MESS-2 and Lobster Hepatopancreas TORT-1, NRC-INMS, Canada) along with data on recovery, coefficient of variation (CV: standard deviation/average 100) and the limit of detection (LOD: the standard deviation of the blank 3).

Metal	NRC certified value	This study	Recovery (%)	CV (%)	LOD (mg l ⁻¹)
MESS-2					
Cu	39.3 \pm 2.0	41.2 \pm 0.6	105	1.5	0.064
Fe	6.22 \pm 0.31	7.14 \pm 1.27	115	17.8	0.771
Mn	365 \pm 21	327 \pm 19	90	2.5	0.236
Ni	49.3 \pm 1.8	52.8 \pm 1.9	107	7.0	0.126
Pb	21.9 \pm 1.2	26.6 \pm 0.2	122	0.8	0.183
Zn	172 \pm 16	182 \pm 17	106	1.2	0.027
TORT-1					
Cu	106 \pm 10	103 \pm 2	97	1.9	0.043
Fe	105 \pm 13	113 \pm 12	108	9.7	0.364
Mn	13.6 \pm 1.2	14.5 \pm 0.3	107	1.9	0.030
Ni	2.50 \pm 0.19	2.20 \pm 0.03	88	1.3	0.102
Pb	0.35 \pm 0.13	0.33 \pm 0.10	94	3.0	0.088
Zn	180 \pm 6	155 \pm 4	86	2.6	0.079

Table 2. The percentage contribution of the <63 µm fraction in surficial sediments (%) and the contribution of organic component in the fraction (%) in the Vistula River plume, October 1996-September 1997.

month	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	avg ± STD
Percentage contribution of <63 µm fraction													
SW10	5.1	8.5	5.5	19.0	7.1	7.6	4.7	4.6	83.7	24.1	63.9	24.6	21.6 ± 25.9
SW20	15.7	38.3	21.0	23.1	27.9	20.4	21.1	22.6	24.9	24.1	25.0	12.9	23.1 ± 6.3
SW30	2.4	3.3	1.3	4.3	6.8	6.1	3.8	5.7	4.2	5.7	4.0	3.1	4.2 ± 1.6
SW40	15.9	3.0	12.2	14.0	1.8	11.7	17.7	0.6	0.6	0.3	0.4	7.0	7.1 ± 6.8
SW60	68.0	70.5	71.4	71.4	66.4	73.4	63.1	62.0	67.8	73.4	66.4	78.9	69.4 ± 4.8
Organic component of the <63 µm fraction													
SW10	7.8	9.0	10.5	11.7	10.5	0.4	11.8	3.1	16.8	12.9	13.0	9.0	9.7 ± 4.4
SW20	12.5	11.5	4.1	10.7	11.9	5.4	11.0	7.2	10.9	10.7	8.0	8.6	9.4 ± 2.7
SW30	7.0	8.2	3.2	7.8	6.2	4.9	9.2	5.6	6.7	8.9	6.2	6.5	6.7 ± 1.7
SW40	4.2	7.5	5.5	4.0	6.1	2.1	9.9	3.2	6.2	8.4	8.8	7.1	6.1 ± 2.4
SW60	10.4	6.7	7.6	10.1	8.1	6.5	6.9	4.0	5.2	9.4	6.2	4.3	7.1 ± 2.1

Table 3. Effects of month and site on metal concentrations in the overlying water (dissolved and easily extractable particulate-bound fractions), surficial sediments (easily extractable fraction) and the soft tissues of *Macoma balthica* (two-way ANOVA; * p<0.05, ** p<0.01, *** p<0.001, blank space - not significant \pm 95 % CL). The same effects were evident for the content and the concentration of particulate-bound metals in the water.

	Dissolved		Particulate-bound		Surficial sediments		<i>Macoma balthica</i>	
	month	site	month	site	month	site	month	site
Cu			*			***	***	
Fe						***	***	**
Mn	**			**		***	***	***
Ni						***		**
Pb	*	**	*	**		*	***	
Zn	*					***	***	

Table 4. Summarised results of the linear regression between different bioavailable metal fractions in the environment and metal concentrations in the soft tissue of *Macoma balthica* in the Gulf of Gdansk, southern Baltic Sea. Sediment metals were additionally normalised for the effect of concentrations of labile forms of Fe and Mn, the percentage contribution of <63 µm fraction and its organic component (* p<0.05, ** p< 0.01, *** p<0.001, blank space - not significant ± 95 % CL, - negative relation, + positive relation)

Metal / Fraction	Dissolved	Particulate	Sediment	Normalised sediment			
				Fe conc.	Mn conc.	fraction < 63 µm	organic matter in fraction < 63 µm
Cu					**/+		
Fe							
Mn		*/+ ^a	***/+	***/+			
Ni	*/+						
Pb							*/+ **/+ ^c
Zn		*/+ ^b				**/+	

^a - refers to metal content in the overlying water (µg l⁻¹)

^b - refers to metal concentration in TPM (µg g dry wt⁻¹)

^c - calculated after removal of the result for SW60 from the regression

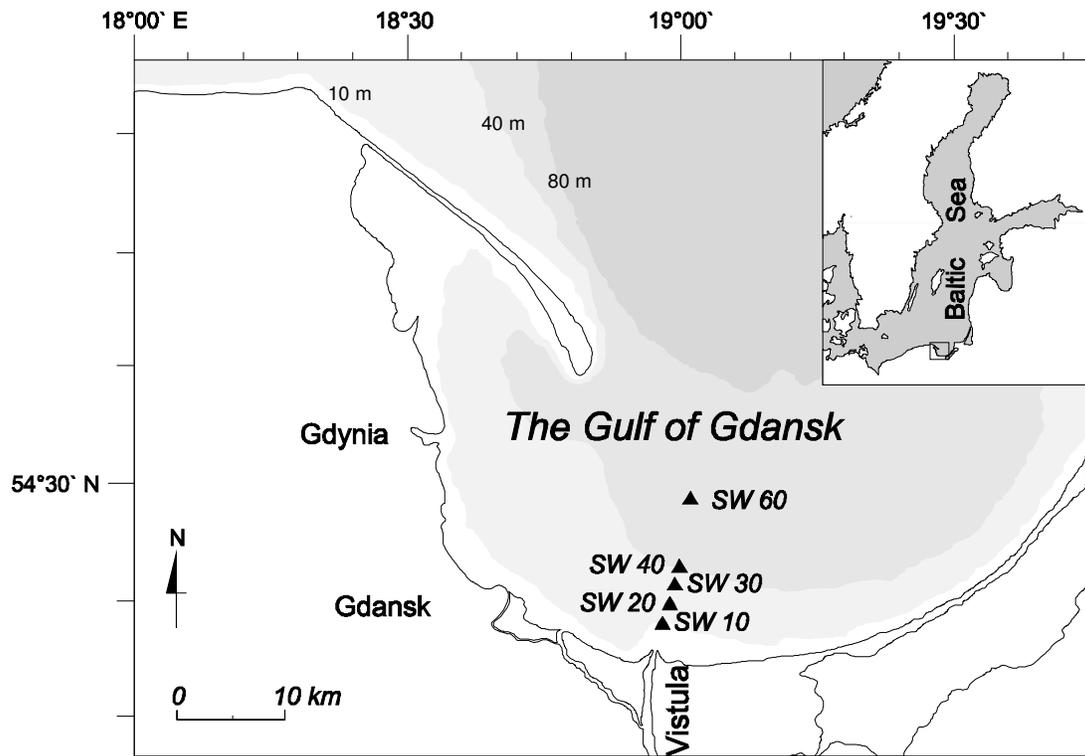


Fig. 1.

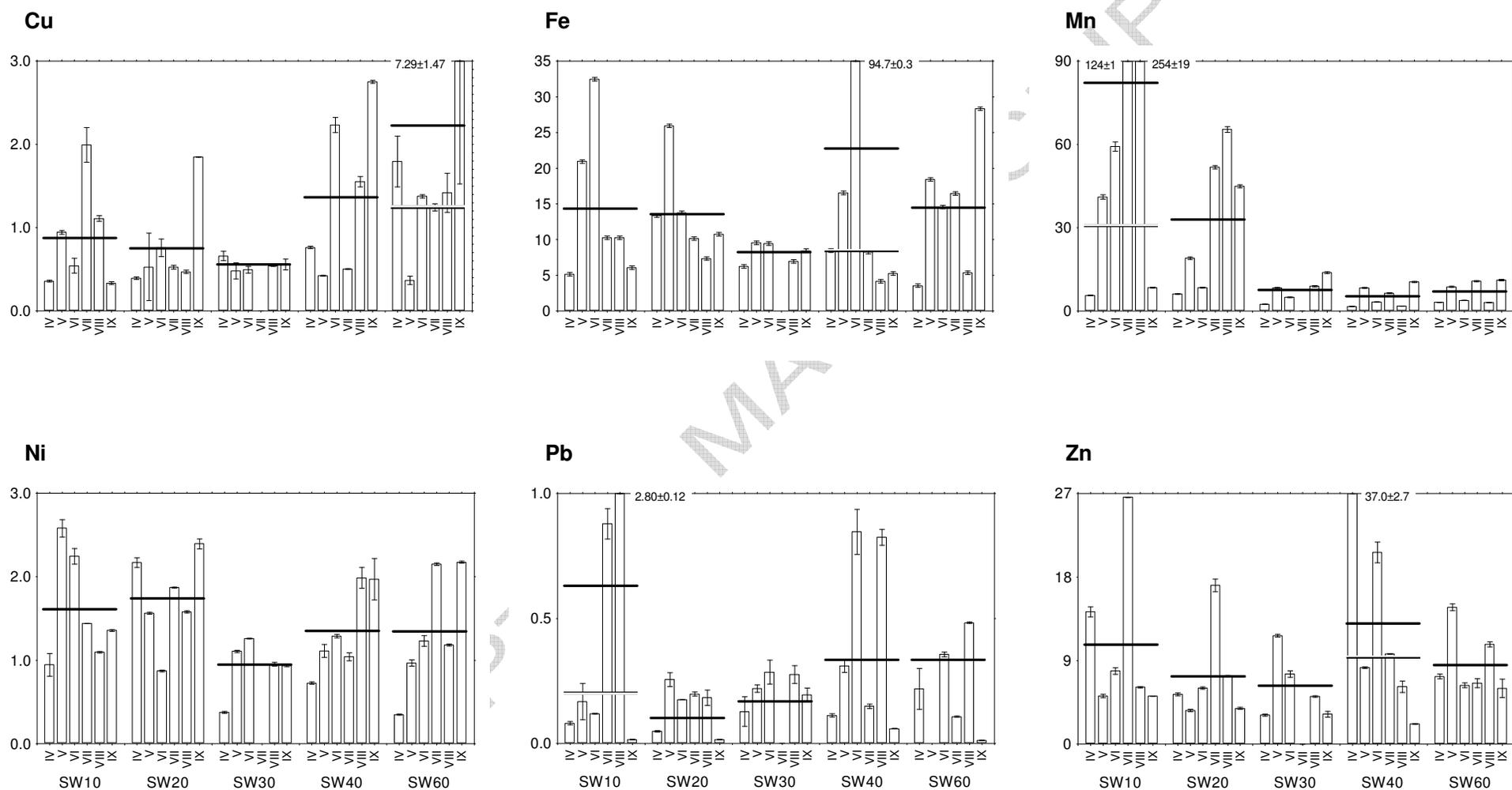


Fig. 2.

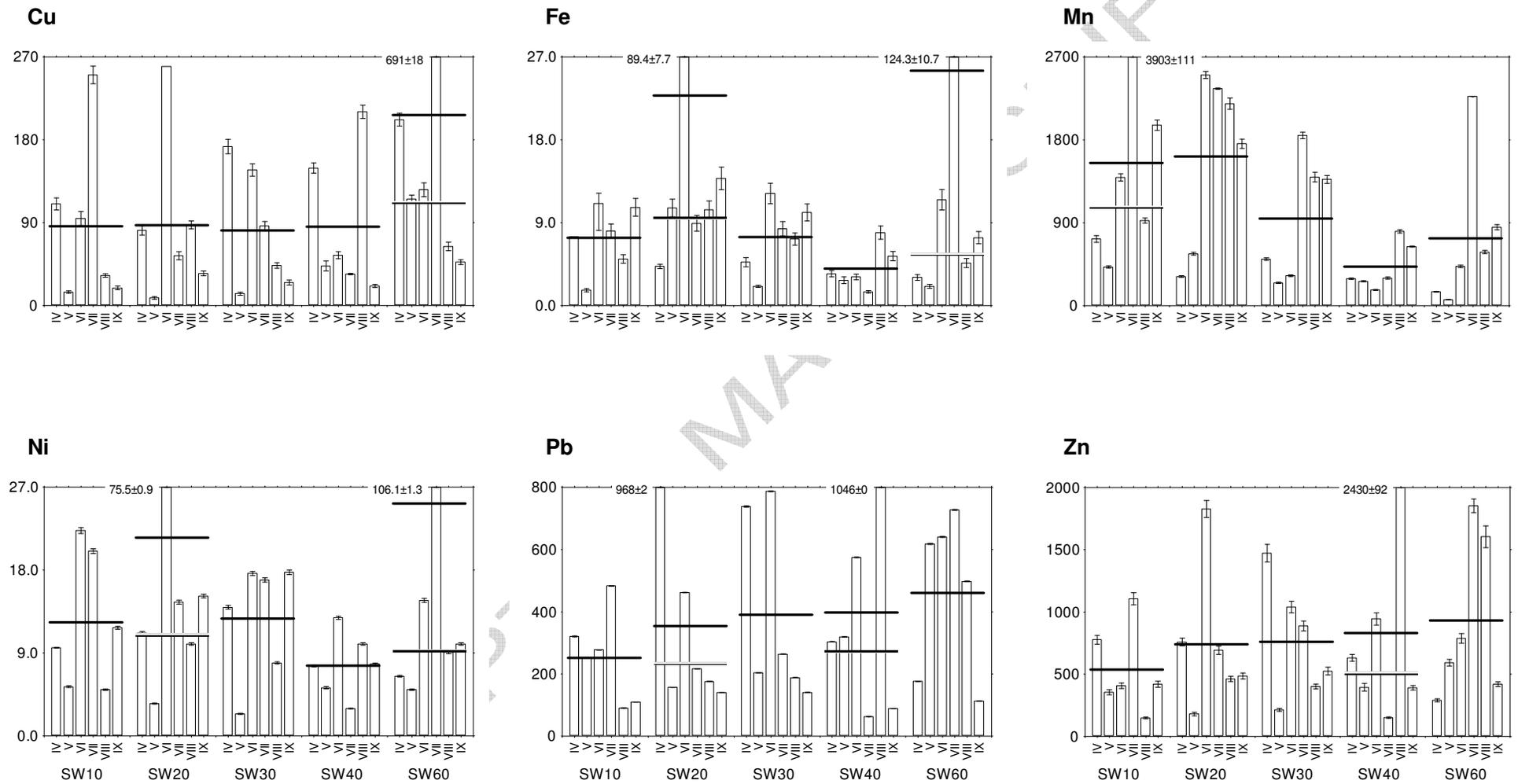


Fig. 3.

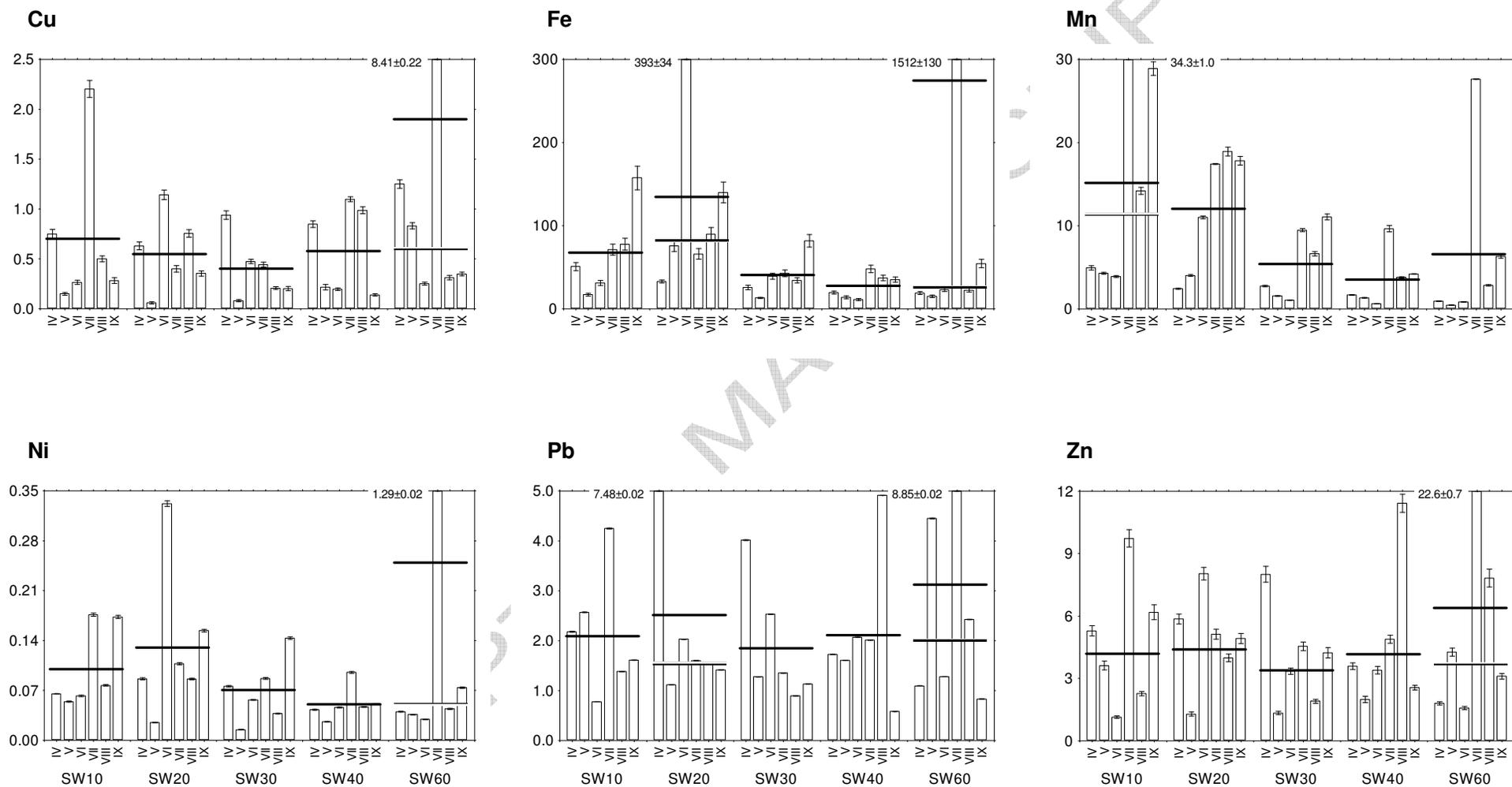


Fig. 4.

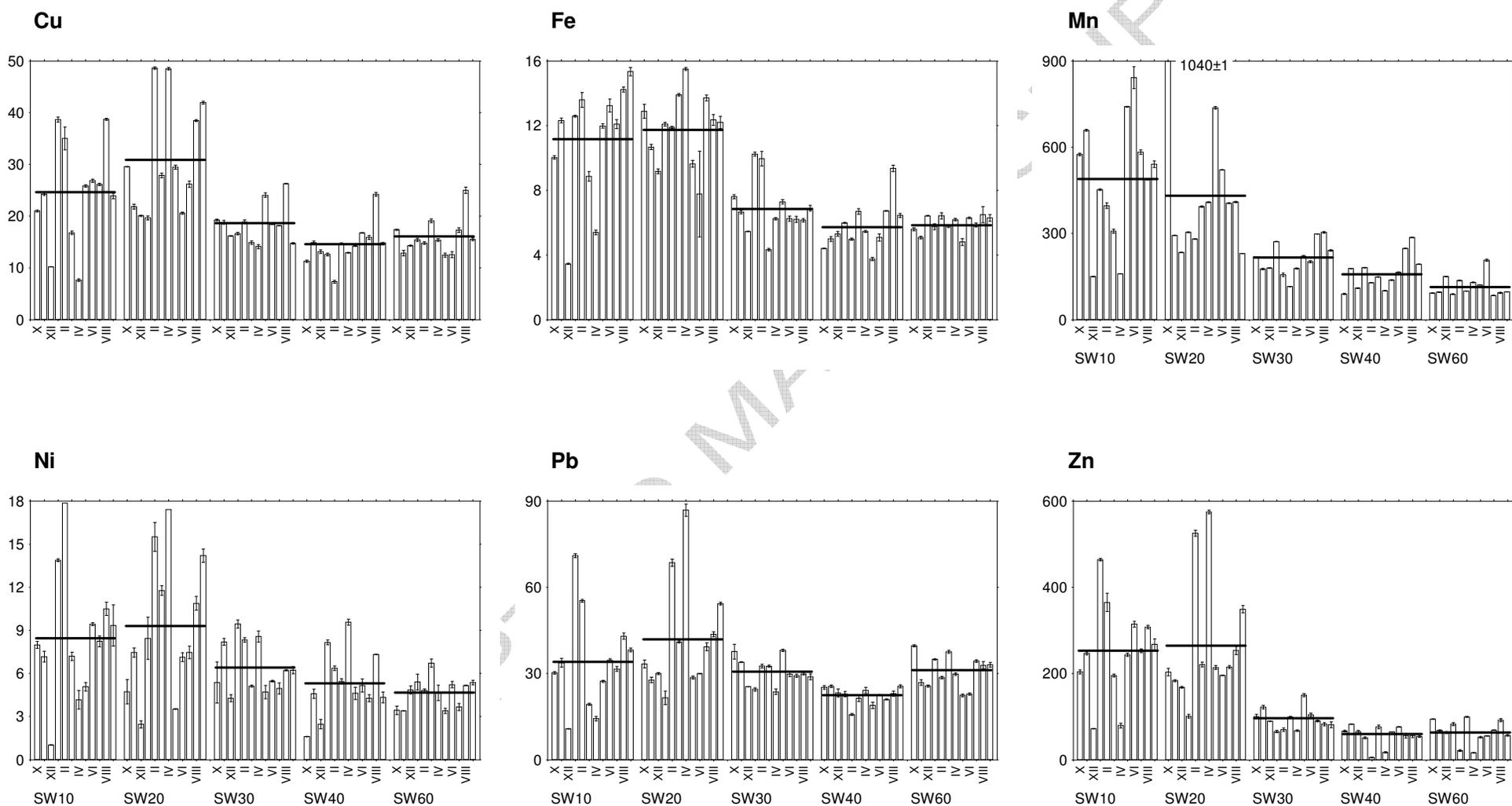


Fig. 5.

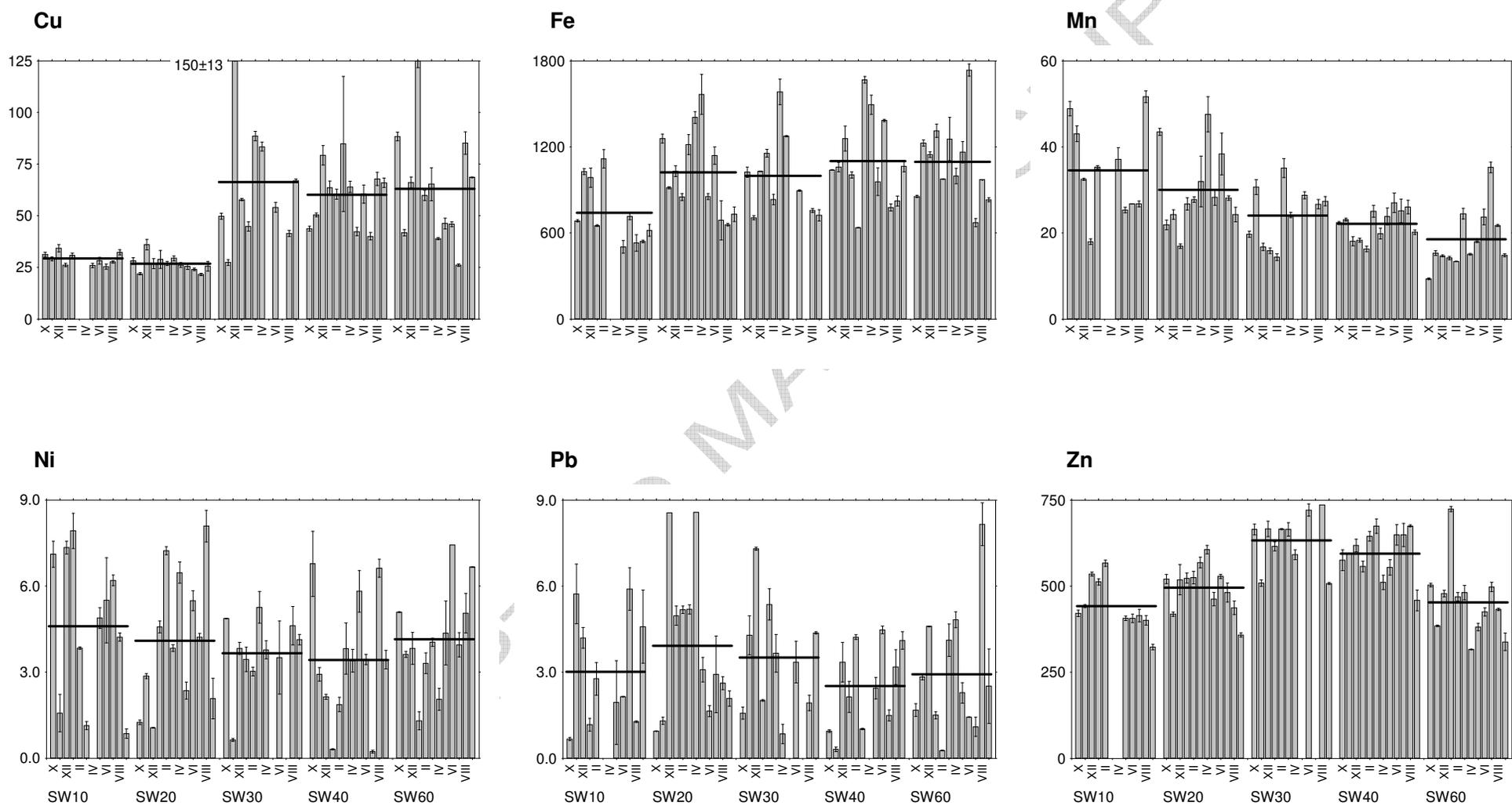


Fig. 6.