

C. FERREIRA DE MIRANDA
M. MANUELA MOTA BATISTA
Departamento de Química
Universidade de Évora
ÉVORA

e
Centro de Electroquímica
e Cinética da Universidade de Lisboa (INIC)
Faculdade de Ciências
LISBOA

M. DE LOURDES PIMENTA DA SILVA
Departamento de Química
Universidade de Évora
ÉVORA



AVAILABILITY INDICES, PHYSICO-CHEMICAL ASPECTS

I — Available Molybdenum in some Alentejo Soils

Available molybdenum was determined in 7 soils of the Evora region using the Lowe and Massey and the Grigg extraction methods. Some likely pertinent soil parameters were also determined. A discussion of the correlation analysis of the data is presented and a tentative description of the experimental results in terms of a physico-chemical model is put forward. The precision of the analytical methods is evaluated and discussed.

INTRODUCTION

Since the creation of Agricultural Chemistry by Liebig in the eighteen forties, chemists and agronomists have attempted to define and to determine the availability of nutrients in soils by using analytical chemistry procedures. This is an ambitious task as it amounts to quantifying the driving force involved in the transfer of chemical species from a polyphased system (the soil) to another polyphased system (the plant) through an interphase where active transport may be operating. Furthermore, some feed-back may occur owing to the complexing action of the plant exudates, and prevailing conditions may be those of non-equilibrium. Availability indices are generally determined by extraction procedures and that makes the situation more difficult, from the conceptual point of view at least, because measurement alters the very variables which are supposed to be measured. Experience shows, however, that this is no sufficient reason for abandoning chemical availability indices but, certainly, there is a strong case for trying to understand the chemistry underlying their definitions.

The time scale of such chemistry may range from the century, for the weathering of the parent-rock materials, to that of some minutes for the kinetics of the extraction procedures used in measuring a particular availability index. Involved are: (i), a *non-mobile form* of the nutrient (consisting e.g. of minerals of the parent-rock materials, precipitates and occlusions) whose level changes slowly over the years or the centuries; (ii), a *mobile form* (comprising complexes, either labile or more or less inert, with colloidal organic matter, and adsorbates on clay minerals), whose level can change in a month-to-years time scale; and (iii), the *available form* whose level is quickly adjusted (say, at the hour-to-days time scale) to a steady state equilibrium, with the mobile form on the one hand, and the plant or other source of uptake, on the other hand. This is summarised in fig. 1 where the parallel between available and extractable forms of a micronutrient is also indicated. The proposed scheme is in agreement with the translocation process advanced by ZUNINO and MARTIN [1] but emphasis is given here to mobility rather than to possible mechanisms.

Knowledge of the mechanisms of the *mobile form* → *available form* conversion is fundamental, both from the standpoint of the agronomist

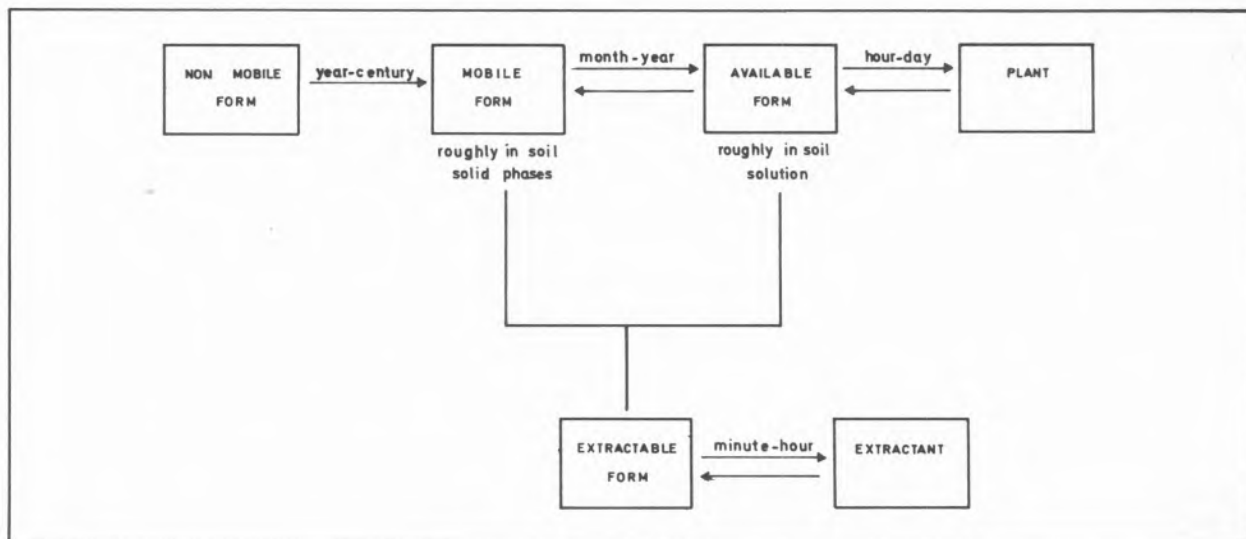


Fig. 1

A simple model for relating nutrient mobility and metrology

who may want to improve the level of available nutrients in a given soil, and from the standpoint of the chemist who seeks good chemical indices of availability. However, such mechanisms are difficult to investigate because, for the systems concerned, it is not possible to fix all variables but one in order to study its influence. Thus, statistical analysis of the data is often resorted to, generally using a linear model which may prove justified for interpolation purposes but less so for the elucidation of mechanisms.

It is important to realize that extraction procedures introduce a distribution equilibrium completely alien to the natural system, which is nevertheless supposed to simulate the distribution equilibrium between available nutrient and plant. Now, if one keeps in mind that transfer of a nutrient from soil to plant involves an acceptor, an interphase and a donor, it is surprising that chemically defined availability indices work at all. Indeed, (i), extractant and plant are altogether different acceptors; (ii) the interphase is drastically modified when soil is put into contact with extractants; and (iii) soil donor properties change as its composition is altered by the extraction procedure itself. Ideally, the transfer-to-plant ability of the available form species should be measured using a standard donor, and the donor capacity of the soil should be measured against a standard acceptor; and the chemical potential of the species should be evaluated without disturbing it. As they stand, availability indices are crude estima-

tions which have to be — and actually are — tested against the real behaviour of groups of plants and soils.

Molybdenum is one of the most important micronutrients, being a determining factor in the natural process of atmospheric nitrogen fixation by microorganisms as well as in nitrogen metabolism, where it plays a role comparable to that of the first transition elements iron, copper and manganese in the metabolism of oxygen. The literature on molybdenum in soils is very extensive and has been recently reviewed [2]. However, data concerning the mobility and the availability of molybdenum and the relevant controlling factors are fragmentary and often conflicting [2]. There is in this field a large scope for the chemist, both from the fundamental point of view and from that of potential applications.

In the present preliminary investigation, we report some results concerning availability indices for molybdenum deficient soils of the region of Evora. The analytical experience we gathered is used in discussing precision and sensitivity of methods, and the statistical analysis of data is tentatively and critically exploited as a tool for obtaining hints about possible physico-chemical interactions.

EXPERIMENTAL

Seven soils of the Evora region were studied. All were reputedly deficient in molybdenum and

though proceeding from neighbouring areas they corresponded to different geomorphological characteristics (Cf. Table 1).

Four *ca* 3 kg field samples of each soil were taken with the usual precautions, down to a depth of 15-20 cm. The samples were air dried, gently ground and screened to 2 mm, then finely ground and sieved to 0.2 mm [3]. The samples were kept in appropriate stoppered bottles. From each sample aliquots of adequate size were taken for the analyses. The following parameters were evaluated: humidity; total molybdenum content; available molybdenum; free iron; organic matter; pH; clay fraction; and qualitative mineral composition of the clay fraction.

Table 1
Nature of the soils investigated in this work

Soil number	Classification
1	Entisol. Typic xerofluvent
2	Entisol. Lithic xerofluvent
3	Alfisol. Typic haploxeralf
4	Entisol. Typic xerorthent (granite)
5	Alfisol. Lithic rhodoxeralf
6	Alfisol. Aquic haploxeralf
7	Entisol. Typic xerorthent (gneiss)

Humidity was determined by loss of weight of a 10 g sample at $105^{\circ}\text{C} \pm 3^{\circ}\text{C}$ until constant weight. Analytical results are reported on a dry weight basis. Total molybdenum determinations were carried out with 2 g soil samples which were melted with sodium carbonate (10 g), followed by the usual classical treatment [4]. Available molybdenum was determined by two methods: (i), the Lowe and Massey method [5] — continuous extraction by boiling water in a Soxhlet apparatus (soil, 50 g; water 250 ml; 16 hours); and (ii), the Grigg method [6] — extraction with an oxalate buffer solution of pH = 3.3 (soil, 40 g; 0.2M oxalic acid plus 0.1M ammonium oxalate, 250 ml; 16 hours with stirring). In all cases molybdenum was estimated spectrophotometrically by the thiocyanate-stannous chloride method which is the most widely used owing to its sensitivity and general freedom from interference [7]. Butyl acetate

[8], amyl alcohol plus carbon tetrachloride (1:1) [9], and isopropyl ether [4] were tested as organic extractants for stability of colour and easy separation of phases; the later was found to be the most convenient.

Free iron was determined in the TAMM'S extract [10] (soil, 10 g; 0.2M oxalic acid plus 0.1M ammonium oxalate, 100 ml; 16 hours with stirring) by spectrophotometry of the 1,2-dihydroxybenzene 3,5-disulphonate (tiron) complex [11].

Organic matter was estimated volumetrically according to ANNE'S method [12].

The pH of soils was measured with the glass electrode in water (soil, 20 g; water 20 ml; 30 minutes stirring; 1 hour decantation) and in calcium chloride 0.01M medium (soil, 10 g; solution 20 ml; 30 minutes stirring, 30 minutes decantation) [13].

The clay fraction in the soils was determined as usual by the pipette sampling method [13]. The qualitative mineral composition of the clay fraction was established by x-ray diffraction.

RESULTS AND DISCUSSION

1 — RESULTS AND PRECISION OF THE ANALYTICAL PROCEDURES

The results of the evaluation of molybdenum levels as well as those of some potentially determining factors are presented in Table 2. Both total and available molybdenum levels are considerably lower than the normal values which are 1-2 ppm and 0.2-0.4 ppm respectively [14]. For a total molybdenum level of 0.5-0.8 ppm, the Lowe and Massey extraction method seems to be more discriminative than Grigg method for defining available levels, in agreement with LOWE and MASSEY [5]. Indeed, availability indices span a single to double interval the first case, whereas in the second one, values cluster within 20% (at most) around the average, fluctuations being thus rather small, though analytically significant.

Independently from difficulties associated with significant soil sampling and adequate soil extraction procedures, molybdenum determination in soil at the ppb-ppm level is a delicate analytical problem. Therefore, assessments of its precision are desirable. Variance in molybdenum determinations (in a given soil sample, by a given extraction procedure, and by a given colorimetric method) arises from

Table 2
Molybdenum levels and likely correlated parameters of soils of the Évora region

Soil	Total Mo (ppb)	Available Molybdenum (Grigg) (Lowe & Massey) (ppb)		Free iron (ppm)	Organic matter (%)	pH water CaCl ₂		Clay fraction (%)	Clay minerals
1	698	55	14	1177	0.79	5.0	4.1	5.3	illite + kaolinite
2	510	55	15	2364	1.11	6.6	5.3	11.9	kaolinite
3	651	54	28	1566	1.52	6.6	5.4	16.1	kaolinite + montmorillonite
4	768	56	28	2113	0.88	6.7	5.5	13.0	kaolinite + montmorillonite
5	719	43	26	1443	3.25	7.1	6.3	13.6	illite + kaolinite
6	730	59	25	1644	1.80	7.6	7.0	11.3	illite + kaolinite
7	545	51	24	1667	1.65	8.3	7.6	19.4	illite + kaolinite

three sources: (i), the instrumental uncertainty of the absorbance reading; (ii), variance associated with the preparation of the colorimetric solution; and, (iii), variance due to the soil extraction procedure.

Uncertainty due to instrument reading is negligible, not more than 0.001 at any point in the absorbance scale. The standard deviation of an absorbance measurement is therefore essentially due to variance of colour development. A direct estimation based on replicate measurements with Mo standards — which were performed for establishing the colorimetric calibration curve — yielded 0.003; this value was corroborated by the external estimation, resulting from the fitting of the calibration curve. Molybdenum concentration c (ppm) in a soil sample of mass M (g) is given as a function of the absorbance measurement, A , by $c = 1.112 \times 10^4 A/M$ where the factor 1.112×10^4 is derived from the calibration curve and the appropriate dilution factors. Therefore, the standard deviation of c arising from the colorimetry is $\sigma_c = 0.003 \times 1.112 \times 10^4 / M = 33.36/M$ (Table 3, column 3a).

On the other hand, for each type of determination (total Mo, Grigg available Mo and Lowe and Massey available Mo) we have 7 averages and variances, one for each soil. CHOCHRAN test [15] showed that the variances were not significantly different from

soil to soil, as it could be anticipated from the fact that the same analytical procedure was used for all soils. The combined estimation of variance comprising thus 28 results is presented for each type of determination on Table 3, column 3b.

Clearly, the variance of the molybdenum determinations is not mainly associated with the soil extraction method; colorimetry itself contributes to roughly 50% of the standard deviation. Precision of the determinations is 3-6% down to the 40 ppb level and 7-14% at the 15 ppb level. Detection limits are of the order of 50 ppb for total molybdenum and 5 ppb for available molybdenum determinations.

As to free iron, when 10 g samples are used, precision is 1-4% at the 1000-3000 ppm level. Variance is practically entirely due to the extraction procedure. The detection limit is 80 ppm.

Organic matter can be estimated at the 1-3% level in 1-2 g samples with a precision of 1-3%; the detection limit is 0.06%.

2 — THE CORRELATION ANALYSIS OF THE DATA

The correlation matrix for our data is presented in Table 4. Except for a significant positive correlation between pH in water and pH in 0.01M CaCl₂, which is trivial, and the correlation between pH and clay

Table 3
Precision of molybdenum, iron and organic matter determination

MOLYBDENUM						
Determination	Weight of sample (g)	Standard deviation (ppb)		Concentration level (ppb)	error (%)	Detection limit (ppb)
Total Mo	2	17(a)	30(b)	500 — 800	4 — 6	60
Available Mo (Grigg)	40	0.8	2	40 — 60	3 — 4	4
Available Mo (Lowe & Massey)	50	0.7	2	15 — 30	7 — 14	4
IRON						
Free Fe	10	0(a)	40 ppm(b)	1000 — 3000 ppm	1 — 4	80 ppm
ORGANIC MATTER						
Organic matter	1-2	0.03 %		1 — 3 %	1 — 3 %	0.06 %

(a) Standard deviation for colorimetry alone

(b) Standard deviation for the whole procedure, including extraction from soil

Table 4
Simple correlation coefficient between pairs of variables

	Mo _T	Mo _G	Mo _{LM}	MO	pH ₁	pH ₂	MA	Fe
Mo _T	1	0.050	0.450	0.146	—0.245	—0.171	—0.381	—0.377
Mo _G		1	—0.186	—0.787*	—0.162	—0.216	—0.323	0.296
Mo _{LM}			1	0.426	0.566	0.513	0.637	—0.008
MO				1	0.442	0.527	0.343	—0.309
pH ₁					1	0.978***	0.813**	0.255
pH ₂						1	0.724*	0.082
MA							1	0.248
Fe								1

* Significant at the 10% level

** Significant at the 5% level

*** Significant at the 1% level

fraction, MA (probably not pertinent in the present context), the only significant correlation is between Grigg available molybdenum (Mo_G) and organic matter. In particular, Lowe and Massey available molybdenum (Mo_{LM}) levels, which definitely vary

from soil to soil, do not seem to be correlated with any of the factors we have considered.

Since the influence of each one of these might be obscured by the variations of the others, the partial correlation coefficients were evaluated according to

the general equation $r_{ij,\dots,p} = -C_{ij}/C_{ii} C_{ij}$, where $[C_{ij}]$ is the inverse of the simple correlation matrix [16]. The results are presented in Tables 5 and 6. At this stage, only organic matter, pH in 0.01M CaCl₂, clay fraction and free iron were taken into account as potentially determining factors for Mo_G and Mo_{LM} levels. (The reasons for eliminating one of the pH indices are obvious, and those for not considering total molybdenum content will be apparent later). Again, only Grigg available molybdenum and organic matter are significantly correlated (at the 10% level).

These results call for some comments as the plots of Mo_G and Mo_{LM} against the potentially pertinent

Table 5
Third order partial correlation coefficients
(excluded variables: Mo_T, Mo_{LM} and pH₁)

	Mo _G	MO	pH ₂	MA	Fe
Mo _G	—	−0.826 *	0.605	−0.530	0.138
MO		—	0.685	−0.411	−0.118
pH ₂			—	0.753	−0.044
MA				—	0.301
Fe					—

* Significant at the 10% level.

Table 6
Third order partial correlation coefficients
(excluded variables: Mo_T, Mo_G and pH₁)

	Mo _{LM}	MO	pH ₂	MA	Fe
Mo _{LM}	—	0.208	−0.024	0.483	−0.112
MO		—	0.407	−0.058	−0.379
pH ₂			—	0.573	0.046
MA				—	0.291
Fe					—

variables do not rule out some trends, though not linear, especially for Mo_{LM} (figs. 2 and 3).

It can be argued that, for our data, the number of fixed variables is high compared to the number of samples and in fact we even had to leave out some variables in order to dispose of at least 2 degrees of freedom for the significance tests concerning the partial correlation matrices. A more relevant point however is that calculation of correlation coefficients is based on a covariance/variance ratio definition. Now, this implies a linear model which may be inadequate. Indeed a linear model cannot be justified on mechanism grounds: even a single chemical reaction or ion exchange equilibrium would rather lead to linear log-log relationships between variables, and several coupled equilibria would

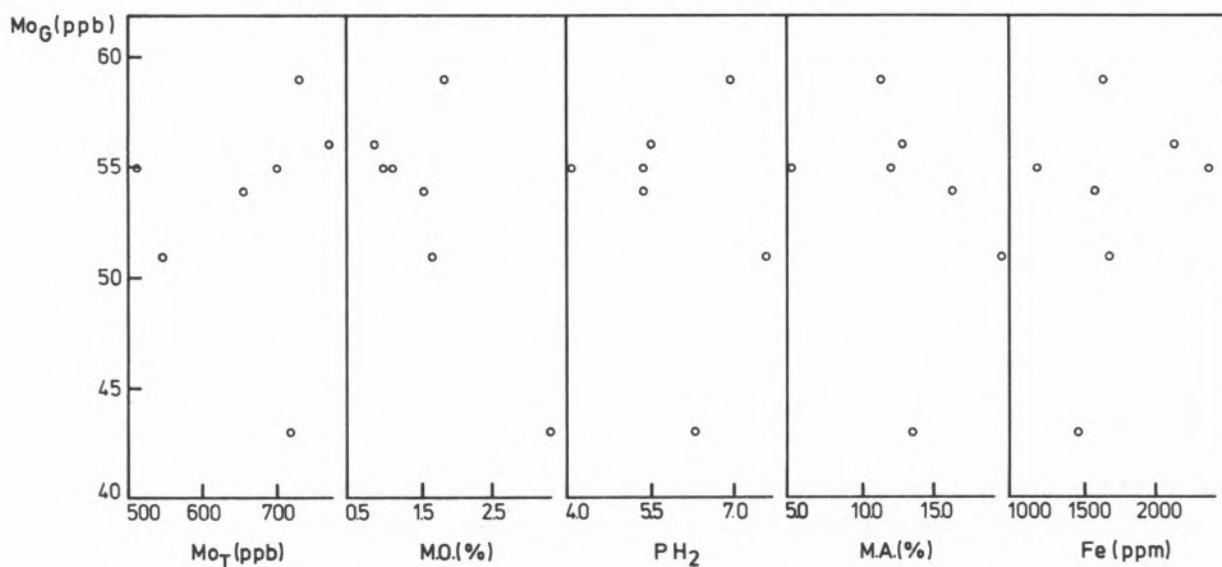


Fig. 2
Plots of Grigg's molybdenum against potentially pertinent variables

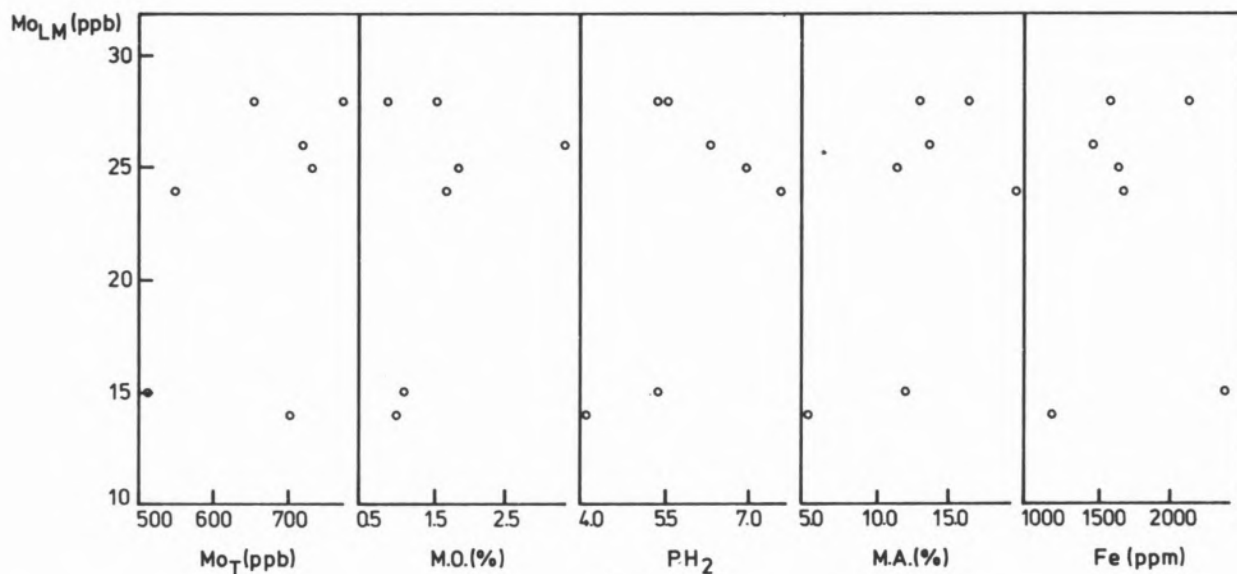


Fig. 3

Plots of Lowe and Massey's molybdenum against pertinent variables

result in more complicated relationships. It is not surprising therefore that we also failed to find significant linear simple and partial correlations between the logarithms of variables.

Single and multiple linear regressions are not reported here since the data interpolation aspects are not pertinent in the present context. On the contrary, principal component analysis may offer some interest and was therefore carried out [17], in spite of the limited number of OTU's, in order to assess their degree of similarity or otherwise (fig. 4):

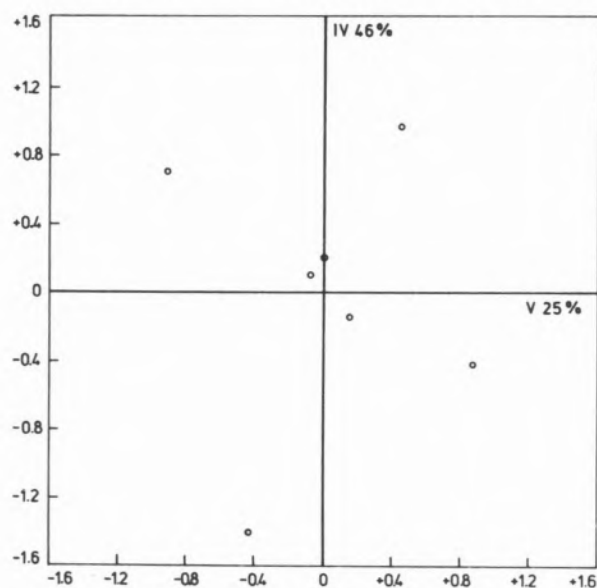


Fig. 4

Ordination plot from principal component analysis

points are fairly scattered in the plane of the principal components as we hoped, though three soils tend to form a cluster.

3 — PHYSICO-CHEMICAL ASPECTS

Within the framework we put forward in the introduction, our results for Grigg available molybdenum, Mo_G , are easily understood on the assumption that mobile molybdenum levels are about the same for all the soils we investigated and that Mo_G is nearly equal to the sum of mobile plus available molybdenum. (This is likely since oxalate ion is a fairly strong complexant for Mo (VI)). In this view, the negative correlation between Mo_G and organic matter then merely reflects the competition between insoluble organic matter and oxalate ion for Mo(VI). A predominant role is thus implicitly attributed to organic matter; this seems to be justified in the present case, considering the relatively low levels of clay fraction (5-20%) and its nature (little or no montmorillonite and low ferric oxide content, 0.2-0.3%). This is not in contradiction with JONES [18] results for a krasnozem soil from Wollongbar (South Wales Australia) which indicated ferric oxide as the chief factor controlling molybdenum mobility: Wollongbar soil contains 72% clay fraction and 15% ferric oxide.

As to Lowe and Massey available molybdenum levels, Mo_{LM} , they correspond, in our view, to the partial dissociation of the complex Mo(VI)-soil

organic matter, the Mo(VI) concentrations in the extract being lower than in the case of a Grigg extract because the amount of competitive complexant in solution is now nearly zero. Actually, since some organic matter and other potentially complexing species may be solubilized by hot water, the final concentration of complexing agent in the solution is not only low but also ill-defined. This is a drawback of Lowe and Massey method (unbuffered extractant medium) and can explain the apparently advantageous discriminative character of the index. 0.01M CaCl₂ might prove a more rational non complexing extractant.

ACKNOWLEDGMENTS

The authors wish to thank Professor J. Lima de Faria of the "Laboratório de Técnicas Físico-Químicas aplicadas à Mineralogia e Petrologia" of the "Junta de Investigação Científica do Ultramar" for the x-ray diffraction analysis of the clay fractions and Mrs. Helena Mafalda Carolino of the Departamento de Fitotecnia of the Universidade de Évora for the determination of the clay fraction in the soils investigated.

REFERENCES

- [1] H. ZUNINO, J.P. MARTIN, *Soil Sci.*, **123**, 65 (1977).
- [2] M.M. MOTA BATISTA, C. FERREIRA DE MIRANDA, "O Molibdénio nos solos", Monograph of the Instituto Macional de Investigação Científica, Lisbon (1981).
- [3] "Methodes d'Analyse Physique et Chimique des Sols". Office de la Recherche Scientifique et Technique d'Outre-Mer, Bondy, France.
- [4] E.R. PURVIS, N.K. PETERSON, *Soil Sci.*, **81**, 223 (1956).
- [5] R.H. LÓWE, H.F. MASSEY, *Soil Sci.*, **100**, 238 (1965).
- [6] J.L. GRIGG, *N.Z.J. Sci. Technol.*, **34**, 405 (1953).
- [7] E.B. SANDELL, "Colorimetric Determination of Traces of Metals". Interscience Publishers, New York, 644 (1959).
- [8] J. RODIER, "L'Analyse de l'Eau", Dunod, Paris, 272 (1976).
- [9] C.M. JOHNSON, T.H. ARKLEY, *Anal. Chem.*, **26**, 572 (1954).
- [10] O. TAMM, *Meddn. St. Skosforsk Inst.*, **19**, 385 (1922).
- [11] J.H. YOE, A.R. ARMSTRONG, *Anal. Chem.*, **19**, 100 (1947).
- [12] P. ANNE, *Ann. Agron.*, **5**, 161 (1945).
- [13] C.A. BLACK, D.D. EVANS, J.L. WHITE, L.E. ENSMINGER (ed.) "Methods of Soil Analysis", American Society of Agronomy, Inc., Publisher, Madison, Wisconsin, USA, 552 (1965).
- [14] B.T. CHENG, G.J. OUELLETE, *Soil Fertil.*, **36**, 207 (1973).
- [15] V.P. SPIRIDONOV, A.A. LOPATKIN, "Tratamiento Matemático de Datos Físico-Químicos", Editorial Mir, Moscow, 100 (1973).
- [16] K.W. SMILLIE, "An Introduction to Regression and Correlation", Ryerson Press, Toronto, Academic Press, London, 58 (1966).
- [17] P.H. SNEATH, R.R. SOKAL, "Numerical Taxonomy", W.H. Freeman and Company, San Francisco, 245 (1973).
- [18] L.P.H. JONES, *J. Soil Sci.*, **8**, 313 (1957).

RESUMO

Determinou-se o teor em molibdénio assimilável em sete solos da região de Évora pelos métodos de extracção de Lowe e Massey e Grigg. Alguns parâmetros susceptíveis de condicionar os níveis de molibdénio assimilável foram igualmente determinados. Apresenta-se uma discussão da análise de correlação dos resultados e sugere-se uma descrição destes em termos de um modelo físico-químico. Apresenta-se também uma avaliação da precisão dos métodos empregados.