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17 **EVOLUTION OF HEAVY METALS IN MARSH AREAS UNDER RICE FARMING**

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25 **Abstract**

26 Total and EDTA-extractable concentrations of Cd, Co, Cu, Ni, Pb, and Zn in rice-farming
27 soils and their distribution in the profile depth were evaluated. The temporal variability of
28 metal concentrations in the superficial horizon was studied during 1991-1995. Results show
29 that the highest metal concentrations correspond to Zn, Pb, and Cu, for total and EDTA-
30 extractable fraction. In general, heavy metals showed an accumulation in soil surface, except
31 for Co and Ni that have their highest values in the deeper horizons. pH and organic matter
32 were the key parameters that regulate the distribution of metals in these soils. All the studied
33 metals were significantly correlated with available P, N, and sand textural fraction. The study
34 of the temporal evolution of these metals shows that the most significant variations
35 correspond to Cd, Cu, Ni, and Zn. Although Cd shows an increase, Cu and Zn tend to
36 decrease in these soils.

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39 *Keywords:* EDTA-extractable fraction, soil profile, temporal variations of metals in soils.

40

41 **INTRODUCTION**

42

43 Traditionally, the soil has been considered as a depository of unlimited quantities of human
44 and animal wastes because of its regenerative and buffer capacity. Now, it is generally agreed
45 that some pollutants (e.g. heavy metals, nitrates, phosphates, pesticides, etc.) are influencing
46 the natural equilibrium of soil functions (Batjes & Bridges, 1993), and could decrease the
47 productivity of soil and its filter and buffer capacity. This situation is especially dangerous in
48 fragile ecosystems such as marsh areas that support rice crops, which is widely used all over
49 the world giving sustenance to great part of the population, mainly in developing and
50 depressed countries.

51 In the case of heavy metals, their fate in soils would be controlled by a complex set of
52 chemical reactions and by a number of physical and biological processes acting within the
53 soil profile. Their initial mobility will largely depend on the form in which the heavy metals
54 are added; this, in turn, depends on their source (Jones & Jarvis, 1981). Once the heavy
55 metals enter the soil, they may then: (a) remain in soil solution and pass into drainage water,
56 (b) be taken up by plants growing on the soil; or (c) be retained by soil in soluble or insoluble
57 forms. Two last options are in continuous change in seasonally wetted soils, such as those
58 that support rice crops, because of the cycles of aerobic and anaerobic conditions that affect
59 the soil redox potential. The presence of sulphur, Fe and Mn oxides, and highly reduced
60 conditions favor the immobilization and precipitation of heavy metals (Evans, 1989; Bourg &
61 Loch, 1995), but when these conditions become oxidizing the heavy metal cations are
62 solubilized gradually and released to the aqueous phase. These conditions make these soils
63 more vulnerable to enhance the solubility of metals (Loch *et al.*, 1993) and to increase their
64 toxicity.

65 Moreover the study of the different forms in which metals could exist in soils, several
66 authors have noted the importance of evaluating metal movement and persistence. These

67 studies, generally, have been carried out in contaminated soils by addition of sewage sludges
68 or organic residues from agriculture or cattle raising (Sposito *et al.*, 1983; Ducaroir *et al.*,
69 1990; Li & Shuman, 1997). However, other types of additions (e.g. fertilizers, pesticides and
70 wastewater effluents) could be considered (Giusquiani *et al.*, 1992; McLaren & Ritchie,
71 1993; Gimeno-García *et al.*, 1996). All these studies show the importance of the precipitated
72 inorganic forms as mechanisms to immobilize the heavy metals, potentially toxic, in
73 agricultural soils. Heavy metal retention time in soils can reach thousands of years even in
74 sandy soils with low retentive properties (Salomons, 1993).

75 In this paper, total and EDTA-extractable concentrations of Cd, Co, Cu, Ni, Pb, and Zn in
76 rice-farming soils of Albufera Natural Park were evaluated as well as their distribution in the
77 profile depth and the influence of soil properties on distribution. Finally, the variation of
78 heavy metals concentration with time was studied during a period of 5 years (1991-1995).

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80

81 **MATERIALS AND METHODS**

82

83 **Study area and sampling**

84 The study was carried out in a rice plot of 3.2 ha, located in the north zone of the Natural Park
85 of La Albufera (Valencia, Spain). This Park covers an area of 21,000 ha, in which 12,000 ha
86 are used for rice crops. This area has undergone a long serie of ecological alterations,
87 beginning by the introduction of rice farming during the last century and going on with the
88 industrialization of the neighboring zones. By the other hand, a network of irrigation channels
89 that transport residual water from the surrounding populations and industries crosses the Park
90 and irrigates the fields. At this moment the area is undergoes a continuous change in land use
91 by drying and draining to adapt it to new types of crops, mainly orchard and citrus.

92 The studied site is typical of the rice crops of the zone. It is located in a low-lying area
93 with a high and variable water table, which appears on the surface in several points due to the
94 topography and the vicinity of the marine water interface. Soils of this zone have been
95 developed from organic black and gray silts and are affected intensely, at the surface, by
96 agricultural practices. They show, as their most important physical and chemical
97 characteristics, an impermeable profile, carbonated, with hydromorphic properties, and high
98 salinity level. According to FAO-UNESCO classification (1988), these soils belong to the
99 Calcareous-gleic Fluvisol type in saline phase. Table 1 shows some physical and chemical
100 characteristics of the studied soils.

101 The analyzed horizons are characterized by having a silt loam/clay-loam texture, alkaline
102 pH, high total carbonate levels, and the incidence of redox phenomena.

103 Random sampling was carried out at different depths according to the horizon distribution
104 and the presence of the water table in 44 points of the studied area with a total of 119
105 samples. Sampling was performed in Spring-Summer, when the soils were not flooded. To
106 study the temporal evolution of the metal, the sampling was repeated yearly from 1991 to
107 1995, in approximately the same dates.

108 After collection, soil samples were stored in polyethylene bags hermetically sealed until
109 analysis. In the laboratory, these samples were air-dried and passed through a 2-mm sieve. A
110 fraction of each sample was homogenized in an agate mortar before analysis.

111 **Apparatus and reagents**

112 All materials for analysis were previously soaked in 30% HNO₃ (v/v) for 24 hours and rinsed
113 with distilled-deionized water. All reagents were of analytical grade and were checked for
114 trace metal contamination. Deionized water was used in preparing stock solutions.

115 The digestion process for soil samples was carried out in a Tecator Digestion System 40,
116 1016 Digester (40 places for tubes of 2.5 cm diameter). The agitation of soil-extractant
117 mixtures was conducted in an automatic shaker, previously adapted.

118 Presence of metals was measured by Flame Atomic Absorption Spectrophotometry (F-
119 AAS) involving direct aspiration of the aqueous solution into an air-acetylene flame.
120 Deuterium background correction was used to measure Cu, Ni, Pb, and Zn. Absorbance
121 measurements of Cd and Co were made by Graphite Furnace Atomic Absorption
122 Spectrophotometry (GF-AAS). For Cd a standard addition technique was employed because
123 matrix interactions were observed.

124

125 **Analytical determinations**

126 Total concentration of metals was determined by a nitric-perchloric acid digestion method
127 (Andreu, 1993). This process was carried out by direct digestion of 1 g of soil sample using
128 HNO₃ and HClO₄ in the oxidation stage. The residue was dissolved in 4 ml of 6M HCl at
129 120°C, filtered and made to a volume to 50 ml with distilled-deionized water.

130 To determine the extractable fraction of metals, 4 g of soil sample were treated with a
131 solution of 0.05M EDTA at pH 7, in a modification to the procedure proposed by the
132 Agricultural Development and Advisory Service of England and Wales (ADAS, 1983).

133 Particle size analysis was performed by a pipette method (Gee & Bauder, 1986). Organic
134 matter concentration was determined by oxidation with K₂Cr₂O₇ (Jackson, 1958). Cation
135 exchange capacity was obtained by extraction with 1M NH₄Ac solution (Rhoades, 1986).
136 Aggregate stability was assessed using a wet-sieving procedure (Primo & Carrasco, 1976).
137 Total and mineral nitrogen were determined by an auto-analyzer using the Bremmer method
138 (Black, 1965). Total carbonates were measured using the Bernard calcimeter method

139 (Duchaufour, 1965). Electrical conductivity was measured in the soil saturation extract
140 (Richards, 1964). For each analytical determination, four repetitions per sample were carried.

141 Relationships between the metal levels and selected soil properties and the effect of depth
142 in the metal distribution were statistically analyzed by multiple correlations, unweighted least
143 squares linear regressions, and step-wise multiple linear regressions. Univariate and
144 multivariate analysis of variance (ANOVA) were applied. Tukey's multiple range test was
145 used at $p=0.05$ to separate means between soil horizons.

146

147

148 **RESULTS AND DISCUSSION**

149

150 **Total and extractable fractions of metals**

151 Table 2 shows the total and extractable concentrations of the studied metals in the soil
152 samples. All the values determined are, in general, within the range described by different
153 authors for agricultural soils (Jen & Chen, 1992; Crisanto & Lorenzo, 1993; Canet *et al.*,
154 1997). Only total concentration of Cu and Zn exceeded these values.

155 Total levels are lower than the maximum established by the European Union (CEC, 1986)
156 for agricultural soils, and Spanish legislation for calcareous soils. They are within the normal
157 range values in soils set by Dutch legislation and the Statens Forurensningstilsyn of Norway
158 (Reimann *et al.*, 1977)

159 EDTA-extractable fraction levels of the studied metals in the analyzed soils (Table 2) are
160 within the range determined by several authors for agricultural and prairie soils (Nielsen,
161 1988; Liang *et al.*, 1990; Sauerbeck, 1991). However, they are lower than the data reported by
162 Baraona and Romero (1997) for rural areas of North Spain.

163 The correlations between the different metals (Table 3), in the total concentrations and in
164 the extractable fraction, were studied. The most significant relationships were also
165 determined in the best fitting equations obtained by the step-wise multiple linear regression
166 analysis (Table 4). Two strong relationships are present in these soils: Cd with Pb, and Co
167 with Ni. The relationships are more significant with respect to their total concentration.
168 Davies (1997) observed similar correlations in contaminated industrial soils of Wales,
169 although the levels detected in his study were lower for Co and Ni and higher for Cd and Pb.

170 Zn shows a significant correlation with all of the studied metals in their total
171 concentration as in the EDTA-extractable fraction. Extractable Pb is significantly
172 correlated with the other metals except Ni, but it is more influenced by Cu and Zn in its
173 total concentration and in the extractable fraction. Co has a negative correlation with Cd in
174 the extractable forms.

175 A high dependence was observed between the total concentration and the
176 extractable fraction for Cu and Zn.

177

178 **Influence of soil properties**

179 Correlation coefficients of the studied metals with soil chemical and physical properties are
180 reported in Tables 5 and 6 respectively. Table 7 shows the best-fit equations for the total
181 concentration and the extractable fraction of the studied metals in relation with soil
182 characteristics.

183 All the studied metals show significant negative correlations with soil pH. Their
184 availability in soils manifests also this negative correlation. This fact has been reported by
185 several authors (Liang *et al.*, 1990; Halen, 1991; Temminghoff, 1995; McBride *et al.*, 1997).
186 These metals have also strong relationships with organic matter concentration, but more
187 significantly with their extractable forms. It has to be considered that these soils are under

188 continuous flooding during the rice grown until its harvest and completely dry the rest of the
189 year. In addition, these soils have a water table highly variable in depth. These processes
190 control the transformation of soil organic matter, which is greatly subjected to the variations
191 in the aerobic or anaerobic soil conditions (Parton, 1987; Olk *et al.*, 1996). These variations
192 influence the evolution and levels of the different forms of organic C, their solubility,
193 characteristics and the incorporation to the soil organic-mineral complex. The distribution
194 and quantities of the different organic matter forms and metabolites have an important effect
195 on the absorption and mobility of heavy metals in soils (Zachara *et al.*, 1994; Li & Shuman,
196 1997).

197 Other soil parameters highly correlated with the studied metals are the available
198 phosphorous and soil nitrogen. It is consequence of the extensive use of N and P fertilizers by
199 the farmers, usually in excess, that occurs in all the area. Electric conductivity is widely
200 correlated with the extractable forms of metals, except Cu, and the same occurs with the
201 available Ca.

202 Highly significant negative correlations are observed between metals and the percentage of
203 the different sand fractions, and between metals and the total sand concentration of these
204 soils. The most correlated sand fraction corresponds to the 0.25 to 0.10 mm of particle size. It
205 is observed for all the studied metals except for extractable Zn, which does not show any
206 correlation with the different soil textural fractions including clay. Likewise, all metals have
207 positive correlations with the silt fraction mainly in the particle size from 0.02 to 0.002 mm,
208 but for extractable Zn. Co, Cu, and Pb also showed positive significant correlations with the
209 clay concentration. These observations agree with the studies of Hlavay *et al.* (1992) and
210 Flores-Vélez *et al.* (1996) on the concentration of metals in the different soil textural fractions
211 and in dusts in different work places.

212 The good correlations between Cu and Pb and between Co and Ni, together with the
213 significant correlations between pH, organic matter, and cation exchange capacity and the
214 total concentration and the extractable fraction of these metals could indicate a similar
215 dissolution-precipitation pattern.

216 Relationships observed among metals and between metals and soil properties are,
217 generally, similar respect to their available percentage in the studied soils (Table 8).

218

219 **Metal availability for plants**

220 The proportion of the EDTA-extractable fraction relative to the total concentration of the
221 metal could be an indicator of the quantity of metal available for plants and could reflect its
222 comparative mobility (Andreu, 1993; He & Singh, 1993). In this way, the percentage of
223 extractable fraction could be used as an estimate of the comparative mobility of the studied
224 metals. According to these data (Table 8), the order of comparative mobility in these soils is:

225
$$\text{Cu} > \text{Cd} > \text{Pb} \gg \text{Zn} > \text{Ni} > \text{Co}.$$

226 This progression shows that the elements potentially most toxic to humans (e.g., Cd and
227 Pb), together with Cu, are the most mobile in these soils. However, the values for Zn and Cd
228 are lower than those obtained by Hamon *et al.* (1997) in sandy loam soils under citrus crops.
229 Co and Ni are the elements most strongly retained.

230 Highly significant correlations occurred between Cd and Co on their available percentage
231 in these soils (Table 8). Availability of Co and Pb is related directly with Ni total
232 concentration, but Zn percentage is the most influenced by Ni. Co and Ni are highly
233 correlated in availability on soils in the same way that was observed to their total
234 concentration and EDTA-extractable fraction.

235

236 **Distribution of metals in the soil profile**

237 Total Cd, Cu, and Pb show their maximum values in the superficial horizons (Table 2),
238 decreasing with depth, as it is reflected by negative correlation coefficients of their
239 concentrations with depth (Table 6). Statistically significant differences are observed for Cd
240 concentration below 85 cm depth, and Cu and Pb shows these differences below 30 cm
241 depth. Figure 1 shows the predicted evolution of these metals with depth and their best-fit
242 equation models. Cd and, more specifically, Cu and Pb are strongly retained in the first
243 centimeters of soil, possibly due to their immobilization by organic matter and phosphates
244 that are accumulated in this surface zone. This fixing effect due to organic matter phosphates
245 is in accordance with the high significant correlations coefficients obtained for these
246 parameters (Table 5). Several authors have observed that Cd tends to remain in the top few
247 centimeters of the soil, and its downward movement is very slow (Loganathan & Hedley,
248 1997). Cu and Pb show great affinity for soil organic matter (Celardin & Chatenoux, 1990;
249 Flores-Vélez *et al.*, 1996). Generally, it is accepted that organic matter complexes have an
250 important role in the mobility and availability of these metals (Alba & Chen, 1995; Herbert,
251 1997).

252 Total Co and Ni show an increase with depth, more intense in the case of Co that was
253 around 50% higher concentration in the deepest horizon than in the first 30 cm of soil. In the
254 case of Ni, this increase is lower and gradual. This course agrees with the positive
255 correlations observed for these metals with depth and with the prediction equations (Table 6
256 and Figure 1). Total Zn shows a variable course in these soils and without any statistical
257 correlation with depth. Its predicted evolution is reported in Figure 1. These observations
258 agree with those of Celardín and Chatenoux (1990) and Herbert (1997), for Swiss forest soils
259 and contaminated podzol soils. For Co, Ni, and Zn, the influence of the movement and
260 distribution of soluble organic compounds and oxides (Fe, Al and Mn), together with the

261 variations in cation exchange capacity in these soils is very important (Giusquiani *et al.*,
262 1992; Gimeno-García *et al.*, 1995; Li & Shuman, 1997). The influence of the cation exchange
263 capacity and organic matter is reflected by its significant correlation coefficients with these
264 parameters (Table 5). The organic matter concentration in the profile decreases with depth
265 and increases in the 3C horizon. This fact could be due to the ploughing in depth that these
266 rice soils receive (*fanguexat*) and to the oscillation of their water table level that promotes
267 frequent changes in the soil redox conditions.

268 The evolution of the EDTA-extractable metal fractions with depth is similar to the total
269 concentration, except for Pb and Cd, which do not show statistical differences between
270 horizons. However, an accumulation of these forms of Pb and Cd is observed within the first
271 40 cm of soil. Co and Ni have a statistically significant increase with depth, mainly in the
272 deepest horizons (>100 cm depth). This tendency in the distribution with depth of the
273 extractable fraction of Co and Ni is more accentuated than in their total concentrations. The
274 predicted evolution of these metals is reported in Figure 2. Extractable Zn also shows similar
275 variability than its total concentration, with a decrease in the zone from 30 to 70 cm and a
276 progressive increase with depth. This distribution of the extractable Zn is parallel to the
277 trends observed for the organic matter concentration, electric conductivity and total N (Table
278 1), which is correspondent to the significant correlation coefficients obtained (Table 5). This
279 course of Zn in its distribution with depth is in accordance with the studies on metals
280 availability of Benbi and Brar (1992) in semiarid calcareous soil of Punjab, and the
281 observations of Mandal and Hazra (1997) in soil under three different rice farming systems.

282 Only Cu, Ni and Zn show significant correlations with depth concerning the percentage of
283 available metal. Their predicted evolution in the soil profile and the best-fit equations
284 obtained are reported in Figure 3. However, for all the studied metals an increase of their
285 availability with depth is observed.

286

287 **Temporal evolution of heavy metals**

288 A study of the five-year period was completed. Annual samplings were performed in the
289 same places and in the same season (spring) during 1991-1995. In this period, the variability
290 of total concentration and extractable fraction for Cd, Co, Cu, Ni, Pb and Zn in the superficial
291 horizon were studied.

292 The evolution of heavy metal concentrations in time can be observed in Figure 4.
293 Generally, total and extractable concentrations of the studied metals have similar courses. Cd
294 shows the most important increase with time, whereas Ni has a slight increase until 1994.
295 This increase in time for Cd is in accordance, with the observations of Robards & Worsfold
296 (1991) in estuarine coastal systems, who reported persistence, or an increase in some cases, of
297 Cd for 2 years.

298 Cu and Zn had a clear tendency to decrease, whereas Pb showed few variations with time.
299 This result is similar to the behavior observed by Tsaplina (1993) in a study of these metals in
300 a meadow biogeocenosis. Hamon *et al.* (1997) also reported a similar tendency for Zn
301 studying the evolution in the availability of this metal for different plant species. However,
302 Co does not show a net variation, except for the intense increase of 1992. Berrow & Burrige
303 (1990), studying the persistence of metals in sewage sludge treated soils for 17 years, found
304 that the amounts of metals extracted by EDTA remained as high as 80 to 90% for Cd, Cu and
305 Zn and around 50 to 60% for Ni and Pb respect to their initial values.

306 The particular flooding/drying regime of these soils, with the variations in the redox
307 conditions, could influence greatly the temporal evolution of the studied metals in them.
308 The high levels of carbonates, the pH and the distribution of organic matter in depth could
309 also affect the adsorption/precipitation patterns of metals in these soils, and then its
310 retention and accumulation.

311

312

313 **CONCLUSIONS**

314

315 Total concentrations and extractable fractions determined in the analyzed soils are lower
316 than the values established by the European and Spanish legislation for calcareous soils.
317 Highly significant correlations have been observed between total concentration of Cd and Pb
318 and for Co with Ni. Zn shows significant correlations with total and EDTA-extractable forms
319 of all the studied metals. The percentage of the extractable fraction suggests that the highest
320 relative mobility correspond to the most toxic elements Cd, Cu, and Pb.

321 pH, organic matter, and the sand and silt fractions, mainly the particles with size of 0.25-
322 0.10 mm and 0.02-0.002 mm respectively , are the parameters with more significant influence
323 on the different metals studied. The effects of the intensive use of fertilizers are reflected by
324 the significant correlations of the metals with N and available P.

325 Total and extractable concentrations of Cd, Cu, and Pb show their maximum values in the
326 surface horizon. Extractable fraction of Co and Ni increased at the deeper horizon. Zn shows
327 a variable distribution but always increasing below 70 cm depth. It could be due to the intense
328 influence that exerts the variability of the water table and the alternation of flood and dry
329 periods. These circumstances greatly affects the distribution of organic matter and the
330 presence of its soluble forms that favors the mobility of some metals, together with the
331 variations in the redox conditions of these soils.

332 The period 1991-1995 is short to predict the evolution of heavy metals in the studied soils.
333 However, a tendency to increase has been observed for Cd, whereas Cu and Zn levels
334 decrease. Co, Ni and Pb tend to maintain these levels in these soils.

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Table 1. Physical and chemical properties of the studied soils

	^a Horizons					
	Ap1	Ap2	AB	2Bg1	2Bg2	3Cr
Depth (cm)	0-30	30-45	45-65	65-85	85-125	> 125
Water content (%)	2.69	2.01	2.68	2.14	0.94	1.01
Aggregate stability (%)	11.14	----	----	----	----	----
pH (H ₂ O)	7.53	7.78	7.80	7.60	7.70	7.50
pH (KCl)	7.15	7.14	7.07	6.87	7.20	7.10
^b E.C. (dS m ⁻¹)	2.05	1.43	1.62	2.51	2.55	3.10
^c CO ₃ (%)	31.46	34.16	37.25	19.89	29.22	34.94
^d O.M. (%)	2.07	1.24	1.22	2.35	2.07	4.07
N _{total} (%)	0.146	0.084	0.060	0.100	0.170	0.170
N _{mineral} (cmol kg ⁻¹)	4.50	2.51	0.66	0.60	1.08	1.13
P ₂ O ₅ (cmol kg ⁻¹)	14.74	5.94	1.80	1.56	0.321	n.d.
^e CEC (cmol kg ⁻¹)	15.09	12.46	17.29	23.10	20.00	23.99
Ca ²⁺ (cmol kg ⁻¹)	10.70	9.00	13.35	18.06	18.88	22.25
Mg ²⁺ (cmol kg ⁻¹)	3.39	2.70	3.24	4.23	0.69	1.04
K ⁺ (cmol kg ⁻¹)	0.50	0.41	0.37	0.33	0.11	0.09
Na ⁺ (cmol kg ⁻¹)	0.51	0.33	0.32	0.48	0.32	0.61
Particle size distribution (%)						
< 0.002 mm	25.72	26.47	32.49	34.30	65.54	35.93
0.02-0.002 mm	8.78	10.41	9.12	17.85	13.80	40.83
0.05-0.02 mm	19.50	19.77	24.38	18.44	17.17	19.74
0.10-0.05 mm	7.21	7.46	8.28	6.37	2.50	2.00
0.25-0.10 mm	18.98	19.45	6.02	8.25	0.75	0.50
0.50-0.25 mm	4.53	3.56	1.87	3.00	0.25	n.d.
1.00-0.50 mm	1.25	1.03	1.12	2.10	n.d.	0.50
2.00-1.00 mm	0.57	0.88	1.20	2.12	n.d.	0.50
Total sand (%)	32.55	32.38	18.48	21.85	3.5	3.5
Total silt (%)	41.33	40.96	48.58	43.39	30.97	60.57

471 ^a Designation of soil profile horizons according FAO (1988). ^b Electric conductivity.

472 ^c Total carbonate concentration. ^d Organic matter. ^e Cation exchange capacity

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Table 2. Total concentrations and EDTA-extractable fractions of metals in the studied soils

	Depth	Element (mg kg ⁻¹)					
		Cd	Co	Cu	Ni	Pb	Zn
Total content							
Ap1	0-30	*0.625 a	9.651 a	28.105 a	24.578 a	48.835 a	65.565 ab
Ap2	30-45	0.490 a	9.695	17.068 b	21.801 a	41.446 a	44.574 a
AB	45-65	0.632 a	9.770 a	17.268 b	25.605 a	41.868 a	57.915 ab
2Bg1	65-85	0.524 a	12.895 ab	18.062 b	29.465 a	39.776 a	67.444 ab
2Bg2	85-125	0.356 ab	19.218 b	15.755 b	34.750 a	46.274 a	80.667 b
3Cr	>125	0.228 b	17.849 b	17.073 b	36.441 a	37.427 a	82.139 b
Extractable fraction							
Ap1	0-30	0.100 a	0.088 a	5.369 a	0.616 a	5.588 a	5.195 a
Ap2	30-45	0.084 a	0.110 a	3.240 a	0.480 a	5.117 a	1.938 ab
AB	45-65	0.042 a	0.164ab	4.012 a	0.702 a	3.875 a	0.801 b
2Bg1	65-85	0.063 a	0.199 b	5.991 a	1.335 ab	4.578 a	1.911 ab
2Bg2	85-125	0.083 a	0.116 ab	3.864 a	1.420 ab	7.280 a	2.284 ab
3Cr	>125	0.078 a	0.369 c	4.227 a	2.110 b	6.884 a	4.466 a

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* Values with different letters indicate the means are significantly different at $p=0.05$ probability level (LSD) within each column.

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Table 3. Correlation coefficients ($p \leq 0.05$) between total content and EDTA-extractable fraction of the studied metals ($n=118$. NS= no significant correlation).

Total Content	Cd	Co	Cu	Ni	Pb	Zn
Cd	1.0000					
Co	NS	1.0000				
Cu	0.3722	NS	1.0000			
Ni	NS	0.8425	NS	1.0000		
Pb	0.5413	NS	0.5839	0.4734	1.0000	
Zn	0.6296	0.7059	0.4118	0.7836	0.6066	1.0000

Extractable Fraction	Cd	Co	Cu	Ni	Pb	Zn
Cd	1.0000					
Co	-0.3747	1.0000				
Cu	NS	NS	1.0000			
Ni	NS	0.4560	0.3955	1.0000		
Pb	0.8710	NS	0.4394	0.5014	1.0000	
Zn	0.7635	NS	0.6548	0.5002	0.6672	1.0000

Total Content	Extractable Fraction					
	Cd	Co	Cu	Ni	Pb	Zn
Cd	NS	NS	0.4620	NS	NS	NS
Co	0.5558	NS	NS	0.5282	0.6953	0.3273
Cu	NS	NS	0.6276	NS	NS	0.5560
Ni	0.5407	NS	0.3550	0.5696	0.7847	0.4078
Pb	0.4501	NS	0.7155	NS	0.5556	0.6642
Zn	0.6269	NS	0.5636	0.7251	0.7691	0.7694

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490 **Table 4. Best fit equations for the interactions between total content and EDTA-**
 491 **extractable fraction of the studied metals (n=118. $P \leq 0.05$).**

	Total content	R ²
Cd	$Cd = e^{(0.880 - (66.559/[Pb_{tot}])}$	0.52651
Co	$Co = -0.753 + 0.456 [Ni_{tot}]$	0.70979
Cu	$Cu = 13.213 (1.093^{[Cu_{ext}]})$	0.47531
Ni	$Ni = 4.057 + 1.535 [Co_{tot}] + 1.063 [Cu_{ext}]$	0.81534
Pb	$Pb = 19.191 + 16.274 [Cd_{tot}] + 0.897 [Cu_{ext}] + 0.193 [Zn_{tot}]$	0.69827
Zn	$Zn = 12.817 + 8.675 [Ni_{ext}] + 1.228 [Ni_{tot}] + 3.107 [Zn_{ext}]$	0.88727
EDTA-Extractable Fraction		
Cd	$Cd = 0.066 - 0.002 [Ni_{tot}] + 0.017 [Pb_{ext}] + 0.021 [Ni_{ext}] - 0.218 [Co_{ext}]$	0.87326
Co	$Co = 0.131 - 0.918 [Cd_{ext}] + 0.101 [Ni_{ext}]$	0.54889
Cu	$Cu = -2.711 + 4.271 [Cd_{tot}] + 0.145 [Cu_{tot}] + 2.291 [Ni_{ext}]$	0.70879
Ni	$Ni = -0.764 + 2.975 [Co_{ext}] + 0.019 [Zn_{tot}]$	0.71506
Pb	$Pb = 4.992 + 54.962 [Cd_{ext}] + 0.049 [Co_{tot}] + 0.302 [Cu_{ext}] + 0.022 [Cu_{tot}] + 0.024 [Pb_{ext}] + 0.642 [Zn_{ext}] + 0.064 [Zn_{tot}]$	0.92149
Zn	$Zn = -3.719 + 51.226 [Cd_{ext}] + 0.306 [Cu_{ext}] - 0.543 [Pb_{ext}] + 0.107 [Zn_{tot}] - 0.214 [Co_{tot}]$	0.91670

492 [X_{tot}] Total content of the indicated metal

493 [X_{ext}] EDTA-extractable fraction of the indicated metal

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Table 5. Correlation coefficients ($p \leq 0.05$) of the total content and EDTA-extractable fraction of studied metals with some soil chemical properties ($n=118$. NS= no significant correlation).

Total Content	W cont. ^a	pH	E.C ^b	Carbonates	O.M. ^c	CEC ^d	Mg ^e	Na ^e	K ^e	Ca ^e	Ntot ^f	Nmin ^g	P ₂ O ₅
Cd	0.6874	-0.5346	NS	0.3598	NS	NS	0.6710	0.3256	0.7643	NS	NS	0.3768	0.3757
Co	-0.5144	-0.3560	0.5777	-0.4265	0.4886	0.5051	-0.5119	NS	-0.3952	0.7152	NS	NS	NS
Cu	0.3860	-0.4544	NS	NS	NS	NS	NS	0.4894	0.4433	NS	0.6543	NS	0.7933
Ni	NS	-0.5627	0.6174	NS	0.6558	0.6860	NS	NS	NS	0.7844	0.4525	NS	0.3340
Pb	0.3410	-0.4279	NS	NS	0.5053	0.5299	NS	0.4430	0.3627	0.4250	0.4990	NS	0.5599
Zn	NS	-0.5780	0.5378	NS	0.6799	0.5294	-0.3490	NS	NS	0.6639	0.4690	NS	0.3882
Extractable Fraction													
Cd	-0.4685	-0.5562	0.4142	-0.3675	0.5090	NS	-0.3629	NS	NS	NS	0.4144	NS	0.4756
Co	NS	-0.3622	0.4258	0.4841	0.3919	0.5519	NS	NS	NS	0.4408	NS	-0.4091	-0.4168
Cu	0.3825	-0.5591	NS	NS	0.6038	0.4897	0.3746	0.5544	NS	NS	0.5093	NS	0.4089
Ni	-0.3440	-0.4810	0.7156	NS	0.7259	0.5668	NS	NS	-0.4487	0.7022	NS	-0.3983	NS
Pb	-0.4104	-0.5924	0.4807	NS	0.6114	0.3693	-0.3827	NS	NS	0.5224	0.4137	NS	0.3979
Zn	NS	-0.5846	0.4118	NS	0.5909	NS	NS	NS	NS	NS	0.5724	NS	0.6066

^a Water content. ^b Electric conductivity. ^c Organic matter. ^d Cation Exchange Capacity. ^e Cations in available form. ^f Total Nitrogen. ^g Mineral Nitrogen.

Table 6. Correlation coefficients ($p \leq 0.05$) between total content and EDTA-extractable fraction of the studied metals and soil physical properties ($n=118$. NS= no significant correlation)

Total Content	Particle size distribution (in mm)								Total sand	Total silt	Estructur alEstabili	Depth
	2.0-1.0	1.0-0.5	0.5-0.25	0.25-0.10	0.10-0.05	0.05-0.02	0.02-0.002	<0.002				
Cd	NS	NS	NS	-0.4517	NS	-0.3223	NS	NS	-0.3391	0.3334	0.3225	-0.3360
Co	-0.4694	-0.4824	-0.6016	-0.4602	NS	NS	0.7206	0.6105	-0.5520	NS	NS	0.5252
Cu	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.4889	-0.4035
Ni	-0.3489	-0.3618	-0.7332	-0.6644	NS	NS	0.6513	0.6696	-0.6916	0.4888	NS	0.3981
Pb	NS	NS	-0.5560	-0.6456	NS	NS	NS	0.5335	-0.5931	0.4588	0.6952	NS
Zn	NS	NS	-0.6095	-0.4811	NS	0.5463	0.4200	0.4803	-0.5377	0.4330	0.3793	NS
Extractable Fraction												
Cd	-0.4038	NS	NS	NS	0.3795	NS	0.3659	NS	NS	NS	0.3257	NS
Co	0.4317	0.3351	-0.3361	-0.4567	NS	NS	0.4244	NS	-0.4265	0.4154	NS	0.6376
Cu	NS	NS	-0.4178	-0.4781	NS	NS	NS	NS	-0.4261	0.4495	0.5265	NS
Ni	NS	NS	-0.5227	-0.4486	NS	NS	0.7215	0.4107	-0.4765	0.4016	NS	0.5627
Pb	-0.4159	-0.4030	-0.5436	-0.4080	NS	NS	0.5615	0.3983	-0.4274	0.3275	NS	NS
Zn	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.6596	NS

Table 7. Best fit equations for the interactions between the studied metals and some soil physical and chemical properties (n=118. p=0.05).

Total content		R ²
Cd	Cd= 3.396-0.423[pH]-0.225[Na]+0.065[K]	0.73217
	Cd= 1.072-0.008[SAf]-0.010[SIC]-0.004[Depth]	0.54915
Co	Co= 5.809-3.569[W]+14.106[K]+0.606[Ca]	0.85332
	Co= -5.224-1.232[SAvC]+0.284[Sif]+0.157[SAt]+0.318[Clay]	0.80091
Cu	Cu= 13.543+4.430[W]-20.560[K]+0.785[P]	0.73687
	Cu= 17.386+4.437[ES]-0.338[ES] ² +0.007[ES] ³	0.44625
Ni	Ni= 11.672-0.664[CEC]+1.762[Ca]+0.381[P]	0.82580
	Ni= 43.707-2.387[SAm]+0.325[Sif]-0.331[SIt]	0.66073
Pb	Pb= 27.865+0.778[CEC]+0.474[P]	0.57607
	Pb= 45.104-0.307[SAf]+0.804[ES]	0.82950
Zn	Zn= 32.391+6899[OM]-3.752[Mg]+1.591[Ca]+0.931[P]	0.78055
	Zn= 70.795+1.050[ES]-4.458[SAm]	0.49150
Extractable Fraction		
Cd	Cd= 0.996-0.021[W]-0.116[pH]+0.002[P]	0.66266
	Cd= -0.005+0.003[ES]-0.023[SAvC]+0.009[[SAvf]+0.002[Sif]	0.73317
Co	Co= -0.059+0.003[CO ₃ ⁻]+0.032[OM]+0.004[CEC]-0.004[P]	0.65972
	Co= -0.270+0.048[SAC]+0.002[SAf]+0.006[SIt]+0.002[Depth]	0.80704
Cu	Cu= -0.061+0.644[W]+1.693[OM]	0.52180
	Cu= 4.710+0.171[ES]-0.064[SAf]	0.46620
Ni	Ni= 0.231+0.133[EC]+0.370[OM]-0.563[Na]-0.075[Nm]	0.83470
	Ni= 0.259+0.042[Sif]	0.52049
Pb	Pb= 106.891+2.775[OM]-12.718[pH]-0.673[Mg]-	0.83077
	83.539[Nt]+0.235[P]	0.38741
Zn	Zn= -0.993+1.653[OM]+0.139[P]	0.52357
	Zn= 1.894+1.478[ES]-0.177[ES] ² +0.005[ES] ³	0.73288

[Na] Available Na (cmol kg⁻¹). [K] Available K (cmol kg⁻¹). [Depth] Soil depth (cm). [Ca] Available Ca (cmol kg⁻¹). [SAt] Total sand fraction (%). [Clay] Total clay fraction (%). [SAm] Medium sand (%particle size 0.50-0.25 mm). [SIt] Total silt (%). [SAvf] Very fine sand (%particle size 0.10-0.05 mm). [CO₃⁻] Total carbonates (%).

Table 8. Percentage of available metals (Extractable/Total Ratio in %) and the interactions between them and with soil properties

	Depth	Cd	Co	Cu	Ni	Pb	Zn
Ap1	0-30	*16.00 ab	0.91 a	19.10 a	2.51 a	11.44 a	7.92 a
Ap2	30-45	17.14 ab	1.13 a	18.98 a	2.20 a	12.35 a	4.35 ab
AB	45-65	6.65 a	1.68 a	23.23 a	2.74 a	9.25 a	1.38 b
2Bg1	65-85	12.02 ab	1.54 a	33.17 a	4.53 ab	11.51 a	2.83 ab
2Bg2	85-125	23.31 ab	0.60 a	24.52 a	4.09 ab	15.73 a	2.83 ab
3Cr	>125	34.21 b	2.07 a	24.76 a	5.79 b	18.39 a	5.44 ab
						R ²	SF
Cd	Cd= 26.211-6.957[W] +62.469[Nt]					0.84432	0.0000
	Cd= -11.597+0.419[SAf]+0.737[SIf]+0.705[SIc]					0.64693	0.0000
	Cd= 25.563+123.508[Cd _{ext}]-27.168[Cd _{tot}]-1.959[Co _%]					0.84988	0.0000
Co	Co= 0.840+0.448[Mg]-0.167[Nm]-0.038[P]					0.89843	0.0000
	Co= 0.904+0.644[SAvc]					0.57310	0.0000
	Co= 2.295-0.335[Cd _%]-12.001[Cd _{ext}]+0.047[Cu _%]+0.209[Ni _%]+ +0.080[Pb _%]-0.069[Ni _{tot}]					0.90247	0.0000
Cu	Cu= 1.643[CEC] ^{0.907}					0.82606	0.0000
	Cu= 1.64(0.967 ^[SAf])					0.73461	0.0000
	Cu= -0.252+4.280[Co _%]+1.546[Cu _{ext}]+0.719[Pb _%]					0.78064	0.0000
Ni	Ni= 1.170-2.740[Nm]+1.281[OM]					0.56044	0.0000
	Ni= 0.458+0.976[SAvc]+0.124[SIf]					0.57324	0.0000
	Ni= -0.064+1.612[Co _%]-10.985[Co _{ext}]+0.034[Cu _%]+3.071[Ni _{ext}]+ +0.256[Zn _{ext}]-0.416[Cu _{ext}]					0.97841	0.0000
Pb	Pb= 181.818-21.847[pH]+0.253[P]-64.165[Nt]+0.489[CEC]-2.673[W]					0.77282	0.0000
	Pb= 8.079-1.413[SAvc]+0.375[SIf]					0.49525	0.0000
	Pb= 10.729-0.076[Cd _%]+0.111[Ni _{tot}]+2.168[Pb _{ext}]-0.272[Pb _{tot}]					0.99288	0.0000
Zn	Zn= 46.258+0.565[EC]-5.781[pH]					0.69364	0.0000
	Zn= 3.069+1.921[ES]-0.201[ES] ² +0.005[ES] ³					0.61348	0.0000
	Zn= 3.134+0.158[Ni _{tot}]+1.270[Zn _{ext}]-0.111[Zn _{tot}]					0.93219	0.0000

* Values with different letters indicate the means are significantly different at $p=0.05$ probability level (LSD) within each column.

[X_%] Percentage of metal in available form.

[W] Soil water content (%). [Nt] Total nitrogen (%). [SAf] Fine sand (%particle size 0.10-0.25 mm). [SIf] Fine silt (%particle size 0.02-0.002 mm). [SIc] Coarse silt (%particle size 0.05-0.02 mm). [Mg] Available Mg (cmol kg⁻¹). [Nm] Mineral nitrogen (cmol kg⁻¹). [P] P₂O₅ (cmol kg⁻¹). [SAvc] Very coarse sand (%particle size 2.0-1.0 mm). [OM] Organic matter content. [CEC] Cation exchange capacity. [EC] Electric conductivity (dS m⁻¹). [ES] Structural stability of aggregates (%).

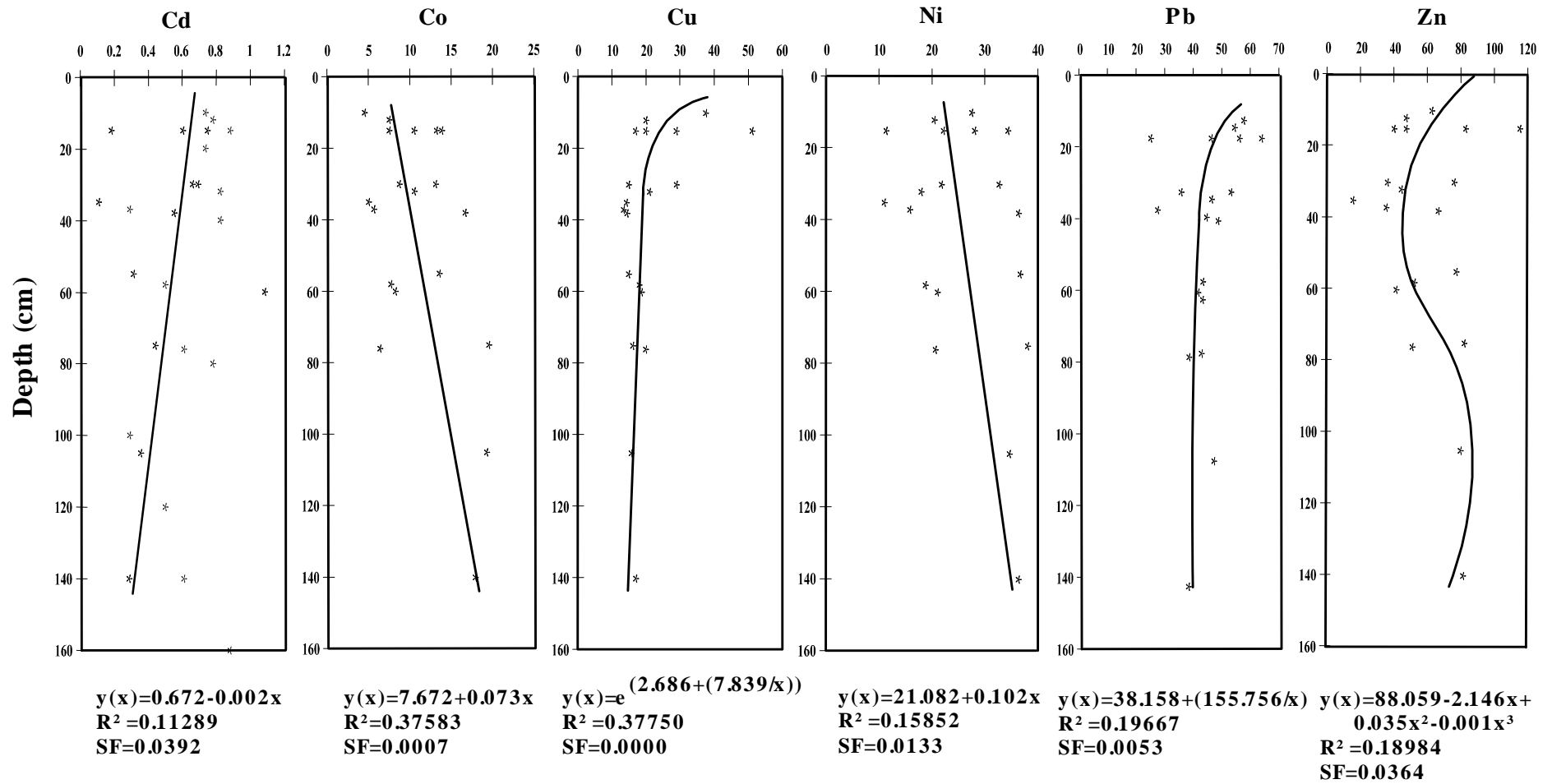


Figure 1. Predicted evolution and best-fit equations of the total content of the studied metals (mg kg⁻¹) with depth.

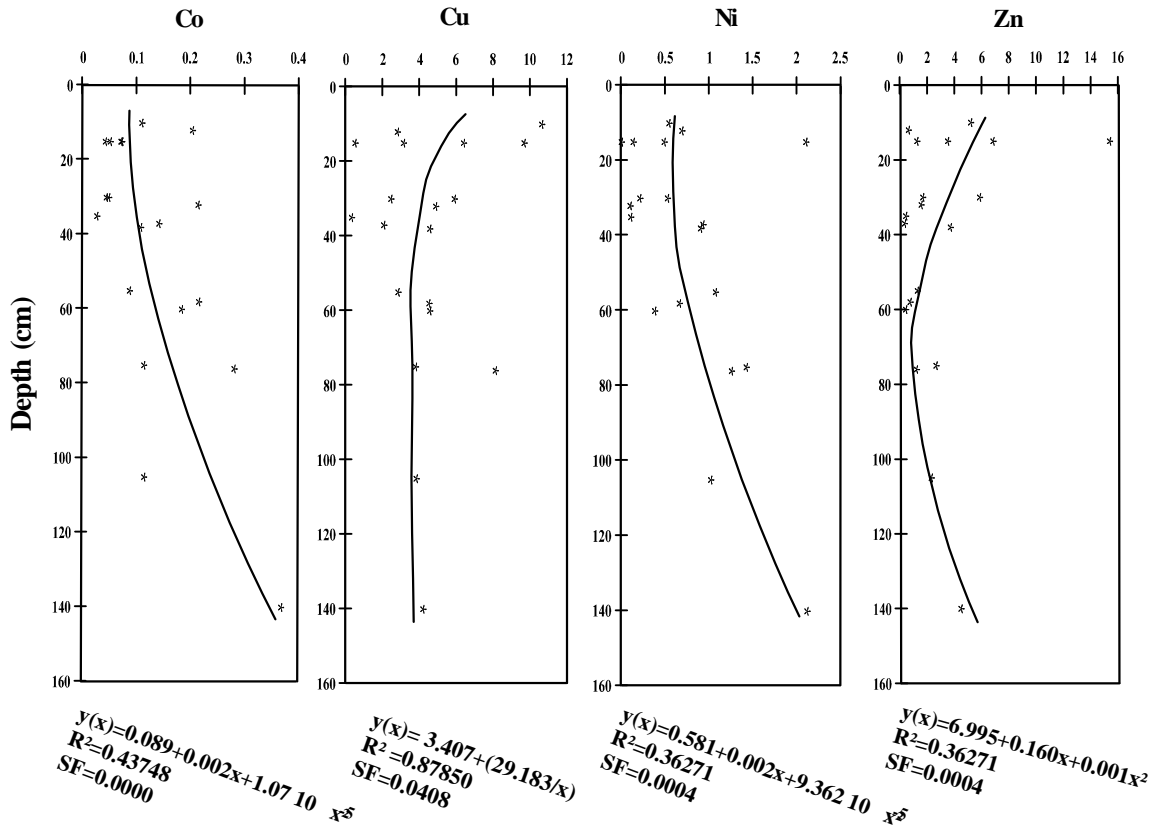


Figure 2. Predicted evolution and best-fit equations of Co, Cu, Ni and Zn extractable fraction (mg kg⁻¹) with depth.

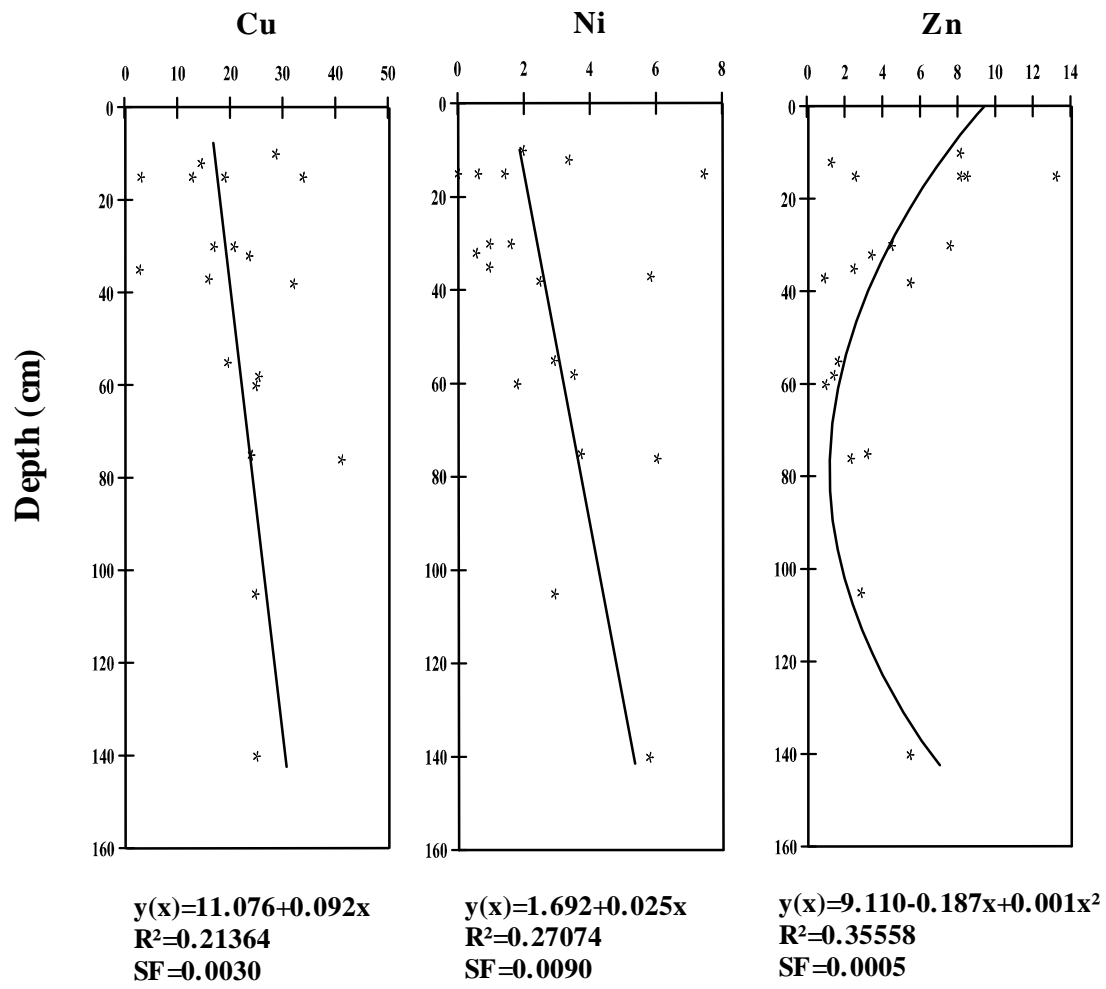


Figure 3. Predicted evolution and best-fit equations of the available percentage of Cu, Ni and Zn with depth.

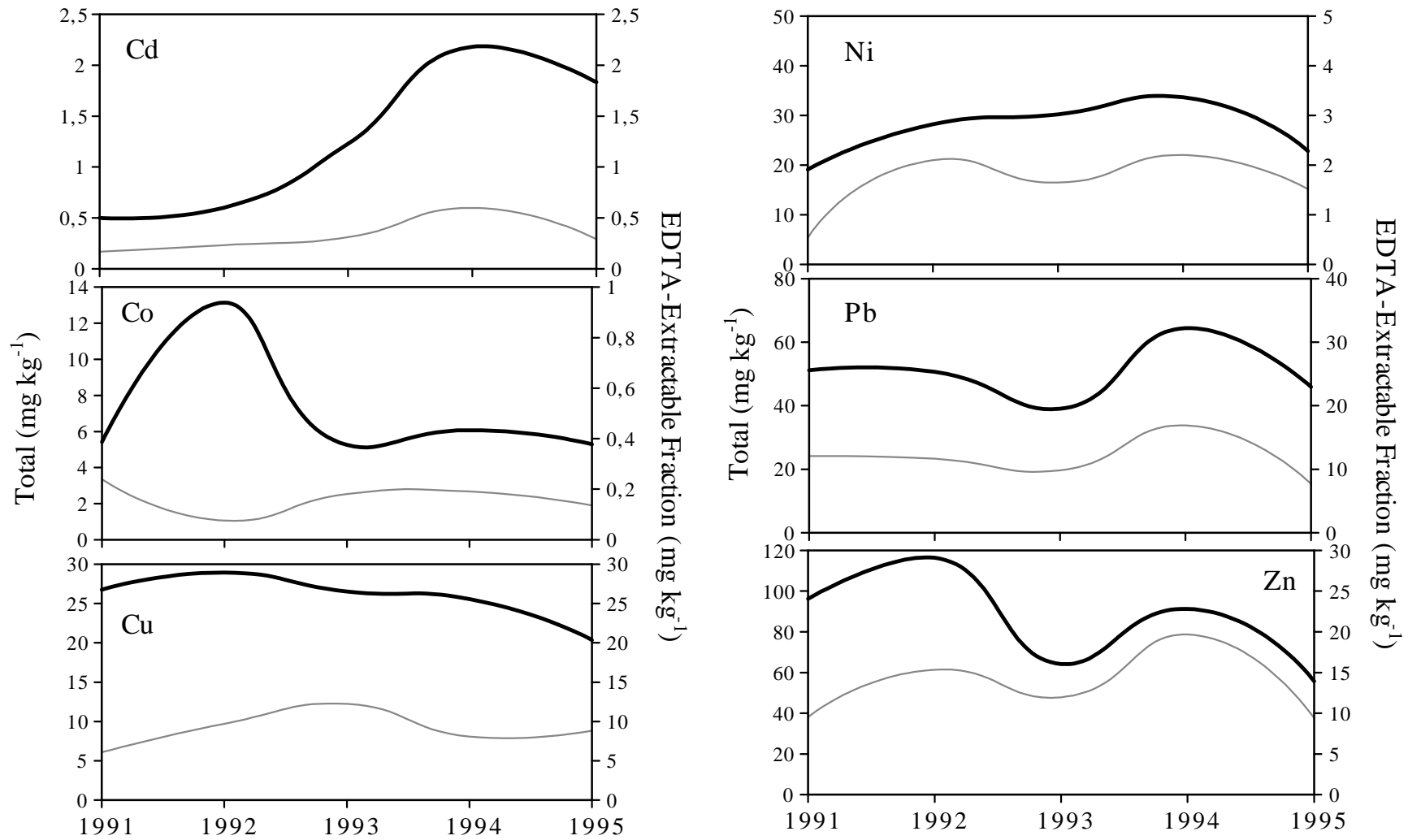


Figure 4. Evolution of total content (□, black line) and extractable fraction (, grey line) of the studied metals between 1991 and 1995.

