



Online Version ISSN: 1314-412X
Volume 4, Number 4
December 2012

AGRICULTURAL SCIENCE AND TECHNOLOGY

2012

An International Journal Published by Faculty of Agriculture,
Trakia University, Stara Zagora, Bulgaria

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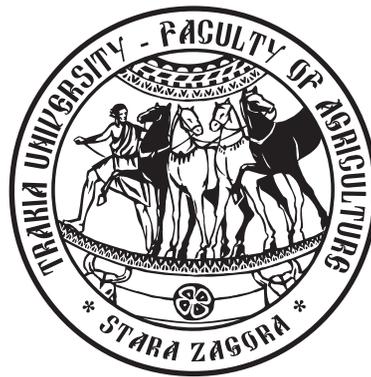
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*AGRICULTURAL
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2012

An International Journal Published by Faculty of Agriculture,
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Application of NIRS as a rapid and alternative method for prediction of heavy metals content in soil

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Abstract. Determination of heavy metals content in soil by acid or microwave mineralization and spectrophotometer measurement via AAS or ICP are sufficiently accurate, but they are time consuming and labour intensive. These methods are not flexible enough for environmental research. Application of near infrared spectroscopy (NIRS) as a tool for ecological assessment and monitoring of soil quality have been investigated recently. The reported results for determination of trace elements in soil by NIRS are controversial. The objective of this study was to investigate the application of NIRS as a rapid and non-expensive method for determination of heavy metals content in soil. A total of 121 soil samples from the 0–20 and 20–40 cm layer were collected from Stara Zagora, Chirpan, Kazanlak and Gurkovo regions. Total Zn, Cu, Pb, Ni and Cd content in the investigated soil samples were determined by AAS using AAnalyst 800 Atomic Absorption Spectrometer, Perkin Elmer. Near-infrared spectra of all soil samples were measured using spectrophotometer NIRQuest 512, within the range from 900 to 1700 nm. PLS regression was used for developing models related tested parameters to the spectra. According to the statistical parameters of a regression procedure the best accuracy of determination was obtained for total Cu content with correlation coefficient $R=0.92$ and $RPD=3.9$ for the calibration set of data and $r=0.77$ and $RPD=2.3$ for the test data. Models for determination of Cd, Pb, Zn and Ni content via near-infrared spectroscopy could be classified as with good to low accuracy of determination, according to the obtained ratio SD/SEC and SD/SEP .

Keywords: heavy metals content, soil, near infrared reflectance spectroscopy, chemometrics

Introduction

Soil contamination with heavy metals is a topical problem in Bulgaria and in the world. Contaminated soils have a potential risk for human health and the sustainable development of ecosystems and therefore soils should be monitored and eco-toxicologically tested. There is a great demand for rapid, non destructive and less time consuming methods for quick control and prediction of soil quality, environmental monitoring, and other precision measurements in agriculture. A lot of numerous publications showed that Near infrared reflectance spectroscopy (NIRS) could be a promising technique for the investigation of soil carbon, total nitrogen, CEC, and clay content. (Mouazen et al., 2006; Viscarra Rossel et al., 2006; Viscarra Rossel et al., 2009; Volkan et al., 2010; Todorova et al., 2011; Genot et al., 2011). That new and alternative method for soil analysis starts to be applied in soil testing labs (Malley and Williams., 2005). It is not surprising because of the many advantages of NIRS in comparison to other spectral methods: no reagents are required, analysis of one sample is ready in about 1 minute, several parameters of the sample can be determined simultaneously. Samples can be analyzed also in the field by developed new NIRS instruments, particularly portable instruments with optical fibres. No special qualification for performing analysis is needed.

There are several papers that investigate possibility of NIRS as a method for ecological assessment and monitoring of soil quality (Cecillon et al., 2009; Brunet et al., 2009). The reported results for

determination of trace elements in soil by NIRS are controversial. The accuracy of determination depends on soil type, spectral region, and range of tested parameter values. Siebllic et al. (2004) and Cozzolino and Morron (2003) reported successful prediction of copper content in soil via NIRS technique with values of correlation coefficient (r) more than 0,80, whereas Thomas Kemper and Sommer (2002) and Islam et al. (2004) obtained poor prediction with $r < 0,60$. According to Chodak et al. (2004) Vis-NIRS has a great potential for determination of zinc content in soil. Authors used one hundred soil samples taken from surface horizon of Cambisols and measured by Foss NIRSystem within the range from 400 to 2500 nm. Excellent prediction of zinc content were obtained with $r=0,96$ and $RPD=3,2$. Similar results reported Siebllic et al. (2004). Morron and Cozzolino (2003) obtained lower correlation coefficient $r=0,75$ using bigger number of soil samples-332, but also low standard error of calibration $SEC=1,2$ mg/kg for prediction of zinc content.

The objective of this study was to investigate the application of NIRS as a rapid and non-expensive method for determination of heavy metals content in soil.

Material and methods

Soil samples

A total of 121 soil samples from the 0–20 and 20–40 cm layer were collected from Stara Zagora, Chirpan, Kazanlak and Gurkovo municipalities during May – July, 2010. Soil types were Calcic

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Vertisols, Haplic Vertisols, Chromic Luvisols, Skeletic Fluvisols and Anthrosols, (World Reference Base for Soil Resources, 1998). The samples were air-dried, crushed and sieved with particle-size less than 2 mm.

Chemical analysis

The sampling, transportation and preservation procedures: single manual sampling, composite sampling, were conducted following EN ISO 10381 requirements. The soil samples preparation and acid mineralization were conducted according to standard EN ISO 11466. The soil samples were treated with *aqua regia* ($\text{HNO}_3:\text{HCl} = 1:3$) for 16 hours at room temperature and heated subsequently for two hours, with condensation and returning of the escaped vapors with a reflux condenser. Total Zn, Cu, Pb and Ni concentrations in the investigated soil samples were determined by AAS using AAnalyst 800 Atomic Absorption Spectrometer, Perkin Elmer, according to EN ISO 11047. Cadmium content was determined by using graphite furnace and measurement at 228,8 nm. All investigations were performed in triplicate with RSD less than 2%.

Spectral analysis

The spectral data of all air dried soil samples were measured using spectrophotometer NIRQuest 512, within the range from 900 to 1700 nm. (Figure 1) The absorbance was recorded as a log $1/R$, where R is diffuse reflectance. PLS regression was used for model building relating content of tested parameters to the spectra. Spectral data were numerically transformed as first derivative using the Savitsky-Golay transformation. (Rinnan et al., 2009) PLS regression was carried out by Unscrambler 9.7 (CAMO Software AS, Norway).

Two-third of the samples were used as calibration set (n=72)



Figure 1. NIRQuest, within the range from 900 to 1700 nm

and the remaining samples as independent validation test set (n=49). Outliers up to 2% of all samples were removed in order to avoid biased prediction. The accuracy of each calibration equation was evaluated based on correlation coefficient between the values of the soil chemical parameter and soil spectra (R), r – coefficient of determination between chemically measured soil parameters and NIRS predicted values of test set samples, SEC/SEP – standard error of calibration or prediction and value of RPD – the ratio of standard deviation of data set SD to the SEC/SEP. According to Viscarra Rossel, 2007, the RPD values were classified in the following way: RPDs between 1.5 and 2.0 indicate poor predictions; RPDs between 2.0 and 2.5 indicate good prediction and RPDs > 2.5 very good /excellent model/ prediction.

Results and discussion

The results for the range, mean values and standard deviation of Cu, Cd, Pb, Zn and Ni mg/kg of soil samples by chemical methods in the respective calibration and test sets are presented in Table 1. The studied soil samples were characterized with wide range of copper content. Most of the samples were with Cu content near background values in Bulgaria, but there were several samples with copper concentration more than the maximum permissible (trigger) values of 150 mg/kg. (Regulation №3/2008. On the admissible content of harmful substances in soils.) The results indicated that all the concentrations of Cd, Zn, Pb and Ni were near background values of soil in Bulgaria, an exception with several samples with Cd, Pb and Zn content more than Precaution values.

The results of the quantitative determination of total Cu, Cd, Pb, Zn and Ni content in tested soil samples by PLS regression are presented in Figures 2, 3, 4, 5 and 6. The correlation coefficients between the measured and NIRS predicted values for all tested trace elements were between 0.72 and 0.92 for both calibration and test set. The best accuracy of determination was obtained for total Cu content with correlation coefficient $R=0.92$ and $RPD=3.9$ for the calibration set and $r=0.77$ and $RPD=2.3$ for the test data. According to statistical parameters that calibration model was characterized as very good model. Similar results were obtained by Chodak et al, 2004 with values of $RPD=3.4$. Figure 2 graphically illustrates the relationships between determined and NIR spectroscopy predicted values of total Cu content in tested soil samples. The values of tested parameters determined by AAS are presented on the abscissa and the values of Cu content predicted by NIRS are presented on the ordinate. Three of the samples with values of copper more than 200 mg/kg were predicted with high accuracy of determination. Therefore that calibration equation could be used successfully to determine copper content in contaminated soils.

Table 1. Range, mean and standard deviation (SD) of soil tested parameters content in the examined samples.

Soil parameter	Calibration set, n=72				Test set, n=49			
	min	max	MEAN	SD	min	max	MEAN	SD
Cu mg/kg	1.68	264.9	36.3	62.1	4.5	263.5	50.2	46.8
Cd mg/kg	0.04	1.27	0.22	0.23	0.04	1.28	0.21	0.24
Pb mg/kg	5.63	82.5	20.2	14.7	5.6	68.6	27.7	15.04
Zn mg/kg	8.54	266.9	77.9	56.3	8.54	250.0	81.7	51.1
Ni mg/kg	1.9	52.0	21.9	11.5	1.09	38.4	20.6	12.3

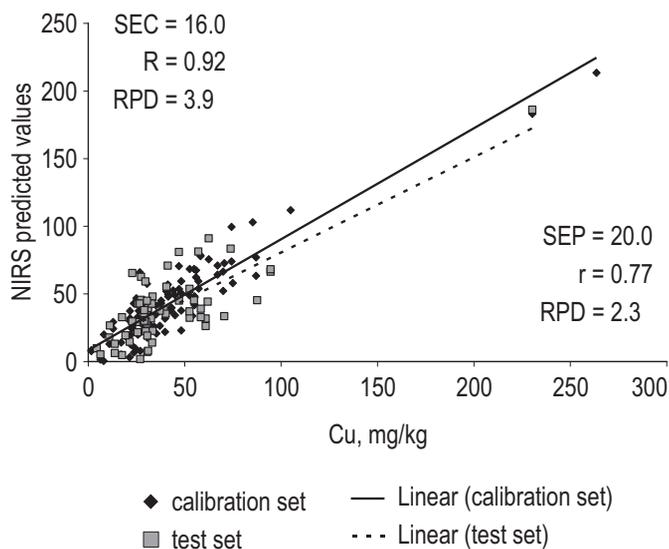


Figure 2. Relation between actual and NIRS predicted values of total Cu content in the tested samples.

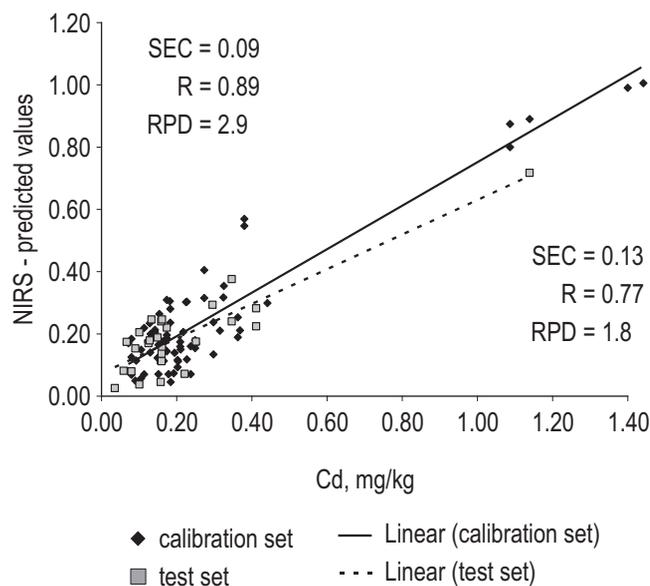


Figure 3. Relation between actual and NIRS predicted values of total Cd content in the tested samples.

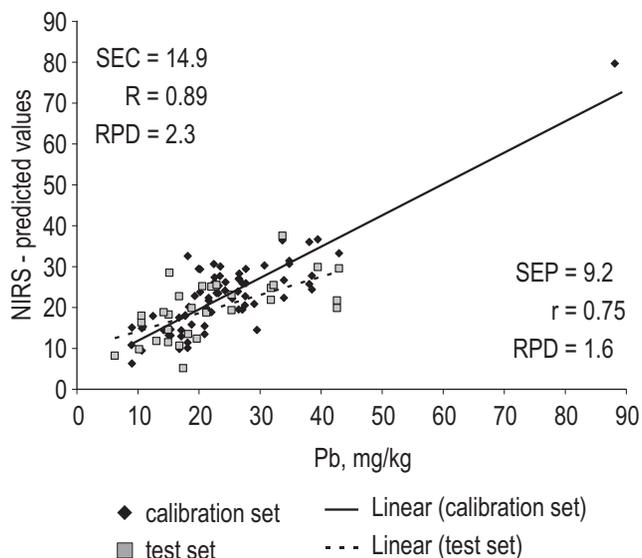


Figure 4. Relation between actual and NIRS predicted values of total Pb content in the tested samples.

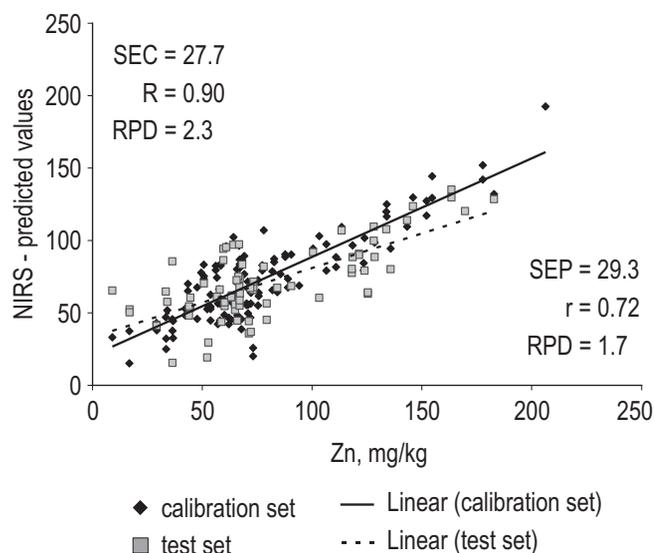


Figure 5. Relation between actual and NIRS predicted values of total Zn content in the tested samples.

The model for prediction for Cd content showed good accuracy of determination, according to the statistical parameters from the regression procedure (Figure 3). The correlation coefficients were $R=0.89$ and $r=0.77$ for calibration set and test set, respectively. Standard error of calibration and standard error of prediction were similar to the values reported by Sieblliec et al. (2004), $SEC=0.06$ and $SEP=0.17$ mg/kg. Probably the reason about lower value of RPD was very small range of concentration of Ca in the investigated samples – from 0.04 to 1.28 mg/kg.

Models for determination of Pb, Zn and Ni mg/kg content via near-infrared spectroscopy could be classified as models with good to low accuracy of determination. The values of R were between 0.89 and 0.90 and values of r were between 0.73 and 0.75. The ratio between SD and SEC were 2,3 for calibration set for Pb, Zn and Ni

content and RPD from 1,5 and 1,7 for test set. The calibration equations obtained for Pb, Zn and Ni determination could be used for dividing new samples into two groups - samples with Pb, Zn and Ni content near background values and samples with above background values of tested parameters.

The full spectral information obtained by NIRQuest 512 in the range 900–1700 nm, transformed as PLS factors was used to determine the studied trace elements content. PLS regression vectors were investigated to find the important spectral information for prediction of tested parameters. Spectral data at wavelengths, for which regression coefficients have high values, are significant for prediction of tested soil parameters. The obtained significant spectral information for Cu, Zn, Pb, Ni and Cd content determination is summarized and presented on Table 2.

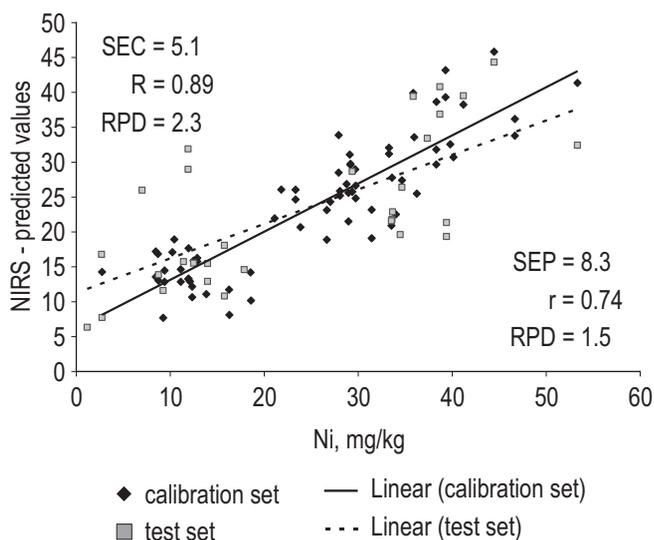


Figure 6. Relation between actual and NIRS predicted values of total Ni content in the tested samples

Table 2. Important spectral information for Cu, Zn, Pb, Ni and Cd content determination.

	Range, nm		
Cu, mg/kg		1259	1422, 1629
Zn, mg/kg	1151-1164		1421, 1618, 1721
Pb, mg/kg	915, 1065	1151-1168	1430, 1632
Ni, mg/kg	909		1411-1436
Cd, mg/kg	907	1270	1430, 1528

spectrum in a specific way (Viscarra Rossel, 2006; Onions, 2009).

The most significant spectral range in every calibration equation for the studied soil parameters was wavelength range from 1411 to 1436 nm. According to Viscarra Rossel (2006) and Onions (2009) absorption around 1400 nm are connected with absorption of O-H – first overtone in free water, absorbed water and O-H functional groups in crystal structure of clay minerals in soil, like kaoline, illite, bentonite. Spectral data at 907, 909 and 915 nm were used in calibration models for Pb, Ni and Cd content predicted. Absorption near 900 and 915 nm is also connected with Mg-OH bending and O-H stretching vibrations in soil minerals, like chlorite.

The next important spectral range in the calibration models for the tested trace elements was wavelength range from 1528 to 1629 nm. According to Gaydon et al. (2009) rock with high-grade sulphide minerals like chalcopyrite (CuFeS_2) and bornite (Cu_5FeS_4) exhibit a strong peak between 1500 and 1600 nm. Absorption around 1630 nm is connected also with aromatics C-H groups in humic acids in soil. (Stenberg et al, 2010). Analysis of important spectral information in calibration equations confirmed that determination of Cu, Zn, Pb, Ni and Cd content was based on NIR spectra of organic matter and humic-clay complex and minerals in the soil. Therefore the relationship between Cu, Zn, Pb, Ni and Cd content in soil and soil spectra was indirect.

Conclusion

Near infrared reflectance spectroscopy with chemometrics

Trace elements in soils can be divided into different fractions – water soluble, exchangeable, oxide-bound, carbonate-bound, organic matter-bound and residual. The residual trace elements are those occluded in the resistant minerals and are non-extractable. Metals can be adsorbed onto surfaces of soil colloids (clay minerals, humic acid, clay-humic complex) through non-specific adsorption (by static electric force) and specific bonds between the ion and the surface). Chelation is a process during which trace elements form stable complexes with organic or inorganic ligands. Organic matter such as humic acids, fulvic acids and organic acids can serve as ligands to chelate trace elements in soil (He et al., 2005).

Not all fractions of heavy metals will influence near-infrared soil spectra. Pure metals do not absorb in the vis-NIR region. However, they can be detected because of co-variation with spectrally active components. For example, they can be complexed with organic matter, associated with hydroxides, sulfides, carbonates, or oxides that are detectable in the vis-NIR, or adsorbed to clay minerals. (Stenberg et al., 2010) Water-soluble metals will change water structure and position of NIR water peaks. Humic acids and clay content in soil have absorption in near infrared region and organic matter-bound and clay minerals bound heavy metals will change soil

technique have high potential for total copper content predicted in samples from different soil units. The ratio SD/SEC and SD/SEP obtained for determination of Cd, Pb, Zn and Ni mg/kg content via near-infrared spectroscopy could be classified as model with good accuracy of determination. The relationship between soil tested parameters content and soil spectra was indirect and based on specific bonds between the metal ion and soil colloids (clay minerals, humic acid, humic-clay complex).

Acknowledgements

This work was supported financially by the Norwegian Collaboration Program, NORWAY GRANTS, Subject: "Assessment, reduction and prevention of air, water and soil pollution in Stara Zagora Region" Ref. No. 2008/115236.

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The introduction must answer the following questions: What is known and what is new on the studied issue? What necessitated the research problem, described in the paper? What is your hypothesis and goal?

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possible for others to repeat the experiment in order to verify results.

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Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

Conclusion: The most important consequences for the science and practice resulting from the conducted research should be summarized in a few sentences. The conclusions shouldn't be numbered and no new paragraphs be used. Contributions are the core of conclusions.

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Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows, IXth International Conference on Production Diseases in Farm Animals, Sept. 11 – 14, Berlin, Germany, p. 302 (Abstr.).

Thesis:

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AGRICULTURAL SCIENCE AND TECHNOLOGY

Volume 4, Number 4
December 2012



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