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Original Research

Soil Reference Materials in Ecotoxicity Testing – Application of the Concept of EURO-Soils to Soils from Poland

B. Maliszewska-Kordybach*, A. Klimkowicz-Pawlas, B. Smreczak

Department of Soil Science, Erosion and Land Protection, Institute of Soil Science and Plant Cultivation – State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland

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Abstract

An attempt to apply the concept of so-called SIM-EURO-Soils, i.e. soils with properties similar to the reference material IRMM-443-EURO-Soils, was undertaken in relation to Polish soils. The aim of SIM-EURO-Soils identification was to find natural soils with specified properties which can be applied as reference materials in ecotoxicity testing and are available in satisfactory amounts. Two hundreds sixty seven soil samples collected from agricultural land in Poland in 2000-04 were included in the evaluation process. The soils were characterized in respect to their basic pedological properties (texture, organic matter content, pH and total nitrogen content) as well as to their level of contamination with heavy metals (Zn, Pb, Cd and Cu) and polycyclic aromatic hydrocarbons (PAHs). It was shown that it is possible to select soils with properties corresponding to SIM-EURO-Soils. One tenth of the studied soils was classified as equivalent to three groups of Pol-SIM-EURO-Soils (soils similar to EURO-Soil-1, EURO-Soil-3 and EURO-Soil-4), with half of them corresponding to low-organic, slightly acidic EURO-Soil-1 identified originally in Sicily. Analysis of the results indicates the necessity for further works aimed at identifying new EURO-soil groups typical for Central and East European countries such as Poland. There is also a need of specification of SIM-EURO-Soils criteria, mainly regarding the level of its contamination and methods of analysis.

Keywords: EURO-Soils, ecotoxicity testing, ecotoxicology, soil reference materials, Polish soil properties

Introduction

The main tasks of ecotoxicology are monitoring and assessing the fate and effects of contaminants introduced in the environment; soil ecotoxicology is particularly difficult due to the problems related to the extreme complexity of soil ecosystems and enormous diversity of soil organisms [1]. Ecotoxicity tests, creating a tool for fulfilling those objectives [2], are applied for two main purposes: legal control of chemicals and soil quality evaluations (including ecological risk assessment – ERA) [2-4]. Additionally, ecotoxicological test methods are also suggested as a good instrument for obtaining indirect information about potential bioavailability of pollutants for specific organisms [5]. In all cases the problem of appropriate soil reference materials is extremely important, since there is a strong association between ecotoxicity of contaminants in terrestrial environment and soil characteristics [4, 6].

*e-mail: bkord@iung.pulawy.pl

Although some standards aiming at the unification of ecotoxicity procedures in soils have already been developed by international (e.g. OECD, ISO TC 190) and national (e.g, US EPA, Environment Canada) bodies, the problem of "soil standards" has not yet been overcome. OECD suggested [7] application of the artificial test substrate imitating soil material (so-called "OECD soil"), which has turned out to be very popular with ecotoxicologists and is applied in many ecotoxicity tests [8]. The substrate, introduced for testing acute effects on earthworms (OECD, 1984), consists of a mixture of sand (70%), kaolinite clay (20%) and ground peat (10%) with the addition of $CaCO_3$ to maintain pH of 6±0.5. Organic matter corresponds to 6.17% and total nitrogen to 0.11% (giving C:N of 32.6) with the water holding capacity (WHC) of about 56% [7, 8]. Actually, depending on the properties of peat the WHC of OECD artificial soil can vary considerably. However, many soil scientists have criticized this substrate as having properties not similar to natural soils. The high content of organic substances in OECD substrate affecting bioavailability of many contaminants and thus changing the results of the biotests was of special concern. In most of the ISO standards for evaluation of the ecotoxic properties of contaminated soils three options for control soil are given: – artificial "OECD soil", – uncontaminated soil of the same textural class as tested soil and "as similar as practicable in all respects", – natural soil with characteristic specified in ISO 11269-2 [9] i.e. fine particles (φ <0.02mm) below 20%, organic matter (OM) < 3.0% (C_{org} <1.5%), pH in the limits of 5 to 7.5. The exception is ISO 22030 [10] standard on chronic toxicity towards high plants, where a recommendation for standard natural soil includes OM content < 5.0%. The OECD guideline for terrestrial plants testing [11] confines the content of organic carbon to < 1.5% and texture to sandy loam, loamy sand and clay loam groups. Nevertheless, the recommended conditions are not unequivocal and, as a result, various field soils are applied often as reference soils. However, testing chemicals introduced to soils or testing contaminated soils for regulatory purposes demands standardized conditions. Hence, if the field soils are going to be accepted as reference soils, their characteristics have to be identified. After preliminary attempts to apply some selected natural soils as reference material in ecotoxicity testing (e.g. so called "LUFA soil 2.2" of pH_{CaCl2} 6.1 and C_{org} of 2.70%) the idea of using European reference soils (so called EURO-Soils) was developed [8, 12, 13]. The standard operating procedures were intended to be set up for the selection of sampling sites, allowing us to choose soils representative for Europe, to unify treatment and characterization of soil samples. The EURO-Soils concept was introduced initially for adsorption/desorption testing in the framework of OECD Test Guideline 106 [14]. However, soon after its successful implementation in 1990, the soils found broader range of application in soil-related measurements [13]. This led to the preparation of a second generation of EURO-Soils (re-sampling at the same points) and their comprehensive characterization with respect to their pedological properties, sorption behaviour, matrix constituents and content of inorganic (heavy metals) as well as organic (polycyclic aromatic hydrocarbons, pesticides) contaminants [12, 15-17]. Certification procedure in accordance with the severe quality requirements enabled distribution of the second set of six EURO-Soils under the label IRMM-443-EURO-Soils [13, 17]. The broad information on EURO-Soils concept and its application was given by Gawlik et al. [12, 15-17] and by Römbke and Amorim [8]. The basic properties of six selected EURO-Soils (average from duplicate sampling) representing specific regions of the "old EU" (Italy, France, Greece, Germany, Austria, and the UK) are given in Table 1.

Table 1. Basic properties of EURO-Soils and the suggested acceptance range for SIM-EURO-Soils [in brackets] (modified after Römbke and Amorim, [8] and Gawlik et al. [13]).

Soil – Origin	Texture (%)	OM (%)	pH		C:N
	Sand – Silt – Clay		H_2O	$CaCl_2$	
ES 1 – Italy (Sicily)	3 – 22 – 75 [Clay]	2.65 [2.0 – 4.0]	6.0	5.0 [5.0 – 6.5]	7.6 [< 10]
ES 2 – Greece (Peloponnesus)	3 – 64 – 23 [Silt]	6.40 [4.0 – 8.0]	8.0	7.4 [6.5 – 7.5]	18.5 [> 20]
ES 3 – UK (Wales)	46 – 37 – 17 [Loam]	6.45 [4.0 – 8.0]	6.0	5.0 [5.0 – 6.5]	13.3 [10 -20]
ES 4 – France (Normandy)	4 – 76 – 20 [Silt]	2.85 [2.0 – 4.0]	7.0	6.5 [6.5 – 7.5]	9.7 [10 -20]
ES 5 – Germany (Schleswig-Holstein)	81 – 12 – 6 [Sand]	15.90 [> 8.0]	4.6	3.2 [3.0 – 4.0]	30.7 [> 20]
ES 7 – Austria (Lungau)	46 – 35 – 19 [Loam]	11.7 [> 8.0]	5.2	4.4 [3.5 – 4.5]	14.3 [10 -20]

There was an ES 6 soil identified as well. However, since it derives from the BC horizon of soil ES 4 and its application is very restricted – it was not included in the evaluations.

The first attempts to utilize the EURO-Soils as a reference material for ecotoxicity tests was not very successful, mainly due to the limited amount of the homogenous soil material and the lack of experience in the behaviour of some test species in those soils. As a result, Römbke and Amorim [8] introduced the concept of application of soils with properties similar to the original EURO-Soils (suggesting the name of "SIM-EURO-Soils"). The selected main properties (texture, pH, organic matter content and C:N ratio), critical for SIM-EURO-Soils identification, aimed to reflect ecological condition for soil organisms and to control environmental availability of contaminants in soils. Simultaneously, the acceptance ranges for a soil to be similar to the existing EURO-Soil were proposed [8] – Table 1. Following their conception, the authors [8] undertook an effort to select ten SIM-Soils derived from Germany and Portugal and to apply them in ecotoxicity tests [18]. It has to be stressed that the EURO-Soils and SIM-EURO-Soils identified till now are representative for the West Europe and there is lack of information if the idea can be applied for the greates part of the soils from Central and Eastern European countries.

The aim of our studies was to investigate the opportunity of the extension of the concept of SIM-EURO-Soils to soils from Poland. Our approach included an assessment of the possibility of identification of soils similar to the original EURO-SOILS in a wide set of soil data covering various regions of the country and differing in their characteristic. Two hundred sixty seven different soils representative of arable lands in Poland were included in the studies.

Experimental Procedures

Sampling Area Selection

The sampling procedure was focussed on identification of soils fulfilling SIM-EURO-Soils classification in the wide set of soil samples representing properties typical for Poland. The selection of the sampling areas was aimed to reflect typical Polish soils, taking into consideration regional environmental conditions and levels of industrialization. Two series of samples (Series I and Series II) were included in the studies.

Series I (216 sampling points distributed rather uniformly over the country) comprised the samples taken in the year 2000 during the realization of the programme "*Monitoring of the chemistry of arable soils in Poland*" [19, 20]. Series II (51 samples taken in the year 2004) included soils deriving from two parts of the country: the eastern part (rural regions) and the southwestern part (more urbanized and industrialized areas). GPS (geographic position system) technique was applied for determining the geographic position of each sampling point of Series II.

All soil samples were taken from the surface layer (0 – 30 cm) of agricultural land (mostly arable fields). Soil materials were transported to laboratory, air dried at about 20°C, well mixed, sieved to pass a 2 mm sieve-mesh and stored in the dark at 12–16°C for a period not exceeding 6 months before further characterization.

Determination of Soil Properties

Soil characteristics included the determination of particle size distribution, soil organic matter content, pH, total nitrogen content and content of contaminants (PAHs, Zn, Pb, Cd and Cu).

Soil particle size distribution was established by an aerometric method [21]. Organic carbon (C_{org}) content was determined by sulfochromic oxidation of organic carbon [22], followed by titration of the excess $K_2Cr_2O_7$ with $FeSO_4(NH_4)_2SO_4 \cdot 6\ H_2O$ as it was described by Tiurin [23]. Organic matter content (OM) was calculated on the basis of the relationship OM=1.724* C_{org}. pH was determined potentiometrically in 1:2.5 (m/V) suspension of soil in 1 $mol \cdot L^{-1}$ KCl solution and in water [24]. Total nitrogen content (N_t) was establish by modified (TiO_2 as Se substitution) Kjedahl method [25].

Sixteen PAH (Σ16PAH) compounds (US EPA list) were determined by extraction with dichloromethane in Soxtec apparatus (Bûchi Extraction System B-811) for at least 5 hours (35 cycles). The extracts were concentrated under vacuum on a rotary evaporator and cleaned up on glass mini-columns filled with silica gel (1 g) suspended in dichloromethane. PAHs were eluted with a mixture of CH_2Cl_2/n-hexane (2:3 v/v). The eluat was concentrated and analyzed on Agilent 6890N gas chromatograph equipped with Agilent 5973 Network mass spectrometer (70 eV) and 7683 B series autosampler and DB-5 MS fused-silica capillary column 30 m x 0.25 mm I.D. x 0.25µm film and with 10 m guarded column (J&W Scientific, USA). Helium was used as a carrier gas and GC oven was programmed within the range of 60–290°C. Resolution of PAH compounds has been achieved according to ISO 18287 standard [26]. The precision of the method expressed as the mean relative standard deviation (RSD) was in the range of 2–24% for individual PAHs, and 8% for the sum of 16 PAH compounds. The mean recovery of 16 PAHs from the reference soil was 71%, the recovery for individual compounds was in the range of 53–112%. Analysis details are given elsewhere [27].

Total Zn, Cd, Pb and Cu levels were determined by the *aqua regia* digestion [28] procedure (2 h hot digestion in 1:3 v/v mixture of concentrated nitric and hydrochloric acids, followed by refluxing in 3 M hydrochloric acid). The filtrates were analyzed using atomic absorption spectroscopy (AAS) apparatus (Perkin Elmer 450). The pretreatment and determination procedures from ISO 11466 [28] and ISO 11047 [29] were applied. Quality control included duplication of every 10th sample and analysis of soil reference materials (NIST 2709 and NIST 2710) every 20 samples. Precision of the method defined as percentage relative standard deviation (RSD) was <2.5% for all analyzed metals.

Statistic

The precision of the results was expressed by the standard deviation values. The standardized skewness and standardized kurtosis parameters were used to determine whether the sample comes from a normal distribution. One-way analysis of variance (ANOVA) was applied, after variance check (Bartlett's test), for the evaluation of the effect of tested factors on soil characteristics (Tukey HSD method at $\alpha \leq 0.05$ level).

Results and Discussion

Soil Characterization

The statistical evaluation of the main properties of the soils from Series I and Series II is given in Table 2. Since significant departures from normality were noted for most data, all soil properties were log transformed prior to further evaluations.

Soils included in Series I were mostly light (median content of the fraction ø<0.02 mm 21%), low organic (median OM content 1.8%) and slightly acidic (median pH_{KCl} 5.4) with rather uniform distribution of those parameters (coefficient of variability – CoV – within the limits of 15–62%). Although the general level of contamination of the soils with organic and inorganic pollutants (median content of PAH, Zn and Pb of 0.34, 35 and 12.8 $mg \cdot kg^{-1}$, respectively) was rather low, the variability of the results was high (CoV of 135–792%) due to some extremely high values noted in the selected sampling points in a highly industrialized region (e.g. Silesia voievodeship) [19]. Nevertheless, the content of those contaminants in 75% of soil samples (upper quartile values) was much below the limit values set by Polish regulations for the top layer of agricultural soils [30].

Table 2. Statistical evaluation of Polish soil properties.

Parameter	Median	SD [e]	Quartile		Range	CoV [f] (%)
			Lower	Upper		
I Series (n = 216)						
Fr ϕ<002 mm (%)[a]	6	7	3	9	1 – 48	89
Fr ϕ<02 mm (%)[b]	21	15	14	36	1 – 84	62
OM (%)[c]	1.8	0.8	1.5	2.2	0.8 – 5.7	41
pH_{KCl}	5.4	0.8	4.8	6.0	3.7 – 7.4	15
C/N	11.0	2.3	9.5	12.8	7.1 – 19.5	21
Σ16PAH ($\mu g \cdot kg^{-1}$) [d]	341	666	188	535	54 – 6,680	135
Cd ($mg \cdot kg^{-1}$)	0.24	6.2	0.18	0.35	0.07 – 90.87	792
Zn ($mg \cdot kg^{-1}$)	35.0	344.5	23.5	51.7	7.7 – 5,012.0	483
Pb ($mg \cdot kg^{-1}$)	12.8	79.2	9.9	19.5	4.3 – 1,073.3	335
II Series (n = 51)						
Fr ϕ<002 mm (%) [a]	4	4	3	7	1 – 18	80
Fr ϕ<02 mm (%) [b]	22	11	16	30	7 – 53	46
OM (%) [c]	2.0	1.2	1.6	2.6	0.9 – 8.5	56
pH_{KCl}	5.3	1.1	4.4	6.2	3.7 – 7.4	21
pH_{H2O}	6.2	1.0	5.4	7.1	4.5 – 8.2	17
C:N	9.3	2.9	8.1	11.6	1.0 –18.6	29
Σ16WWA ($\mu g \cdot kg^{-1}$)[d]	253	356	120	488	73 – 1,800	97
Cd ($mg \cdot kg^{-1}$)	0.62	1.2	0.56	1.17	0.01 – 7.85	112
Zn ($mg \cdot kg^{-1}$)	42.7	136.9	19.1	91.4	9.1 – 667.3	158
Pb ($mg \cdot kg^{-1}$)	26.8	121.1	9.67	85.9	6.1 – 720.2	177
Cu ($mg \cdot kg^{-1}$)	6.2	11.5	3.8	10.0	1.0 – 64.7	124

[a] fraction ϕ<0.002 mm content, [b] fraction ϕ<0.02 mm content, [c] organic matter content, [d] sum of the content of 16 PAH compounds according to USEPA list, [e] standard deviation, [f] coefficient of variation.

Basic properties of the soils from Series II had also rather uniform distribution; coefficients of variations for fraction ø<0.02 mm, OM, pH_{KCl} and C:N were in the range of 20–55%. Median values for fraction ø<0.02 mm was 22% with upper quartile of 30.0%. For OM median was 2.0%, while with upper quartile was equal to 2.64%. Medians for pH_{KCl} and C:N corresponded to 5.3 and 10.0, while their upper quartiles were 6.3 and 12.0, respectively. Soils from both parts distinguished in Series II (East rural part and industrial/urbanized South-West part) did not differ significantly (one way ANOVA, at α≤0.05 level) in respect to their basic properties. However, a statistically significantly higher level of contamination was noted in the South-West part in regard to the content of PAH (median: 528 $\mu g \cdot kg^{-1}$ *versus* 132 $\mu \cdot kg^{-1}$ in the East part) and the content of Cu (median: 8.9 $mg \cdot kg^{-1}$ *versus* 4.0 $mg \cdot kg^{-1}$ in the East part).

Generally, the properties of soils from both series were very similar (Table 2), which indicates that the analyzed data set represents rather well Polish soils from agricultural areas.

Table 3 shows the example of the division of tested soils (Series II) according to the classes as proposed for the SIM-EURO-Soils [8]. Half of the soils had organic matter content < 2.0%, while the other 49% of samples were characterized by the OM in the limits of 2.0–4.0%. C:N ratio <10 was found in the greatest part of the samples (61%), while the other part of soils represented C:N ratio within the limits of 10–20. Distribution of soils according to their pH values was more regular and covered all relevant classes.

Identification of EURO-Soils

Identification of the similar to EURO-Soils (SIM-EURO-Soils) was based on the approach of Römbke and Amorim [8] applied in the selections of SIM-EURO-Soils from Germany and Portugal. According to their suggestion, the investigated soil can be described as "similar" if three out of four selected properties (OM, pH, C:N and texture) fall into the "accepted range" defined for each of six original EURO-Soil (Table 1). However, in the case of Polish soils characterized according to the national standards, the texture was a parameter which had to be excluded *a priori* from the "fitting" procedure of studied soils. The Polish classification system of soil texture [31] is not fully compatible with the FAO system [32], since it is based on different particle-size classes and textural classes. Information on the content of the fractions ø<0.02 mm and ø<0.002 mm given in Table 3 reflects national textural classes, as determined in most Polish laboratories. Thus, the "similarity" of studied soils to EURO-Soils was evaluated in all cases on the basis of three parameters: OM, pH and C:N.

It has to be mentioned that OM content was below 2% (i.e. the lowest limit for SIM-EURO-Soils – Table 1) in 66% of the soils from Series I and in 51% of the soils from Series II (Table 3). This means that, taking into consideration only OM parameter, 171 of the 267 soils under study did not fit any of the SIM-EURO-Soils classes. Thus, further recognition of SIM-EURO-Soils was restricted to 96 soil samples.

Twenty nine soils from both series were categorized as having their properties in the ranges corresponding to three SIM-EURO-Soil classes – Table 4. The codename applied for those soils included the prefix "Pol" followed by the number of the SIM-EURO-Soil (e.g. Pol-SIM-ESx).

Fifteen soils from Series I and three soils from Series II were classified as Pol-SIM-ES1. The original ES1 was described (FAO classification) as a clay texture group [32], whereas according to Polish classification system [31] the selected soils represent mostly loams (11 soils), silts (3 soils), clays (2 soils) and sand (1 soil) – Table 4. Soils from this group derived from Series I originated from all regions of the country (different geographical positions – Table 4), while soils from Series II were located in the western part of Poland and concentrated in small area close to the town of Tarnowskie Gory in a Silesia. OM content in Pol-SIM-ES1 class was within the limits of 2.01–3.04% with median value of 2.48% (original ES1 – 2.65%), pH_{KCl} was within the limits of 5.2–6.4 with median of 6.0 (ES1 – 5.0), while C:N ratio varied from 7 to 10 with median value of 9 (ES1 – 7.6) – Table 5. While the physicochemical soils properties were uniform (from class definition), the selected soils represented various

Table 3. Percentage of the soil samples (Series II) in groups of soils distinguished according to their properties specific for SIM-EURO-Soils groups (n = 51).

OM[a]		pH_{KCl}		C:N	
Range (%)	Percentage	Range	Percentage	Range	Percentage
< 2.0	51	3.0 – 4.5	25	< 10	61
2.0 – 4.0	43	4.5 – 5.0	20	10 – 20	39
4.0 – 8.0	6	5.0 – 6.5	35	> 20	0
> 8.0	0	6.5 – 7.5	20		

[a] organic matter

Table 4. Basics physicochemical properties of Pol-SIM-EURO-Soils and their locations.

Soil Code	Texture[a]	Position		OM[b]	pH_{KCl}	C:N	Σ16 PAH[c]	Zn [d]	Pb [e]
		Latitude-N	Longitude-E						
Series I									
1-Pol-SIM-ES1	sandy loam	52°43′47″	18°15′50″	2.1	5.9	7.3	531	26.8	9.6
2-Pol-SIM-ES1	heavy loam	49°33′55″	21°41′10″	2.14	6.4	8.3	313	95.3	20.1
3-Pol-SIM-ES1	medium loam	49°51′40″	20°48′30″	2.15	5.4	8.4	728	81.7	44.2
4-Pol-SIM-ES1	medium loam	50°19′15″	21°05′05″	2.2	5.2	8.7	333	78.3	24.0
5-Pol-SIM-ES1	silty light loam	54°13′38″	18°52′15″	2.01	5.8	8.8	483	43	8.3
6-Pol-SIM-ES1	medium loam	51°42′35″	16°03′20″	2.73	5.3	8.9	175	73.3	26.5
7-Pol-SIM-ES1	silty medium loam	49°57′25″	20°21′40″	2.51	6.3	9.1	269	60.0	21.9
8-Pol-SIM-ES1	clayey silt	50°10′25″	20°13′15″	2.56	6.2	9.2	128	56.7	18.7
9-Pol-SIM-ES1	loamy silt	50°04′08″	20°06′25″	2.82	6.2	9.3	6 680	168.3	40.7
10-Pol-SIM-ES1	loamy silt	50°04′60″	19°33′30″	2.22	5.2	9.5	232	113.3	36.7
11-Pol-SIM-ES1	silty medium loam	49°37′15″	19°09′10″	2.96	5.9	9.5	663	98.3	20.1
12-Pol-SIM-ES1	silty clay	53°43’ 29”	18°55’ 38”	2.38	6.3	9.5	749	80.3	16.3
13-Pol-SIM-ES1	silty clay	49°45′42″	22°35′25″	2.45	5.4	9.6	155	50.1	19.1
14-Pol-SIM-ES1	heavy loam	54°09′11″	21°16′00″	2.19	5.6	9.9	630	51.7	14.0
15-Pol-SIM-ES1	silty light loam	52°16′04″	19°22′58″	3.04	6.1	9.9	210	25.7	16.3
1-Pol-SIM-ES3	silty medium loam	54°11′38″	19°12′50″	5.68	6.5	11.0	574	61.7	32.4
2-Pol-SIM-ES3	clay	49°43′10″	20°19′20″	5.49	5.7	11.1	452	73.3	14.1
3-Pol-SIM-ES3	heavy loam	53°52′20″	18°48′45″	4.31	5.1	12.0	4 577	101.3	49.0
4-Pol-SIM-ES3	loamy silt	51°07′25″	23°33′20″	4.39	5.3	13.6	295	121.7	32.7
5-Pol-SIM-ES3	silty medium loam	49°48′55″	19°01′30″	4.15	6.0	14.4	1 209	208.7	45.7
1-Pol-SIM-ES4	silty light loam	50°53′10″	19°15′25″	2.18	7.0	10.8	459	38.3	16.9
2-Pol-SIM-ES4	sandy loam	49°40′25″	19°11′30″	3.82	7.0	13.0	2 559	128.3	20.3
Series II									
16-Pol-SIM-ES1	silty light loam	50°25′38″	18°50′48″	2.73	6.4	8.3	704	174.3	188.4
17-Pol-SIM-ES1	heavy loamy sand	50°27′45″	18°48′10″	2.52	6.3	7.0	592	87.1	66.5
18-Pol-SIM-ES1	light loam	50°25′29″	18°50′04″	3	6.4	8.7	1136	667.3	720.2
3-Pol-SIM-ES4	light loam	51°16′01″	21°55′22″	2	7.3	11.6	113	26.22	18.29
4-Pol-SIM-ES4	silty heavy loamy sand	51°15′30″	21°55′38″	2.64	7.2	10.9	91	17.39	9.14
5-Pol-SIM-ES4	loamy silt	51°14′12″	21°57′02″	3.16	7.3	12.2	181	28.36	15.24
6-Pol-SIM-ES4	heavy loamy sand	51°26′18″	22°02′38″	2.51	7.1	11.23	121	37.18	12.19

[a] texture according Polish standard BN-78/9180-11 (1978), [b] organic matter content (%); [c] content of 16 PAHs according to US EPA list (µg· kg^{-1}), [d] content of Zn (mg· kg^{-1}), [e] content of Pb (mg· kg^{-1}).

Table 5. Statistical evaluation of the properties of the selected groups of Pol-SIM-ESx soils.

Parameter	Mean	Median	SD [e]	Upper Quartile	Range	CoV [f] (%)
Pol-SIM-ES 1 (n=18)						
Fr ϕ<02 mm (%)[a]	39	39	13	46	16 – 63	34
OM (%)[b]	2.48	2.48	0.33	2.73	2.01 – 3.04	13
pH_{KCl}	5.9	6.0	0.4	6.3	5.2 – 6.4	8
C:N	9	9	1	10	7 – 10	9
Σ 16PAH (μg·kg^{-1})[c]	817	507	1487	703	128 – 6680	182
Cd (mg·kg^{-1})	1.1	0.5	1.8	0.8	0.2 – 7.9	163
Zn (mg·kg^{-1})	112	79	144	98	26 – 667	127
Pb (mg·kg^{-1})	72	21	166	41	8 – 720	228
Pol-SIM-ES 3 (n=5)						
Fr ϕ<02 mm (%)[a]	53	50	20	67	34 -80	37
OM (%)[b]	4.80	4.39	0.72	5.49	4.15 – 5.68	15
pH_{KCl}	5.7	5.7	0.6	6.0	5.1 – 6.5	10
C:N	12	12	1.5	14	11 – 14	12
Σ 16PAH (μg·kg^{-1})[c]	1421	574	1797	1209	295 – 4282	126
Cd (mg·kg^{-1})	1.0	0.8	0.9	0.9	0.3 – 2.7	95
Zn (mg·kg^{-1})	113	101	58	122	62 – 209	51
Pb (mg·kg^{-1})	35	32	14	46	14 – 49	39
Pol-SIM-ES 4 (n=6)						
Fr ϕ<02 mm (%)[a]	24	26	7	31	16 – 32	27
OM (%)[b]	2.72	2.58	0.67	3.16	2.00 – 3.82	25
pH_{KCl}	7.2	7.2	0.1	7.3	7.0 – 7.3	2
C:N	12	12	1	12	11 -13	7
Σ 16PAH (μg·kg^{-1})[c]	587	151	975	459	91 -2559	166
Cd (mg·kg^{-1})	0.8	0.7	0.5	1.0	0.3 – 1.7	58
Zn (mg·kg^{-1})	46	33	41	38	17 – 128	89
Pb (mg·kg^{-1})	15	16	4	18	9 -20	26

[a] fraction ϕ<0.02 mm content, [b] organic matter content, [c] sum of the content of 16 PAH compounds according to US EPA List, [e] standard deviation, [f] coefficient of variation.

levels of contamination with PAH (128 – 6680 μg·kg^{-1} corresponding to CoV of 182%) and heavy metals: Zn (26 – 667 mg·kg^{-1} with CoV of 127%), Cd (0.2–7.9 mg·kg^{-1} with CoV of 163%) and Pb (8–720 mg·kg^{-1} with CoV of 228%).

The next group included five soils representing Pol-SIM-ES3 class. All Pol-SIM-ES3 soils derived from Series I and were collected in various parts of the country (Table 4). Soils from this group included three loams, one clay and one silt according to Polish classification system. Original ES3 had a loam texture according to FAO system [12]. Median values for OM, pH and C:N in Pol-SIM-ES3 were of 4.39%, 5.7 and 12.0, respectively (Table 5). The same values in original ES3 corresponded to 6.45%, 5.0 and 13.3. Variability of physicochemical properties of Pol-SIM-ES3 was very low (CoV of 10–15%), whereas soil contamination level differed significantly, particularly for PAHs (range 295 – 4282 μg·kg^{-1}, CoV of 126%) and Cd (range 0.3 – 2.7 mg·kg^{-1}, CoV of 95%).

Six other soils were classified as Pol-SIM-ES4; three of them were described as loams, two as sands and one as silt (Polish system), whereas original ES4 represented silt (FAO classification) [12]. Two Pol-SIM-ES4 were identified in samples from Series I and four in Series II (Table 4). The soils from the second series were collected in the eastern part of Poland and originated from small Pulawy area in Lublin region. The basic properties of Pol-SIM-ES4 were uniform (CoV of 2-27%) with median values for OM of 2.72% (ES4 – 2.85%), for pH of 7.2 (ES4 – 6.7) and for C:N of 12 (ES4 – 9.7). Similarly as in the case of the previous classes, diverse levels of contamination of Pol-SIM-ES4 soils were observed; this was particularly visible for PAHs (CoV of 166% corresponding to the range 91–2559 $\mu g \cdot kg^{-1}$) and Zn (CoV of 89% with the range 17–128 $mg \cdot kg^{-1}$) – Table 5.

Problems Related to Pol-SIM-EURO-Soils Identification

The presented data indicates that it is possible to find soils similar to EURO-Soils in Poland, i.e. in a European country different from those where the six original EURO-Soils were identified: Italy, Greece, UK, France, Germany and Austria and, additionally, in Portugal [8]. The majority of soils identified in Poland was in Pol-SIM-ES1 group corresponding to slightly acidic Vertic Cambisoil from Sicily characterized by low OM content and low C:N ratio (Table 1). Two other identified classes, i.e. Pol-SIM-ES3 (similar to Distric Cambisoil from Wales in the UK) and Pol-SIM-ES4 (similar to Ortic Luvisoil from Normady in France) were less numerous. Soils with organic matter content > 8% or with C:N ratio >15 (similar to ES2, ES5 and ES6) were not found in the analyzed data set.

The process of identification of Polish soils corresponding to SIM-EURO-Soils indicates some problems.

Methodologies of Determination of Soils Properties.

Original EURO-soils were characterized in respect to their pedological properties (grain size distribution, pH, total and organic carbon, cation exchange capacity) according to German standard methods of VDLUFA, while their texture and soil units were classified along with FAO procedures [12]. For the second edition of EURO-Soils applied procedures followed respective ISO standards [17]. Römbke and Amorim [8] did not recommend methods of determination of SIM-EURO-Soil properties (texture, OM, pH and C:N); however, it can be assumed that the authors followed current methodologies used for EURO-Soils description. In these studies soils were characterized with methods used in most of Polish laboratories, which are not always compatible with the methodologies applied in other countries. As has been mentioned, the national texture classification system [31] is different than the FAO system [32]. EURO-Soils were characterized by pH_{CaCl2}, while for Polish soils pH was measured in KCl solution. Nevertheless, both methods are comparable [24], thus, soil pH_{KCl} information was considered suitable. Organic carbon content in Polish soils was determined by titration with Mohr salt after sulfochromic oxidation. This practice [23] is not very popular in West-European countries, where C_{org} values are often calculated on the basis of "losses at ignition" procedure. For total nitrogen determinations in Pol-SIM-ES soils the Kjedahl method was used, but with some modifications in relation to relevant ISO standard. Differences in C_{org} and N_{total} values may affect C:N ratio and, consequently, influence identification of SIM-EURO-Soils.

This indicates that, if the concept of SIM-EURO-Soils is going to be extended to other countries, methodologies for the determination of key parameters applied for its identification have to be clearly specified. Employment of relevant ISO TC190 (*International Standardization Organisation, Technical Committee 190 "Soil Quality"*) methods can be recommended.

Contamination of Soil Samples

The main purpose of identification of SIM-EURO-Soils is their application for ecotoxicity tests as reference materials. Hence, it is assumed *a priori* that their level of contamination is low. The original EURO-Soils were analyzed extensively in respect to the content of broad range of inorganic and organic pollutants [12, 13] and it was concluded that "no unusual contamination could be observed" [12], although in some cases higher contents for some chlorinated hydrocarbons was observed [16]. The authors of SIM-EURO-Soils concept did not refer to this problem [8]. This study shows (Tables 4 and 5) that it is possible to identify Pol-SIM-EURO-Soils fulfilling the key selection criteria but having high content of PAHs and heavy metals. Six soils did not fulfill Polish soil quality standards [30]: 9-Pol-SIM-ES1 (PAHs), 16-Pol-SIM-ES1 (Pb), 18-Pol-SIM-ES1 (PAHs, Zn and Pb), 3-Pol-SIM-ES3 (PAHs), 5-Pol-SIM-ES3 (PAHs and Zn). Nevertheless, it has to be pointed out that the median content of Zn and Pb in Pol-SIM-ES1, Pol-SIM-ES3 and Pol-SIM-ES4 (Table 5) was lower than in original EURO-Soils. Zn content in two series of ES1 was 132.9 and 121.0 $mg \cdot kg^{-1}$, respectively [12], while the median value for Pol-SIM-ES1 was 79 $mg \cdot kg^{-1}$ (Table 5). Slightly higher values were noted for PAHs: their median content in soils Pol-SIM-ES1 and Pol-SIM-ES3 was 507 and 574 $\mu g \cdot kg^{-1}$, respectively (after exclusion of highly contaminated samples the corresponding values were: 333 and 452 $\mu g \cdot kg^{-1}$). All the same, they were still on the same level as those considered "not unusual" for original EURO-Soils (e.g. 530 $\mu g \cdot kg^{-1}$ in ES3) [12]. Generally, the presented data confirm low levels of contamination of Polish agricultural soils. However, they indicate also the necessity of setting up "contamination limits" in SIM-ES identification. This can be a difficult task since the problem "how clean is the clean" is a matter of different approaches (regulations, risk assessment, etc.) on global and national levels.

Specification of New SIM-ES Classes

Practical aspects of ecotoxicity studies (legal control of chemicals and risk assessment) demand realistic approaches to different soil environment conditions. Polish soils are characterized by low organic matter content: 64% of soils under study had OM content < 2.0%, i.e. they are beyond any of the existing SIM-ES criteria. If the concept of using SIM-EURO-Soils as reference materials in ecotoxicity studies will find a wider application, then selected soils have to be representative for the regional environmental conditions. This implies the need for the selection of new groups of EURO-Soils (and relevant SIM-EURO-Soils) characterizing soils typical for this part of Europe.

Conclusions

The first attempt of application of the concept of SIM-EURO-Soils to Polish soils, undertaken in these studies, shows that it is possible to select soils with properties corresponding to EURO-Soils. Ten percent of all data set (267 soil samples) were classified as equivalent to three groups of SIM-EURO-Soils. Over half of those selected soils exhibited properties comparable to low-organic, slightly acidic soil ES1 identified originally in Sicily. However, the analysis of the results indicates the necessity of further specify SIM-EURO-Soil criteria, mainly regarding the level of contamination as well unification of analytical methods. Further efforts should aim at identifying new EURO-Soil groups reflecting in a better way properties of soils from Poland and other Central/East European countries.

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