

4 Analytical and statistical methods

The problems of soil pollution with heavy metals and organic pollutants are subject of investigation in all countries of Europe including Bulgaria. Their correct solution requires the application of very precise analytical methods and reproducibility of the different pollutants determination in soil and the definition of reliable criteria for the estimation of the pollution degree that are compatible with the concrete conditions.

4.1 Pedological analysis

Morphological description of investigated soil profiles was made according to standard methods presented in “MANUAL FOR SOIL SURVEY AND MAINTAINING DIGITAL SOIL MAP OF BULGARIA” (1995). Colour was recorded as hue, value and chroma (MUNSELL COLOUR CHARTS 1973). About 3 kg soil samples from main soil horizons was taken for analysis. The soil samples were dried at room temperature for several days. After drying large stones and roots were removed and then soil samples were homogenised and sieved through a 2.0 mm sieve.

For characterisation the main physical, physico–chemical, and chemical properties of investigated soils were used following analyses (TOTEV et al. 1987):

- The pH is determined by a pH-meter glass electrode in a soil suspension. The suspension was prepared by shaking 1 part of soil with 2.5 parts of distilled water;
- Soil moisture – individual soil moisture values are determined gravimetrically;
- Total carbonates – is determined by “volumetric calcimetry”;
- The total organic matter (OM) content was obtained by wet oxidation (KONONOVA 1963);
- Texture of analysed soil profiles was determined according to the FAO (1987) method, with separation of following fraction sizes: coarse sand (2.0 - 0.2 mm); fine sand (0.2 – 0.02 mm); silt (0.02 – 0.002 mm); clay (<0.002 mm). Soil samples were ultrasonically dispersed prior to size and density separation of primary particles (ETROPOLSKY and ATANASSOV 1976).
- Extraction of soil fraction – approximately 10 g from each soil fraction (coarse sand; sand; slit and clay) were extracted from master soil horizons, followed by determina-

tion of total chemical and heavy metals content. Before separation soil samples were ultrasonically dispersed, and the extraction was carried out in distilled water.

4.2 Analytical methods for determination of heavy metals

The rapid and accurate determination of heavy metals in environmental matrices such as soil has led to the development and continuous improvement of various analytical methods concerning both sample digestion and the choice of most appropriate instrumental techniques to obtain exhaustive chemical information in shortest time possible.

At present, there are various official methods which are applying different acid mixtures and microwave heating systems. In this respect official methods for soil analysis in many countries report the use of aqua regia for the solubilisation of various analytes (NEN 6465 1981; Metodo Ufficiale no. 13 1994; DIN EN ISO 11885:04.98).

The instrumental analysis has lately evolved from monoelemental spectroscopic techniques (FAAS and GFAAS) to multielemental ones (ICP-AES and ICP-MS) which allow the analysis of a large number of analytes in a reasonably short time and achieve wider sample characterisation. The successful use of these techniques depends on various conditions: good detection limits, wide dynamic range, compatibility with different acid mixtures for sample dissolution and low matrix effects.

Optical atomic emission techniques, e.g. those using plasma source such as ICP-AES, are suitable to perform simultaneous or fast sequential determination of a large number of elements in a single sample solution at trace level. On the other hand, mass-spectrometry methods based on ion counting instead of photon counting involve an extremely fast methodology with high sensitivity (SANZ-MEDEL 1997).

At present, the estimation of the degree of soil pollution with heavy metals in Bulgaria is based on their total contents determined by total soil digestion in a mixture of HF-HClO₄ according the Bulgarian Government Standards (BGS) (17.4.4.02-80; 17.4.4.03-80; 17.4.4.04-80; 17.4.4.05-80). This method is comparatively expensive, dangerous for the lab staff and harmful for the environment. Comparison between two methods of soil digestion for the determination of heavy metal contents: HF-HClO₄ standardized in Bulgaria and the aqua regia method standardized in Germany (DIN EN ISO 11885:04.98) was made by

BOJINOVA et al. (1996). The aqua regia method (DIN EN ISO 11885:04.98) had a comparative degree of extraction for elements Cd, Cu, Ni, Pb, and Zn, it is cheaper, safer and there is no need of special laboratory equipment.

In this study the total content of Cd, Cu, Ni, Pb, and Zn was determined as well in whole soil as in particle-size fractions extracted from soil horizons according to DIN EN ISO 11885:04.98. The determined total concentrations of investigated elements are presented in appendix Tables A5 and A6 (appendix).

TOTAL CHEMICAL ANALYSIS

Refractory materials such as ores, slags and minerals are often very resistant to acid attack, making the preparation of a sample solution difficult and time consuming. Selection of an effective method of disintegrating the sample matrix to a reproducible form is a prerequisite for speedy, precise and accurate analysis.

Fusion with a molen alkali metal borate flux provides a rapid and simple means of dissolving chemically stable materials to yield glass-like solid solutions. This method eliminates any inhomogeneity of particle size, density or composition in the sample. Borate fluxes perform a dual function in atomic optic spectrographic analysis. First, they provide a simple and effective means of disintegrating the sample into a glass-like solid of uniform composition. Secondly, a borate flux acts as a spectrochemical “buffer”. In this investigation there determined the total chemical content in studied soils (see appendix Table A7) after heating of mix soil/lithium meta-borate at a ratio 1:3, and measuring total metals content by ICP-AES (KAMBUROVA et al. 1991).

4.3 Analytical methods for determination of organic compounds

For determination of organic pollutants in solid matrices, the appropriate selection of sensitive and selective measurement techniques involves, in general, the use of adequate extraction procedures followed by a careful clean-up of the extracts and extensive pre-concentration prior to injection of the sample in a chromatographic system in order to determine as many compounds as possible without mutual interferences.

Different drying (thermal, freeze-drying, chemical drying), extraction (Soxhlet, sonication, KOH-digestion, mechanical shaking, supercritical fluid extraction (SFE), accelerated sol-

vent extraction (ASE), microwave assisted extraction (MAE)) and detection techniques (high resolution gas–chromatography–mass-spectrometry (HRCG-MS), liquid chromatography - fluorescence (LC-FD)) were applied to real samples with respect to the final determination of organic pollutants.

Thermal and chemical drying were equally suited, whereas applying freeze-drying naphthalene was partly lost. Thermal drying at ambient temperature showed generally no losses of the compounds (BERSET et al. 1999).

The main challenge in obtaining fast and reliable analytical results in organic pollutant determinations is finding appropriate extraction procedures that are suitable for application to complex matrices. The goal is to extract many pollutants in a single operation and to develop robust and fast clean-up procedures.

More recently other extraction techniques have been developed trying to reduce extraction time and the amount of solvent. Some of these methods like SFE, ASE and MAE (BERSET et al. 1999) have successfully been applied to the determination of organic pollutant in environmental matrices like soils and sediments. The aim of every extraction technique in analytical chemistry is to remove effectively the analyte from its matrix, rapidly, with minimal solvent usage and quantitatively. The choice of extraction technique is frequently decided upon based on initial capital cost, operating costs, simplicity of operation, amount of organic solvent required and sample throughput. The cold-liquid extraction is still well established method. The extraction time for the group sonication, mechanical shaking, ASE and SFE was very short compared to Soxhlet extraction. But ASE and SFE have high cost and need high time for method development.

The use of mass spectrometry detection systems available for liquid and gas chromatography offers tremendous advantages for both the selective detection of compounds in complex, non totally resolved mixtures and for the sensitive determination of organic pollutants, providing fast sequential information on multiple compounds with a high level of sensitivity (KRAHN et al. 1993; DAVIS et al. 1993).

Determination of priority organic pollutants was carried out according E DIN ISO 10382: 02.98 and E DIN ISO 13877: 06.95. For quality assurance in all used methods procedural blanks and control samples were processed in the same manner as a real samples.

4.4 Statistical methods

Data sets for investigated soils contain values that are extremely high or low, i.e. they are outliers in the statistical sense. Also, because the data are not normally distributed, the application of parametric statistical methods results in distorted reflections of reality. Application of non-parametric statistical methods for quantitative description of widely varying values give information which can often be interpreted better than the result from normal parametric methods. These methods do not require calculation of the usual parameters such as the mean or standard deviation, and therefore they are also called non-parametric. Non-parametric tests have in common that they do not use the values of the quantitative variables but their ranks and they are based on counting.

Two sets of numbers (x_1, x_2, \dots, x_{N_x}) and (y_1, y_2, \dots, y_{N_y}) from independent measuring series, with n_x and n_y measurements were examined with non-parametric test (Wilcoxon). It applies to check if the x 's and y 's are drawn from the same distribution. The null hypothesis is that both members of the pair are drawn from the same distribution. If this is true, the differences are equally likely to be positive or negative and should be distributed symmetrically (MASSART et al. 1988).

To perform the test one:

- Ø computes the list of differences $\delta_j = (x_j - y_j)$;
- Ø sorts the absolute values of the differences $\{|\delta_j|\}$ into ascending order;
- Ø adds up the ranks assigned to the positive differences or the ranks assigned to the negative differences. This sum is called T.
- Ø $T = \min (T+, T-)$
- Ø $T_{crit} = (n, \alpha)$.

Null hypothesis is to be rejected, if $T_{min} \leq T_{crit}(n, \alpha)$. If null hypothesis were true, it would be expected that the sum of all ranks for positive differences (T_+) would or close to the sum for negative differences (T_-).

The check for mutual, linear dependency of two variables is made by the calculation of the Spearman correlation coefficient r_s ($-1 \leq r_s \leq +1$). This coefficient measures the relationships between two variables that are both measured on ordinal scales and can therefore, be used as a variable alternative to the Pearson correlation, which requires the conditions for the use of parametric analyses to be satisfied. The Spearman correlation is interested in obtaining a measure of the consistency of the relationship across X and Y values (HARTUNG et al. 1987). For two – dimensional ordinal scaled features (X,Y) also to the n investigation units observed developments, one can calculate then the Spearman struggle correlation coefficient r_s , which corresponds to the normal coefficient of correlation r_{XY} ranks.

$$r_s = \frac{\sum_{i=1}^n R(x_i)R(y_i) - n\bar{R}(x)\bar{R}(y)}{\sqrt{(\sum_{i=1}^n R(x_i)^2 - n\bar{R}(x)^2)(\sum_{i=1}^n R(y_i)^2 - n\bar{R}(y)^2)}}$$

When transform r_s with $d_i = R(x_i) - R(y_i)$ substitution:

$$r_s = 1 - \frac{6 \sum_{i=1}^n d_i^2}{n(n^2 - 1)}$$