

Article

The Effect of Sample Preparation and Measurement Techniques on Heavy Metals Concentrations in Soil: Case Study from Kraków, Poland, Europe

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Abstract: Accurate and reliable laboratory results are an extremely important and integral part of conducting scientific research. Many factors influence the results obtained, including the type of determination method, accuracy and precision of measurements, and laboratory equipment used for the tests. This paper presents the results of measurements of heavy metal concentration in soil using two methods for adding soil components into solution and different laboratory methods and types of measuring equipment. The first method used was hot digestion of soil samples with a mixture of concentrated HNO₃ and HClO₄, after prior ashing of organic matter (IUNG method). The second method was a two-stage decomposition, where soil samples were hot digested, initially with oxidizing acid (HNO₃) and subsequently with non-oxidizing acid (HF) (two-stage decomposition). The concentrations of selected heavy metals (Cr, Cu, Fe, Mn, Ni, Pb and Zn) were determined in solutions obtained by both digestion methods. The solutions obtained from soil decomposition were determined twice using atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP MS) methods in different laboratories using different types of spectrometers. In most cases, the measured concentrations of heavy metals are the highest for the two-stage solution samples and the measurements of their concentrations carried out using ICP. The exceptions are the measurements of Cu and Ni concentrations. In the case of Cu, lack of significant differentiation in concentrations of this metal may result from different forms of occurrence of Cu in soil than in the case of other metals. For Ni, however, a reversal of the trends seen for the other metals is observed and the type of spectrometer used for measurements is important. There may be an interference of the spectrometric spectrum of Ni with the spectra of the other determined metals. However, this is not clear at the present stage of the study. In conclusion, the results of this study indicate that the choice of soil sample preparation and the type of spectrometer used for measurements can, in many cases, determine the value of laboratory results, even if it is in an expected range of standard material. Research of published papers proved that most of them show only results based on one selected course of methodology without comparison with others. The novelty of the paper is the comparison of the measurements of heavy metal concentration in soil using two methods for adding soil components into solution and different laboratory methods and types of measuring equipment. Additionally, the article includes a discussion of the importance of methodology. We believe that the conclusions may help to better understand how sample preparation and measurement methods applied may influence the results obtained.

Keywords: heavy metals; soil; determination methodology; sample preparation; spectrometry



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1. Introduction

Accurate and reliable laboratory results are essential for scientific research. Results can be affected by many factors, including the test methods used, the accuracy and precision of the test performed, and the laboratory equipment used [1]. Measurements of concentrations of various chemical compounds/elements are carried out, among others, to study the composition of soils. Soil is a complex three-phase solid-liquid-gas system, in which continuous processes of decomposition and synthesis of mineral and organic compounds and their transfer between different soil horizons take place. The infiltration of compounds into the soil is made possible by infiltrating water. Rainwater percolates through the different soil layers, dissolving and/or transporting dissolved substances and suspended solids to deeper soil layers. From an agronomic point of view, the soil must be protected and used in such a way that it is possible to continuously produce sufficient quantities of high-quality plant raw materials [2]. Heavy metals play an important role here as excessive accumulation of these elements in soils may cause far-reaching, often irreversible chemical degradation of soils. In the short term, negative effects of heavy metal pollution may be hardly noticeable, however, their gradual accumulation may lead to exceeding the sorption capacity of soils and, as a consequence, release of those metals into the environment. This can be a source of significant contamination of the water-soil environment and consequently the food chain. Contaminants accumulating in soils may also affect soil organisms and/or plants, which may lead to a reduction in biodiversity or decrease in soil vegetation cover, and consequently to an increase in water and aeolian erosion processes [3,4]. In times of rapid economic development, the content of heavy metals in soil can be significant. The biggest amount of heavy metals are emitted to the environment in the areas of mining and metallurgical processing of metal ores, cement production and industrial combustion of fossil fuels. The environment can also be a source of toxic substances. An example is Pb, which can be leached from the rock environment or derived from geothermal H₂S sources [4,5]. All over the world, the problem of heavy metal contamination of soils is common, which is a frequent subject of scientists' research, e.g., in Europe [6,7] or in Asia, including, among others, China [8,9], in Mongolia [10], in Tibet [11], India [12], and Pakistan [13].

Heavy metal ions are retained in the surface layer of soils mainly due to sorption, and their movement into the soil profile is relatively slow. Excessive accumulation of heavy metals in the surface layer of soils is one of the basic causes of chemical degradation of soils and pollution of soil-soil and surface waters. Heavy metals occurring in the soil lead to an imbalance in the recycling of soil nutrients and a decline in soil quality [13,14]. Furthermore, heavy metals are characterised by the easy accumulation in soils of different types [15,16]. The occurrence of elevated concentrations of heavy metals in agricultural soils and consequently also in food poses a threat to animal and human health. For example, too high concentrations of Cu can cause liver cirrhosis and chronic anaemia. Apart from affecting soft tissues, heavy metals can also accumulate in bone tissue, Pb being one example. Cr is also worth mentioning. Its deficiency in the body may be manifested by diabetic symptoms, while compounds of this element on high oxidation levels (+4, +6) are directly toxic and carcinogenic for humans. Based on a comprehensive analysis of heavy metal hazards, the United Environmental Protection Agency has listed eight heavy metals (Pb, Cd, Cr, Hg, As, Cu, Zn, Ni) as major contaminants of the groundwater environment [17]. Due to the high danger of heavy metals contained in soils, numerous studies have been undertaken on soil contamination with these elements e.g., [18–20]. There are many indicators determining the level of heavy metal contamination in soils, such as the pollution index [21], geo-accumulation index [22], Nemerow integrated pollution index [23], enrichment factor [24], and potential ecological risk index [25].

There are many methods used for the determination of heavy metal content in dissolved soil samples. For example, pulsed differential stripping voltammetry (DP ASV) is one of the most sensitive instrumental methods that can achieve very low limits of quantification for metal cations [26–28]. For surface samples, X-ray analysis (XRD) is used

to determine the elemental composition. The percentages of the main mineral components of soils are determined by means of pattern analysis, the number of radiation pulses and the intensity of reflections [5]. Spectroscopy methods based on the interpretation of spectra produced by the interaction of radiation with matter, especially atoms (atomic spectrometry), are widely used. Methods based on atomic spectra can be divided into absorption and emission methods. An example of the former is atomic absorption spectrometry (AAS). The principle of this method is based on determining the relation between absorption of electromagnetic radiation for a particular line of the determined element and its concentration, which is quantitatively expressed by Lambert-Beer law [5,13,26]. Atomic emission methods include, among others, the historically first atomic excitation technique: flame photometry. The concentration of elements in solutions after the previous extraction can also be determined using the technique of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) [29]. The inductively coupled plasma mass spectrometry (ICP-MS) technique was developed by Gray in 1978, and the first instruments to use it was built in 1978 [30,31]. The first instruments for its application appeared several years later than the ICP-OES technique. ICP-MS spectrometers enable fast multi-element analysis (up to 70 elements within a few minutes). The technique is characterised by high sensitivity, selectivity, low limit of quantification and rapidity of determinations. Inductively coupled plasma analytical instruments are widely used for the determination of trace amounts of metals in environmental samples such as drinking water, wastewater and groundwater. It can also be used for the determination of trace amounts of metals in petrochemical samples, soil samples, or food products.

Environmental studies require a wide variety of fieldwork and laboratory determinations. In order for the results to be a basis for further considerations and interpretation they should be characterised by sufficient accuracy and reliability. During both the field and laboratory stages of the study, there are possibilities of errors that may distort the results obtained. In order to control the precision of the determinations it is recommended to take duplicate samples and to assess the precision of laboratory determinations. Unfortunately, even if these recommendations are followed, it may be difficult to obtain fully valid test results since, depending on the preparation and measurement technique/apparatus used, significantly different results may be obtained for the same environmental samples [32]. In Poland, the information on soil contamination is scarce and selective. Usually, the information concerns larger agglomerations, i.e., Warszawa, Łódź, or Wrocław [33–36], and mainly characterise concentrations of heavy metals in soils. The information on soil contamination in towns of medium and small size (<100,000 inhabitants) is sporadic. There is also a lack of literature reports on sample preparation and measurement techniques, which may influence the variability of heavy metal determination results in soil. In this context, the methodology of determinations, the accuracy and precision of the measurements performed, and the laboratory equipment used for the studies are important. Taking into account the above observations, the aim of this study was to determine the influence of the method of sample preparation and the applied measurement methods on the variation in the determination of heavy metal concentrations in soils. The study area was the area directly adjacent to the Arcelor Mittal metallurgical plant near Kraków in southern Poland. This area is characterised by a significantly polluted soil environment as a result of many years of operation of the steelworks. Soil samples prepared by two methods were analysed and the solutions obtained were determined using two analytical methods and different types of spectrometers. The contents of heavy metals in soil determined using two selected methods commonly used in this type of study, i.e., AAS and ICP-MS, were compared.

Wide research of the published articles proves that most of them show only results based on one selected course of methodology without comparison with others. Many of them are based only on the influence of preparation methodology in plants, i.e., on *Pleurozium schreberi* moss samples prior to use in active biomonitoring studies [37]. Four different methodologies were tested across four different sample locations with the conclusion that of the four methods used to prepare mosses for later exposure in active biomonitoring,

the best method is averaging with simultaneous conditioning of mosses in demineralised water. Another published paper shows evaluation and comparison between a conventional acid digestion method and a microwave digestion system for heavy metals determination in *Mentha* samples by ICP-MS [38]. The concentrations of selected heavy metals were measured in the stems and leaves of the *Artemisia herba-alba* medicinal plant and soil samples were evaluated by Massadeh et al. [39]. Results showed different results in heavy metal concentrations of washed and unwashed parts of plants, which is still the preparation method. The results of this study [39] revealed that there is a correlation between heavy metal levels in medicinal plants and their concentrations in soil. Usually, authors show that results depend on the preparation but choose only one measurement method.

The novelty of the paper is the comparison of the measurements of heavy metal concentration in soil using two methods for adding soil components into solution and different laboratory methods and types of measuring equipment. Additionally, the article includes a discussion of the importance of the methodology. We believe that the conclusions may help to better understand how sample preparation and measurement methods applied may influence the results obtained.

Innovation of our research is the comparison of the selected methods which shows how the methodology is important. Results are highly dependent on the preparation and measurement methods applied. We believe that the conclusions based on the comparison may help to better understand how the methodology applied may influence the results obtained.

2. Study Area

The study area covers 6200 ha and is located directly at the eastern border of the Arcelor Mittal (formerly W. Lenin) steelworks in the Kraków area (southern Poland, Central Europe). The study area was subjected to almost 70 years of the direct impact of the steelworks. The steelworks mainly emit dust-gas pollutants, which reach the ground surface in the form of wet and dry precipitation (Figure 1). The main pollutants are gaseous sulphur compounds and heavy metals (Cr, Cu, Fe, Mn, Ni, Pb, Zn) occurring in various chemical forms (including elemental form). The technological development of the steelworks and changes in the volume of steel production directly affected the emission of pollutants. In the initial years of the steelworks existence (mainly 1950s to 1980s), the emission of pollutants to the environment was very high. At present, the modern technologies applied have considerably reduced it. However, the pollutants emitted during many years of the steelworks existence still constitute a serious environmental problem and the explanation of the processes they undergo in the water and soil environment is a key issue for the assessment of the condition of the natural environment in the studied area. Among the pollutants emitted by the steelworks, heavy metals are potentially the greatest hazard to the environment. The following were selected for detailed analyses: Cr, Cu, Fe, Mn, Ni, Pb and Zn, while the main source of these metals in the study area was/is the emission from the steelworks.

The study area is characterised by high homogeneity of soil types—the parent rocks of soils are mainly Quaternary loess sediments related to the Vistula glaciation. Soils developed on outcrops of Cretaceous marls (Figure 2) occur only subordinately in small areas of the NE part of the study area. Besides the mentioned primary loess deposits, alluvial and deluvial deposits occur in narrow stream valleys. Very small fragments of stream valleys are occupied by mud and peat deposits. The areas of particular types and subtypes calculated on the basis of planimetric measurements (Table 1) confirm the domination of soils developed on loess.

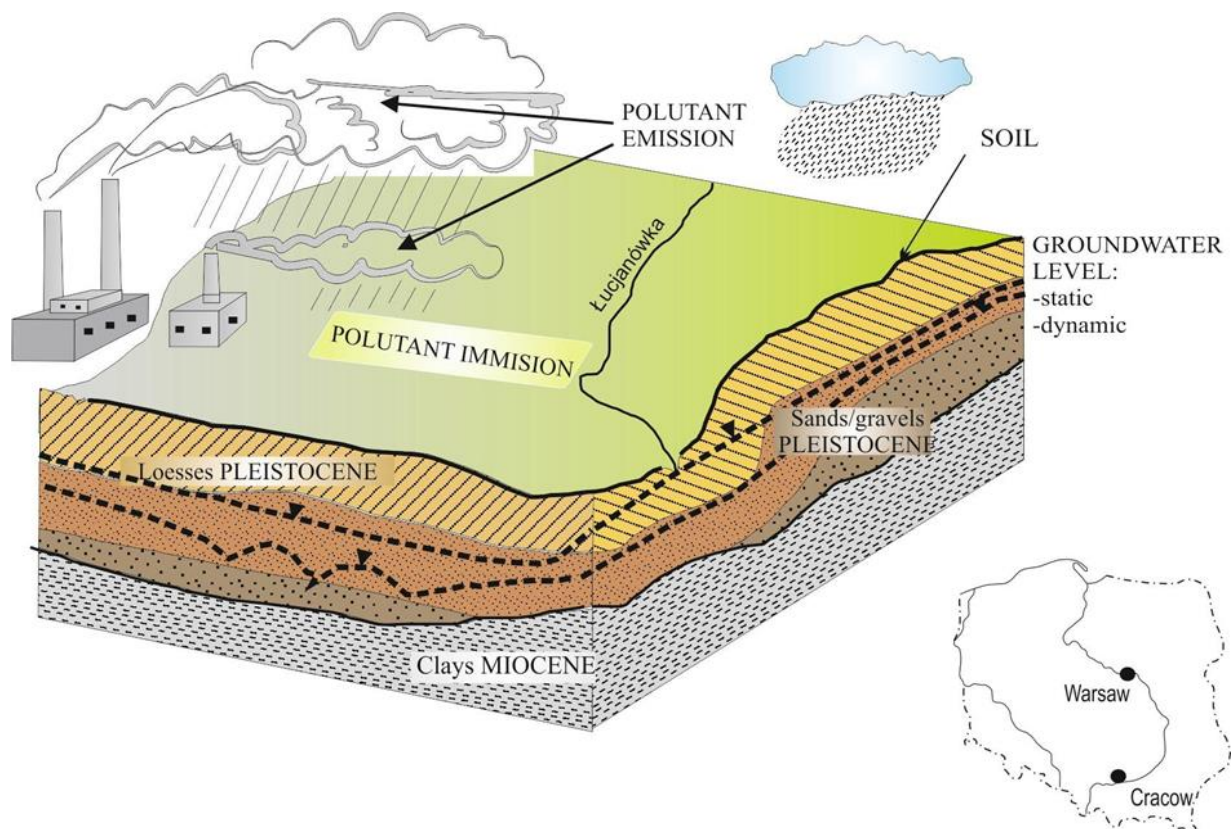


Figure 1. Study area layout and location.

Table 1. Soil types of the study area [40].

Soil Types and Subtypes	Share [%]
brown soils developed from loess and loess loam formations	22.4
proper deluvial brown soils developed from loessial and loessial clay formations	3.0
alkaline lignite soils developed from loess and clay formations	2.3
proper chernozem developed from ordinary loess formations	0.9
proper deluvial chernozems developed from loess and loess loam formations	13.4
degraded chernozem developed from loess and loess loam formations	51.4
silts formed from alluvial deposits of silt and clayey silt composition	5.1
clayey loess soils	0.7
rendzina soil formed from chalky marls	0.5
mud-peat and peat-silt soils	0.3
Total	100.0

Only in the NE part of the study area are soils developed on marl outcrops (Cretaceous), but they occupy only 0.5% of the study area (Figure 2).

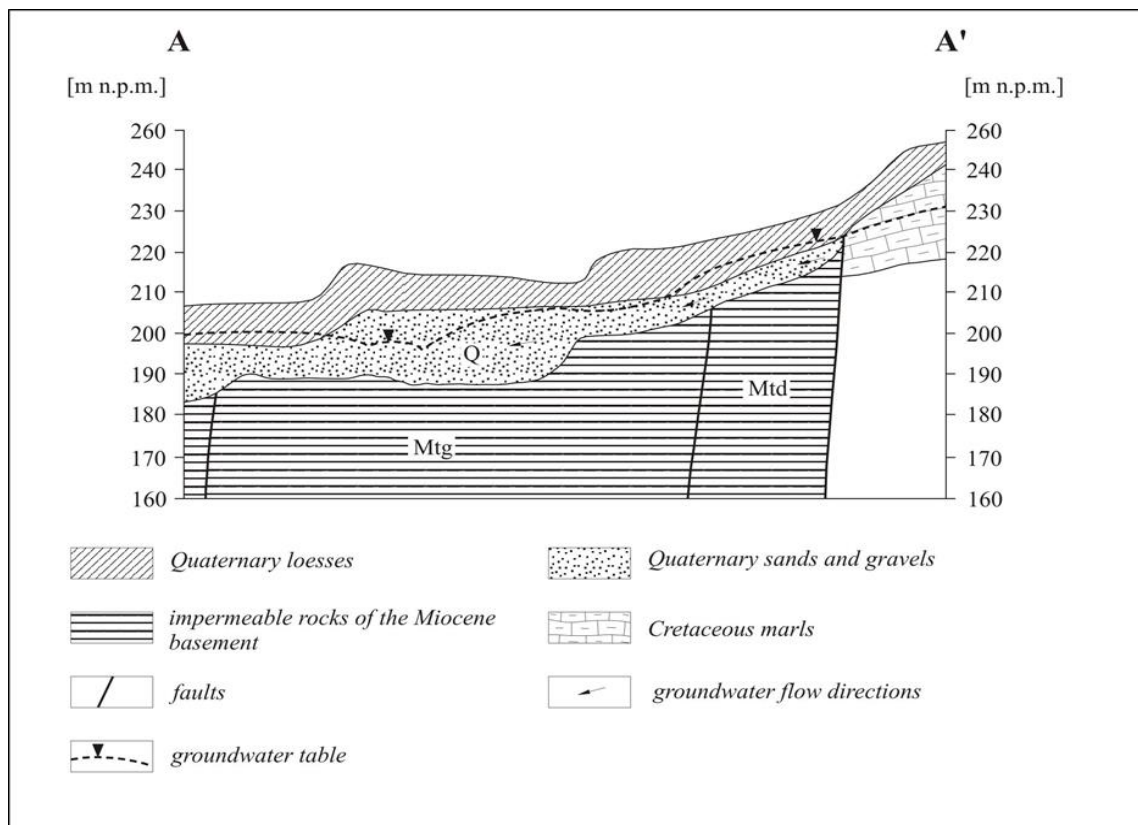


Figure 2. Simplified hydrogeological cross-section A-A' (acc. to [41] modified).

There is also an underground water intake located in the immediate vicinity of the steelworks, which is used as a source of drinking water. This intake consists of 13 wells exploiting a shallow valley water-bearing horizon. The aquifer is mainly composed of alluvial sediments of various grains. Within the studied area, the aeration zone of the aquifer is formed mainly by poorly permeable loess sediments with a thickness of several metres on average. Consequently, the aeration zone is characterised by high retention capacity due to its considerable thickness and poor permeability properties, which determines the relatively long infiltration time of rainwater. The average infiltration velocity value obtained on the basis of interpretation of natural (SO_4^{2-} ions) and artificial (Br^- ions) tracer movements in the aeration zone profile is $0.53 \text{ m} \cdot \text{year}^{-1}$ [42–47]. As a result, at least some part of heavy metals may be absorbed. It is significant because infiltration waters are the basic source of supply for the aquifer system in the study area. The study area is predominantly used for agricultural purposes. Arable fields cover the vast majority of the area. The agricultural character of land use has a major impact on soil conditions, especially on pH and humus content. This determines the ability of the soil to retain contaminants. Heavy metals are subject to a number of different physical and chemical reactions in the soil layer, which can cause significant delays in their migration into the aquifer system and often even stop their migration [30]. Heavy metal concentrations in soils also depend on the type of soil itself. In the studied area the soils are mainly characterised by neutral and alkaline reactions, which favour retention of heavy metal migration [48]. Precipitation with $\text{pH} < 5$ occurs sporadically only in April and May, which is not a significant factor that could trigger the leaching of metals from the soil cover.

3. Materials and Methods

An area of over 60 km^2 was sampled (Figure 3). Samples were collected in a kilometre grid using a bucket sampler. There were 65 samples taken from the arable soil layer (0–20 cm).

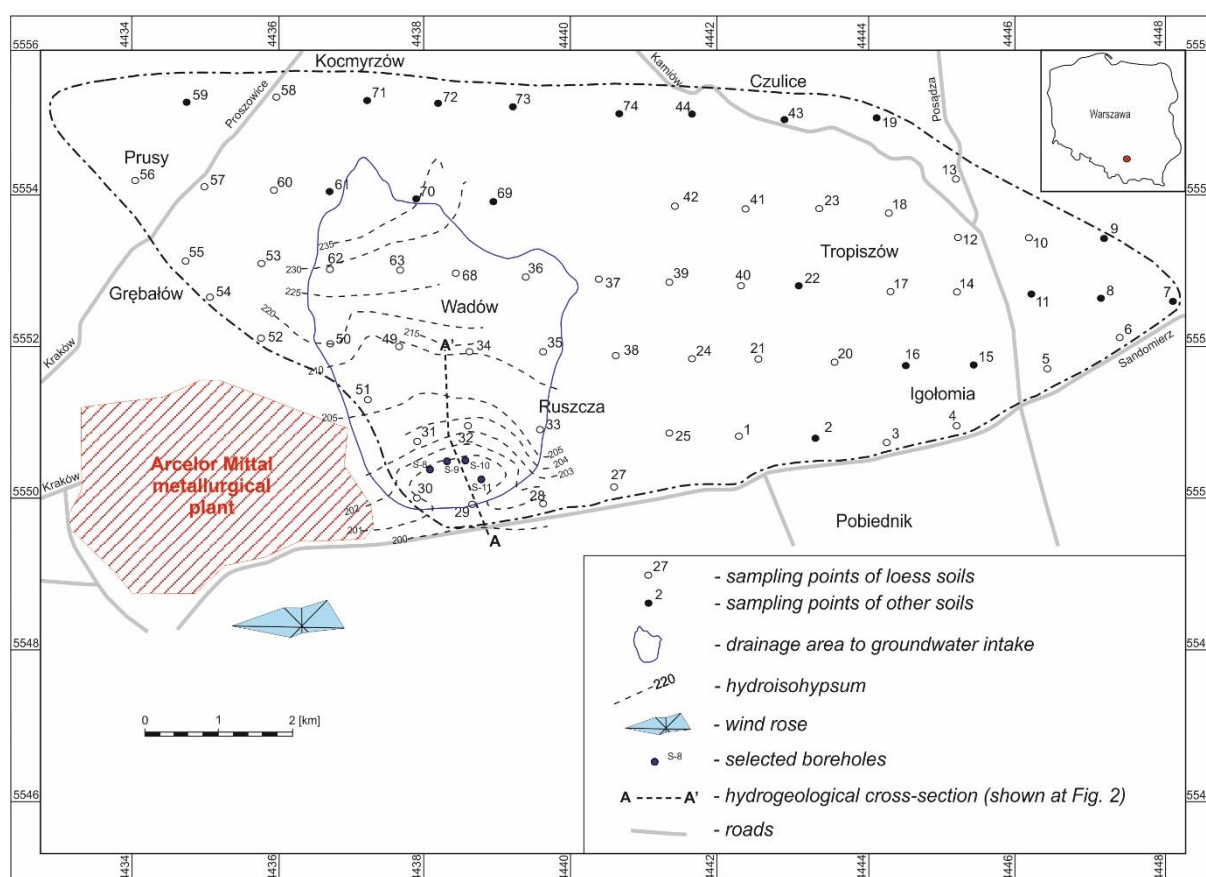


Figure 3. Sampling sites.

At 9 points, duplicate samples were taken following the same procedure as for the other soil samples. Duplicate samples accounted for 13% of all soil samples collected. Additionally, the standard material as San Joaquin soil was used and the results were sufficient. This preliminary test was necessary for further investigation. Results were in the range of expected values but some differences were observed (Table 2). It was an impulse to create the presented manuscript.

Table 2. Standard material analyses [40].

mg/kg	Standard San Joaquin Soil	AAS ₁	ICP-MS ₁
Cr	60–115 (79)	67	78.8
Cu	26–40 (32)	27	29.3
Fe	-	19.18	29.528
Mn	-	282	292.9
Ni	65–90 (78)	84	79.9
Pb	12–18 (13)	13	14.4
Zn	87–120 (100)	87	97.6

3.1. Sampling and Methods for Decomposition of Mineral Soil Components

The samples collected in linen bags in an air-dry state were transported to the Institute of Soil Science, Plant Cultivation and Fertilisation in Puławy, where preliminary operations were performed, consisting of manual grinding of air-dry samples in a porcelain mortar and sieving them through a sieve with a mesh diameter of 1 mm (which is equivalent to separation into so-called earthy and skeletal parts). The samples were then crushed in

a 12-min cycle in an electric agate mortar to a grain diameter of <0.1 mm. The soil dust obtained as a result of grinding was stored in paper bags in an air-dry state. The material thus prepared was used for further studies. The concentrations of heavy metals in the soils of the study area were determined using two methods of transferring components from soil to solution:

- with the IUNG method of hot digestion of soil material with a mixture of concentrated HNO_3 and HClO_4 after incineration of organic matter [49];
- with a two-step decomposition method, i.e., oxidative acid digestion followed by non-oxidative acid digestion (HNO_3 and HF , respectively) in a microwave oven [50].

Complete decomposition of soil minerals is a relatively difficult operation, so cold or hot leaching of soil components with mineral acids and mixtures is often used. The chemical components brought into solution by strong acids during hot leaching are assumed to be close to their total content in the soil [49]. In soils with a similar grain size distribution, the relation between the amount of chemical components in the digestion solution and their total content is relatively constant. To determine the so-called total content of individual elements in the soil, the method of hot digestion of soil material with a mixture of concentrated HNO_3 and HClO_4 was adopted. Acid digestion of organic soils is preceded by the ashing of the organic matter. Soil ashing releases components bound in the organic matter. Digestion of the incineration residue with a mixture of HNO_3 and HClO_4 causes decomposition of most of the soil minerals and the passage of the constituents into solution. In the following part of the article, this decomposition is referred to as the IUNG method and was carried out at the Department of Soil Science and Soil Erosion of the IUNG in Puławy. The second method of decomposition was a two-stage decomposition, i.e., digestion with oxidizing acid and then with non-oxidizing acid (HNO_3 and HF , respectively), hot, in a microwave, which was carried out in the laboratory of the Department of Hydrogeology and Water Protection of the AGH University of Science and Technology (AGH) in Kraków.

3.2. Methods for the Determination of Heavy Metals in Soil and Assessment of the Precision of These Determinations

The concentrations of selected heavy metals (Zn, Pb, Cu, Fe, Mn, Ni, Cr) were determined in solutions obtained by both digestion methods. The solutions obtained from the decomposition by the IUNG method were determined twice: by the AAS method (AA500 flame, PG Instruments, Leicestershire, UK) in the IUNG laboratory and by the ICP MS method (ELAN 6100 Perkin Elmer, Waltham, USA) in the AGH laboratory. Solutions obtained from two-stage decomposition were analysed only on ICP MS in the AGH laboratory.

To assess the precision of determinations based on results of determinations in duplicate samples, hydrogeochemical simplified methods were applied [1], i.e., correlation plot and percentile diagram. Duplicate samples should constitute at least 6% of the total number of normal samples and should be taken randomly [51]. The described graph for estimating precision is based on percentiles 50, 90, and 99 of the $\sigma_c = 0.05$ c function, plotted on a logarithmic grid. The diagrams (Figure 4) should be viewed considering the position of individual points in relation to the lines limiting the likely percentage of differences between samples for the assumed model (in this case for a precision of 5%). If more than 50% of the points are below the 50% line and less than 10% are above the 90% line, it can be considered that the assumed precision has been achieved.

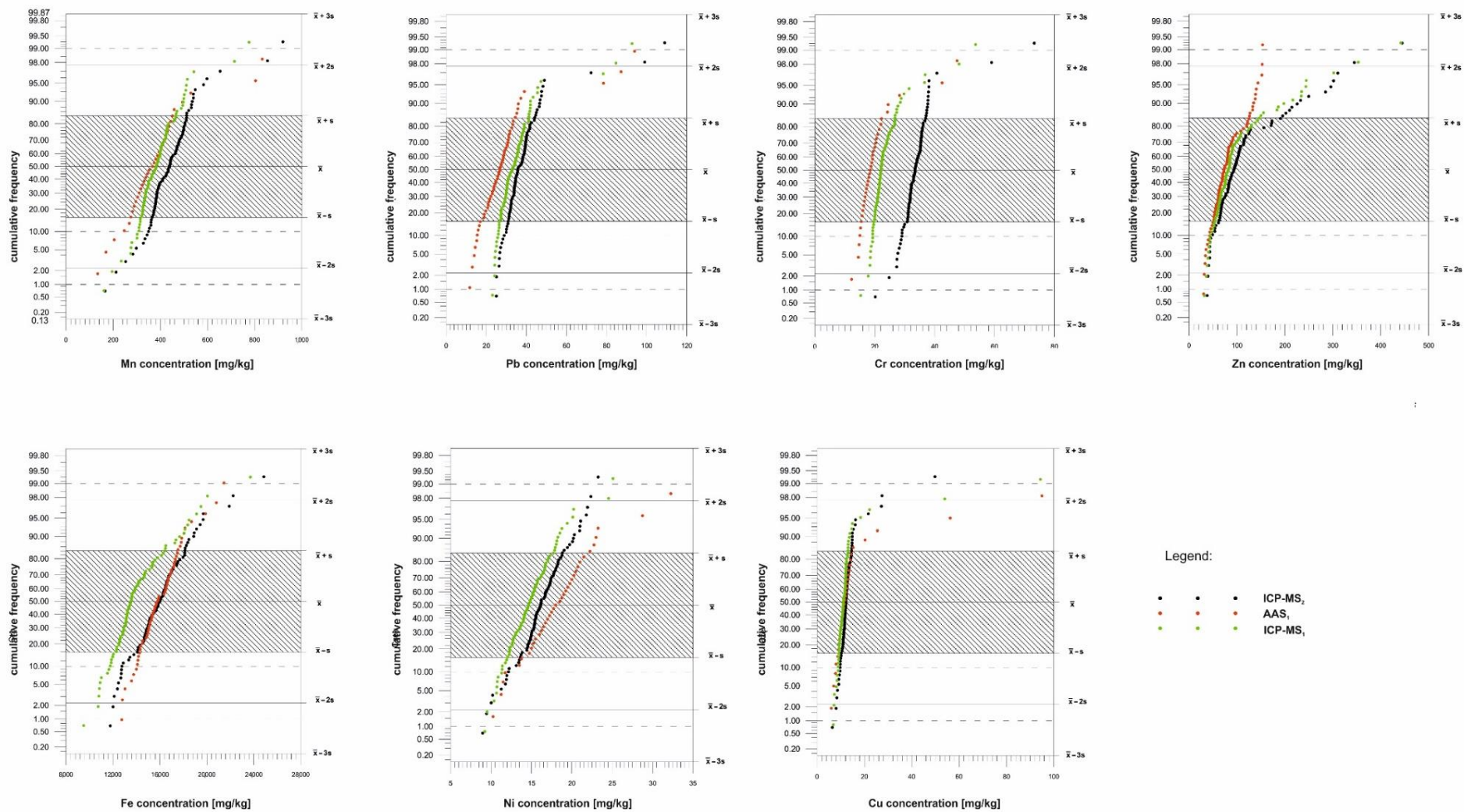


Figure 4. Frequency plot of cumulative heavy metal content determined by different methods in soil samples.

In order to determine whether the variation in heavy metal concentrations in individual soil samples statistically significantly differs depending on the analytical method used and/or sample preparation, analysis of variance with Repeated Measures Anova (hereafter referred to as RMA) was applied. In this analysis, the null hypothesis of equality of means of individual measurements is tested. Rejection of the null hypothesis indicates the presence of significant differences between the means in successive repeated measurements. RMA, apart from the assumptions typical for the analysis of variance (normality, homogeneity of variance), also requires the assumption of sphericity of variance to be met. This assumption was checked using the Mauchly test to determine the equality of variances for differences between individual measurements. If a significant violation of sphericity is found, it is recommended to perform multivariate tests, which are much more resistant to violation of sphericity.

4. Results and Discussion

The soil organic matter is characterised by significant sorption capacity, consisting of binding various components, including heavy metals, which limits their migration in the soil environment and their toxicity. The results of the content of humus in the samples indicated that the vast majority of soils were characterised by medium and high humus content (Table 3). Very high and low humus soils were of minor importance (in the order of a few percent).

Table 3. Content of hummus in soil samples.

Content of Hummus %	<1.0 Low	1.01–2.00 Medium	2.01–3.50 High	>3.51 Very High
percent of samples %	9.2	40.2	47.2	3.4

The pH value is a very important parameter characterising the soil's ability to retain heavy metals. The results of the pH in KCl for soil samples are presented in Table 4. The general pH of the analysed soils was mostly neutral and slightly acidic.

Table 4. pH values in soil samples.

pH (in KCl)	<4.5 Very Acidic	4.6–5.5 Acidic	5.6–6.5 Slightly Acidic	6.6–7.2 Neutral	>7.2 Alcaline
percent of samples %	9.2	17.2	26.4	34.6	12.6

The concentrations of heavy metals in soil samples depend on many factors and are time-varying. The assessment of heavy metals accumulation and the self-cleaning ability of the soil environment as a heavy metal storage medium is a complex issue. The process of migration of pollutants can be divided into two stages. The first one is related to the direct supply of pollutants as a result of increased anthropopressure and their accumulation in the environment. The second stage is the duration of the operation of the processes to which the stored pollutants are subject after the anthropopressure has ceased or markedly diminished. Due to the slow migration of some pollutants, such as the heavy metals considered, the system can naturally reduce the mass, toxicity, concentration and mobility of pollutants without human intervention. Such entrusting to nature of some or all of the self-cleaning processes has been defined by the Office of Solid Waste and Emergency Response (OSWER) of the EPA as Natural Attenuation (also known as intrinsic remediation or natural restoration) [52].

Soil air is characterised by an increased content of CO₂, which, by changing the gas balance of infiltrating waters, leads to an increase in the content of HCO₃[−] ions in the waters percolating through the unsaturated zone [53]. Solid phases, usually amorphous and/or cryptocrystalline, are also formed in the soil zone. For this reason, they are more

soluble than the crystalline phases. In general, the soil also experiences a radical change in pH and Eh conditions, which affects the migration capacity of many elements along with infiltration waters. Water migration of elements in the soil zone is also dependent on the amount and nature of the organic substance, which creates various types of complex compounds, protective colloids, etc. In the area covered by the research, brown soils and chernozems, characterized by a high degree of enrichment in organic matter, predominate. Sorption processes take place in the soils, in which colloids, clay minerals and organic matter participate. This study focuses only on the physicochemical processes occurring in the soil, with the omission of biological processes. However, it is worth noting their significant role in modifying the chemistry of the soil.

Being aware of the complexity of the processes, the ranges of concentrations of analysed metals in soils determined in the study were measured and are presented in Table 5.

Table 5. Ranges of heavy metal concentrations in soils in the study area [mg/kg].

	IUNG Distribution Designation on the AAS (AAS ₁)	IUNG Distribution Determination on ICP-MS (ICP-MS ₁)	Two-Step Decomposition Determination on ICP-MS (ICP-MS ₂)	Geochemical Background to Loess Soils (According to Kabata-Pendias A., 1993)
Zn	30.5–138	32.1–442	37.8–445	30–360 65 on average
Pb	11.8–94	23.1–92.8	25.0–109	19–49 25 on average
Cu	6–95	6.9–94.4	6.4–49.8	4.0–53.0 19.0 on average
Cr	15.3–47.5	15.3–53.7	20.2–73.3	14.0–80.0 38.0 on average
Ni	10.3–32.3	9.5–25.1	8.9–23.3	10–104 25 on average
Fe	12,756–21,460	9510–20,055	11,771–24,854	Fe (%) 0.8–2.78 on average 1.2
Mn	134–832	161–776	166–920	380–700 560 on average

Graphical comparison of metal concentrations determined by individual methods and spectrometer types from soil samples prepared from both IUNG and two-stage decomposition is shown in Figure 4.

It can be observed that both the type of decomposition method and laboratory determination methods noticeably affected the determined concentration value of almost all the analysed heavy metals. Generally, heavy metal concentrations were higher for the two-stage decomposition than for the IUNG method. The exception was Ni, where samples prepared by the IUNG method and determined by AAS were higher than in the other methods of preparation and/or laboratory determinations. In the case of Cu, sample preparation with both methods leads to practically the same results regardless of the method of laboratory determination.

In order to confirm whether the variation in heavy metal concentrations in individual soil samples statistically significantly differs depending on the applied method of laboratory determinations and/or sample preparation, analysis of variance with repeated measurements (RMA) was applied. The results of normality tests (Shapiro-Wilk) indicate that in most cases (>92%), the analysed data are characterised by a distribution more or less deviating from normal. Therefore, an attempt was made to normalise the data using the Box-Cox transformation. Unfortunately, this did not give satisfactory results and only for two metals (Zn and Fe), a normal distribution was obtained for determinations performed

by all methods (AAS₁, ICP-MS₁ and ICP-MS₂). At the same time, the results of Mauchly's test indicate a significant violation of sphericity for all analysed data and therefore further analysis was carried out using Pillais, Hotelling and Roy's multivariate tests. The results of these tests indicate statistically significant variation in the concentrations of all heavy metals depending on the analytical method and/or sample preparation used. For in-depth data analysis, conservative post-hoc tests (Scheffé's test) were additionally performed. These tests showed that Cu concentrations in soil samples do not differ significantly depending on the chosen laboratory test method and/or sample preparation. In contrast, the other results of the post-hoc tests are consistent with the results of the multivariate tests. This ambiguity in the results may be due to the fact that even if the multivariate tests are significant, this may only apply to a limited part of the data population. In this case, the significant variation in Cu concentrations shown by the multivariate test may be the result of the presence of anomalous concentrations of this metal in some soil samples. Conservative post-hoc tests, such as the Scheffé test, are more resistant to the occurrence of anomalous values and, except for Cu, no lack of differentiation of concentrations of the analysed metals depending on the method of their determination was observed. Similar observations can be made from the analysis of the expected values of the marginal averages (Figure 5).

Similar results were presented by Jablonska et al. [54]. These authors noted that even the method of mineralisation can influence the test results, with metals such as As, Cd or Zn. As they state, when dealing with the solution of soil samples for determination of Co, Mn and Cu, total mineralization with both HF/HCl/HNO₃ and H₂O₂/HF/HNO₃ mixtures can be successfully applied due to the lack of significant differences in obtained analyses by ICP-MS and AAS techniques, however, in the case of heavily contaminated samples, it is best to use total distribution with H₂O₂/HF/HNO₃. Additionally, at extremely high metal contents, the absorption of fluorescent radiation is equally important as well as the primarily determined metal, which may be the cause of the so-called matrix effect consisting of the change of chemical composition of the sample matrix [54].

The precision determined from the duplicate samples for the determination of heavy metals is shown using a probabilistic plot in Figure 6.

The precision of heavy metal determinations was found to be satisfactory for all metals determined on both AAS and ICP-MS except Cr determined on AAS. For all determinations, more than half of the points lay below the 50% line. For Mn, Cr, Cu and Pb, individual points were just above the 99% line but did not represent more than 10% of all samples. This indicated adequate precision of determinations of metal concentrations in the studied soil samples. Only the determination of Cr concentrations by AAS showed precision slightly lower than expected (Figure 6). Concentrations of other metals were determined with precision equal to 5%.

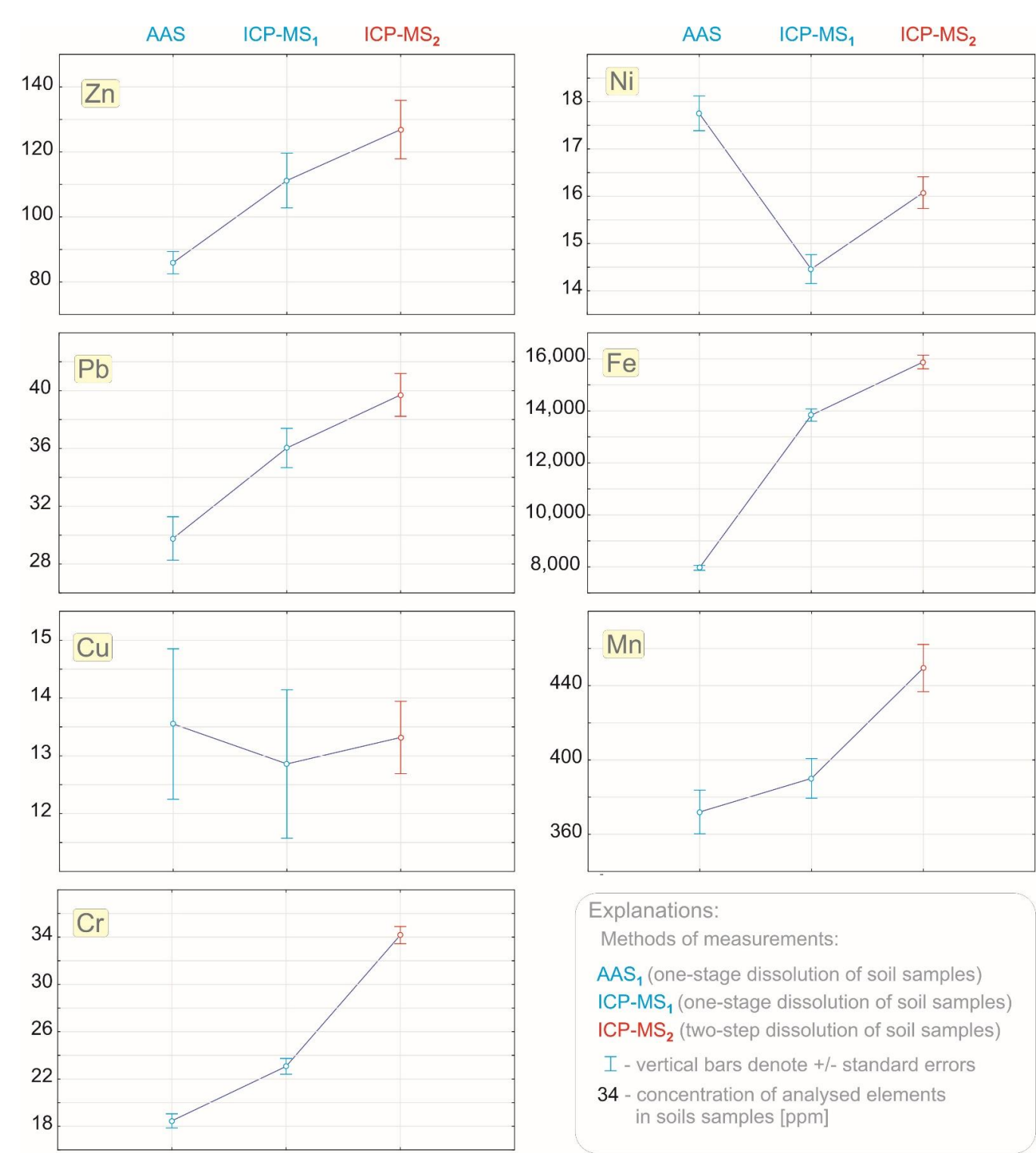


Figure 5. The Least-squares means for analysed metals.

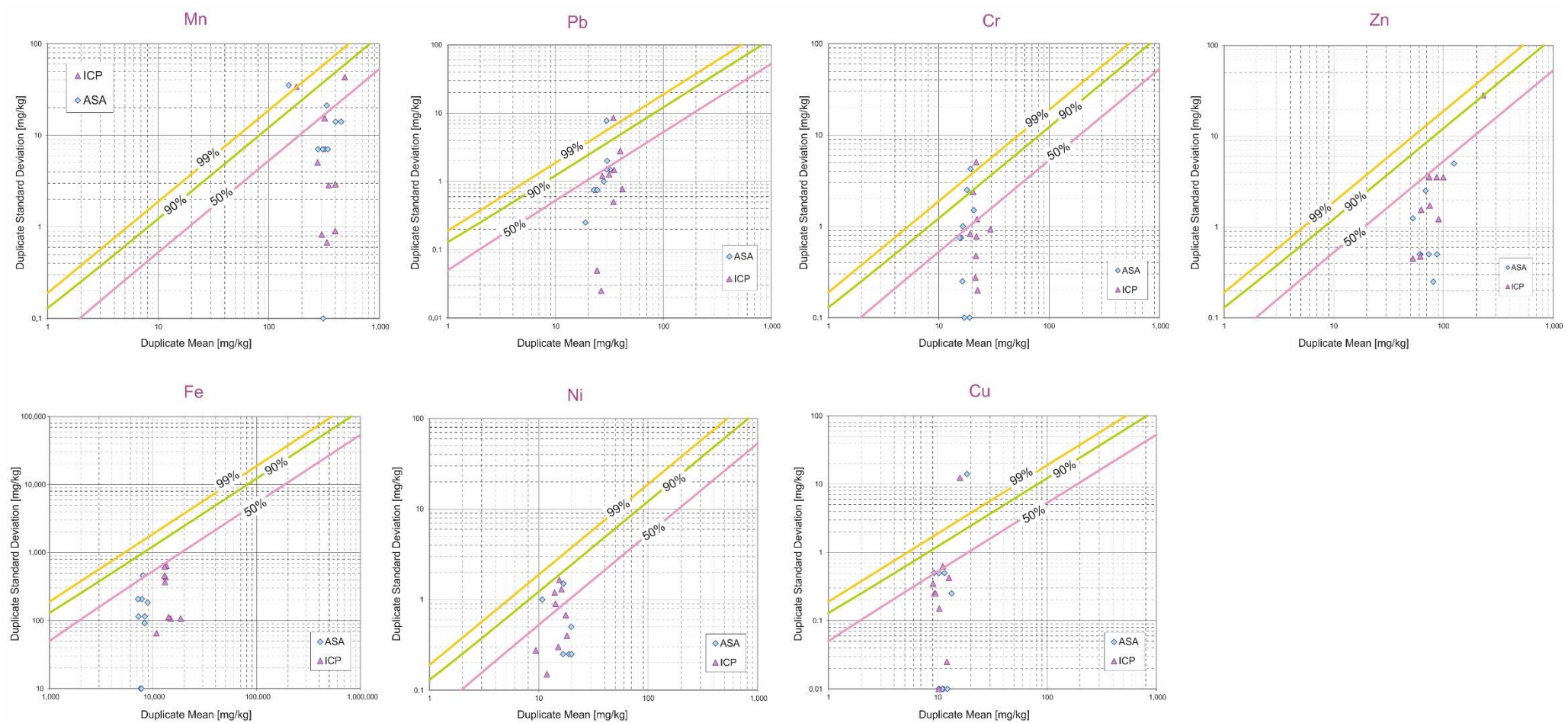


Figure 6. Precision of heavy metal determinations.

5. Conclusions

The results of the conducted studies indicate the occurrence of significant statistical differentiation of heavy metal concentrations in soil depending on the sample preparation method and/or the applied determination method. In most cases, the concentrations of heavy metals are the highest for samples digested in two stages, and their concentrations are measured using ICP-MS. However, this does not apply to Cu and Ni. In the case of Cu, the lack of significant differentiation of this metal concentration may result from different forms of Cu occurrence in the soil than the other metals. Unfortunately, the forms of occurrence of the analysed metals in soils are not known at present and it requires further studies. For Ni, however, a kind of reversal of trends seen in the case of other metals is observed and the highest concentrations are observed in the case of AAS determinations, while the lowest in the case of two-stage dissolution samples and their concentrations measured with ICP-MS. As in the case of Cu, the speciation of this metal may be important, but it seems more likely that in the case of Ni, the type of spectrometer used for the measurements may be important. It is possible that in the case of AAS, the spectrometric spectrum of Ni interferes with the spectra of the other determined metals, however, this is not fully clear at the present stage of the study, and further studies in this direction are planned.

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