Manganese and Zinc in Acidic Agricultural Soils From Central Spain: Distribution and Phytoavailability Prediction With Chemical Extraction Tests

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Abstract: The extractability and distribution of manganese (Mn) and zinc (Zn) were evaluated in acidic agricultural soils from Central Spain. Both single (0.1 M hydrochloride [HCl] and 0.05 M ethylenediaminetetraacetate [EDTA]) and sequential extraction procedures (SEP) (modified Tessier procedure and Community Bureau of Reference [BCR] protocol) were applied to 29 representative soils that belong to the Alfisol, Inceptisol, and Entisol orders. Average relative Mn extractabilities with respect to the total content (16.6% for HCl and 31.9% for EDTA) were higher than those of Zn (7.7% for HCl and 6.5% for EDTA). Manganese was mainly released in the oxide-bound phase of both SEP (33.1% for modified Tessier and 48.9% for BCR), whereas Zn was predominantly found in the residual fraction (49.1% for modified Tessier and 31.4% for BCR). Significant correlations were only found between the amounts of extractable Zn and the oxide-bound fraction in both SEP. Few relationships were established between Zn fractions extracted by the BCR procedure and those obtained with the Tessier method. Both metal concentrations in spring barley (*Hordeum vulgare* L., cv Beka) grown in 11 selected soils and the calculated soil-plant transfer coefficients (soil-plant concentration factor (CF), mean values of 31.2 for Mn and 196 for Zn) were poorly (or not at all) correlated with the different extracted soil fractions (single and sequential). A number of empirical equations have been obtained by regression analyses to predict the Mn and Zn uptake by barley, with soil metal forms and some soil characteristics as components (pH for Mn and organic matter for Zn). Values of R^2 in the equations were relatively low (<68%). Single-extraction techniques produced worse results than SEP for the evaluation of Mn phytoavailability. The modified Tessier procedure provided better predictions of Zn uptake by plants than BCR, but not better than those obtained with the HCl extraction method.

Key words: Availability, fractionation, micronutrient, Alfisol, Inceptisol, Entisol

Knowledge of the distribution of trace elements in soils and their availability to plants is important for maintaining and improving crop production and food quality. Several of these trace elements are micronutrients required in small amounts by plants or animals for normal nutrition and health (Wu et al., 2006). Manganese (Mn) and zinc (Zn) play important roles in plants, and deficiencies in soils may markedly limit the production of cereals (Cakmak et al., 1999; Jiang, 2005). The distribution of metals among soil components is important for

assessing the potential of soils to supply sufficient micronutrients for plant growth and retain toxic quantities of metals (Adriano, 2001).

The quantification of chemical forms is normally based on the use of chemical reagents, often involving sequential extractions (Bermond, 2001; D'Amore et al., 2005). Among the proposed sequential extraction procedures (SEP), there are some that are widely used, particularly to deal with sediments and polluted soils (Li and Shuman, 1997; Ma and Rao, 1997; Gleyzes et al., 2002; Kubová et al., 2004; Guo et al., 2006). However, depending on the objectives of the analyses, it is also interesting to apply leaching tests based on single extractions to considerably reduce the amount of experimental work required (Perez-Cid et al., 2002). Different solutions have also been used to extract micronutrients from agricultural soils by means of a single extraction procedure. These solutions must be capable of removing the chemical forms of soil microelements that are available to plants through processes that increase their solubility, for example, lowering pH or adding chelating agents. The extracting solutions currently used therefore tend to contain a chelating agent, such as ethylenediaminetetraacetate (EDTA) or diethylenetriaminepentaacetate (DTPA), or an acid reagent such as dilute hydrochloric acid (HCl), acetic acid (HOAc), or Mehlich 1 (Lindsay and Norvell, 1978; Zhu and Alva, 1993; Jones, 2001; Wang et al., 2004; Meers et al., 2007; Menzies et al., 2007).

In the literature covering the sequential chemical extraction of metals, there is great diversity in reported SEP values. The most widely applied SEP is based on the original work on sequential extraction proposed by Tessier et al. (1979), which allows the separation of extractable metals into four fractions: exchangeable, acid soluble, reducible, and oxidizable. Although this SEP and its modifications were initially developed for sediments, they have often been applied to soils (Lopez-Sanchez et al., 1993; Barona and Romero, 1996; Abollino et al., 2002; Su and Wong, 2003; Yu et al., 2004; Inaba and Takenaka, 2005; Hseu, 2006). The advantage that all of these methods offer when they are applied to agricultural soils is that they separate the exchangeable fraction (extracted with 1 M $MgCl_2$) that is usually associated with the absorption by plants. In an account of the harmonization of extraction techniques, the European Community Bureau of Reference (BCR), now known as the Standards, Measurements and Testing Programme of the European Commission, Rauret et al. (1999) established a common procedure (the BCR method) for studying the fractionation of trace elements and heavy metals in environmental solid samples (sediments, soils, waste residues, etc.). The BCR SEP allows the separation of extractable metals into three (acid soluble, reducible, and oxidizable) fractions. This extraction procedure has been applied to soils by several authors (Mossop and Davidson, 2003; Sutherland and Tack, 2003; Kubová et al., 2004; Zemberyova et al., 2006).

Iron (Fe) and Mn oxides are known to be important soil components that control the retention of trace metals in soils (Jeanne, 1968; Alloway, 1995; McBride, 1994; Cambier and

Charlatchaka, 1999; Cornu et al., 2005). Some researchers therefore prefer to use longer and more complex SEP than Tessier or BCR for soils (Shuman, 1985; Mandal et al., 1992; Ma and Uren, 1997; Krishnamurti and Naidu, 2002; Arias-Estevéz et al., 2007). These longer and more complex methods allow the possibility of differentiating various oxide forms within the reducible fraction: easily reducible (Mn oxides); moderately reducible (amorphous Fe oxides); and poorly reducible (crystalline Fe oxides) forms. These amorphous and microcrystalline structures are both sources and sinks for metals in the natural environment and contribute to a *dynamic* trace element fraction in the soil (Tack et al., 2006).

The SEP methods should not be universally applied to all soils but need to be evaluated on a site-specific basis for each soil (La Force and Fendorf, 2000). Even so, standardization of these procedures is the only way to achieve comparability when using sequential extractions (Quevauviller, 1998). The use of uncomplicated and widely applied methods, such as Tessier or BCR, could be convenient from an operational point of view and could help to promote the harmonization. A trend toward the extension of the use of these methods has been observed in the last few years.

Metal phytoavailability in soils depends on factors such as soil properties and plant species (Soon and Bates, 1982; Sauerbeck and Hein, 1991; Davies, 1992; Smith, 1994; Fageria et al., 2002; Hough et al., 2003; Tazisong et al., 2004). Many experiments have been performed to determine how soil parameters influence the transport of trace metals within soil-crop ecosystems and to establish relationships between the behavior

of metals, soil properties, and the results of single- or multiple-extraction tests (Jeng and Singh, 1993; Adriano, 2001; Adamo et al., 2003; Pueyo et al., 2003). These relationships can be used to predict the ability of crops to extract metals from the soil. Several studies have shown that the phytoavailability of metals is specially related to soil pH, suggesting that this characteristic might improve estimates of soil-available micronutrients (McBride et al., 1997; Miner et al., 1997; Krishnamurti and Naidu, 2002; Watmough et al., 2005; Wu et al., 2006). In acidic soils, fundamental chemical properties for plant nutrition (e.g., cation exchange and buffer capacity) are largely governed by organic matter content (Moody et al., 1997). In cereal-growing areas of Central Spain, soil organic matter levels are typically below 10 g kg^{-1} (Monturiol and Alcalá, 1990), and when soils are also acidic, micronutrient deficiencies are very likely. Much work still remains to be done with agricultural soils to develop a better knowledge of extraction procedures and to improve the understanding about micronutrient limitations and trace metal toxicity. This work will then help to improve the predictions for different kinds of soils, metals, and plants.

The main aims of the present study, which was performed on 29 typical uncontaminated soils from Central Spain (acidic with low organic matter content), were (i) to apply two sequential and two single chemical extraction procedures to elucidate the Mn and Zn distributions and the potentially available quantities of both metals in this type of soil (The two SEP applied were a modification of the Tessier SEP and the protocol designed by the BCR. The single procedures were the one proposed by the BCR that uses 0.05 M EDTA as reagent and

FIG. 1. Geographical location and soil classifications of the 29 acidic soils in the Madrid region (Spain) used for the study.

TABLE 1. Selected chemical and physical properties of the soil samples (range, mean, and median values)^a

Soil properties ^b	Range value	Mean value	Median value
pH _w (1:2)	5.19–6.88	6.11	6.07
Clay, g kg ⁻¹	82–429	211	202
EC, mS cm ⁻¹	15.3–176	55.8	40.5
CEC, cmol _c kg ⁻¹	0.85–27.7	12.7	13.4
Available P, mg kg ⁻¹	5.91–86.3	42.5	41.5
OC, g kg ⁻¹	1.28–9.98	4.01	3.65
N, g kg ⁻¹	0.30–1.40	0.74	0.80
Total Mn, mg kg ⁻¹	61.4–496	155	135
Total Zn, mg kg ⁻¹	8.16–28.2	16.4	13.4

^aData from Obrador et al. (2007).

^bEC: electric conductivity; P: extractable P.

another that extracts metals with 0.1 M HCl), (ii) to study the influence of different soil properties, (iii) to evaluate the ability of the four methods applied to predict Mn and Zn uptake by a greenhouse barley crop, and (iv) to compare the results obtained with findings from other chemical extraction methods and with those for other types of soil (Alvarez et al., 2006; Obrador et al., 2007).

MATERIALS AND METHODS

Study Area and Soil Samples

The study was carried out in the Madrid region of Central Spain (Fig. 1). Samples from the surface horizons (0–20 cm) of 29 acidic cultivated soils from this area were used for the study. These soils were characterized in previous work using standard analytical determinations (Obrador et al., 2007). The study zone is a typical cereal-growing area. All of the soils studied have been subjected, at the surface, to intensive agricultural practices. As shown in Table 1, most of the characteristics of the selected soils (Ap horizons) displayed a wide range of variation. The soils had *medium to high* clay content, with the organic carbon (OC) and nitrogen values ranging from *very low to low*, whereas their available phosphorus contents had the greatest variation (*very low to very high*). Very low cation exchange capacity (CEC) values were found in some soils, but mean value was similar to that obtained by Perez et al. (2000) for other soils in the same region of Spain. The electric conductivity values obtained showed great variation, but all of the soils could be considered to be nonsaline. In the case of pH, the selected soils can be classified as *acidic* or *moderately acidic*, with their pH corresponding to Class III: Red Mediterranean and Brown non-Calcic Spanish soils. These pH values are tolerated by most crops. According to the U.S. Department of Agriculture classification (Soil Survey Staff, 2006), these soils belong to the Alfisol, Inceptisol, and Entisol orders (Fig. 1). The total Mn and Zn concentrations in most of the soils were not high (as would be expected in acidic soils), but some differences were observed between both metals. Manganese concentrations ranging from 61.4 to 496 mg kg⁻¹ (mean value, 155 mg kg⁻¹) fall within the interval (20–3000 mg kg⁻¹) found by Lindsay (1979) and nearly within that (80 to 1300 mg kg⁻¹) suggested by McBride (1994). Zinc concentrations ranged from 8.16 to 28.2 mg kg⁻¹ (mean value, 16.4 mg kg⁻¹). In some soils, Zn levels were lower than the smallest values within the intervals proposed by Lindsay ([1979] 10–300 mg kg⁻¹) and McBride ([1994] 17–125 mg kg⁻¹). The mean value was also lower than the smallest value

suggested by McBride (1994). The range of Zn values reported for Spanish soils by Perez et al. (2000) was wider than those shown in Table 1, whereas the lower level was similar.

Single-Extraction Procedures

The two single-extraction procedures applied are both commonly used as bioavailability predictors. One of them, proposed by the BCR, extracts metals with a solution of 0.05 M EDTA (Pueyo et al., 2001). A 5-g soil sample was transferred to a 250-mL extraction bottle to which 50 mL of 0.05 M EDTA solution was added. The mixture obtained was shaken in an end-over-end shaker operating at 30 r.p.m. for 1 h. The other single-extraction method extracts metals with a 0.10 M HCl solution (Reed and Martens, 1996). A 5-g soil sample was transferred to a 50-mL Erlenmeyer flask to which 20 mL of 0.1 M HCl solution was added. The mixture was shaken in a reciprocal shaker at 180 cycles min⁻¹. Both extracts were removed immediately from the residue. Separation was performed by filtration through Whatman 42 filter paper. The filtrates were then stored at 4 °C until they were analyzed.

Sequential-Extraction Procedures

Procedure A

A modified Tessier sequential extraction method (proposed by Elliot et al., 1990) was applied. The organically bound fraction was leached before dissolving the carbonate and iron oxide phases. This permits the destruction of the organic phases that could otherwise have entrapped the minerals and thus helped to ensure a better extraction for subsequent phases. The metal fractions were sequentially extracted from the soils (1 g) using a 50-mL borosilicate centrifuge tube with the following solutions and extracting conditions: 16 mL of a 1-M MgCl₂ solution adjusted to pH 7.0 for 1 h (exchangeable phase [EX]); 40 mL of a 0.1-M Na₄P₂O₇ solution for 24 h (organic-bound phase [ORG]); 16 mL of a 1-M NaOAc solution adjusted to pH 5.0 with HOAc for 5 h (acidic phase [AC]); 40 mL of a 0.175-M (NH₄)₂C₂O₄/0.1-M H₂C₂O₄ solution for 4 h conducted in the dark (oxide-bound phase [OX]). In each of the above steps, the samples were agitated on an end-over-end shaker at room temperature. The EX form is generally considered to be readily mobile and easily bioavailable. The ORG, AC, and OX fractions may be more or less labile, depending on the physical and chemical properties of the soils.

Procedure B

This is the BCR procedure (Rauret et al., 1999). The metal fractions were sequentially extracted from the soils (1 g) in a 50-mL borosilicate centrifuge tube with the following solutions and extracting conditions: 40 mL of a 0.11-M HOAc solution for 16 h (EX + AC); 40 mL of a 0.5-M NH₂OH HCl (pH 1.5 with HNO₃) solution for 16 h (reducible phase [RED]); 10 mL of an 8.8-M hydrogen peroxide (H₂O₂) for 1 h at room temperature and then 1 h at 85 °C to reduce the volume and a further aliquot of 5 mL of 8.8-M H₂O₂ for 1 h at 85 °C; then 50 mL of a 1-M NH₄OAc (adjusted to pH 2.0 with HNO₃) solution for 16 h (oxidizable phase [OXD]). In each of the above steps, the samples were agitated using an end-over-end shaker at room temperature.

All soil samples were extracted and analyzed in triplicate using the different procedures. The residual fraction was calculated as the difference between the total concentration and the sum of the other extracted fractions. The residual fraction is incorporated into the crystalline lattices of the soil minerals and is therefore considered the most inactive form. There were no delays between the addition of the extracting solution and the

beginning of the shaking process. Between each successive extraction, separation was undertaken by centrifugation (15 min, 1770 g), decantation, and filtering on Whatman 40 filter paper. The supernatant was decanted and stored at 4 °C until it was analyzed.

In all extracts, concentrations of Mn and Zn were determined by air-acetylene atomic absorption spectrophotometry. The detection limits for Mn and Zn were 10^{-3} mg L $^{-1}$ and 2×10^{-3} mg L $^{-1}$, respectively. Standard solutions for metals were prepared for each extraction in background solutions of the extracting agents.

Pot Experiment: Phytoavailable Mn and Zn

The Mn and Zn concentrations in barley (*Hordeum vulgare* L., cv Beka) stems and leaves were used to represent the phytoavailable Mn and Zn. Plants were grown in polyethylene pots that contained 2000 g of 11 different soils (a representative selection from the 29 soils studied) with a wide range of potentially phytoavailable Mn and Zn and with different physical and chemical properties. After a growth period of 8 weeks, the aboveground portion of the plant (including leaves and stems) was cut, and Mn and Zn concentrations were determined as described elsewhere (Obrador et al., 2007). The range of variation in plant concentrations was greater for Mn (20.5–69.2 mg kg $^{-1}$) than for Zn (21.5–44.2 mg kg $^{-1}$). These concentrations were within the range levels considered *sufficient or normal* for both metals (Kabata-Pendias, 2001). A quantitative evaluation of the relationship between the element uptake by the plants and its total content in the soil was made for each metal and soil by calculating a soil-plant transfer coefficient (concentration factor [CF]):

$$CF(\%) = \frac{\text{Plant concentration}}{\text{Soil concentration}} \times 100$$

Data Analysis

Descriptive, simple, and stepwise multiple regression analyses and other statistical studies were conducted with the Statgraphics-Plus version 5.1 software (Manugistic, Inc., Rockville, MD). Multifactor variance analyses were carried out to determine the main effects of different parameters, and multiple comparisons of variables were made using Duncan's separation of means procedure. A probability level of $P \leq 0.05$ was chosen to establish a statistical significance.

RESULTS AND DISCUSSION

Extractability and Metal Distribution in Soil Fractions

The results of single and sequential extractions for both metals are presented in Figs. 2 to 5. The amounts of Mn extracted with HCl ranged from 5.1 to 72.2 mg kg $^{-1}$, whereas using EDTA, they ranged from 6.3 to 161.0 mg kg $^{-1}$. The range therefore spans from low to very high levels (Loué, 1988). In the case of Zn, the ranges for extracted amounts were 0.57 to 4.5 mg kg $^{-1}$ (HCl) and 0.40 to 4.7 mg kg $^{-1}$ (EDTA). These values spanned from critical to very high levels. The EDTA- and HCl-extractable levels of both micronutrients in the analyzed soils were within the ranges determined by several different authors for agricultural soils (Sauerbeck, 1991; Andreu and Gimeno-García, 1999). However, they were lower than for data reported by Barona and Romero (1997) for rural areas of northern Spain. There were positive and highly significant correlations between the two methods used to estimate available Mn and Zn ($P < 0.0001$). Average relative Mn and Zn extractabilities with

respect to total contents were, respectively, 16.6% and 7.7%, using HCl, whereas using EDTA, they were 31.9% and 6.5%. For both reagents, the percentages of extracted Zn were numerically lower than those for Mn, which is contrary to what usually occurs in bioaccumulation by plants (Kabata-Pendias, 2001). Furthermore, the behavior of the two reagents was different for each of the two metals. The HCl reagent extracted a much lower proportion of Mn than EDTA, whereas the relative extractabilities of Zn were similar (rather than being significantly different, $P < 0.002$).

For Mn and the modified Tessier SEP (Fig. 2), the concentrations in the ORG were generally the lowest (ranging from 3.8 to 21.3 mg kg $^{-1}$), whereas in the OX and RES fractions, they were usually higher than in the other fractions (ranging from 6.1 to 282.0 mg kg $^{-1}$ and from 21.0 to 108.0 mg kg $^{-1}$, respectively). Compared with alkaline soils (Alvarez et al., 2006), Mn contents in the most potentially available fraction (EX) were certainly noteworthy (3.3–73.5 mg kg $^{-1}$). These high concentrations could be attributed to the acidic nature of the soils and/or the soil texture. At low pH values, the Mn level in the solution was so high that much of Mn remained in the exchange complex of the soils. When MgCl $_2$ was added, the Mg cation replaced the adsorbed Mn. In all extracted fractions, very high values of concentration were obtained for some soils. This led to mean values being higher than median values. This was particularly evident in the case of the EX fraction, for which 20 values (from a total of 29) were below the mean. Multifactor variance analysis showed significant differences between fractions ($P < 0.0001$). The mean values of Mn concentrations (milligrams per kilogram) in the different fractions were ordered as follows according to Duncan's separation of averages method: OX (58.6) > RES (43.7) > AC (24.0) $\geq$ EX (19.8) $\geq$ ORG (9.6).

FIG. 2. Distribution of solid-phase Mn fractions using a modified Tessier sequential-extraction scheme (Mn fractions: exchangeable [EX], organic [ORG], acidic [AC], oxide [OX], residual [RES]), and HCl- and EDTA-extractable Mn of the studied soils. Average of three replications; coefficients of variation, all fractions ranging from 2.12% to 6.13%; HCl extraction ranging from 0.78% to 4.43%; and EDTA extraction ranging from 0.55% to 2.74%.

FIG. 3. Distribution of solid-phase Mn fractions using the BCR sequential extraction scheme (Mn fractions: exchangeable plus acidic [EX + AC], reducible [RED], oxidizable [OXD], residual [RES]), and HCl- and EDTA-extractable Mn of the studied soils. Average of three replications; coefficients of variation, all fractions ranging from 1.63% to 7.91% (see Fig. 2 for HCl and EDTA extractions).

There was also an analogous order for mean values of metal content extracted in each fraction expressed as percentage of total content (ranging from 33.1% for OX to 6.7% for ORG). This order occurred in most soils, with the exception of the two highest fractions in which this order was reversed in half of the soils. For the BCR procedure (Fig. 3), the Mn organic-bound fraction (OXD) and the fraction that was extracted first (EX + AC) exhibited the lowest values of Mn concentration (ranging from 4.6 to 38.0 mg kg⁻¹ and from 3.0 to 80.8 mg kg⁻¹, respectively). In contrast, the oxide-bound fraction (RED) usually had the highest value, which ranged from 11.5 to 345.0 mg kg⁻¹. Multifactor variance analysis also showed significant differences between fractions for this procedure ($P < 0.0001$). The mean values for Mn concentration (milligrams per kilogram) in the different fractions were ordered as follows: RED (82.9) > RES (30.4) ≈ EX + AC (24.7) ≈ OXD (17.7). There was also an analogous order for the mean values of the percentages of total content (which ranged from 48.9% for RED to 12.8% for OXD). In both procedures, Mn was primarily released in the oxide-bound phase, but the quantities of metal extracted with Procedure A were much smaller. On the other hand, the organic-bound fraction was the smallest with both procedures, but higher Mn percentages were released with the H₂O₂ oxidant agent (Procedure B) than with a pyrophosphate solution (Procedure A). In the case of Mn forms, the order of extraction was analogous in both procedures, and the residual quantities were not significantly different.

For Zn and the modified Tessier protocol (Fig. 4), the contents in the first two extracted fractions (EX and ORG) were similar and ranged as follows: EX, from 0.42 to 2.3 mg kg⁻¹; and ORG, from 0.72 to 2.3 mg kg⁻¹. As in alkaline soils, these two fractions exhibited lower concentrations than the others, but in the soils of this study, the values were usually much higher than in those soils (Alvarez et al., 2006). As in alkaline soils, the highest fraction was always the RES, with values ranging

from 3.5 to 14.9 mg kg⁻¹. Multifactor variance analysis showed significant differences between fractions ($P < 0.0001$). The mean values for Zn concentrations (milligrams per kilogram) in the different fractions were ordered as follows: RES (8.2) > AC (4.0) > OX (1.6) ≈ ORG (1.3) ≈ EX (1.3). The same order was also found for mean values for the amount of metal extracted in each fraction with respect to total content (with values ranging from 49.1% for RES to 8.5% for EX). For the BCR procedure (Fig. 5), the smallest fractions were also the first two extracted: EX + AC, from 0.50 to 3.2 mg kg⁻¹; and RED, from 2.3 to 7.4 mg kg⁻¹. In contrast, the organic-bound fraction (OXD) was generally the largest, with values ranging from 2.8 to 7.6 mg kg⁻¹. Multifactor variance analysis also showed significant differences between fractions ($P < 0.0001$). In this case, the mean values for Zn concentration (milligrams per kilogram) in the different fractions were ordered as follows: RES (5.6) ≈ OXD (5.6) > RED (3.8) > EX + AC (1.4). The same order was also found for mean values for the amount of metal extracted in each fraction with respect to total content, although the percentage in OXD (35.0%) was higher than in RES (31.4%). For Zn, the order of the mean values in the fractions with Procedure B differed from that obtained with Procedure A, and the results also differed from the values yielded by Procedure B in alkaline soils (Alvarez et al., 2006), exception in the case of the first extracted fraction (always the one with the lowest value).

Table 2 presents the bivariate correlation matrix between the different fractions extracted by modified Tessier and BCR SEP and the total soil content of each metal. For Mn with Procedure A, significant relationships were obtained between the different fractions (except RES). A very similar result was obtained with Procedure B. All of the quantities extracted with the two procedures were proportional to the total amount for soils.

FIG. 4. Distribution of solid-phase Zn fractions using a modified Tessier sequential-extraction scheme (Zn fractions: exchangeable [EX], organic [ORG], acidic [AC], oxidized [OX], residual [RES]), and HCl- and EDTA-extractable Zn of the studied soils. Average of three replications; coefficients of variation, all fractions ranging from 3.07% to 8.32%; HCl extraction ranging from 1.03% to 4.57%; and EDTA extraction ranging from 0.57% to 3.70%.

FIG. 5. Distribution of solid-phase Zn fractions using the BCR sequential-extraction scheme (Zn fractions: exchangeable plus acidic [EX + AC], reducible [RED], oxidizable [OXD], residual [RES]), and HCl- and EDTA-extractable Zn of the studied soils. Average of three replications; coefficients of variation, all fractions ranging from 1.58% to 8.92% (see Fig. 4 for HCl and EDTA extractions).

These correlations were highly significant ($P < 0.001$) in several cases. In contrast, for Zn, only the EX and ORG fractions correlated (Procedure A). No significant relationships between fractions were found with Procedure B. Positive and highly significant correlations ($P < 0.001$) were only observed between the total Zn content and the RES fraction of Procedure A and the RES fraction of Procedure B. Several statistically significant correlation coefficients were observed for Mn between the fractions extracted by each of the SEP. The most statistically sig-

nificant values occurred between the concentrations in the EX, ORG, AC, and OX fractions (extracted by Procedure A) and the concentrations extracted in the first two steps of Procedure B (EX + AC and RED). In contrast, the results of the two SEP for Zn had few significant correlations. The quantities of Zn released in the EX fraction by Procedure A were correlated ($P = 0.011$) with those extracted in the first step of Procedure B (EX + AC). The amounts of Zn extracted in the RES fraction by Procedure A were correlated with the amounts of metal released in the same fraction of Procedure B.

To compare the results of the two SEP methods tested, the fractions relating to each method were subdivided into four equivalent groups (acid-exchangeable, organic-bound, oxide-bound, and residual; Table 3). The metal partitioning results showed that the distributions obtained with the two procedures were significantly different for the two metals ($P < 0.0001$). The modified-Tessier SEP (Procedure A) yielded lower percentages of the total amounts within the organic- and oxide-bound fractions for both of the two metals analyzed ($P < 0.0001$). However, this method tended to yield higher values for the acid-exchangeable and residual fractions ($P < 0.0001$).

The low values obtained in the oxide-bound fraction (Procedure A) could be explained by the fact that the oxalate/oxalic acid-buffered solution used to isolate this fraction (in the dark and at ambient temperature) only attacks amorphous iron phases with a low degree of crystallinity. As oxalate action is light sensitive, the UV catalytic effect has been used to speed up the attack on the crystallized iron phases (Pickering, 1986). Shuman (1985) suggested a further step after the extraction of amorphous Fe oxides. The reagent proposed combines the effects of the oxalate/oxalic acid solution, temperature, and a supplementary reducing compound (ascorbic acid). Despite the lower organic matter content of these soils, substantial percentages of metals (particularly Zn) were released in the oxidizable fraction when H_2O_2 was used as an oxidant reagent (Procedure B). This could be explained by the fact that H_2O_2 can simultaneously oxidize organic materials and soil sulfides.

Even when all of the soils were acidic, significant quantities of both metals were extracted with 1 M NaOAc-HOAc at pH 5.0 (Procedure A). This probably occurred because most

TABLE 2. Correlation matrixes comparing the extractable Mn (below-left) and Zn fractions (top-right) with modified Tessier scheme (Procedure A) and BCR scheme (Procedure B) ($n = 29$)

Fractions [†]	Procedure A					Procedure B				
	EX	ORG	AC	OX	RES	EX + AC	RED	OXD	RES	Total
Procedure A						Zn metal				
EX		0.63**	-0.05	0.31	-0.34	0.46*	-0.04	0.08	-0.21	-0.10
ORG	0.73***		-0.08	0.39	0.01	0.35	0.14	0.97	0.08	0.16
AC	0.60**	0.88***		0.13	-0.03	-0.25	0.23	0.43	0.51*	0.56*
OX	0.48*	0.66**	0.84***		0.04	0.02	0.25	0.28	0.21	0.31
RES	0.01	0.29	0.45	0.42		-0.07	0.12	0.42	0.76***	0.76***
Procedure B						Mn metal				
EX + AC	0.67***	0.91***	0.90***	0.70***	0.44		-0.18	-0.12	-0.21	-0.12
RED	0.61**	0.75***	0.89***	0.94***	0.41	0.75***		0.08	0.03	0.27
OXD	0.46	0.59**	0.62**	0.66**	0.37	0.51*	0.73***		0.44	0.65**
RES	0.01	0.07	0.17	0.25	0.55*	0.18	0.03	-0.11		0.93***
Total	0.61**	0.79***	0.92***	0.95***	0.56*	0.83***	0.95***	0.69***	0.30	

***, **, and * denote significance at 0.01%, 0.1%, and 1% levels, respectively.

[†]Metal fractions: EX, exchangeable; ORG, organic; AC, acidic; OX, oxide; RES, residual; RED, reducible; OXD, oxidizable.

TABLE 3. Average percentage of total Mn and Zn extracted in the soils with modified Tessier scheme (Procedure A) and BCR scheme (Procedure B) ($n = 29$)

Metal	Procedure	Solid-Phase Metal Fractions			
		Acid Exchangeable	Organic Bound	Oxide Bound	Residual
Mn	A	27.6	6.74	33.1	32.6
	B	15.5	12.8	48.9	22.7
Zn	A	32.4	8.51	9.99	49.1
	B	9.43	35.0	24.2	31.4

soils have relatively high pH (>6) and may therefore contain small amounts of carbonate minerals (Chlopecka et al., 1996). Moreover, metals extracted with this reagent were probably not only derived from a carbonate-bound fraction, but also from specifically adsorbed and easily reducible Mn oxide-bound fractions (Ma and Uren, 1997). Another possible explanation could be that when Procedure A was used, metals were able to form low-solubility pyrophosphates in the previous step: a pH of 5.0 in the third step would have contributed to their solubilization. All these could explain the fact that the quantities of metals (particularly Zn) extracted in the acidic phase by Procedure A (1 M buffered acetate solution, pH 5.0) were rather high in comparison with those released with Procedure B (0.11 M HOAc).

Comparison Between Single- and Sequential-Extraction Procedures

Table 4 presents the bivariate correlation matrix between single and sequential extracted fractions for both metals and between single-extracted amounts and total soil contents. Manganese extracted from the soil by HCl and EDTA was both related to total soil content and also to all of the fractions extracted by each SEP ($P < 0.0001$). The analysis showed few significant correlations between Zn-extractable and sequentially extracted quantities of metal. Significant correlations were only found between HCl- and EDTA-extractable Zn and the oxide-bound fraction in both SEP (OX for Procedure A and RED for Procedure B). Several authors have already suggested that the adsorption of metals by soil oxides plays an important role in controlling the solubility of certain metals (Shuman, 1998; Catlett et al., 2002; Kabata-Pendias, 2004).

The mean values for Mn concentrations (milligrams per kilogram) obtained from the single-extraction procedures and the first two fractions extracted in each one of the two SEP

applied (the most potentially available fractions) were ordered as follows: EDTA (51.7) $>$ EX + ORG (29.4) $\approx$ HCl (25.2) $\approx$ EX (19.8) for Procedure A; and (EX + AC) + RED (107.0) $>$ EDTA (51.7) $>$ HCl (25.2) $\approx$ EX + AC (24.7) for Procedure B. This occurred for most of the soils, as observed in Figs. 2 and 3. For Zn, none of the single-extraction procedures extracted any more Zn than was extracted in the first steps of the two procedures. The mean values for Zn concentration (milligrams per kilogram) were ordered as follows: EX + AC (1.4) $\approx$ EX (1.3) $\approx$ HCl (1.2) $\approx$ EDTA (1.0). The same pattern was repeated for most of the soils (Figs. 4 and 5).

Relationships Between Chemical Extractability and Soil Properties

Few significant correlations were found between soil properties and soil metal forms in terms of percentage of total content. In the case of Mn, positive relationships were found between clay content in soil and the following: the HCl-extractable quantities ($r = 0.43$, $P < 0.02$), the ORG-bound fraction of Procedure A ($r = 0.50$, $P < 0.01$), and the EX + AC fraction of Procedure B ($r = 0.48$, $P < 0.01$). This suggests that in these soils, Mn lability may be related with silicate clay content. Soil pH had a negative relationship with respect to the percentages of Mn in the EX fraction of Procedure A ($r = -0.44$, $P < 0.02$). In the case of Zn, there was a significant negative correlation between the first fraction that was extracted using the modified Tessier procedure (EX) and soil pH ($r = -0.40$, $P < 0.03$), which suggested that the most mobile metal fraction (exchangeable form) was negatively related to the soil pH. Significant negative relationships were also observed between soil CEC and the amounts of Zn released in the first step of each of the two SEP applied: EX ($r = -0.40$, $P < 0.03$) and EX + AC ($r = -0.58$, $P < 0.001$). The soil CEC was also negatively correlated with the amounts of extractable Zn: HCl ($r = -0.59$, $P < 0.001$) and EDTA ($r = -0.54$, $P < 0.002$). Sthai and James (1991) reported a gradual decrease in Zn activity as soil pH increased, which they attributed to increased CEC of the soils. Meers et al. (2006) suggested that the main factors influencing Zn solubility in soil are pH and soil texture, either represented by CEC or by the sand fraction. No correlations between soil OC content and organic-bound metal fractions (ORG fraction for Procedure A and OXD fraction for Procedure B) for either of the metals were obtained. This result is different from that reported in a previous study involving the same soils but with a modified Shuman SEP. In that case, despite the lower organic content of the soils, a highly significant correlation was obtained between the soil OC and the organically complexed fraction (extracted

TABLE 4. Pearson correlations between HCl and EDTA extractions, sequential fractions, and total soil concentrations of Mn and Zn with modified Tessier scheme (Procedure A) and BCR scheme (Procedure B) ($n = 29$)

Metal [†] / Extraction	Procedure A					Procedure B				
	EX	ORG	AC	OX	RES	EX + AC	RED	OXD	RES	Total
Mn										
HCl	0.79***	0.93***	0.80***	0.61**	0.19	0.91***	0.70***	0.56*	-0.01	0.73***
EDTA	0.80***	0.92***	0.92***	0.77***	0.30	0.84***	0.87***	0.64**	0.04	0.87***
Zn										
HCl	0.23	0.34	-0.06	0.50*	0.06	0.28	0.60*	0.04	-0.07	0.13
EDTA	0.28	0.38	-0.01	0.50*	-0.04	0.22	0.65**	-0.11	-0.08	0.08

***, **, and * denote significance at 0.01%, 0.1%, and 1% levels, respectively.

[†]Metal fractions: EX, exchangeable; ORG, organic; AC, acidic; OX, oxide; RES, residual; RED, reducible; OXD, oxidizable.

with 0.7 M NaOCl). Furthermore, all of the chemometric multivariate relationships (regression and principal component analysis) showed that, as well as pH, soil OC was an important soil property concerning the distribution of forms Mn and Zn in acidic soils.

Accumulation of Mn and Zn in Plant

Table 5 presents the values for the calculated soil:plant CF obtained with data from the greenhouse experiment; the results for metals extracted using the two single-extraction procedures and in the most potentially available fraction of each of the two SEP (percentage of total). The magnitudes of the CF coefficients were always much greater for Zn than for Mn (mean values of 31.2 for Mn and 196.0 for Zn). Similar results have been reported by other authors (Mohamed et al., 2003). The CF values were positively correlated with plant metal concentrations only in the case of Mn ($r = 0.75$, $P = 0.008$). Manganese and Zn CF values were not significantly interrelated, which suggested that soil properties modify the phytoavailability of the two metals in different ways. For Mn, the most significant correlation was obtained between the CF values and soil pH ($r = -0.73$, $P = 0.011$), whereas for Zn was between the CF coefficients and soil CEC ($r = -0.77$, $P = 0.005$).

The CF values were not significantly correlated with the percentage of the two metals extracted from the soils by the two single-extraction procedures in any of the cases. However, significant relationships existed between Zn plant concentrations and the amounts of HCl ($r = 0.70$, $P = 0.017$) and the EDTA ($r = 0.60$, $P = 0.049$)-extractable Zn. This result is comparable to that reported in a previous study (Obrador et al., 2007) involving the same soils for other single reagents (0.43 M HOAc, DTPA-triethanolamine [TEA], and Mehlich-3).

Significant and positive relationships were found between the CF values and the percentages of Zn extracted in the first step of each of the two SEP: EX fraction of Procedure A ($r = 0.70$, $P = 0.016$) and EX + AC fraction of Procedure B ($r = 0.65$, $P = 0.031$). However, no significant correlations were found with the same fractions in the case of Mn. With respect to the

concentrations (milligrams per kilogram) in the different sequentially extracted fractions, only the quantities of Zn extracted from soils in the ORG-bound fraction of Procedure A correlated with its concentration in plant tissues ($r = 0.70$, $P = 0.017$). However, when a modified version of the Shuman (1985) procedure was applied, significant relationships were also obtained between the soil amorphous Fe oxides fraction and concentrations of both Mn and Zn in plants.

CONCLUSIONS

In the acidic soils investigated here, the extractable amounts of Mn and Zn and the distribution of metals in the fractions obtained depended on the metal in question, the procedure applied, and the soil tested. For both of the single reagents used, the percentages of extracted Zn compared with the total quantity were much lower than for Mn. The distribution between the different metal fractions showed that Mn was mainly released in the oxide-bound phase of both SEP, whereas Zn was predominantly found in the RES fractions (the fraction associated with the mineral portion and mainly associated with aluminosilicate minerals). These results are comparable to the findings of a previous study involving the same soils and other single- and sequential-extraction tests. Bivariate relationships between soil properties and different metal forms suggested that soil pH is one of the main influencing factors. The soil texture (expressed as clay content) and soil CEC also exhibited significant correlations with the distribution of soil metal, but the role of organic matter was not evident. In contrast, in a previous study involving the same soils, soil OC content and soil pH were the main variables that influenced the predictability of the most potentially available amounts of metals, whether this was estimated on the basis of soil-extractable metal (HOAc, DTPA-TEA, and Mehlich-3) or based on the first two extracted fractions of modified Shuman SEP. Both high relative extractability and large amounts of metal were observed in the most potentially available fractions, which contrasted with the results obtained in alkaline soils.

TABLE 5. Plant/soil concentration coefficients (CF), HCl and EDTA extractables, and first extracted fraction of each sequential extraction (all expressed as percentages) for the soils used in the greenhouse experiment

Parameter ^a	Soil number ^b										
	1	2	6	7	11	12	15	20	26	27	29
Mn											
CF	32.3	54.9	24.4	11.7	20.6	27.0	27.7	67.2	20.1	43.2	13.9
HCl	5.15	6.39	19.5	16.2	12.8	14.4	21.8	19.4	20.7	24.9	15.8
EDTA	15.2	9.10	38.7	43.5	21.8	48.2	29.1	8.90	51.5	48.5	30.4
EX ^c	5.19	3.79	10.5	9.18	6.88	28.6	10.7	7.86	6.76	17.7	6.99
EX + AC	10.9	5.98	19.7	19.8	21.8	10.7	16.7	12.3	23.3	26.1	13.0
Zn											
CF	251	146	139	120	202	207	140	204	254	315	180
HCl	10.4	3.21	4.48	8.59	8.54	7.60	10.4	14.0	25.8	13.9	13.4
EDTA	8.61	2.82	3.60	9.93	5.27	9.16	4.86	7.72	26.8	12.6	10.3
EX	8.44	6.88	3.55	3.45	6.25	8.61	2.27	13.3	11.1	12.0	12.2
EX + AC	18.1	5.19	4.53	7.46	11.4	10.9	2.70	22.3	10.2	14.8	11.0

^aMetal fractions: EX, exchangeable; AC, acidic.

^bIn accordance with the number of soils in Fig. 1.

^cFirst extracted fraction of modified Tessier scheme (Procedure A).

First extracted fraction of BCR scheme (Procedure B).

TABLE 6. Multiple regression models between metal concentration in plant (*Y*) against HCl and EDTA extractions, solid-phase metal fractions with modified Tessier scheme (Procedure A) and BCR scheme (Procedure B), and soil properties (*n* = 11)

Extraction	Equation [†]	R ² , %
Mn		
HCl	$Y = 193 - 25.7 \times \text{pH} - 0.33 \times \text{Mn-HCl}$	56.2 ($P < 0.04$)
Procedure A	$Y = 181 - 23.0 \times \text{pH} - 0.17 \times \text{Mn-EX}$	55.1 ($P < 0.05$)
	$Y = 188 - 26.1 \times \text{pH} - 0.27 \times \text{Mn-OX}$	67.8 ($P < 0.02$)
Procedure B	$Y = 192 - 25.1 \times \text{pH} - 0.17 \times \text{Mn-(EX + AC)}$	54.6 ($P < 0.05$)
	$Y = 183 - 24.7 \times \text{pH} - 0.14 \times \text{Mn-RED}$	60.2 ($P < 0.03$)
Zn		
HCl	$Y = 20.4 - 5.09 \times \text{Zn-HCl}$	48.8 ($P < 0.02$)
EDTA	$Y = 23.1 - 4.08 \times \text{Zn-EDTA}$	36.5 ($P < 0.05$)
	$Y = 19.3 - 1.30 \times \text{OC} - 2.50 \times \text{Zn-EDTA}$	53.6 ($P < 0.05$)
Procedure A	$Y = 14.0 - 11.60 \times \text{Zn-ORG}$	48.5 ($P < 0.02$)
	$Y = 13.4 - 1.61 \times \text{OC} - 6.96 \times \text{Zn-EX}$	62.3 ($P < 0.03$)
	$Y = 12.4 - 1.29 \times \text{OC} - 4.30 \times \text{Zn-(EX + ORG)}$	63.6 ($P < 0.02$)
Procedure B	$Y = 13.4 - 1.27 \times \text{OC} - 1.73 \times \text{Zn-(EX + AC + RED)}$	53.7 ($P < 0.05$)

[†] Metal fractions: EX, exchangeable; ORG, organic; AC, acidic; OX, oxide; RED, reducible.

An interesting way to the different extraction methods is to determine their ability to predict the phytoavailability of the metals. Table 6 presents the best-fit regression (single and multivariate) equations obtained from this study to predict the absorption of metals by barley plants. For Mn, plant metal concentrations and soil-extractable and sequential-extracted fractions showed a lack of significant correlations. The ability of the single- and sequential-extraction methods tested in the present study to predict the phytoavailability of Mn in the soils studied did not come up to initial expectations. To obtain significant regression equations, it was necessary to include the soil pH values; even so, the R^2 values remained low (ranging from 54% to 68%). More valuable results were obtained in a previous study in which these soils were analyzed by applying a modified version of the Shuman SEP. In that case, significant relationships were obtained between soil amorphous Fe oxide fraction and plant Mn concentration. The additional introduction of soil pH as variable improved the equation, which was able to explain 80% of the variation in barley metal concentrations. More valuable results were also obtained when the two SEP (modified Tessier and BCR) were applied to alkaline soils in which plant Mn concentration correlated with the first two extracted fractions for both procedures. For these soils, the inclusion of soil clay content improved the equation, which was subsequently able to explain 95% of the variation for the modified-Tessier procedure and 69% of the variation for the BCR procedure. In this study, it was not possible to predict plant Mn concentrations based on only a single extraction. The same result occurred with EDTA in alkaline soils or with any other single reagents (0.43 *M* HOAc, DTPA-TEA, and Mehlich-3) in the same acidic soils. Moreover, the inclusion of general soil properties in the equations as components only significantly improved predictions of the phytoavailability of Mn for barley in the case of EDTA extraction from alkaline soils (including soil clay content) and HCl extraction from acidic soils (including soil pH). Even so, the R^2 values were low in both cases (<60%). Single-extraction techniques were therefore not as good as SEP for evaluating the phytoavailability of Mn.

Single-extraction methods provided better information for metal uptake by plants for Zn than for Mn. The phytoavailability of Zn for barley was significantly predicted using only HCl-

or EDTA-extractable amounts (Table 6). Similar behavior was observed in a previous work with other frequently used single reagents (0.43 *M* HOAc, DTPA-TEA, and Mehlich-3). The inclusion of soil properties as variables in the equations did not always improve the relationships obtained with respect to the amount of Zn taken up by the plants. In the case of the SEP, the best-fit equation obtained with our data set that included only one fraction as a component was the one that related the Zn plant concentration with the soil Zn ORG-bound fraction extracted in the second step of the modified-Tessier procedure. More regression equations were obtained that described Zn plant concentration as a function of soil-extracted fractions, but to achieve statistical significance, it was necessary to include more than one fraction and/or soil OC content. Even in such cases, the resulting R^2 values remained low (<65%). A comparison of the results obtained in this study indicated that for the soil studied, the Tessier-modified procedure was better at predicting Zn uptake by plants than the BCR procedure, but it was not better than the HCl extraction method. Unlike Mn, more valuable results were not obtained when a modified version of the Shuman SEP was applied to these acidic soils. For these soils, the best-fit regression equation describing plant Zn concentration was obtained in a previous work with DTPA-extractable amounts and general soil properties (soil pH and extractable phosphorus) as components. Single-extraction techniques therefore provided for the soils studied (acidic soils that are low in organic matter) rapid and reliable evaluations of Zn phytoavailability. For alkaline soils, the single EDTA extraction (plus CaCO_3 and total contents) was also able to explain 70% of the variation in Zn concentrations in barley.

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