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APPLICABILITY OF BIOCHEMICAL INDICES TO QUALITY ASSESSMNET OF SOIL PULLUTED WITH HEAVY METALS*

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Abstract

The objective of this study has been to test a series of soil quality biochemical indices so as to select the one(s) that would mainly rely on enzymes participating in the processes of carbon and nitrogen transformations and reflect objectively the quality of soil. Moreover, an ideal index should be comparable to yields of crops.

A vegetative experiment with 5 replications was set up on two soils: loamy sand and sandy loam, which had been polluted with cadmium, copper and zinc. Once the soil moisture content was raised to the level of 60% capillary water retention capacity, the following plants were sown: oat, spring oilseed rape and yellow lupine. Twice during the growing season, the activity of soil enzymes such as dehydrogenases, catalase, urease, ß-glucosidase, acid phosphatase, alkaline phosphatase and arylsulphatase was determined. Next, based on the soil enzymatic activity as well as the content of clay and organic carbon, 21 indices of the biochemical soil activity (BA_1 to BA_{21}) were proposed, which were divided into two groups: simple and complex ones. In addition, coefficients of correlation between yields of plants and biochemical soil quality indices were calculated.

The experiment has demonstrated that the activity of enzymes should be expressed in units of the product of a catalyzed reaction in 1 h time calculated per 1 kg d.m. of soil, i.e. the activity of dehydrogenases in ěmol TFF, catalase – mol O_2, alkaline phosphatase and arylsulphatase – mmol PNP, while that of urease in mmol $N\text{-}NH_4^+$. Introduction of uniform units facilitates comparison of the results and quality assessment of different soils, irrespective of the author or research centre they originate from. It has also been shown that among the 21 tested biochemical indices of soil quality assessment, the best ones are BA_{20} = %C × (Deh + Pal + Pac + Ure), derived from the content of organic

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carbon and activity of four enzymes: dehydrogenase, alkaline phosphatase, acid phosphatase and urease, and BA_{21} = Deh + Kat + Pal + Pac + Ure + Glu + Aryl, calculated from the activity of seven enzymes: dehydrogenases (Deh), catalase (Cat), acid phosphatase (Pac), alkaline phosphatase (Pal), urease (Ure), β-glucosidase (glu) and arylsulphatase (Aryl).

Key words: cadmium, copper, zinc, soil enzymes, biochemical index of soil quality.

ZASTOSOWANIE WSKAŹNIKÓW BIOCHEMICZNYCH DO OCENY JAKOŚCIOWEJ GLEBY ZANIECZYSZCZONEJ METALAMI CIĘŻKIMI

Abstrakt

Celem pracy było przetestowanie wskaźników biochemicznej jakości gleby i zaproponowanie takiego, który byłby oparty głównie na enzymach uczestniczących w procesach transformacji węgla i azotu oraz odzwierciedlał obiektywnie stan gleby. Ponadto byłby porównywany z plonowaniem roślin.

Doświadczenie wegetacyjne wykonano w 5 powtórzeniach na dwóch glebach: piasku gliniastym i glinie piaszczystej, które zanieczyszczono kadmem, miedzią i cynkiem. Po doprowadzeniu gleby do wilgotności równej 60% kapilarnej pojemności wodnej wysiano następujące rośliny: owies, rzepak jary oraz łubin żółty. Dwukrotnie w czasie wegetacji roślin określono aktywność enzymów glebowych: dehydrogenaz, katalazy, ureazy, β-glukozydazy, fosfatazy kwaśnej, fosfatazy alkalicznej i arylosulfatazy. Następnie wykorzystując aktywność enzymów glebowych, zawartość iłu i węgla organicznego zaproponowano 21 wskaźników biochemicznej aktywności gleby (BA_1 – BA_{21}), które podzielono na wskaźniki proste i złożone. Obliczono również współczynniki korelacji między plonem roślin a biochemicznymi wskaźnikami jakości gleby.

Stwierdzono, że aktywność enzymów powinna być wyrażona w jednostkach produktu katalizowanej reakcji w czasie 1 h w przeliczeniu na 1 kg s.m. gleby, tj.: dehydrogenaz – µmol TFF, katalazy – mol O_2, fosfatazy alkalicznej, fosfatazy kwaśnej i arylosulfatazy – mmol PNP, natomiast ureazy – mmol $N\text{-}NH_4^+$. Używanie zunifikowanych jednostek umożliwia porównywanie wyników i ocenę jakości różnych gleb, niezależnie od autora i ośrodka badań. Wykazano również, że spośród 21 przetestowanych biochemicznych wskaźników jakości gleb najlepszymi wskaźnikami są: BA_{20} = %C × (Deh + Pal + Pac + Ure), obliczany na podstawie zawartości węgla organicznego i aktywności czterech enzymów: dehydrogenaz, fosfatazy alkalicznej, fosfatazy kwaśnej i ureazy, oraz BA_{21} = Deh + Kat + Pal + Pac + Ure + Glu + Aryl, obliczany na podstawie aktywności siedmiu enzymów: dehydrogenaz (Deh), katalazy (Kat), fosfatazy kwaśnej (Pac), fosfatazy alklalicznej (Pal), ureazy (Ure), β-glukozydazy (Glu) i arylosulfatazy (Aryl).

Słowa kluczowe: kadm, miedź, cynk, enzymy glebowe, biochemiczny wskaźnik jakości gleby.

INTRODUCTION

Work on constructing biochemical indices has continued for over 100 years as the earliest measurements of soil enzymatic activity were taken in 1905-1910 and involved determination of the activity of catalase and peroxidase (ZAHIR et al. 2001). The first soil quality index which included the activity of soil enzymes was developed by Hofmann and Seegener in 1950 (NANNIPIERI et al. 2002). In Poland, back in the early 1980s, MYŚKÓW (1981) proposed two soil quality indices (M). One was derived from counts of bacteria, actinomyces and fungi, and another one, which was based on the content of organic carbon and activity of dehydrogenases. Several years later, the same author and his co-workers (MYŚKÓW et al. 1996) suggested another biological index of soil fertility, which additionally included soil adsorption capacity. STEFANIC et al. (1984) proposed a fertility index (BIF) calculated from the activities of dehydrogenases and the activity of catalase. In the same year, BECK (1984) elaborated a soil quality index (EAN) based on the activities of dehydrogenases, catalase, alkaline phosphatase, protease and amylase.

GARCIA and HERNANDEZ (1997) took the content of microbiological carbon as well as the activities of β-glucosidase and arylsulphatase to suggest a biological and biochemical soil quality index (SOM), which made it possible to determine changes in organic matter contained in degraded soils. In the same year, KUCHARSKI (1997) presented a biochemical soil fertility index (M_W), which he had derived from the content of organic carbon (%C) and the activities of dehydrogenases, urease, acid phosphatase and alkaline phosphatase.

TRASAR-CEPEDA et al. (1998) demonstrated that the content of total nitrogen (N_c) in soil can be described with a linear function which comprised 5 parameters: microbiological biomass, mineralized nitrogen, activity of phosphatase, activity of β-glucosidase and urease. TRINCHERA et al. (1999) proved that the quality of soil can be evaluated according to the degree of its humification and the amount of humified matter. The same authors also suggest that the ratio of biomass carbon to total carbon content, expressed in %, could be another good indicator of soil quality. They found out that the content of microbiological biomass carbon (C_m) in the 0 to 20 cm deep layer of arable soil corresponded to 1.33% of the total organic carbon (TOC) content, and to 2.09% of TOC in fallow land. In the deeper layer (20-40 cm), these proportions changed, so that they contained 2.06% of C_m relative to TOC in arable soil and just 0.16% in fallow land. Other authors, such as SCHARENBROCH et al. (2005), also recommended to compare the C_m to TOC ratio. They claim that the said ratio is a good indicator of changes in ecosystems. For example, it identifies the pool of carbon which is mineralizable.

DICK et al. (2000) suggest that the status of mineral soils should be assessed according to the determined activities of soil enzymes. Chemical determinations, in their opinion, are less helpful. They suggest using the activity of alkaline phosphatase (Pal) and acid phosphatase (Pac) to determine whether liming is needed. They claim that if the Pal to Pac ratio is 0.5 or more, soils have proper reaction, but if it falls below 0.5, soils should be limed.

De la Paz Jimenez et al. (2002) worked out a formula that facilitated interpretation of changes in the content of carbon according to the index of microbiological activity (the activity of dehydrogenases and the activity of 3 enzymes responsible for transformations of sulphur, phosphorus and carbon). Koper and Piotrowska (2003) believe that an index derived from the content of organic carbon, total nitrogen, activity of dehydrogenases, alkaline phosphatase, protease and amylase would be suitable for assessment of soil fertility.

Puglisi et al. (2005) worked out a soil alteration index (SAI) based on 15 determinations of fatty acids found in soil phospholipids (PLFA). Their studies demonstrated that SAI was useful in classification of soils depending on their degree of transformation.

A year later, Puglisi et al. (2006) presented three other indices of soil quality based on the activity of seven enzymes (AI 1): arylsulphatase, β-glucosidase, acid phosphatase, urease, invertase, dehydrogenases and phenyloxidase, four enzymes (AI 2): β-glucosidase, acid phosphatase, urease and invertase, three enzymes (AI 3): β-glucosidase, acid phosphatase and urease. In the same year, Bastida et al. (2006) proposed a microbiological degradation index (MDI).

This wide range of microbiological and biochemical indices reported in the literature encouraged us to undertake the present study with an aim of finding out which soil fertility index would assess the condition of soil most objectively. The research was based on the assumption that a good soil quality index should rely on enzymes engaged in the transformation of carbon and nitrogen and should be comparable to yields of crops. Therefore, it was decided to test a series of biochemical indices of soil fertility and find out which one was optimal.

MATERIAL AND METHODS

In order to achieve the above objective, a vegetative (pot) experiment was set up on two soils: loamy sand and sandy loam, which had been polluted with heavy metals. A more detailed description of the characteristics of the soils can be found in Table 1.

The trials were run in a glasshouse at the University of Warmia and Mazury in Olsztyn. Polyethylene pots of the capacity of 3.5 dm^3 were used. The trials were replicated five times. Prior to putting into the pots, soil (3 kg per pot) was mixed in a polyethylene container with macronutrients and heavy metals according to the experimental design. Once portions of the soil were transferred to pots, water was added to bring the soil moisture content to 60% of capillary water holding capacity. Next, the following plants were sown: cv. Kasztan oat, cv. Huzar spring oilseed rape and cv.

Table 1

Physical and chemical properties of soils

Type of soil	pH_{KCl}	Hh	S	T	V (%)	Content					
						C_{org}	N_c	K_w	Na_w	Ca_w	Mg_w
		(mmol(+)g kg^{-1})				(g kg^{-1})		(mg kg^{-1})			
Loamy sand (pg)	6.7	7.8	98.7	106.5	92.7	11.0	0.97	180	28	1429	80
Sandy loam (gp)	6.8	5.2	131.4	136.6	96.2	9.9	1.14	168	57	2214	50

Hh – hydrolytic acidity, S – sum of exchangeable cations, T – total soil adsorption capacity, V – base saturation, C_{org} – organic carbon, Nc – total nitrogen, w – appropriate exchangeable cations

Mister yellow lupine. After emergence, the plants were thinned leaving 12 oat, 8 oilseed rape and 5 yellow lupine plants per pot.

The experimental variables were:

1) type of soil: loamy sand and sandy loam;
2) type of heavy metal: Cd^{2+}, Cu^{2+}, Zn^{2+};
3) quantity of the heavy metal in mg kg^{-1} d.m. of soil:
   Cd^{2}: 0, 50, 150;
   Cu^{2+} and Zn^{2+}: 0, 150, 450;
4) species of the crop: oat, spring oilseed rape and yellow lupine.

Aqueous solutions of $CdCl_2$, $CuCl_2$ $2H_2O$, $ZnCl_2$ were added to batches of soil previously weighed out. They were thoroughly mixed with the soil, which was then placed in pots. When the soil moisture content reached 60% of capillary water holding capacity, the following plants were sown: oat, spring oilseed rape and yellow lupine.

The same fertilization doses were applied to all the treatments (in mg kg^{-1} of soil): 100 (except yellow lupine, which was not fertilized with nitrogen), P – 35, K – 100, Mg – 20. Nitrogen was applied as $CO(NH_2)_2$, phosphorus as KH_2PO_4, potassium as KH_2PO_4 + KCl and magnesium as $MgSO_4 \cdot 7H_2O$.

The plants were grown for 50 days. Then, they were harvested and both green and dry matter yields were determined. On harvest (day 50) and while the plants were growing (day 25), soil samples were taken to determine the activity of soil enzymes: dehydrogenases (EC 1.1) using TTC as substrate (Öhlinger 1996), catalase (EC 1.11.1.6), urease (EC 3.5.1.5), β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1) and arylsulphatase (EC 3.1.6.1) according to Alef and Nannpieri (1998). All the enzymatic determinations, except the activity of catalase, were performed on a Perkin-Elmer Lambda 25 spectrophotometer.

Finally, indices of the biochemical soil activity ($BA_1 - BA_{21}$) were proposed based on the determined activities of soil enzymes, content of clay and content of organic carbon. The following formulas were used for this purpose:

$BA_1 = \log_{10}C\ Deh,$

$BA_2 = \log_{10}C\sqrt{Deh},$

$BA_3 = \log_{10}clay\sqrt{Deh},$

$BA_4 = Deh + Cat,$

$BA_5 = Deh + Ure,$

$BA_6 = Deh + Glu,$

$BA_7 = Deh + Pac,$

$BA_8 = Deh + Pal,$

$BA_9 = Deh + Aryl$

$BA_{10} = \log_{10}C\sqrt{Deh + Cat + Pal},$

$BA_{11} = \log_{10}clay\sqrt{Deh + Cat + Pal},$

$BA_{12} = \log_{10}C\sqrt{Deh + Cat + Pal + Pac},$

$BA_{13} = \log_{10}clay\sqrt{Deh + Cat + Pal + Pac},$

$BA_{14} = \log_{10}C\sqrt{Deh + Cat + Pal + Pac + Ure},$

$BA_{15} = \log_{10}clay\sqrt{Deh + Cat + Pal + Pac + Ure},$

$BA_{16} = \log_{10}C\sqrt{Deh + Cat + Pal + Pac + Ure + Glu},$

$BA_{17} = \log_{10}clay\sqrt{Deh + Cat + Pal + Pac + Ure + Glu},$

$BA_{18} = \log_{10}C\sqrt{Deh + Cat + Pal + Pac + Ure + Glu + Aryl},$

$BA_{19} = \log_{10}clay\sqrt{Deh + Cat + Pal + Pac + Ure + Glu + Aryl},$

$BA_{20} = \%C\ (Deh + Pal + Pac + Ure),$

$BA_{21} = Deh + Cat + Pal + Pac + Ure + Glu + Aryl,$

where:

BA – biochemical activity,
Deh – activity of dehydrogenases (μmol TFF kg^{-1} d.m. h^{-1}),
Cat – activity of catalase (mol O_2 kg^{-1} d.m. h^{-1}),
Ure – activity of urease (mmol $N\text{-}NH_4^+$ kg^{-1} d.m. h^{-1}),

Glu – activity β-glucosidase (mmol PNP kg^{-1} d.m. h^{-1}),
Pac – activity of acid phosphatase (mmol PNP kg^{-1} d.m. h^{-1}),
Pal – activity of alkaline phosphatase (mmol PNP kg^{-1} d.m. h^{-1}),
Aryl – activity of arylosulphatase (mmol PNP kg^{-1} d.m. h^{-1}),
C – content of C_{org} in g kg^{-1} d.m. of soil,
%C – content of C_{org} in d.m. of soil in %,
clay – content of clay fraction in %.

Additionally, coefficients of correlations between the crop yields and biochemical indices of soil quality were calculated.

RESULTS AND DISCUSSION

The adamant search for an optimal index of soil quality undertaken by researchers from different parts of the world (Myśków 1981, Beck 1984, Stefanic et al. 1984, Myśków et al. 1996, Garcia, Hernandez 1997, Kucharski 1997, Trasar-Cepeda et al. 1998, Garcia-Gil et al. 2000, De la Paz Jimenez et al. 2002, Koper, Piotrowska 2003, Puglisi et al. 2005, Winding et al. 2005, Puglisi et al. 2006, Fu et al. 2009, Li et al. 2009, Kucharski 2010, Wyszkowska et al. 2010, Kucharski et al. 2011, Grzebisz et al. 2012, Medyńska-Juraszek, Kabała 2012, Wyszkowski, Sivitskaya 2012, Giacometti et al. 2013) proves how important it is to find a suitable model that would reflect objectively soil condition but also take into account all the factors which influence its fertility. Constructing microbiological and biochemical indices of soil quality should rely mainly on enzymes engaged in process of carbon and nitrogen transformations.

The biochemical indices of soil quality presented in this paper were divided into two categories: simple ones derived from two parameters (Tables 2–4) and complex ones, calculated from a higher number of parameters (Tables 5–7). To make the results obtained by different authors comparable, it was decided to express the activity of each enzyme in micromoles or millimoles of the reaction product in kg of d.m. of soil in a specific time unit.

Despite different values of these indices, the authors looked at the sensitivity to heavy metal pollution and correlation with crop yields instead of the absolute value of each analyzed index so as to indicate the best one. When oat was grown (Table 2), the biggest differences between unpolluted soils and the ones polluted with a dose of 150 mg kg^{-1} soil of heavy metals were observable for the indices BA_1 and BA_9. Both of these indices were on average 70% lower for the polluted soils than for the control ones. The smallest differences in this context were demonstrated by the indices BA_2 (49%) and BA_3 (50%).

Table 2

Simple biochemical indices of the quality of soil contaminated with heavy metals under oat

Type of soil	Dose heavy metal (mg kg^{-1})	Simple biochemical indices of the quality of soil*								
		BA_1	BA_2	BA_3	BA_4	BA_5	BA_6	BA_7	BA_8	BA_9
Loamy sand	Cadmium									
	0	17.10	4.16	0.75	17.16	18.27	17.57	19.01	20.14	17.22
	50	11.21	3.39	0.60	11.11	12.15	11.59	12.62	12.65	11.17
	150	5.08	2.30	0.40	5.04	5.45	5.56	6.47	5.89	5.07
	Copper									
	0	17.10	4.16	0,75	17.16	18,27	17.57	19.01	20.14	17.22
	50	2.62	1.64	0.29	2.78	3.37	3.06	4.27	4.75	2.73
	150	0.35	0.60	0.11	0.48	0.72	0.82	1.32	1.28	0.45
	Zinc									
	0	17.10	4.16	0.75	17.16	18.27	17.57	19.01	20.14	17.22
	50	13.82	3.76	0.67	13.73	14.37	14.03	15.29	16.47	13.72
	150	8.23	2.92	0.51	8.17	8.56	8.45	9.54	10.55	8.15

cont. Table 2

Sandy loam	Cadmium								
	0	18.10	4.45	2.15	17.00	19.66	17.48	19.10	21.99	17.05
	50	12.61	3.74	1.78	11.77	14.12	12.22	13.60	15.18	11.72
	150	6.10	2.57	1.25	6.03	7.50	6.48	7.58	8.71	5.90
	Copper									
	0	18.10	4.45	2.15	17.00	19.66	17.48	19.10	21.99	17.05
	50	7.07	2.77	1.35	6.91	7.71	7.24	8.56	11.17	6.90
	150	2.09	1.51	0.73	2.23	2.65	2.50	3.65	5.27	2.17
	Zinc									
	0	18.10	4.45	2.15	17.00	19.66	17.48	19.10	21.99	17.05
	50	13.82	3.85	1.89	13.29	15.46	13.59	15.17	17.95	13.27
	150	9.53	3.18	1.58	9.35	11.04	9.64	11.22	13.34	9.21

*$BA_1 = \log_{10} C\ Deh$, $BA_2 = \log_{10} C \sqrt{Deh}$, $BA_3 = \log_{10} clay \sqrt{Deh}$, BA_4 = Deh + Cat, BA_5 = Deh, Ure, BA_6 = Deh + Glu, BA_7 = Deh + Pac, BA_8 = Deh + Pal, BA_9 = Deh + Aryl

BA – biochemical index of soil quality, Deh – dehydrogenases, Cat – catalase, Ure – urease, Glu – β-glucosidase, Pac – acid phosphatase, Pal – alkaline phosphatase, Aryl – arylsulphatase, C – organic carbon on g kg^{-1} of soil, clay – content of clay fraction in %.

Table 3

Simple biochemical indices of the quality of soil contaminated with heavy metals under spring oilseed rape

Type of soil	Dose heavy metal (mg kg^{-1})	Simple biochemical indices of the quality of soil*								
		BA_1	BA_2	BA_3	BA_4	BA_5	BA_6	BA_7	BA_8	BA_9
Loamy sand	Cadmium									
	0	13.81	3.84	0.65	13.19	13.71	13.65	15.37	16.42	13.21
	50	9.89	3.21	0.56	9.71	10.21	10.16	11.32	11.34	9.73
	150	6.66	2.61	0.46	6.72	7.22	7.16	8.29	7.80	6.77
	Copper									
	0	13.81	3.84	0.65	13.19	13.71	13.65	15.37	16.42	13.21
	50	3.72	1.96	0.34	3.81	4.11	4.18	4.99	5.88	3.81
	150	0.34	0.59	0.10	0.51	0.70	0.84	1.49	1.40	0.52
	Zinc									
	0	13.81	3.84	0.65	13.19	13.71	13.65	15.37	16.42	13.21
	50	8.98	3.06	0.53	8.88	9.31	9.21	10.68	11.83	8.83
	150	4.67	2.22	0.38	4.61	4.88	4.94	5.91	6.42	4.57

cont. Table 3

Sandy loam	Cadmium									
	0	13.13	3.82	1.82	12.22	14.60	12.62	14.45	18.11	12.38
	50	12.40	3.70	1.77	11.62	14.10	12.01	13.49	15.66	11.66
	150	7.67	2.90	1.40	7.35	8.95	7.49	8.78	10.18	7.39
	Copper									
	0	13.13	3.82	1.82	12.22	14.60	12.62	14.45	18.11	12.38
	50	5.49	2.41	1.20	5.55	6.64	5.78	7.23	9.86	5.49
	150	2.23	1.54	0.76	2.41	2.73	2.60	3.82	5.47	2.28
	Zinc									
	0	13.13	3.82	1.82	12.22	14.60	12.62	14.45	18.11	12.38
	50	10.41	3.40	1.62	9.81	12.14	10.41	11.91	13.75	9.75
	150	8.26	2.99	1.45	8.00	9.91	8.28	9.95	11.22	7.94

*explanations see Table 2

Table 4

Simple biochemical indices of the quality of soil contaminated with heavy metals under yellow lupine

Type of soil	Dose heavy metal (mg kg^{-1})	Simple biochemical indices of the quality of soil*								
		BA_1	BA_2	BA_3	BA_4	BA_5	BA_6	BA_7	BA_8	BA_9
Loamy sand	Cadmium									
	0	13.14	3.65	0.65	13.17	14.20	13.62	15.70	16.27	13.49
	50	5.65	2.40	0.43	5.76	6.18	6.18	7.40	7.19	5.74
	150	4.20	2.10	0.36	4.21	4.49	4.53	5.74	5.04	4.19
	Copper									
	0	13.14	3.65	0.65	13.17	14.20	13.62	15.70	16.27	13.49
	50	2.27	1.53	0.27	2.40	2.77	2.76	3.76	4.23	2.41
	150	0.33	0.58	0.10	0.49	0.70	0.82	1.49	1.28	0.43
	Zinc									
	0	13.14	3.65	0.65	13.17	14.20	13.62	15.70	16.27	13.49
	50	7.90	2.88	0.50	7.71	8.29	8.19	9.62	10.43	7.69
	150	3.71	1.98	0.34	3.68	3.94	4.29	4.75	5.22	3.66

cont. Table 4

Sandy loam	Cadmium									
	0	13.65	3.88	1.86	12.76	15.43	13.08	14.78	18.01	12.76
	50	9.12	3.16	1.52	8.69	11.34	9.14	10.52	12.66	8.67
	150	5.71	2.51	1.20	5.50	6.87	5.69	7.22	8.32	5.49
	Copper									
	0	13.65	3.88	1.86	12.76	15.43	13.08	14.78	18.01	12.76
	50	4.18	2.15	1.03	4.16	5.05	4.46	5.94	8.59	4.13
	150	2.11	1.51	0.73	2.28	2.75	2.57	3.84	5.76	2.15
	Zinc									
	0	13.65	3.88	1.86	12.76	15.43	13.08	14.78	18.01	12.76
	50	8.91	3.12	1.51	8.58	11.09	8.92	10.57	12.39	8.50
	150	8.35	3.02	1.46	8.04	10.18	8.30	9.80	11.38	7.91

*explanations see Table 2

Table 5

Complex biochemical indices of soil quality contaminated with heavy metals under oat

Type of soil	Dose heavy metal (mg kg^{-1})	Complex biochemical indices of soil quality*											
		BA_{10}	BA_{11}	BA_{12}	BA_{13}	BA_{14}	BA_{15}	BA_{16}	BA_{17}	BA_{18}	BA_{19}	BA_{20}	BA_{21}
Loamy sand	Cadmium												
	0	4.56	0.82	4.79	0.86	4.93	0.89	5.00	0.90	5.99	0.91	24.17	24.77
	50	3.68	0.65	3.92	0.69	4.08	0.72	4.17	0.74	5.12	0.74	16.61	16.74
	150	2.58	0.45	2.90	0.50	3.01	0.52	3.13	0.54	4.08	0.55	8.98	9.18
	Copper												
	0	4.56	0.82	4.79	0.86	4.93	0.89	5.00	0.90	5.99	0.91	24.17	24.77
	50	2.29	0.41	2.65	0.47	2.81	0.50	2.90	0.51	3.88	0.52	7.71	8.16
	150	1.23	0.22	1.60	0.28	1.73	0.30	1.87	0.33	2.87	0.33	2.86	3.37
	Zinc												
	0	4.56	0.82	4.79	0.86	4.93	0.89	5.00	0.90	5.99	0.91	24.17	24.77
	50	4.19	0.74	4.41	0.78	4.52	0.80	4.58	0.81	5.52	0.82	20.32	20.21
	150	3.40	0.60	3.64	0.64	3.73	0.65	3.80	0.67	4.73	0.67	13.83	13.67

cont. Table 5

Sandy loam	Cadmium												
	0	5.19	2.50	5.48	2.64	5.81	2.80	5.91	2.85	6.54	2.87	34.47	29.63
	50	4.39	2.08	4.69	2.23	5.04	2.40	5.15	2.45	5.79	2.47	26.06	21.86
	150	3.27	1.59	3.60	1.75	3.89	1.90	4.02	1.96	4.83	1.98	15.16	14.05
	Copper												
	0	5.19	2.50	5.48	2.64	5.81	2.80	5.91	2.85	6.54	2.87	34.47	29.63
	50	3.69	1.79	4.00	1.95	4.17	2.03	4.27	2.08	5.07	2.10	17.50	15.89
	150	2.57	1.25	2.95	1.43	3.09	1.50	3.20	1.55	4.07	1.57	9.51	8.87
	Zinc												
	0	5.19	2.50	5.48	2.64	5.81	2.80	5.91	2.85	6.54	2.87	34.47	29.63
	50	4.60	2.26	4.88	2.40	5.18	2.54	5.26	2.58	6.01	2.61	27.07	24.38
	150	3.95	1.96	4.26	2.11	4.53	2.24	4.61	2.29	5.43	2.30	20.53	19.05

$BA_{10} = \log_{10}C\sqrt{Deh+Cat+Pal}$, $BA_{11} = \log_{10}clay\sqrt{Deh+Cat+Pal}$, $BA_{12} = \log_{10}C\sqrt{Deh+Cat+Pal+Pac}$,

$BA_{13} = \log_{10}clay\sqrt{Deh+Cat+Pal+Pac}$, $BA_{14} = \log_{10}C\sqrt{Deh+Cat+Pal+Pac+Ure}$, $BA_{15} = \log_{10}clay\sqrt{Deh+Cat+Pal+Pac+Ure}$,

$BA_{16} = \log_{10}C\sqrt{Deh+Cat+Pal+Pac+Ure+Glu}$, $BA_{17} = \log_{10}clay\sqrt{Deh+Cat+Pal+Pac+Ure+Glu}$,

$BA_{18} = \log_{10}C\sqrt{Deh+Cat+Pal+Pac+Ure+Glu+Aryl}$, $BA_{19} = \log_{10}clay\sqrt{Deh+Cat+Pal+Pac+Ure+Glu+Aryl}$,

BA_{20} = %C (Deh + Pal + Pac +Ure), BA_{21} = Deh + Cat + Pal + Pac +Ure + Glu + Aryl)

Table 6

Complex biochemical indices of soil quality contaminated with heavy metals under spring oilseed rape

Type of soil	Dose heavy metal (mg kg^{-1})	Complex biochemical indices of soil quality*											
		BA_{10}	BA_{11}	BA_{12}	BA_{13}	BA_{14}	BA_{15}	BA_{16}	BA_{17}	BA_{18}	BA_{19}	BA_{20}	BA_{21}
Loamy sand	Cadmium												
	0	4.35	0.74	4.66	0.79	4.75	0.81	4.83	0.82	5.63	0.83	22.83	20.80
	50	3.55	0.62	3.82	0.67	3.92	0.68	4.02	0.70	4.93	0.71	15.37	15.07
	150	2.89	0.51	3.19	0.57	3.30	0.59	3.40	0.61	4.39	0.61	10.78	11.36
	Copper												
	0	4.35	0.74	4.66	0.79	4.75	0.81	4.83	0.82	5.63	0.83	22.83	20.80
	50	2.56	0.45	2.84	0.50	2.93	0.51	3.04	0.53	4.01	0.54	8.46	8.83
	150	1.30	0.23	1.71	0.30	1.82	0.32	1.97	0.35	2.99	0.35	3.16	3.81
	Zinc												
	0	4.35	0.74	4.66	0.79	4.75	0.81	4.83	0.82	5.63	0.83	22.83	20.80
	50	3.61	0.63	3.90	0.68	4.00	0.70	4.07	0.71	4.98	0.72	15.92	15.54
	150	2.73	0.47	3.03	0.52	3.11	0.53	3.21	0.55	4.12	0.56	9.67	9.33

cont. Table 6

Sandy loam	0	4.77	2.27	5.09	2.42	5.42	2.58	5.50	2.62	6.13	2.65	30.17	25.18
	50	4.42	2.11	4.72	2.26	5.08	2.43	5.17	2.47	5.84	2.50	26.40	22.39
	150	3.55	1.71	3.84	1.85	4.14	1.99	4.21	2.03	4.99	2.05	17.32	15.14
	Copper												
	0	4.77	2.27	5.09	2.42	5.42	2.58	5.50	2.62	6.13	2.65	30.17	25.18
	50	3.39	1.69	3.72	1.85	3.93	1.96	4.02	2.00	4.89	2.02	15.38	14.65
	150	2.57	1.27	2.92	1.45	3.05	1.51	3.14	1.55	4.04	1.57	9.13	8.86
	Zinc												
	0	4.77	2.27	5.09	2.42	5.42	2.58	5.50	2.62	6.13	2.65	30.17	25.18
	50	4.17	1.99	4.53	2.16	4.89	2.33	5.02	2.39	5.67	2.41	24.44	20.82
	150	3.70	1.80	4.06	1.97	4.38	2.13	4.47	2.17	5.25	2.19	19.37	17.30

*explanations see Table 5

Table 7

Complex biochemical indices of soil quality contaminated with heavy metals under yellow lupine

Type of soil	Dose heavy metal (mg kg^{-1})	Complex biochemical indices of soil quality*											
		BA_{10}	BA_{11}	BA_{12}	BA_{13}	BA_{14}	BA_{15}	BA_{16}	BA_{17}	BA_{18}	BA_{19}	BA_{20}	BA_{21}
Loamy sand	Cadmium												
	0	4.12	0.74	4.45	0.80	4.59	0.82	4.67	0.84	5.67	0.85	20.98	21.70
	50	2.78	0.50	3.11	0.55	3.22	0.57	3.32	0.59	4.31	0.60	10.20	10.80
	150	2.40	0.42	2.77	0.48	2.87	0.50	2.97	0.51	3.91	0.52	8.14	8.20
	Copper												
	0	4.12	0.74	4.45	0.80	4.59	0.82	4.67	0.84	5.67	0.85	20.98	21.70
	50	2.16	0.38	2.51	0.44	2.63	0.46	2.73	0.48	3.73	0.49	6.75	7.28
	150	1.21	0.22	1.63	0.29	1.74	0.31	1.88	0.34	2.90	0.34	2.88	3.56
	Zinc												
	0	4.12	0.74	4.45	0.80	4.59	0.82	4.67	0.84	5.67	0.85	20.98	21.70
	50	3.44	0.59	3.76	0.65	3.88	0.67	3.97	0.69	4.85	0.69	15.07	14.43
	150	2.46	0.42	2.74	0.47	2.82	0.48	2.98	0.51	3.90	0.52	7.95	8.04

cont. Table 7

	Cadmium											
	0	4.73	2.26	5.04	2.41	5.39	2.58	5.47	2.62	6.10	2.64	29.82	24.97
	50	3.96	1.91	4.28	2.06	4.69	2.26	4.80	2.31	5.51	2.33	22.38	19.47
	150	3.25	1.55	3.61	1.73	3.89	1.86	3.97	1.90	4.74	1.92	15.33	13.24
	Copper												
Sandy loam	0	4.73	2.26	5.04	2.41	5.39	2.58	5.47	2.62	6.10	2.64	29.82	24.97
	50	3.30	1.58	3.67	1.76	3.88	1.86	3.98	1.91	4.76	1.93	15.18	13.38
	150	2.68	1.30	3.07	1.49	3.22	1.57	3.34	1.62	4.19	1.64	10.33	9.65
	Zinc												
	0	4.73	2.26	5.04	2.41	5.39	2.58	5.47	2.62	6.10	2.64	29.82	24.97
	50	3.91	1.89	4.26	2.06	4.65	2.25	4.75	2.29	5.48	2.31	21.90	19.25
	150	3.74	1.81	4.06	1.97	4.42	2.14	4.50	2.18	5.25	2.20	19.73	17.31

*explanations see Table 5

The indices calculated for soils contaminated with cadmium, copper and zinc which were cropped with spring oilseed rape (Table 3) were similar to the ones calculated for soil under oats. The BA_1 index indicated most evidently the differences between polluted and unpolluted soils. Its value in polluted treatments was 63% lower than in unpolluted ones. The indices BA_4, BA_5 and BA_9 followed.

Average differences in the range of 70% between values of the indices achieved for polluted and unpolluted soils under yellow lupine were shown by the indices BA_1, BA_4 - BA_6 and BA_9. (Table 4). The values of these indices showing the differences between polluted and unpolluted soils, as average ones for the whole experiment, were approximately the same, irrespective of the plant species and soil type. They all ranged between 65% to 68% and were positively correlated with yields of the crops (Table 8).

Similar values of the indices of soil biochemical activity pointed to a smaller difference between the biochemical activity of the soils polluted with heavy metals and unpolluted ones. In soils under oats (Table 5), spring oilseed rape (Table 6) and yellow lupine (Table 7), the largest differences in the soil enzymatic activity, reaching on average 53-60%, could be captured with the indices BA_{20} and BA_{21}. The index BA_{21} was positively correlated with yields of crops at p=0.01 and the index BA_{20} – at p=0.05 (Table 9).

Our analysis of the results suggests that the sensitivity of every index is primarily dependent on the activity of dehydrogenases and content of carbon. Consequently, the most sensitive were BA_1 among simple indices and BA_{20} and BA_{21} in the group of complex ones. BA_1 informs about an average, independent from the species of crop or type of soil, 68% depression in the biological activity of soil polluted with highest rates of heavy metals. The analogous percentages for the other two indices are 58% (BA_{20}) and 56% (BA_{21}). These two indices were the most sensitive ones among complex indicators, while the other ones in this group, when determined for the most seriously contaminated soil, declined in the range of 29 to 41%. And although they were positively correlated with the yield of plants, their poor sensitivity to contamination makes them less useful for soil quality assessment. Simple indices, despite their obvious advantage such as low cost, should be approached with caution, because they fail to account for a wider range of enzymes which together shape the total enzymatic activity of soil. In conclusion, indices B_{20} and B_{21} are recommended.

Table 8

Coefficients of the correlations between plant yields and simple biochemical indices of soil quality

Type of soil	Plant species	Simple biochemical indices of the quality of soil ***								
		BA_1	BA_2	BA_3	BA_4	BA_5	BA_6	BA_7	BA_8	BA_9
Loamy sand	oat	0.62	0.63	0.64	0.62	0.61	0.61	0.62	0.68	0.62
	oil seed rape	0.73*	0.78*	0.78*	0.30	0.29	0.30	0.30	0.41	0.30
	yellow lupine	0.69	0.60	0.61	0.48	0.47	0.49	0.45	0.52	0.47
Sandy loam	oat	0.73*	0.75*	0.76*	0.74*	0.71*	0.73*	0.74*	0.79*	0.74*
	oil seed rape	0.36	0.40	0.41	0.53	0.54	0.55	0.57	0.62	0.53
	yellow lupine	0.73*	0.67	0.66	0.43	0.43	0.43	0.44	0.46	0.42
Independent from the plant										
Loamy sand		0.61**	0.59**	0.59**	0.49*	0.48*	0.48*	0.47*	0.52*	0.48*
Sandy loam		0.44*	0.43*	0.44*	0.45*	0.41	0.45*	0.45*	0.46*	0.45*
Independent from the plant species and type of soil										
		0.50**	0.47**	0.17**	0.51**	0.43**	0.42**	0.45**	0.47**	0.37**

*correlation coefficients significant at p=0.05, ** at p=0.01
*** explanations see Table 2

Table 9

Coefficients of the correlations between plant yields and complex biochemical indices of soil quality

Type of soil	Plant species	Complex biochemical indices of soil quality ***											
		BA_{10}	BA_{11}	BA_{12}	BA_{13}	BA_{14}	BA_{15}	BA_{16}	BA_{17}	BA_{18}	BA_{19}	BA_{20}	BA_{21}
Loamy sand	oat	0.70	0.70	0.70	0.70	0.70	0.70	0.69	0.69	0.69	0.69	0.68	0.67
	oil seed rape	0.79*	0.81*	0.78*	0.80*	0.78*	0.80*	0.78*	0.80*	0.79*	0.80*	0.74*	0.77*
	yellow lupine	0.67	0.69	0.69	0.71*	0.70	0.72*	0.69	0.71*	0.71*	0.72*	0.74*	0.75*
Sandy loam	oat	0.79*	0.81*	0.79*	0.81*	0.76*	0.78*	0.75*	0.77*	0.77*	0.77*	0.73*	0.76*
	oil seed rape	0.43	0.45	0.45	0.48	0.46	0.48	0.47	0.49	0.48	0.49	0.42	0.46
	yellow lupine	0.80*	0.78*	0.80*	0.79*	0.76*	0.75*	0.76*	0.75*	0.76*	0.75*	0.79*	0.78*
Independent from the plant													
Loamy sand		0.63**	0.63**	0.62**	0.62**	0.62**	0.62**	0.61**	0.62**	0.61**	0.61**	0.62**	0.61**
Sandy loam		0.43*	0.45*	0.42*	0.45*	0.39	0.42*	0.39	0.42*	0.41	0.42*	0.39	0.42*
Independent from the plant species and type of soil													
		0.44**	0.11	0.42**	0.09	0.39**	0.08	0.38*	0.08	0.41**	0.07	0.38*	0.43**

*correlation coefficients significant at p=0.05, ** at p=0.01
*** explanations see Table 5

CONCLUSIONS

1. Activity of enzymes should be expressed in units of the product of a catalyzed reaction in 1 h time calculated per 1 kg d.m. of soil, i.e. the activity of dehydrogenases in ěmol TFT, catalase – mol O_2, alkaline phosphatase, acid phosphatase and arylsulphatase – nmol PNP, and urease – mmol $N\text{-}NH_4$. By using unified units we are able to compare results and to assess quality of different soils, regardless of the author and research centre.

2. Among the 21 analyzed biochemical indices of soil quality, the best ones were BA_{20} = %C × (Deh + Pal + Pac + Ure), calculated from the content of organic carbon and activity of four enzymes: dehydrogenases (Deh), alkaline phosphatase (Pal), acid phosphatase (Pac) and urease (Ure), as well as BA_{21} = Deh + Cat + Pal + Pac + Ure + Glu + Aryl, calculated from the activity of seven enzymes: dehydrogenases (Deh), catalase (cat), acid phosphatase (Pac), alkaline phosphatase (pal), urease (Ure), β-glucosidase (Glu) and arylsulphatase (Aryl).

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