Analysis of soils by means of Pfeiffer's circular chromatography test and comparison to chemical analysis results

Article *in* Biological Agriculture and Horticulture · August 2016

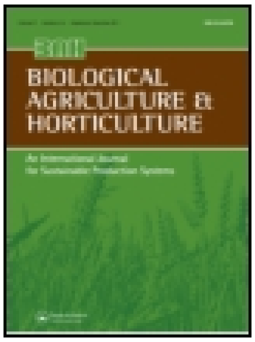
DOI: 10.1080/01448765.2016.1214889

5 authors, including:

Maria Olga Kokornaczyk
Society for Cancer Research, Arlesheim, Switzerland

Fabio Primavera
Basalti Orvieto s.r.l. Orvieto - Italia

Lucietta Betti
University of Bologna



Taylor & Francis
Taylor & Francis Group

Biological Agriculture & Horticulture

An International Journal for Sustainable Production Systems

ISSN: 0144-8765 (Print) 2165-0616 (Online) Journal homepage: http://www.tandfonline.com/loi/tbah20

Analysis of soils by means of Pfeiffer's circular chromatography test and comparison to chemical analysis results

Maria Olga Kokornaczyk, Fabio Primavera, Roberto Luneia, Stephan Baumgartner & Lucietta Betti

To cite this article: Maria Olga Kokornaczyk, Fabio Primavera, Roberto Luneia, Stephan Baumgartner & Lucietta Betti (2016): Analysis of soils by means of Pfeiffer's circular chromatography test and comparison to chemical analysis results, Biological Agriculture & Horticulture

To link to this article: http://dx.doi.org/10.1080/01448765.2016.1214889

Published online: 03 Aug 2016.

BIOLOGICAL AGRICULTURE & HORTICULTURE, 2016
http://dx.doi.org/10.1080/01448765.2016.1214889

Taylor & Francis
Taylor & Francis Group

Analysis of soils by means of Pfeiffer's circular chromatography test and comparison to chemical analysis results

Maria Olga Kokornaczyk[a,b], Fabio Primavera[c], Roberto Luneia[d], Stephan Baumgartner[b,e] and Lucietta Betti[a]

[a]Department of Agricultural Sciences, University of Bologna, Bologna, Italy; [b]Society for Cancer Research, Hiscia Institute, Arlesheim, Switzerland; [c]Agronomist – Soil Scientist, Freelancer, Arezzo, Italy; [d]Chemistry laboratory "Analysis Srl", Todi (PG), Italy; [e]Institute of Integrative Medicine, University of Witten-Herdecke, Herdecke, Germany

ABSTRACT

Pfeiffer's circular chromatography (PCC) is an analytical method relying on paper chromatography principles and applied mainly to test the quality of soils. The output of PCC are colored patterns formed on circular filter papers, pretreated with a photosensitive substance, and imbibed with a NaOH aqueous extract of the soil sample. In this study (performed in frames of the project 'Panis cum Carne' financed by the Umbria Region, Italy) 16 soil samples from differently managed fields and planted with different crops were analysed by means of PCC and standard chemical analysis. The PCC pattern evaluation consisted of three approaches: (i) measurement of pattern zones, (ii) visual scoring of pattern characteristics by schooled evaluators, and (iii) computerised texture analysis. Subsequently the collected PCC data were correlated with the chemical soil characteristics; there were strong significant correlations with the contents of organic matter, total nitrogen, and assimilable phosphorus and bromine. The results of this study provide evidence that PCC patterns may provide an overall and reliable view on the soil state.

ARTICLE HISTORY
Received 22 December 2015
Accepted 17 July 2016

KEYWORDS
Pfeiffer's circular chromatography; soil quality; soil analysis; pattern evaluation; texture; organic matter

Introduction

According to its definition 'chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves in a definite direction' (IUPAC 1997). Nowadays devices for chromatographic analysis are the third most commonly used after scales and devices for pH measurements (Lorke 2010). First chromatographic experiments date back to 1850; however as the birth of chromatography the experiment of Tswett conducted in 1903 is considered. Tswett succeeded for the first time to fully separate two chlorophyll compounds by changing slightly the previous protocol and adding pure solvent after sample imbibition (Ettre & Sakodynskii 1993; Abraham 2004).

Ehrenfried Pfeiffer (1899–1961) developed the circular chromatography test (Pfeiffer's Circular Chromatography [PCC]) for analyzing the quality of soils, composts, and crops (Pfeiffer 1984). Nowadays, PCC together with the ascending chromatography of Kolisko (called also capillary dynamolysis or 'Steigbild') (Kolisko & Kolisko 1978; Zalecka et al. 2010), constitute a unique group

CONTACT Maria Olga Kokornaczyk m.kokornaczyk@vfk.ch

of chromatographic analytical methods, which do not aim at the compound separation, but use the characteristics of patterns formed through the chromatographic process as indicator of the overall sample quality. In fact, PCC experimental procedure does not include the above mentioned protocol modification introduced by Tswett. PCC, together with the above mentioned ascending chromatography of Kolisko, and methods based on evaporation induced crystallisation (copper-chloride crystallisation method (Piva et al. 1994; Busscher et al. 2010; Baumgartner et al. 2012) and droplet evaporation method (Kokornaczyk, Dinelli et al. 2011; Kokornaczyk, Parpinello 2014; Kokornaczyk, Trebbi et al. 2014) make up the so-called picture-forming methods (Kokornaczyk et al. 2011). All these approaches aim at the analysis of the sample quality through the formation of patterns and their subsequent evaluation. Generally, in all picture-forming methods the pattern's complexity and regularity are considered indicators of high quality, whereas reduced pattern formation and irregularity have been found in samples of poor quality (Kokornaczyk et al. 2012).

There are experimental studies reporting on PCC's applicability in quality analysis of soils (Pfeiffer 1984; Khemadi et al. 2008), composts (Hassold-Piezunka 2003), and agricultural products (Knorr 1982; Geier & Seitz 2006; Maeder et al. 2007). In particular, Khemadi et al. (2008) reported on the development and possible use of a large computerised database linking PCC pattern characteristics (e.g. inner-, middle-, and outer-zone, spikes, and color) with the soil sample's chemical composition. Based on the assumption that samples that produce similar PCC patterns also have similar composition, the software is thought to display the matching soil composition by comparing the pattern of the sample to be analysed with patterns available in the database. As described in Hassold-Piezunka (2003) PCC may be used also to determine the quality and maturity of composts. It could be shown that different pattern parameters, e.g. breadths and colors of the zones, length of spikes, contrasts between adjacent zones, and color intensity of borderlines, differ between the compost samples and correlate with some of the compost's chemical components. Studies regarding PCC's applications in food quality analysis mainly concentrated on the differentiation of crops deriving from different field management systems (conventional, organic, and biodynamic). In (Maeder et al. 2007) such differences could be detected in wheat samples grown in a long term experimental trial; PCC was combined with other picture-forming methods and the pattern evaluation consisted in blind grouping performed visually by an evaluator. In (Knorr 1982) collard plants grown under different cultivation conditions (conventional and organic) and different fertilisation levels were analysed by means of PCC; for pattern evaluation a sensory analysis rating test with unstructured scale was adopted performed by a panel of 50 students. Significant differences were obtained between the two cultivation types and strong correlations were found between the sensory data and protein or nitrate-nitrogen levels.

Besides being studied in scientific circles PCC, characterised by a rather simple methodology, is often used by farmers themselves, mainly working in the biodynamic context. There is also an Italian group called INTERLAB, associating producers, scientists, and interested people, which carries out PCC experiments contemporaneously in different laboratories, mainly with the aim of exchanging experiences and focusing on some aspects of the methodology (INTERLAB 2011–2015).

In the present study, PCC was applied to analyse 16 soil samples derived from differently managed fields (biodynamic, organic or conventional agriculture) planted with different crops. The aim was to develop methodologies for the PCC pattern evaluation, to link pattern characteristics with soil chemical analyses, and to test the PCC's sample differentiation potential.

Materials and methods

Samples and analyses

Sixteen soil samples (Table 1) were provided by the biodynamic farm 'Le Due Torri' (Spello, Perugia, Italy). The samples were collected in early spring 2014 from biodynamically, organically, and conventionally managed fields planted with different crop types. The samples were collected at a depth of 10–35 cm, dried, and ground. To avoid interference from accidental variations, every sample was

Table 1. List of soil samples analysed in the study.

No.	Crop	Field management
1	Olive tree (*Olea europaea L.*)	Biodynamic
2	Olive tree (*O. europaea L.*)	Biodynamic
3	Grapevine (*Vitis vinifera L.*)	Biodynamic
4	Grapevine (*V. vinifera L.*)	Organic
5	Grapevine (*V. vinifera L.*)	Organic
6	Barley (*Hordeum vulgare L.*)	Biodynamic
7	Barley (*H. vulgare L.*)	Biodynamic
8	Alfalfa (*Medicago sativa L.*)	Biodynamic
9	Broad been (*Vicia faba L.*)	Biodynamic
10	Millet (*Panicum miliaceum L.*)	Biodynamic
11	Rye (*Secale cereale L.*)	Biodynamic
12	Oat (*Avena sativa L.*)	Biodynamic
13	Wheat (*Triticum aestivum L.*)	Biodynamic
14	Wheat (*T. aestivum L.*)	Organic
15	Wheat (*T. aestivum L.*)	Conventional
16	Flax (*Linum usitatissimum L.*)	Organic

Note: For each sample crop and field management system are reported.

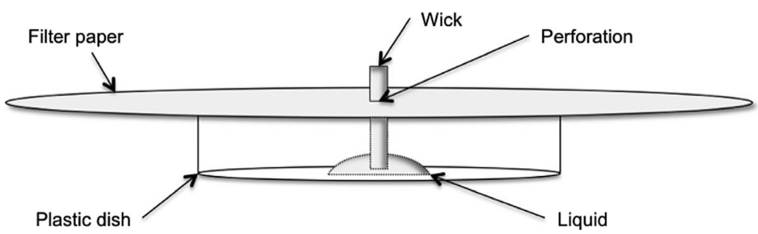


Figure 1. Graphical representation of the performance of the Pfeiffer's circular chromatography tests: a circular filter paper sheet with a wick insert into the central perforation is located on a plastic dish in order that the wick's end is immerged in the solution (liquid phase) to be imbibed.

derived from five sub-samples taken following a preformed grid and mixed together. The samples were coded by a person not involved in the experiment and were sent by post for all analyses. The samples were decoded after data evaluation.

PCC and data elaboration were performed at the University of Bologna, Italy; whereas the chemical compound analysis was conducted at the laboratory 'Analysis srl' (Todi, Perugia, Italy).

Pfeiffer's circular chromatography test

Ten gram of soil were placed in a flask containing 100 ml of 1% aqueous solution of NaOH for extraction. The flask was agitated by hand at the beginning of the extraction, and after 15 min, 1, and 2 h. After the last agitation the flask was left for another 2 h for extraction and sedimentation. After a total of 4 h the supernatant was collected. In the meantime, as depicted in Figure 1, a circular Whatman 1 filter paper (150 mm diameter) was pierced in the middle and a cylindrical wick, folded up from a 15 mm × 15 mm filter paper, was inserted into the perforation. The circular filter paper was put on a plastic dish (60 mm diameter) in order to rest on its edges; whereas the wick was manipulated in order to touch the dish's bottom. The so prepared filter paper was imbibed twice: (i) in the dark, with 0.6 ml of a 0.5% aqueous solution of $AgNO_3$, and, after the filter paper dried, (ii) in artificial light, with 1.2 ml of the supernatant collected after the soil extraction, by placing the imbibition liquid at the bottom of the dish. After the second imbibition the filter paper was left in the light for ca. 12 h to dry and to let the forms and colors fully develop. After this time the wick was detached. All the experimental steps were made at room temperature.

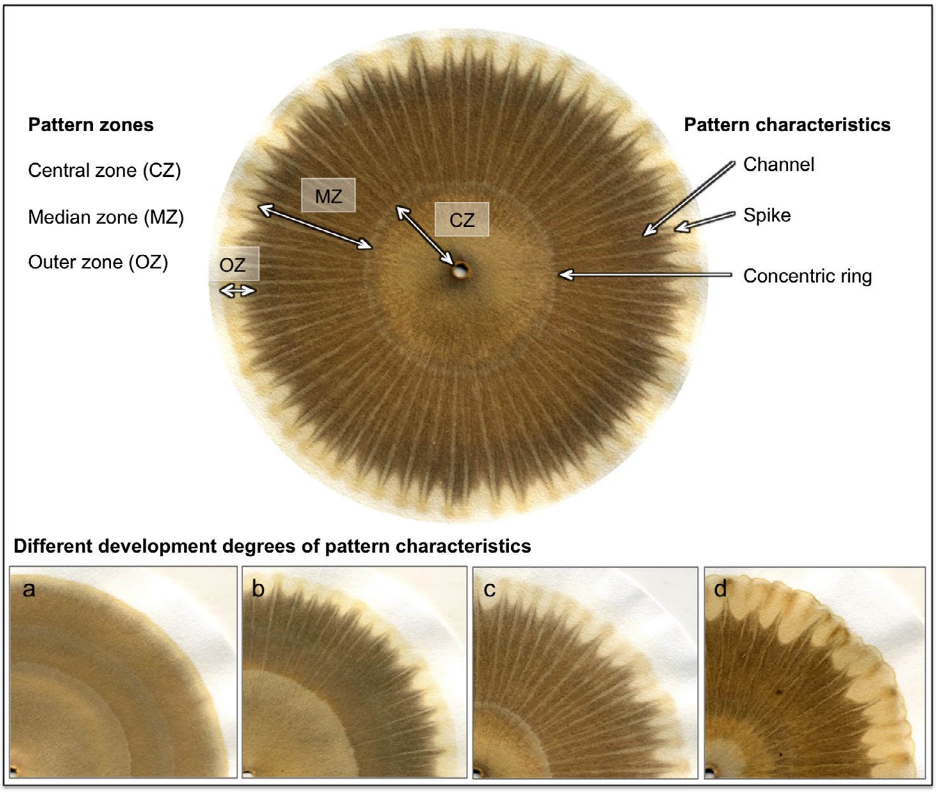


Figure 2. Example of a PCC pattern with described zones and pattern characteristics (on the top) and pattern sections (on the bottom) illustrating, from (a) to (d), an increasing development of radial features, (e.g. channels and spikes) and color intensity, and a decrease of concentric features (e.g. concentric rings).

From each soil sample 3 PCC patterns (filter papers) were prepared, the whole experiment was repeated three times on different days (in total nine PCC patterns per sample). PCC patterns were scanned (resolution 3440 × 3440 pixel) in color; the PCC paper patterns were stored in the dark.

Visual check of the PCC patterns

All PCC paper patterns obtained in the present experimentation were subjected to a visual check, when pattern zones (central-, median-, and outer-zone; denoted as *CZ*, *MZ*, and *OZ*, respectively) and differentiating pattern characteristics were identified and described (Figure 2).

Measurement of PCC pattern zones

Zone measurement (Figure 2) was performed on PCC paper patterns by means of a ruler. On each pattern the following measurements were performed: total radius, *CZ* radius, *MZ* breadth, and *OZ* breadth; as the border between *MZ* and *OZ* the bases of the spikes were considered. All measurements were taken in vertical and in horizontal, and the mean values were calculated.

Visual PCC pattern evaluation

The visual PCC pattern evaluation was performed on PCC paper patterns by two schooled evaluators and consisted in scoring of the development degree of the differential pattern characteristics (*channels,* *spikes, color intensity*, and *concentric rings*; Figure 2) selected upon the results of the visual check. A maximum of 5 points was accredited to characteristics *channels, spikes*, and *color intensity*. For *channels* and *spikes* 1 point was accredited when these characteristics were absent and 5 points when their development was full; for *color intensity* 1 point was accredited when the color was visually sensed as blurred and 5 points when it was sensed as intense. Intermediate points (2, 3, or 4) were accredited in cases of intermediate characteristic development. For the characteristic *concentric rings* (score from 1 up to 4 points) the number of rings was counted and 1 point was accredited to each ring visible on the pattern.

Computerised PCC image analysis

The computerised analysis consists in the measurement of the pattern's texture and was performed on scanned PCC images (3500 × 3500 pixels) by means of the software *ImageJ* (Collins 2007) with the installed plug-in *Texture Analyser* (Cabrera 2003–2005). Out of each image a rectangular region of interest (1000 × 200 pixels) of the median zone was randomly selected (in such a way as not to contain parts of the central or outer zone [*OZ*]), cropped, and converted to 8-bit type (pixels only in gray scale). On such prepared image selections the texture analysis was performed. In particular the parameter *entropy* was considered for further analysis, since it is sensitive to differences in brightness intensity between the pixels and thus also to the presence or absence of channels.

Chemical compound analysis

The following soil compounds and characteristics were selected and analysed in accordance to the Italian Official Regulations on soil analysis (MIPAF 1992, 1999): organic matter (Wolkley and Black method), total nitrogen (Kjeldahl method), cation exchange, assimilable phosphorus, assimilable bromine, exchangeable calcium, exchangeable magnesium, pH, sand, clay, and silt. All the parameters were analysed for the 16 soil samples.

Statistical analysis

The data-set was analysed by means of analysis of variance (ANOVA) followed by the *post hoc* multiple mean comparison with LSD test using the CoStat statistical software (v. 6.4, CoHort Software). Also the visual evaluation data were subjected to the ANOVA analysis, even though they were not continuous (the corresponding statistical output should be therefore interpreted only in a generic way). In addition, the Bravais–Pearson linear coefficient of correlation r was computed to determine the degree of correlation between the PCC data (visual evaluation, pattern zones measurements, and computerised analysis) and the chemical soil parameters.

Results

PCC pattern description

All PCC patterns obtained in the present experimentation consisted of three ring-shaped zones located around the central perforation (Figure 2): (i) the central zone (*CZ*), located nearest to the hole and characterised by rather light color, then (ii) the darker middle zone (*MZ*), and, on the pattern periphery (iii) the very light and in many cases only weakly visible outer zone (*OZ*).

The visual check of the pattern set showed that the most prominent differences concerned the presence of concentric and radial pattern features, which appearance seemed to be inversely related (e.g. the stronger the concentric features, the lesser and weaker the radial ones, and vice versa; Figure 2). As shown in Figure 3(a), patterns with strongly marked concentric features contained up to four concentric rings located in *CZ* and *MZ*. In these patterns the coloration seemed blurred

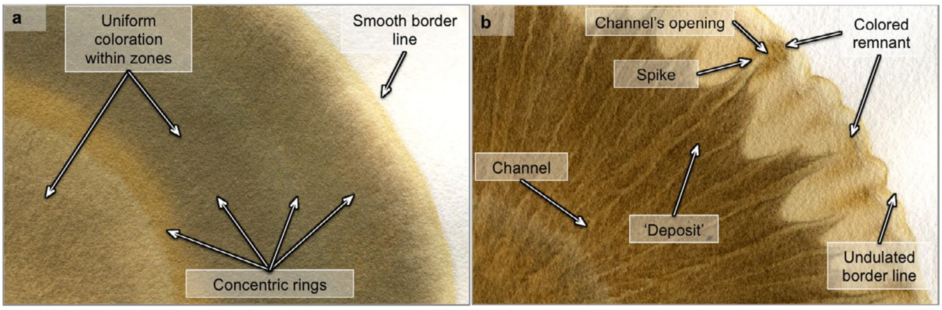


Figure 3. Examples of pattern sections showing strongly marked (a) concentric and (b) radial features.

Table 2. Results of the three PCC pattern evaluation assessments: zone measurement, visual evaluation, and texture analysis.

		Zone measurements				Visual ranking (1–5 points)				Texture	
		OZ (mm)		*CZ* (mm)		Channels		Concentric rings		Entropy	
	N	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Olive tree BD 1	9	13.08 a	0.46	2.00 f	1.90	5.00 a	0.00	1.00 e	0.00	8.81 ab	0.12
Olive tree BD 2	9	6.48 b	0.51	22.44 f	1.01	4.89 a	0.07	1.39 e	0.16	8.94 a	0.13
Vineyard ORG 1	9	4.74 c	0.45	29.89 de	0.88	3.11 c	0.07	2.56 d	0.16	8.63 bc	0.11
Vineyard BD	9	4.37 c	0.75	28.44 e	1.04	3.72 b	0.15	2.56 d	0.24	8.62 bc	0.10
Vineyard ORG 2	9	4.37 c	0.27	32.44 c	0.86	2.94 cd	0.07	2.75 cd	0.16	8.53 cd	0.10
Alfalfa BD	9	3.04 de	0.31	34.62 a	0.28	2.62 de	0.28	3.31 a	0.16	8.36 de	0.06
Millet BD	9	3.07 d	0.28	31.44 cd	0.61	2.22 ef	0.26	2.89 bcd	0.11	8.32 ef	0.06
Barley BD 1	9	2.33 ef	0.14	33.11 abc	0.89	1.83 fg	0.31	3.28 ab	0.22	8.26 ef	0.05
Wheat BD	9	2.74 de	0.13	32.11 c	0.85	1.67 gh	0.07	3.06 abc	0.16	8.36 de	0.02
Wheat CONV	9	2.96 de	0.21	31.56 cd	0.80	1.61 ghi	0.07	3.06 abc	0.16	8.41 de	0.05
Broad Been BD	9	2.92 de	0.28	29.17 e	0.50	1.61 ghi	0.23	3.39 a	0.26	8.28 ef	0.04
Barley BD 2	9	2.67 de	0.15	34.50 ab	0.53	1.39 ghij	0.18	3.11 ab	0.17	8.40 de	0.11
Flax ORG	9	1.85 f	0.25	31.75 c	0.86	1.39 ghij	0.07	3.11 ab	0.16	8.29 ef	0.05
Rye BD	9	2.74 de	0.19	32.83 bc	0.52	1.28 hij	0.07	3.33 a	0.16	8.42 de	0.08
Oat BD	9	2.33 ef	0.21	32.67 c	0.42	1.17 ij	0.07	3.11 ab	0.16	8.16 f	0.05
Wheat ORG	9	2.74 de	0.26	32.06 c	0.46	1.11 J	0.07	3.11 abc	0.16	8.26 ef	0.04

Notes: Different letters indicate statistical differences at $p < 0.05$.
Legend: *N* – number of patterns; *SE* – standard error.

and changed only with the distance from the patterns center, whereas OZ was thin and little visible, surrounded by a smooth borderline. On the contrary, patterns with strongly marked radial features (Figure 3(b)) contained channels starting in most of the cases in *MZ*, but sometimes already in *CZ*, and running towards the pattern's periphery; in-between the channels darker 'deposits' were located. Spikes were formed on the outer ends of channels; moreover, near to the channel openings, inside the well developed *OZ*, colored remnants were often present. The coloration of these patterns was normally intense.

Based on these observations, the PCC patterns were evaluated by (i) measuring the zones, (ii) scoring the development degree of concentric (number of *concentric rings*) and radial (*channels, spikes*) pattern features and the *color intensity* (visual evaluation approach), and (iii) computerised texture analysis of the *MZ* (*entropy*).

Differentiation of the soil samples

The results of the soil sample differentiation by means of the PCC are shown in Table 2. Since all measured parameters, except the *MZ breadth*, correlated strongly with each other (positively and negatively), the values of two negatively correlating parameters with highest sample differentiation

Table 3. Results of *F*-tests of analysis of variance of the pattern evaluation assessments: zone measurements, visual evaluation, and computerised analysis.

	1. Sample		2. Day		1*2 Interaction	
	p	*F*	*p*	*F*	*p*	*F*
Zone measurements						
Total radius	<0.0001	11.89	<0.0001	73.89	0.0004	2.55
Central zone	<0.0001	31.32	0.0002	9.42	<0.0001	2.98
Middle zone	<0.0001	10.74	<0.0001	23.25	<0.0001	9.44
Outer zone	<0.0001	62.73	0.0002	9.69	<0.0001	3.31
Visual evaluation						
Channels	<0.0001	53.33	<0.0001	23.27	0.0002	2.72
Spikes	<0.0001	47.86	0.0295	3.66	<0.0001	3.79
Concentric rings	<0.0001	22.07	<0.0001	11.71	<0.0001	3.48
Color	<0.0001	25.57	0.0243	3.87	0.0044	2.09
Texture analysis						
Entropy	<0.0001	7.56	0.0135	4.51	0.7679	0.77

Note: Effects of main factor, experimentation day and interaction are given.

potential (highest F values; Table 3) deriving from the evaluation approaches (i) measurement of the PCC pattern zones (e.g. *CZ radius*, *OZ breadth*), (ii) visual evaluation (*channels*, *concentric rings*), and (iii) the parameter deriving from the textural analysis (*entropy*), were chosen to be presented here.

Principally, the pattern evaluation revealed differences between the different cultivations. Soils from olive groves produced patterns with significantly widest *OZ* (there was also a significant difference between samples olive tree BD1 and olive tree BD2), followed by the soils from vineyards; whereas soils planted with millet, alfalfa, grains, beans, and flax produced patterns with significantly narrower *OZ* (Table 2). Within the last group highest *OZ breadth* values were observed for millet and alfalfa and lowest for barley, oat, and flax. Similar sequences also appeared for the values of the parameters *channels* and *entropy*. Furthermore, the parameter *channels* enabled a significant differentiation between biodynamic and organic soils deriving from vineyards and wheat cultivations, however in case of the wheat cultivations there was no difference between soils planted with biodynamic and conventional wheat (Table 2). Values of the parameters *CZ radius* and *concentric rings* showed an inverse sequence in comparison to other parameters (Figure 4).

The results of the *F*-test of two-way ANOVA with independent factors (sample and experimentation day) of the PCC pattern evaluation results (zone measurement, visual evaluation, texture analysis) are shown in Table 3. Factors sample and day were strongly significant in all three approaches; however in most cases the *F*-values for sample influence were higher than those for the day influence (except for the measurements of the *total radius* and *MZ breadth*). The interaction between sample and analysis day was significant for all parameters of the zone measurement and visual evaluation approach, but the F values were lower than those of sample and day influence (except for *spikes*); whereas for *entropy* the interaction between day and sample was not significant. As regards the soil sample differentiation potential, the highest significance for the factor sample showed *OZ breadth* and the development degree of *channels*; whereas *entropy*, *MZ breadth* and *total radius* had the lowest *F*-values. In case of *MZ breadth* the day factor had a higher significance level than the factor sample.

Correlations between parameters deriving from the PCC pattern evaluation

Strong and significant correlations were found between parameters derived from the three pattern evaluation approaches (zone measurement, visual analysis, computerised analysis). Firstly, the correlations found between parameters derived from the same evaluation approach are presented below and then the correlations between the parameters from the different evaluation approaches.

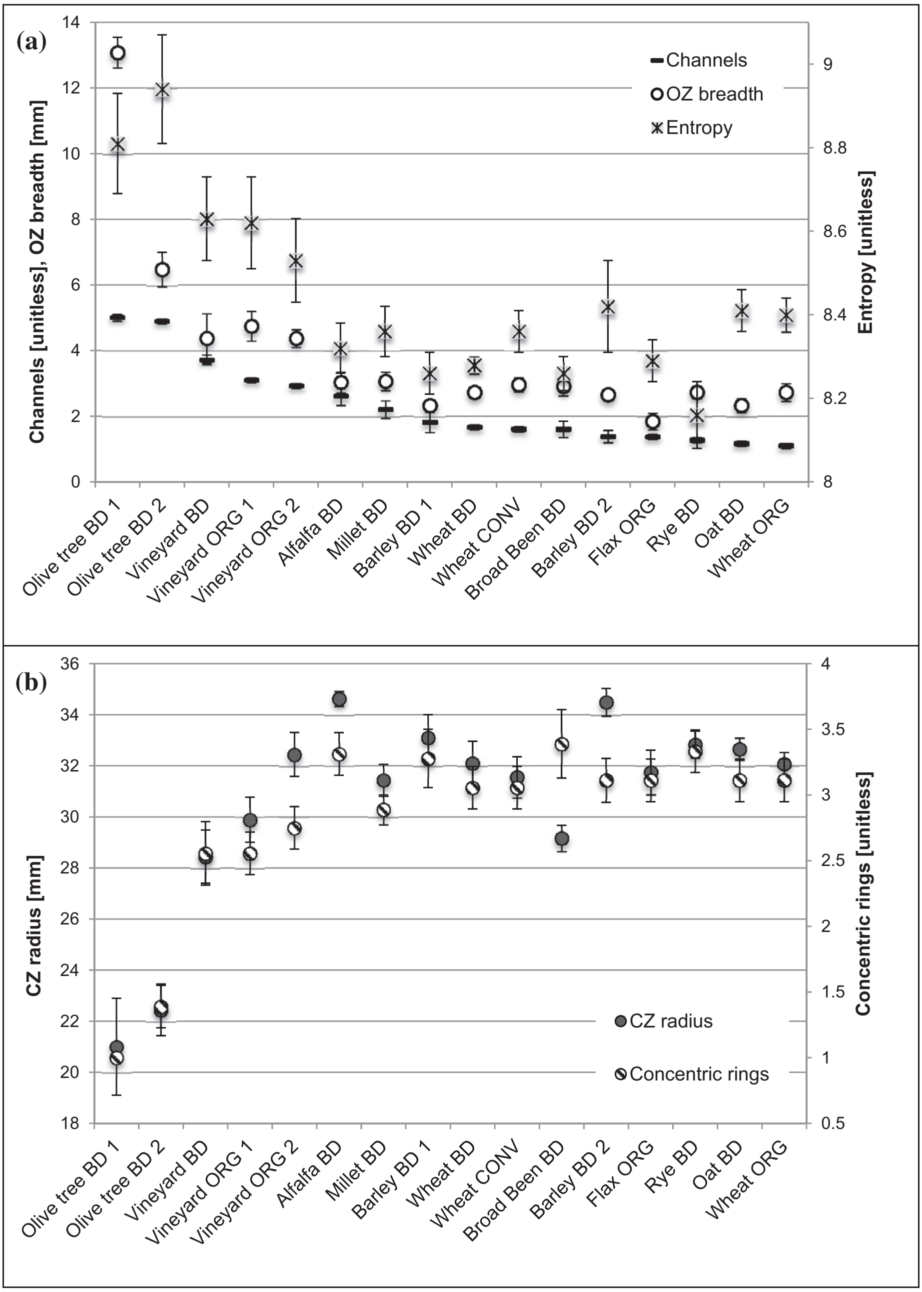


Figure 4. Graphical representation of the mean values of PCC pattern parameters (a) outer zone breadth (*OZ breadth*), development degree of channels (*Channels*), and *Entropy*, and (b) radius of central zone (*CZ radius*), and number of concentric rings (*Concentric rings*).

As regards to the parameters derived from the PCC pattern zone measurement, a significant negative correlation was found between the *CZ radius* and *OZ breadth* ($r = -0.85$). The *CZ radius* created a significant positive correlation with the pattern *total radius* ($r = 0.80$), whereas *OZ breadth* a negative one ($r = -0.67$). Worthy of attention is also the fact, that *MZ breadth* did not create any significant correlations with other pattern measures.

Table 4. Correlations between parameters derived from different PCC pattern evaluation approaches.

	Visual evaluation				Texture analysis
	Channels	Spikes	Color	Concentric rings	Entropy
Zone measurements					
Total radius	−0.63**	−0.74 **	−0.70 **	0.77 **	−0.79 **
Central zone	−0.83 ***	−0.86 ***	−0.77 **	0.92 ***	−0.81 ***
Middle zone	−0.14 ns	−0.24 ns	−0.39 ns	0.22 ns	−0.25 ns
Outer zone	0.83 ***	0.85 ***	0.86 ***	−0.91 ***	0.76 **
Texture analysis					
Entropy	0.74**	0.67**	0.72**	−0.81***	–

Legend: ns = $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

All parameters derived from the visual pattern evaluation were strongly correlated. The correlations between the *concentric rings* and all other pattern characteristics (e.g. *channels*, *spikes*, and *color intensity*) were negative and amounted to $r = -0.90$; -0.91; -0.93, respectively ($p < 0.0001$), whereas *channels*, *spikes* and *color intensity* created between each other strong positive correlations (r = from 0.91 to 0.97; $p < 0.0001$).

Correlations found between parameters derived from different evaluation approaches are shown in Table 4. In particular, *channels, spikes* and *color intensity* correlated positively with the *OZ breadth* and *entropy* and negatively with *total radius* and *CZ radius*; on the contrary, the *concentric rings* created significant negative correlations with the *OZ breadth* and *entropy* and positive ones with *total radius* and *CZ radius*.

Correlations between PCC data and chemical soil analysis

Results of the chemical soil analysis are shown in Table 5. The soil samples differed greatly in contents of organic matter (from 14.60 to 66.58 g kg^{-1}), total nitrogen (from 1.39 to 4.09 g kg^{-1}), phosphorus (from 2 to 55 mg kg^{-1}) and bromine (from 0.68 to 3.93 mg kg^{-1}), whereas there were no big differences observed in the pH values (from 6.90 to 7.34; neutral range). The samples differed also in their compactness (different sand, silt, and clay contents; from 180 to 660 g kg^{-1}, from 160 to 440 g kg^{-1}, and from 180 to 400 g kg^{-1}, respectively).

As shown in Table 6 there were strong, significant correlations between PCC pattern parameters derived from the three evaluation approaches and many of the here considered soil chemical compounds and characteristics, e.g. pH, organic matter content, total nitrogen content, assimilable phosphorus and bromine, as well as the contents of sand, clay and silt. Correlations between cation exchange capacity, exchangeable calcium and magnesium contents and PCC data were all weak (r ranged from −0.41 to 0.44) and were not significant (data not shown). Overall, organic matter content, total nitrogen, phosphorus, bromine and sand created positive correlations with *OZ breadth*, radial features (*channels* and *spikes*), *color intensity*, and texture parameter *entropy*, and negative ones with pattern *total radius*, *CZ radius*, and *concentric rings*. On the contrary, pH, silt, and clay correlated negatively with *OZ breadth*, *channels, spikes*, *color intensity*, and *entropy* and positively with *total radius, CZ radius*, and *concentric rings*.

Discussion

Differentiation of soil samples by means of PCC test

In these experiments significant differences were found in soil samples derived from fields planted with different crops. It was possible to distinguish between soils from the olive groves, vineyards, fields planted with alfalfa (parameter *channels*), and beans, different kinds of grains, oat and flax (Table 2). These results were compatible with the recent knowledge on grain cultivation, which appears to be rather taxing for the soil and is therefore always of concern within crop rotations. Alfalfa on the other

Table 5. Mean values of the soil chemical components and characteristics.

	O. M. (g kg^{-1})	N tot (g kg^{-1})	Cat.Ex.Cap (meq 100 g^{-1})	Ass. P (mg kg^{-1})	Ex. Mg (mg kg^{-1})	Ex. Ca (mg kg^{-1})	Ass. Br (mg kg^{-1})	pH	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})
Uncertainty (%)	0.10	7.30	6.20	2.70	8.10	9.10	3.60	0.03	3.10	3.60	4.20
Olive tree BD 1	66.58	4.09	36.37	43	261	6263	3.93	7.08	660	160	180
Olive tree BD 2	41.33	2.68	27.02	55	126	4845	1.64	6.90	360	380	260
Vineyard BD	31.81	1.99	32.82	30	306	5749	1.51	6.93	180	440	380
Vineyard ORG 1	26.70	1.88	23.83	23	99	4389	1.51	6.98	400	400	200
Vineyard ORG 2	28.04	2.30	28.73	25	88	5398	1.28	7.24	460	300	240
Millet BD	29.20	1.86	30.48	31	176	5586	1.00	7.15	320	440	240
Alfalfa BD	18.36	1.68	25.06	14	281	4416	1.11	7.04	480	300	220
Broad Been BD	22.04	1.74	30.70	22	241	5528	1.11	7.11	340	320	340
Rye BD	21.21	1.45	24.39	10	112	4531	0.68	7.33	340	420	240
Wheat BD	19.23	1.53	25.28	17	111	4718	0.87	7.24	420	320	260
Wheat ORG	18.15	1.58	28.49	13	83	5429	0.59	7.28	280	380	340
Wheat CONV	18.06	1.49	24.92	13	99	4699	0.59	7.40	400	360	240
Barley BD 1	25.07	1.51	37.88	10	280	6914	1.07	7.00	280	320	400
Barley BD 2	20.08	2.90	24.98	8	146	4591	0.70	7.08	260	420	320
Oat BD	14.60	1.39	24.97	2	92	4723	0.73	7.34	340	400	260
Flax ORG	21.04	1.73	25.84	16	98	4865	0.78	7.27	340	340	320

Notes: Measurement results and % of measurement uncertainty are given.
Legend: O. M. – organic matter; N tot – total nitrogen; Cat.Ex.Cap – cation exchange capacity; Ass. – assimilable; Ex. – exchangeable.

Table 6. Correlations (Pearson's *r* values) between the results of PCC pattern evaluation (zone measurement, visual and computerised analysis) and chemical soil analysis.

	O. M.	N tot	Ass. P	Ass. Br	Sand	Clay	Silt	pH
Zone measurements								
Total radius	−0.68**	−0.63**	−0.72**	−0.57*	−0.32	0.32	0.20	0.26
Central zone	−0.88***	−0.69**	−0.87***	−0.81***	−0.40	0.25	0.39	0.44
Middle zone	−0.23	−0.51*	0.03	−0.33	−0.52*	0.54*	0.31	−0.12
Outer zone	0.96***	0.86***	0.72**	0.97***	0.68**	−0.47	−0.62*	−0.36
Visual analysis								
Spikes	0.85***	0.73**	0.88***	0.82***	0.45	−0.42	−0.31	−0.64**
Channels	0.86***	0.71**	0.89***	0.83***	0.44	−0.34	−0.37	−0.67**
Color	0.84***	0.79**	0.79**	0.82***	0.52*	−0.43	−0.42	−0.52*
Concentric rings	−0.92***	−0.81***	−0.86	−0.85***	−0.50*	0.40	0.40	0.45
Texture analysis								
Entropy	0.75**	0.71**	0.81***	0.69**	0.30	−0.30	−0.19	−0.56*

Legend: O. M. – organic matter; N tot – total nitrogen; Ass. – assimilable; ns = $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

hand, having the capacity to enrich the soil in nitrogen, is known to improve the soil's general state. Vineyards and olive groves being long-time cultivations are known to have, above all in biodynamic or organic management, a balanced and fertile soil (Vavoulidou et al. 2009).

Concerning the differentiation of soils derived from different management systems, there was a significant difference between the biodynamic and organic cultivation in case of vineyards and wheat; however in case of wheat there was no difference between the biodynamic and conventional management. Only the parameter *channels* enabled a significant differentiation of soils derived from differently managed fields, thus proving to represent one of the most sensitive PCC pattern evaluation criteria.

In this experiment the comparison between different soil management systems was possible only in a small number of samples (three samples from vineyards and three samples from wheat cultivations) and therefore these results are not decisive and should be repeated.

Increasing the number of repetitions might increase the method's differentiation potential. The present study's design considered a rather small number of repetitions, which enabled us to work with a large number of samples contemporaneously and to gain so a better data-set for the correlations of PCC out-come with chemical soil analysis results (main focus of the experimentation).

As shown by the results of the *F*-test (Table 3) in all pattern evaluation approaches the influence of the experimentation day was significant. Similar results concerning the day factor influence in pattern developing methods have been reported in other studies (Steffen 1983; Baumgartner et al. 2012; Kokornaczyk, Trebbi et al. 2014). In case of the present study, this influence might be reduced by increasing the control of the external conditions during the experimentation (temperature, relative humidity). However, since the self-organisation process is not yet a fully understood phenomenon, the day influence might also be related to other factors that were not considered here.

PCC pattern features and their relation to soil composition

Above all our findings indicate the occurrence of an inverse relationship between the concentric and radial PCC pattern features. This inverse relationship could be observed already during the visual check of the pattern lot (Figure 2), and could be further confirmed by correlations found between the parameters derived from the three pattern evaluation approaches (zone measurement, visual evaluation, and computerised texture analysis) (Table 4, Figure 4). In this way two groups of parameters could be identified:

(i) parameters related to concentric pattern features (*total radius*, *CZ radius*, number of *concentric rings*), and

(ii) parameters related to radial pattern features (*OZ breadth*, *channels*, *spikes*, *color intensity*, and the texture parameter *entropy*),

correlating with each other within one group positively, and in-between the groups negatively.

Considering the correlations found between the PCC pattern parameters and chemical soil analysis results, it could be noticed that radial features were related to positive soil characteristics (high contents of organic matter, total nitrogen, phosphorus, bromine, and sand, e.g. low soil compactness), whereas the concentric pattern features to those indicating poor soil quality (high contents of clay and silt, e.g. high soil compactness, and low contents of organic matter, total nitrogen, phosphorus and bromine). Based on these results, it can be suggested that radial pattern differentiation in PCC is a sign of high soil quality, whereas domination of concentric features indicates an unfertile soil. It can be asserted that the correlations reported here confirm the sensibility of PCC patterns towards soil quality, as also confirm the validity of the three pattern evaluation approaches applied here. It should be further evaluated if other PCC pattern parameters, e.g. the color within specific zones, studied by other authors (Hassold-Piezunka 2003; Khemani et al. 2008), correlate with the soil components and possibly enter the interaction between the concentric and radial pattern features.

Overall the correlations between the results of soil chemical analysis and visual evaluation were stronger than those with the texture analysis parameter, suggesting that the visual evaluation approach was in this study more sensitive to the soil composition than the computerised one. With regards to the pattern zone measurements strongest correlations with the chemical soil composition were found for *OZ breadth* and *CZ radius*. The *MZ breadth* did not create any significant correlations with the other parameters analysed here. This might be caused by the difficulty that was encountered when measuring the pattern's *MZ*, especially in patterns with well-developed, long spikes. As *MZ breadth* we considered the distance from the border between *CZ* and *MZ* to the spikes basis, whereas spikes we considered already part of the *OZ*. This kind of measurement might contain some errors due to large differences in the lengths of spikes and their departing point within one pattern. However the same difficulty concerned also the measurement of the *OZ breadth*, which created strong correlations with other parameters.

Finally, the pH values of all of the soil samples analysed here were in neutral range; and therefore it should be further tested if the results are valid also for acid and alkaline soils.

Differentiation of PCC patterns into concentric and radial pattern features

From the results of this study, it seems that the pattern differentiation in PCC might be considered a result of two opposing processes, e.g. the differentiation into concentric and radial pattern features. While the first basically depends on chromatography's ability to separate compounds and thus might be considered a 'passive' character, the second seems to be driven by interactions between the different compounds and so representing an 'active' self-organisation process. Furthermore, the differentiation of the pattern into radial features raises the pattern complexity, a feature, which also in other picture developing methods (e.g. copper chloride crystallisation, droplet evaporation method, capillary dinamolysis) is considered a sign of high sample quality (Steffen 1983; Baumgartner et al. 2012; Kokornaczyk, Trebbi et al. 2014).

Differentiation into concentric features

In general, in paper chromatography compound separation is expressed by color differentiation. In case of PCC, due to its methodological protocol (during the second imbibition the sample extract is not followed by pure solvent) no complete compound separation takes place. Additionally, the filter paper is pretreated with $AgNO_3$, a photosensitive substance, which oxidates to silver hydroxide, and almost immediately also to the insoluble yellowish-brown silver oxide when exposed to light (Brinton 1982). Therefore, it might be assumed that in PCC different colors, when located as a function of the distance from the pattern's center, may depend on the following phenomena: (i) moving or separating of $AgNO_3$ and its oxidation products by the soaking sample extract, (ii) partial separation of the sample extract compounds, while moving through the filter paper, and formation of zones of different compound concentrations, and (iii) reactions between sample extract compounds and $AgNO_3$ and/or its oxidation products.

The main result of the concentric pattern differentiation is the creation of pattern zones; the presence of such zones has already been reported elsewhere (Brinton 1982; Pfeiffer 1984) and seems to constitute a typical feature of PCC patterns. Additionally concentric rings located within the pattern's *MZ* and/or *CZ* (parameter *concentric rings*) can be formed. With regards to the *OZ breadth*, based on the positive correlations found between this parameter and radial pattern characteristics, it could be considered a radial character, rather than concentric, even though *OZ* is a pattern zone.

Differentiation into radial features

The formation of flow ducts in other chromatographic methods has been reported (Unger et al. 2008); this phenomenon was usually associated to the creation of quicker passage channels of small sized particles. Also in PCC the creation of similar flow-ducts (e.g. channels), as also 'deposits' lying between the channels, might be related to the sizes of particles present in the liquid phase. Knorr (1981, 1982) hypothesised that the weakly soluble silver hydroxide, one of the $AgNO_3$ oxidation products, may partially block the chromatographic paper pores causing the passage of larger organic particles to be slowed down or hindered. Subsequently the small sized particles have to force their passage through the 'deposits' leading so to the formation of channels. Correlations found in the present study between the organic matter content and parameter *channels* seem to confirm this hypothesis.

From a visual examination of the arrangement of radial features (Figure 3(b)) it seems that the channels, while being percolated by the liquid phase, lead to the formation of spikes and small colored remnants in the *OZ* placed in front of the channel openings, probably created out of compounds, which percolated the flow ducts. Taking into consideration the high positive correlations found between the radial pattern characteristics and the *OZ breadth*, actually the whole *OZ* might be considered a product of an enhanced percolation of the liquid phase from the *CZ* directly to the patterns periphery through the pattern's channel-system. This flow dynamic seems to be further confirmed by significant negative correlation found between *CZ radius* and *OZ breadth*.

Conclusions

Our results show that PCC patterns may be considered an information source reflecting the overall soil state. In particular, strong radial differentiation and intense coloration of the patterns seem to be signs of good soil quality, whereas concentric pattern differentiation and blurred colors indicate an unfertile soil. Furthermore, PCC being a cost saving and easy to perform analysis might partially replace the soil chemical analysis; primarily in cases where a comparison of the expected fertility of soil samples is needed, rather than exact compound measurements.

The present study also revealed a need for detailed experimentation focused on the PCC pattern formation processes and regions of precipitation of the various compounds (soil extract compounds, $AgNO_3$ and its oxidation products) within the PCC pattern.

Acknowledgements

A special thanks goes to Dr. Fabio Ciri and Prof. Biancamaria Torquati for the efficient coordination of the project. Furthermore, we would like to thank Paolo Pistis and Dr. Mariola Aldè for the interesting exchange of opinions and precious methodological suggestions.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Umbria Region/Italy in frames of the project 'Panis cum Carne'.

References

Abraham MH. 2004. 100 years of chromatography – or is it 171? J Chromatogr A. 1061:113–114.
Baumgartner S, Doesburg P, Scherr C, Andersen J-O. 2012. Development of a biocrystallisation assay for examining effects of homoeopathic preparations using cress seedlings. eCAM. 2012:1–14. doi:http://dx.doi.org/10.1155/2012/125945.
Brinton WF. 1982. A qualitative method for assessing humus condition. In: Knorr D, editor. Sustainable food systems. Westport (CT): AVI Publishing:416 p.
Busscher N, Kahl J, Andersen J-O, Huber M, Mergardt G, Doesburg P, Paulsen M, Ploeger A. 2010. Standardization of the biocrystallization method for carrot samples. Biol Agric Hortic. 27:1–23.
Cabrera J. Texture analyzer [Internet]. c2003–2005; [cited 2015 Dec 9]. Available from: http://rsb.info.nih.gov/ij/plugins/texture.html
Collins TJ. 2007. ImageJ for microscopy. BioTechniques. 43;Suppl:S25–S30.
Ettre LS, Sakodynskii KI. 1993. M. S. Tswett and the discovery of chromatography I: early work (1899–1903). Chromatographia. 35:223–231.
Geier U, Seitz L. 2006. Bildoptimum und Bildtypen im Rundchromatogramm (Chroma) bei der Untersuchung von Pflanzen [Picture-optimum and picture types in circular chromatogram in the study of plants]. Elemente der Naturwissenschaft. 85: 57–78 (in German).
Hassold-Piezunka N. 2003. Eignung des Chroma-Boden-tests zur Bestimmung von Kompostqualität und Rottegrad [Suitability of the chroma soil tests to determine compost quality and degree of decomposition]. Dissertation for a doctor degree presented at the Department of Mathematic and Natural Sciences. Oldenburg: Carl-von-Ossietzky-University (in German).
INTERLAB [Internet]. c2011–2015; [cited 2015 Dec 9]. Available from: https://sites.google.com/site/sitointerlab/
IUPAC. 1997. Compendium of chemical terminology. 2nd ed. Oxford: Blackwell Scientific.
Khemani D, Joseph MM, Variganti S. 2008. Case based interpretation of soil chromatograms. In: Althoff HD, Bergmann R, editors. Advances in case based reasoning. Proceedings of the 9th European conference in case based reasoning; 2008 Sep 1–4; Trier, Germany; p. 587–599.
Knorr D. 1981. Feasibility of circular chromatographic method for protein determination. Nutr Health. 1:14–19.
Knorr D. 1982. Use of a circular chromatographic method for the distinction of collard plants grown under different fertilizing conditions. Biol Agric Hortic. 1:29–38.
Kokornaczyk MO, Dinelli G, Marotti I, Nani D, Benedettelli S, Betti L. 2011. Self-organized crystallization patterns from evaporating droplets of common wheat grain leakages as a potential tool for quality analysis. Sci World J. 11:1712–1725.
Kokornaczyk MO, Dinelli G, Betti L. 2012. Approximate bilateral symmetry in evaporation-induced polycrystalline structures from droplets of wheat grain leakages and fluctuating assymetry as quality indicator. Naturwissenschaften [The Science of Nature]. 100:111–115.
Kokornaczyk MO, Parpinello GP, Versari A, Rombolà AD, Betti L. 2014. Qualitative discrimination between organic and biodynamic Sangiovese red wines for authenticity. Anal Methods. 6:7484–7488.
Kokornaczyk MO, Trebbi G, Dinelli G, Marotti I, Bregola V, Nani D, Borghini F, Betti L. 2014. Droplet evaporation method as a new potential approach for highlighting the effectiveness of ultra high dilutions. Complement Ther Med. 22:333–340.
Kolisko E, Kolisko L. 1978. Agriculture of tomorrow. 2nd edn. Bournemouth, UK: Kolisko Archive.
Lorke J. 2010. Untersuchung zum Zusammenhang von Bild und Verständnis der Fachmethode Chromatographie [Investigation on the relationship between image and understanding of the chromatography method] [dissertation]. Bochum: Ruhr University.
Mäder P, Hahn D, Dubois D, Gunst L, Alföldi T, Bergmann H, Oehme M, Amadò R, Schneider H, Graf U, et al. 2007. Wheat quality in organic and conventional farming: results of a 21 year field experiment. J Sci Food Agric. 87:1826–1835.
MIPAF. 1992. Gazzetta Ufficiale della Repubblica Italiana [Official Gazette of the Italian Republic]. Serie Generale n. 121:58–87.
MIPAF. 1999. Gazzetta Ufficiale della Repubblica Italiana [Official Gazette of the Italian Republic]. Serie Generale. 248:1–50.
Pfeiffer E. 1984. Chromatography applied to quality testing. Wyoming, USA: Bio-Dynamic Farming and Gardening Association:44 p.
Piva M-T, Lumbroso S, Sieso V, Monnin E, Mion E, Blanc F, de Bornier BM. 1994. Cupric chloride crystallization with human blood – study of pictures obtained in different pathologies. Elemente der Naturwissenschaft. 61:25–39.

Steffen W. 1983. The physico-chemical basis of capillary dynamolisis. Sci Forum 4:3–7; [cited 2016 Jun 21]. Available from: http://www.sciencegroup.org.uk/sf/scienceforum4.pdf
Unger KK, Skudas R, Schulte MM. 2008. Particle packed columns and monolithic columns in high-performance liquid chromatography – comparison and critical appraisal. J Chromatogr A. 1184:393–415.
Vavoulidou E, Coors A, Dozsa-Farksa K, Rombke J. 2009. Influence of farming practice, crop type and soil properties on the abundance of Enchytraeidae (Oligochaeta) in Greek agricultural soils. Soil Org. 81:197–212.
Zalecka A, Kahl J, Doesburg P, Pyskow B, Huber M, Skjerbaek K, Ploeger A. 2010. Standardization of the Steigbild method. Biol Agric Hortic. 27:41–57.