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Is Mehlich 3 soil extraction a suitable screening method for determination of some risk elements?

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Abstract: Legislation limits for risk elements (As, Be, Cd, Co, Cr, Cu, Ni, Pb, V, Zn) in agricultural soil in the Czech Republic are given for the content of elements extracted by *aqua regia*. This extraction is time consuming and environmentally unfriendly. The regular soil survey is based on Mehlich 3 universal soil extractant and covers the simultaneous determination of macronutrients, sulphur, and micronutrients by inductively coupled plasma optical emission spectrometry (ICP-OES). Our study focused on the possibility to use Mehlich 3 extractant also for preliminary screening for the determination of the risk elements simultaneously with the other elements by the ICP-OES method. Mehlich 3 was confirmed as a reliable screening method for Cd regarding sensitivity, specificity and precision (> 0.8) when the cut-off value of 0.27 mg/kg in Mehlich 3 extracts was used for the prediction of soils to be above or below the legislation limit, which in turn is based on *aqua regia* extraction. Very good results were obtained for Be and promising results were received for As, Cu and V. But the available data were not sufficient for the determination of reliable cut-off values for Co, Cr, Ni, Pb and Zn.

Keywords: soil contamination; heavy metals; cut-off values; cadmium; statistical learning

The systematic soil testing scheme in the Czech Republic was established in 1961, founded on a long tradition of soil testing (Klement et al. 2012, Zbiral and Smatanová 2019). Mehlich 3 extractant (Mehlich 1984) has been used for this purpose since 1999 and it was chosen not only for its simple and universal use but also for its ability to produce relatively good correlations between the amount of nutrients extracted from the soil and the plant response. Mehlich 3 extractant has been studied, modified, and recommended by numerous authors (e.g. Wolf and Baker 1985, Sims 1989, Wendt 1995, Eckert and Watson 1996, Zbiral and Němec 2000) and it is now a widely recommended and applied extracting procedure. Critical values in the Czech soil testing scheme were recalculated for P, K, Mg and Ca from the values established for the formerly used traditional extractants using the relationships between these extractants and Mehlich 3 (Zbiral 2001). The same procedure was also applied later

for sulphur (Kulhánek et al. 2018, Zbiral et al. 2018), and for Cu, Zn, Mn, Fe, B (Zbiral 2016). During more than 20 years the Mehlich 3 proved to be a suitable method for large-scale soil testing where a high sample throughput is a crucial point. This is true especially if the simultaneous determination of all elements by inductively coupled plasma optical emission spectrometry (ICP-OES) has been used for the final analysis of the soil extracts.

The determination of some risk elements by widening the scope of the Mehlich 3 method was studied as a rapid and cost-effective alternative by several authors. They preferably focused on Cu and Zn because these elements are also important micronutrients and only at high concentrations they are classified as risk elements (e.g. Mehlich 1984, Ostatek-Boczynski et al. 2012, Pradhan et al. 2015). Sims et al. (1999) compared several routinely used extractants (Mehlich 1, Mehlich 3 and DTPA) with the EPA 3050 method for determination of Cu, Zn, Cd, Pb and Ni.

He found better correlations if multiple regressions including more soil properties were applied. Minca et al. (2013) recommended Mehlich 3 method as a strong estimator of a total and bioaccessible lead in urban soils. They found a very good agreement between Mehlich 3 and the standard US EPA methods. Monterroso et al. (1999) evaluated Mehlich 3 extractant for mine soils. Significant correlations were found between Fe, Cu, Zn and Cd extracted by Mehlich 3 and DTPA. For other elements (Mn, Ni, Co and Pb) there was also an acceptable correlation if pH was included in the relationship.

The Czech Soil legislation defines inter alia prevention values for risk elements, namely As, Be, Cd, Co, Cr, Cu, Hg, Ni, Pb, V, Zn. These prevention values are defined as the content of the individual element determined in *aqua regia* soil extract (ISO 54321, 2020). The system of evaluation of the results according to the legislation is based on hierarchical limit values and reflects also bioavailability of the elements and includes more soil properties in the evaluation. The detailed description and explanation of the prevention values and indication limits as defined by the soil protection legislation in the Czech Republic was presented by Vácha et al. (2016).

The key task for a successful application of Mehlich 3 as a screening method is finding cut-off values for the reliable classification of samples into two categories related to *aqua regia* extraction, below the legislation limit and above it. Techniques of statistical learning for binary classification by means of continuous data offer a suitable tool to perform this task when the underlying assumptions for linear regression (e.g. homoscedasticity or normality) are not fulfilled. A detailed description of this approach can be found e.g. in Irizarry (2019). Briefly, samples are classified as positive or negative depending on if the concentration (*aqua regia*) is below or above the legislation limit. Then the cut-off values are set up based on the sensitivity and specificity which characterise the efficiency of the procedure regarding the true positive and true negative ratios obtained. The meanings of these terms is given in results and the discussion and formulas for their calculations in material and methods.

The main goal of our study was to find relationships between *aqua regia* and Mehlich 3 extraction and, if possible, to define the screening prevention values for risk elements determined in Mehlich 3 extractant derived from the prevention values for *aqua regia* soil extraction.

MATERIAL AND METHODS

Soil samples from the 2017 sampling campaign were chosen. The samples represented most major soil types, climatic regions, and proportions of agronomic cultures (Prášková et al. 2020). The soil samples were air-dried, gently crushed and a fraction under 2 mm was used for the analysis. Descriptive statistics of the samples are presented in Table 1. Only the samples above the limit of quantification

Table 1. Descriptive statistics of the soil samples

Element	N	STAT	M3	AR	AR/M3
			(mg/kg)		
As	381	MED	0.400	10.1	25.3
		MIN	0.150	2.748	
		MAX	4.540	72.8	
Cd	488	MED	0.156	0.236	1.52
		MIN	0.100	0.150	
		MAX	1.698	2.512	
Cr	141	MED	0.215	31.59	97.7
		MIN	0.046	5.607	
		MAX	1.401	225.3	
Cu	595	MED	3.379	17.72	5.09
		MIN	0.415	4.024	
		MAX	81.2	235.8	
Ni	598	MED	2.020	20.93	10.6
		MIN	0.230	2.998	
		MAX	12.17	176.4	
Pb	584	MED	6.530	19.96	3.01
		MIN	0.540	4.930	
		MAX	27.31	96.24	
Zn	598	MED	4.701	70.310	13.5
		MIN	1.372	15.30	
		MAX	137.2	305.4	
Co	598	MED	1.310	10.20	7.41
		MIN	0.220	1.528	
		MAX	6.490	46.52	
Be	345	MED	0.300	1.168	3.70
		MIN	0.200	0.432	
		MAX	1.460	3.778	
V	598	MED	1.500	43.29	28.1
		MIN	0.310	7.313	
		MAX	7.780	251.9	

N – number of samples; M3 – Mehlich 3 extraction; AR – extraction by *aqua regia*; MED – median; MIN – minimum value; MAX – maximum value

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for Mehlich 3 soil extracts were chosen for the statistical evaluation.

Soil extraction and analysis. All samples were analysed under the same analytical conditions in one laboratory to minimise differences from the analytical measurement. The soil samples were extracted by the Unified Working Procedures ÚKZÚZ 30068 method (Zbiral 2016) and by ISO 54321 (ISO, 2020). Risk elements in the extracts were determined using ICP-OES Spectroblue (Spectro GmbH., Kleve, Germany) by the JPP ÚKZÚZ 30074 method (Zbiral 2016). The emission lines and the limits of quantification are summarised in Table 2.

Statistical evaluation

Comparison of extraction efficiency. The ratio of the results reported for *aqua regia* extraction and for Mehlich 3 extraction were calculated individually for every sample and then the median of the individual results was presented as the final extraction efficiency ratio (Table 1).

Determination of the cut-off values. The estimation of the cut-off values for Mehlich 3 was performed according to the procedure described by Irizarri (2019) by means of the R 4.0.3 software (R Core Team 2020, RStudio Team 2020). The whole data set was split into two parts, the training set and the test set, both containing approximately the same number of samples. All calculations to determine the cut-off value were given on the training set. At first, *aqua regia* data was binarised comparing the results with the legislation limit which led to the classification of samples as negative (below the legislation limit for the preventive values) or positive (above the legislation limit for the preventive values). A row covering potential cut-off values in Mehlich 3 was set up. Each of these values were used

as a cut-off criterion for the following calculations. If the concentration in Mehlich 3 was below the cut-off limit, the sample was predicted as negative. If the opposite was true, the sample was evaluated as positive. Total counts of true negative (TN), true positive (TP), false negative (FN) and false positive (FP) cases comparing the outcome of the screening method with the reference data were recorded. The package *purrr* (Henry and Wickham 2020) was used for programming the procedure. Then sensitivity (true positive rate; $TP/(TP + FN)$), specificity (true negative rate, $TN/(TN + FP)$) and precision (positive predictive value, $TP/(TP + FP)$) were calculated. The first deciding criterion was sensitivity with the aim to reach the minimum value of 0.80. For the final determination of the optimum cut-off value, specificity and precision were taken into account and the receiver operating characteristic (ROC) curve was consulted.

In ROC curves, sensitivity (y -axis) is plotted against the false positive rate FPR ($FP/(FP + TN)$), which can be expressed as $(1 - \text{specificity})$, on the x -axis. Finally, validation was performed by application of the selected cut-off values on the test data set. The package *dplyr* (Wickham et al. 2020) was used for a data manipulation and the package *lattice* (Sarkar 2008) was used for preparation of plots.

RESULTS AND DISCUSSION

Extraction efficiency. *Aqua regia* extracts nearly the total contents of many elements from soils. Mehlich 3 is a much weaker extractant than *aqua regia*. In our study a comparison of *aqua regia* and Mehlich 3 extraction clearly showed (as expected), that Mehlich 3 can extract only a relatively small fraction of the *aqua regia* extractable risk elements. The ratio between the extracted amounts is very different

Table 2. Inductively coupled plasma optical emission spectrometry (ICP-OES) limits of quantification for Mehlich 3 and *aqua regia* extracts and the wavelength used for the measurement

Element	λ (nm)	M3	AR	Element	λ (nm)	M3	AR
		(mg/kg)				(mg/kg)	
As	189.042	0.2	1.5	Cu	324.754	0.3	1.5
Cd	228.802	0.1	0.15	Zn	213.856	1.0	5.0
Pb	220.353	0.5	2.0	Co	228.616	0.2	0.5
Cr	267.716	0.3	1.5	V	292.464	0.3	0.5
Ni	231.604	0.2	1.5	Be	313.042	0.2	0.05

λ – wavelength; M3 – limit of quantification for Mehlich 3 extraction; AR – limit of quantification for *aqua regia* extraction

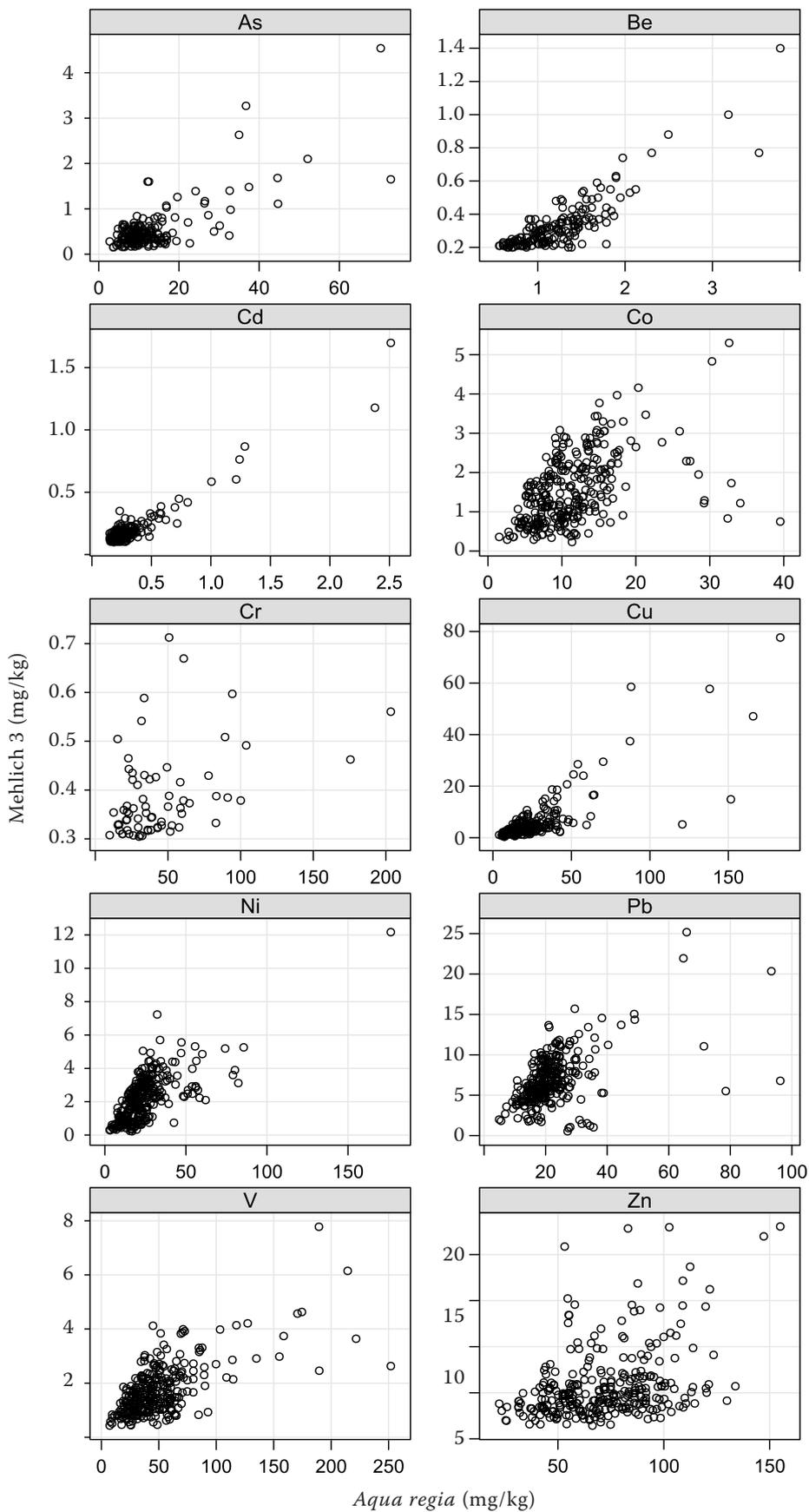


Figure 1. Scatterplots showing relationships between concentrations in *aqua regia* and Mehlich 3. Data from the training set are displayed

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for the individual elements (Table 1). For Cd this ratio was 1.52 and for Cu, Pb and Be it was between 3 and 6. For Co and Ni the ratio was between 7 and 11, for As and V it was more than 25 and for Cr we found the ratio nearly 100. For the elements with a higher ratio, the results for the Mehlich 3 extractant were often below the limit of quantification and therefore not suitable for the statistical evaluation. The final number of samples is given in Table 1.

Cut-off values. As the first choice of statistical evaluation we used linear regression, but we encountered some basic problems. The first was that the relationships between both methods were not linear on the whole range of concentrations for some elements (e.g. Co, Pb, V) (Figure 1). Additionally, regression diagnostics showed that assumptions of homoscedasticity and normality of residuals were not met in most cases, and also revealed some outliers (data from regression diagnostics is not shown). Because of that, statistical approach based on binary classification of soils according to the reference method and evaluation of sensitivity, specificity and precision was used to search for cut-off values.

At first, the cut-off values corresponding to the sensitivity of 0.8 were found for each element. Sensitivity was selected as the first criterion because it is the probability that a positive sample will be correctly classified using Mehlich 3. Since an important aim of any screening method is to save resources, specificity was evaluated as the second criterion. It means the probability that a negative sample will be properly classified using Mehlich 3 which in turn reflects the proportion of samples for which costly and laborious analyses will be correctly avoided. We chose the value of 0.8 as the minimum acceptable limit of specificity.

It is worth noting that there is always a kind of trade-off. If we set up the cut-off value to optimise sensitivity, we will pay by lower specificity and *vice versa*. To overcome this difficulty, so-called receiver operating characteristic curves displaying both sensitivity and specificity, precisely the value 1-specificity (false positive rate), were used to find the optimal cut-off values.

We can see that cut-off values corresponding to sensitivity equal or higher than 0.8 and to specificity of the same value corresponding to FPR of 0.2, can be found for As, Be, Cd, Cu and V (Figure 2). The selected cut-off values along with the sensitivity and specificity obtained using the training set are shown in Table 3. These results were confirmed by validation analysis; only sensitivity for As and V were

slightly lower, between 0.7 and 0.8. The shape of ROC curves confirmed the appropriateness of the suggested procedure for As, Be, Cd, Cu and V (Figure 2). Generally, if a ROC curve has an "L" shape, it indicates that a screening method is a reliable tool for classification.

Another important parameter, mainly from the practical point of view, is precision. It is the probability that a sample is positive if the concentration in Mehlich 3 is higher than the cut-off value. If we take the results from validation, we can see that only Cd gives good results (0.800). Approximately half of positively classified samples in Mehlich 3 will be confirmed in *aqua regia* for Be and Cu and this proportion is even lower for As (0.350). In the case of V, the precision is so low (0.268) that the efficiency of use of Mehlich 3 would have to be considered.

The ROC curves (Figure 2) shows the impossibility of choosing reliable cut-off values for Co, Cr, Ni, Pb and Zn. In the case of Co and Zn, it may be explained by a weak relationship between both extraction methods (Figure 1). Better results were obtained for Ni with a sensitivity of 0.79 and 0.80 and specificity of 0.70 and 0.69 (training set, test set). However, a very low precision of 0.15 and 0.08 for the training and test set resp. precludes the practical use of Mehlich 3 as a screening method. Mehlich 3 is a very weak extractant for Cr with the ratio between itself and *aqua regia* around 100, which resulted in few samples above the limit of quantification in Mehlich 3 (141 in total with 13 positive samples). The extremely low number of positive samples in the case of Pb where only 8 samples out of 584 were positive did not enable any reliable calculations either.

From the practical point of view, Mehlich 3 works very well for Cd as discussed here using the validation results. We proposed the cut-off value of 0.27 mg/g giving satisfactory characteristics for all three evaluated, namely sensitivity, specificity, and precision. Concerning As, Be, Cu and V, sensitivity and specificity are higher than 0.8 or close to this value but precision remains weak.

It has to be stressed that the current study enabled only a rough estimation of the efficiency of the use of the suggested method, caused mainly by the low number of positive samples.

Our results clearly show that the scope of Mehlich 3 extractant can include the prediction of soils potentially contaminated by cadmium – the most critical element in agricultural soils due to the various inputs of this element (sludge, compost, mineral fertilisers)

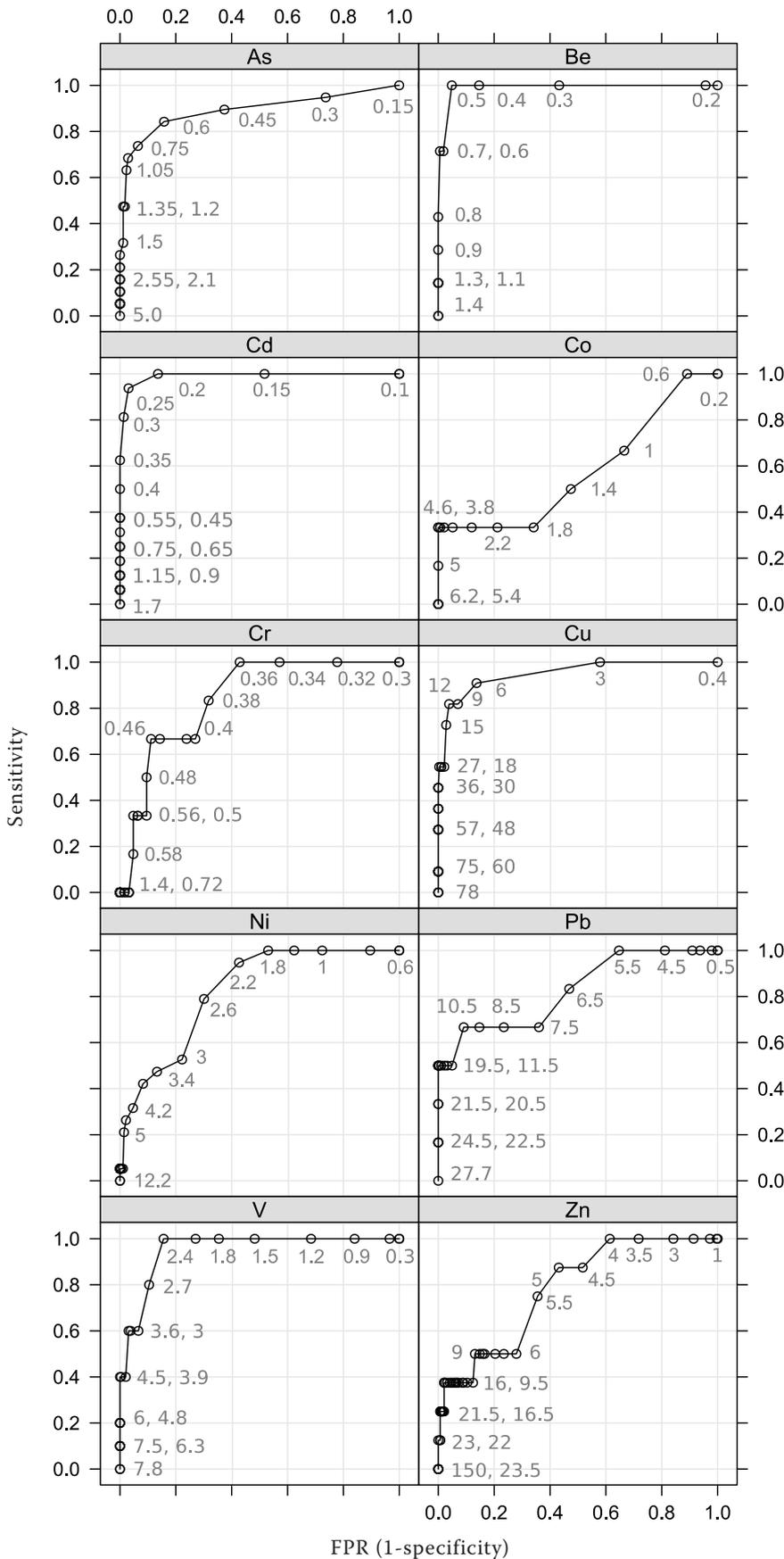


Figure 2. Receiver operating characteristic curves for individual elements displaying predictive ability to classify samples (*aqua regia*) using Mehlich 3 extraction. Figures inside plots show cut-off values. If more values overlap, the highest and the lowest values are displayed. FPR – false positive rate

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Table 3. Proposed cut-off values along with corresponding values of sensitivity, specificity, precision and prevalence for the training and test sets

Element	Cut-off value (mg/kg)	Sample set	Sensitivity	Specificity	Precision	Prevalence
As	0.6	training	0.842	0.842	0.372	0.100
		test	0.737	0.849	0.350	0.099
Be	0.5	training	1.000	0.951	0.467	0.041
		test	0.909	0.939	0.500	0.063
Cd	0.27	training	0.938	0.978	0.750	0.066
		test	0.889	0.991	0.800	0.037
Cu	13	training	0.818	0.965	0.474	0.037
		test	0.833	0.972	0.556	0.040
V	2.6	training	0.900	0.882	0.209	0.034
		test	0.786	0.895	0.268	0.047

and its bioavailability and toxicity. Extraction by means of Mehlich 3 can be recommended also for Be and Cu with the caveat that approximately half of the samples predicted as positive will be confirmed using *aqua regia*. The applied statistical approach enables the establishment of limits according to the possible risks resulting from the false negative determinations, and on the other hand, to minimise the number of false positive samples.

This will improve the cost-efficiency of the whole soil testing by minimising the number of samples determined by the time consuming, expensive, and environmentally unfriendly *aqua regia* extraction.

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