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Original Research

Earthworms Diversity (Oligochaeta: Lumbricidae) and Casting Chemical Composition in an Urban Park from Western Romania

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Abstract

This paper aimed to establish the species diversity of earthworms (Oligochaeta: Lumbricidae) and the chemical composition of earthworm surface castings within an urban park from west side of Romania (Timişoara City): Plevnei Park (45°44'58''N, 21°13'38''E). Eight lumbricid earthworm species have been identified: *Lumbricus terrestris, Aporrectodea rosea, Aporrectodea longa, Aporrectodea caliginosa, Allolobophora chlorotica, Dendrobaena veneta, Dendrobaena octaedra, Dendrodrilus rubidus*. Based on species incidence, several ecological indicators have been calculated: the constancy of earthworm species in the sampling areas, the Sørensen similarity of sampling points as species composition, and the Jaccard similarity (coenotic affinity) of the earthworm species within the sampled points. The surface castings have been analyzed for the following physical-chemical parameters: pH, total organic carbon (TOC), total nitrogen (N), plant available phosphorus (P), plant available potassium (K), and calcium water soluble (Ca). Because it is not yet clearly elucidated the casting behavior (surface or subsurface) of earthworms, and their feeding behavior, habitat behavior and casting behavior are not always clearly separated traits, and because until now it is insufficiently known how the environmental conditions affect the surface or subsurface casts production in earthworms, or how these conditions determine more than a single casting behavior for one species, there had been difficult to conclude which of the found species is responsible for the surface casting within the studied urban park. To find possible correlations existing between the physical-chemical parameters of the earthworm castings, Pearson, Spearman, and Kendall correlations ($p<0.05$) were calculated. The study revealed significant

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positive correlations between these chemical parameters of earthworm castings: pH-K, pH-TOC, and TOC-Ca.

Keywords: epigeic, endogeic, anecic, Sørensen similarity, Jaccard similarity, pH, phosphorus, potassium, total organic carbon, calcium

Introduction

The geographical distribution of earthworm diversity (Oligochaeta: Lumbricidae) has been generally little studied in Romania [1-5]. The already recorded data focused on several zones of the country, like Carpathians areas, Transylvania, and rarely other areas [4] and are insufficient, although this zoological group has been described for Romania until the present through 80 species and subspecies [5]. The urban diversity of earthworms in Romania is even fewer known. No data available was found until now by a current research.

Why is it important to know more about earthworm diversity in urban settlements? According to United Nations Department of Economic and Social Affairs (2018), the urban is the environment where today lives 55% of human population [6]. The same forecast says that until the year 2050, this percentage will increase towards 68%, which seems to be reachable if consider the rapidly grown of urban population of the world in only few decades, from 751 milion in 1950 to 4.2 bilion in 2018 [6]. For Europe, the scenario in even surprising: until 2020, about 80% of the population will live in cities, although only 4% of Europe's surface is occupied by urban settlements [7]. With such an anthropic pressure, the already affected urban biodiversity (179 species targeted by the Directive Habitats are linked to urban ecosystems [7]) and resources penury will face further issues.

To preserve the biodiversity in urban areas and to know more about it must become thus a priority in order to complete the information about the urban landscape.

In urban areas, the connection of human with the nature is through green spaces of the urban parks. The social ecology showed that human kind cannot be separated from its native natural environment [8-9] and therefore making their urban habitats more welcoming in terms of nature is an indicator of wellness [8, 10-11]. To assess the biodiversity of green urban spaces may be a difficult task because of methodological issues and because of anthropogenetic factors affecting it [10].

As a biodiversity component, earthworms accomplish important roles for soil ecosystems and global ecosystems, the widely known being the increasing of soil fertility, the improvement of soil physical-chemical properties, and the waste management (through vermicompostation process) [12-22]. These organisms are worldwide spread, being found, excepting few zones, in almost all parts of the planet. Earthworms (Annelida: Oligochaeta) deposit several tons per hectare per month of castings enriched in nutrients [18]. Although the benefits of earthworm castings have been often emphasized [15, 20, 22-29], there have been authors reclaiming also their negative impacts on fields covered by small vegetation (like sports fields) as reduced photosynthesis or weed invasion, when excessive amount [30], reduction of root biomass [18] or alteration of forest soils when are invading species in soils lacked by native earthworms [31]. This paper aimed to establish the species diversity of earthworms (Oligochaeta: Lumbricidae) together with several ecological features (constancy, Sørensen similarity, Jaccard coenotic affinity) and the chemical composition of earthworm castings within an urban park from Timişoara, a city from the western side of Romania.

Material and Methods

The researches took place in an urban park of Timişoara, the Plevnei Park. Timişoara (45°44'58''N, 21°13'38''E) is a city located in Romania (Europe), in the southeast of Pannonian Plain which extends for approximately 130 square km. The relief of the administrative territory of the city and of the periurban settlements belongs to Plain of Timişoara, the highest elevation is 95 m in the north-eastern side and the lowest point is 84 m in the western side of the city [32]. Timişoara has a moderate temperate continental climate, which is characteristic to the southeastern side of the Pannonian Plain, with sub-Mediterranean influences. During the year 2018, the annual average air temperature was 13.33ºC (the annual average temperature of air in 2018 in Romania was 11.57ºC [33-34]), and the annual amount of precipitations was 611.42 mm, closed to the means of the country (698.8 mm in 2018 [33-34]), but the regime of precipitations remains an irregular one, with years with deviations from the mean.

The diversity of earthworms (Oligochaeta: Lumbricidae) was studied in the Plevnei Park from Timişoara, Romania (45°44'52.7"N, 21°13'15.2"E). Known formerly as Gheorghe Doja Park (in Memoriam of the leader of the Peasant Revolt of 1514), this park has been established in 1903. Starting from 1919, its name changed into the nowadays name. This urban green space has been selected for study because of its location within an anthropogenically active urban area, being bordered at its four limits by frequently circulated car roads, especially during day time, and with a nearby (100 m) tramway at west side. The surface of the park is approximately by 5000 square meters.

To study earthworm species diversity and the chemical composition of the surface castings, across the park there have been identified, on the basis of casting presence in the spaces covered with vegetation, six sampling points.

Earthworm sampling has been made using the method of diluted formaldehyde [35] combined with hand sorting. The taxonomic affiliation of sampled earthworms was made on adult specimens according to Sims and Gerard (1999) [36] at species level, and the establishment of the ecological group (epigeic, endogeic, anecic) to which each species belongs was made according to Bouché (1977) [37-38]. The collection of earthworm castings was done at the surface of the soil on the basis of the occurrence of fresh worm castings. Several ecological indicators have been calculated both for the recorded earthworm species and for the six sampling points: the constancy of species in the sampling areas, the index Sørensen to determinate the ecological similarity of sampling points in terms of species composition, and the index Jaccard to determine the coenotic affinity of the earthworm species identified in the sampled points.

The indices Sørensen and Jaccard have been calculated based on species incidence in the sampled points. The constancy of species has been established by relating the number of sampled plots where the species occurred to the total number of plots [39], followed by the subsequent affiliation of each species to one of the five classes of constancy [39]: class I – 1 to 20% frequency; class II – 21 to 40% frequency; class III – 41 to 60% frequency; class IV – 61 to 80% frequency; class V – 81 to 100% frequency (the species affiliation to these classes is: classes I and II – rare species; class III – accessory species; classes IV and V – constant species).

The formulas used to calculate the indices Sørensen [40-41] and Jaccard [41-43] are given below:

Sørensen index: $2Sp_{12} / Sp_1 + Sp_2$, where Sp_{12} is the number of species shared between sampled plots 1 and 2, Sp_1 is the number of species found in the sampled plot 1, and Sp_2 is the number of species found in the sampled plot 2.

Jaccard index: $(S_{12} / S_1 + S_2 - S_{12}) \cdot 100$, where S_{12} is the number of sampled plots containing both species 1 and species 2, S_1 is the number of sampled plots containing species 1, and S_2 is the number of sampled plots containing species 2.

The castings analyses have been performed for the following physical-chemical parameters: pH, total organic carbon (TOC), total nitrogen (N), plant available phosphorus (P), plant available potassium (K), and calcium water soluble (Ca). The following methodologies have been used: the pH values have been established by the potentiometric method in aqueous suspension (pH_{H_2O}), ratio coprolites – solution 1:2.5, according to standard SR 7184-13:2001 PS-03, using a pH-meter Mettler Toledo Seven Easy AB54; the total organic carbon has been determined according to the Walkley procedure (1947) [44] by rapid dichromate oxidation; the total nitrogen content has been determined by the Kjeldahl method [45], digested with H_2SO_4 at 350ºC, catalytic agents: potassium sulphate and copper sulphate; plant-available phosphorus and potassium have been determined by the spectrophotometry (spectrophotometer UV-VIS Cintra 101) and flame spectrometry methods (spectrometer with atomic absorption VARIAN), respectively, in acetate-lactate ammonium solution at 3.7 pH, by the Egner-Riehm-Domingo method [46]; the content of Ca water soluble has been determined according to STAS 7184/7-87 PL-06, by complexometric titration in water extract - ratio 1:5.

The achieved data has been statistically processed (Pearson, Spearman, and Kendall correlations, $p<0.05$) using the software SPSS (Statistical Package for the Social Sciences) in order to find out possible correlations existing between the physico-chemical parameters of the castings composition.

Results and Discussion

Eight earthworm species (Sims and Gerard 1999) [36] have been found in the technosoil of Plevnei Park from Timişoara, Romania. As habitat ecology [37-38],

Table 1. Earthworm species found in the Plevnei Park from Timişoara, Romania.

No.	Earthworm species	Authority	Habitat ecology	Feeding ecology
1	*Lumbricus terrestris*	Linnaeus, 1758	Anecic	Detritivore, macrophage
2	*Aporrectodea rosea*	Savigny, 1826	Endogeic	Geophage, microphage
3	*Aporrectodea longa*	Ude, 1885	Anecic	Detritivore, macrophage
4	*Aporrectodea caliginosa*	Savigny, 1826	Endogeic	Geophage, microphage
5	*Allolobophora chlorotica*	Savigny, 1826	Endogeic	Geophage, microphage
6	*Dendrobaena veneta*	Rosa, 1886	Epigeic	Detritivore, mesophage
7	*Dendrobaena octaedra*	Savigny, 1826	Epigeic	Detritivore, mesophage
8	*Dendrodrilus rubidus*	Savigny, 1826	Epigeic	Detritivore, mesophage

Table 2. The diagram of Jaccard similarity (coenotic affinity) of earthworm species (%).

Earthworm species	*Lumbricus terrestris*	*Aporrectodea rosea*	*Aporrectodea longa*	*Allolobophora chlorotica*	*Aporrectodea caliginosa*	*Dendrobaena veneta*	*Dendrobaena octaedra*	*Dendrodrilus rubidus*
Lumbricus terrestris		100	100	100	50	33.34	16.67	16.67
Aporrectodea rosea			100	100	50	33.34	16.67	16.67
Aporrectodea longa				100	50	33.34	16.67	16.67
Allolobophora chlorotica					50	33.34	16.67	16.67
Aporrectodea caliginosa						25	33.34	33.34
Dendrobaena veneta							50	0
Dendrobaena octaedra								0
Dendrodrilus rubidus								

three of these species are epigeic, three are endogeic, and two are anecic (Table 1). The classification by feeding ecology [47-48] showed five detritivore species and three geophage species (Table 1).

Jaccard similarity of earthworm species was performed in order to establish the coenotic affinity of the species (Table 2). 100% coenotic affinity between six pairs of earthworm species was found: *L. terrestris – A. rosea, L. terrestris – A. longa, L. terrestris – A. chlorotica, A. rosea – A. longa, A. rosea – A. chlorotica, A. longa – A. chlorotica*. The lowest coenotic affinity found between the recorded earthworm species was 16.67% (Table 2), and nule coenotic affinity was found between two pairs of earthworm species: *Dendrobaena veneta – Dendrodrilus rubidus* and *Dendrobaena octaedra – Dendrodrilus rubidus* (Table 2).

The cluster dendrogram of Jaccard similarity (coenotic affinity) of the eight earthworm species (noted as 1 to 8) (Fig. 1) shows four species highly closed: 1 - *Lumbricus terrestris;* 2 - *Aporrectodea rosea;* 3 - *Aporrectodea longa;* 4 - *Allolobophora chlorotica*. Two groups of species are fairly closed (25%): (6,7) and (5,1,2,3,4), while the group of species 6,7 and 1,2,3,4,5 presents 50% coenotic affinity, separately. The species *Dendrodrilus rubidus* (8) is separated entirely from all the others (singleton, fairly close).

The Sørensen similarity of sampling points as species composition has been calculated (Table 3). All pairs of sampling points showed a high similarity as composition of earthworm species, ranging between 72% and 100% (Table 3).

The dendrogram of Sørensen similarity (Fig. 2) shows one samples group with 100% similarity (2,5)

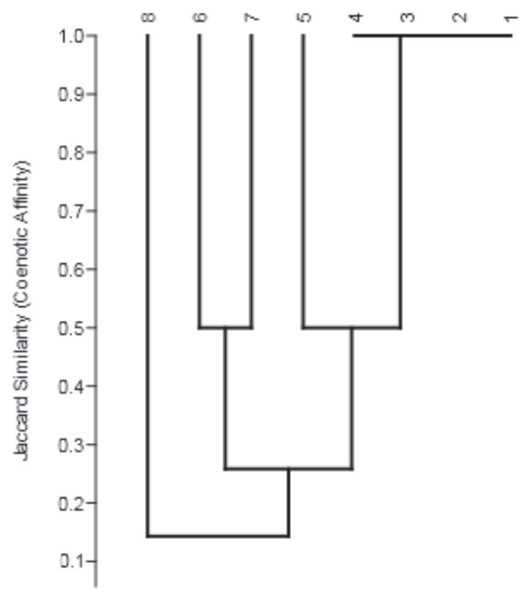

Fig. 1. The dendrogram of Jaccard similarity (coenotic affinity): the earthworm species from 1 to 8 are: 1 - *Lumbricus terrestris;* 2 - *Aporrectodea rosea;* 3 - *Aporrectodea longa;* 4 - *Allolobophora chlorotica;* 5 - *Aporrectodea caliginosa;* 6 - *Dendrobaena veneta;* 7 - *Dendrobaena octaedra;* 8 - *Dendrodrilus rubidus.*

Table 3. The Sørensen similarity (%) of sampling points: the values of Sørensen index ranges between 0 and 1, meaning 0 to 100% similarity.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Sample 1	100%	0.72	0.83	0.83	0.72	0.76
Sample 2	72%	100%	0.88	0.88	1.00	0.80
Sample 3	83%	88%	100%	0.80	0.88	0.90
Sample 4	83%	88%	80%	100%	1.00	0.72
Sample 5	72%	100%	88%	100%	100%	0.80
Sample 6	76%	80%	90%	72%	80%	100%

and a samples group (3,6) with 90% similarity. All hierarchical clusters present a high similarity – over 72%.

In analyzing the chemical composition of earthworm castings, there must be mentioned that not all earthworm species found in the study area cast at the surface. Since it is not yet clearly elucidated the casting behavior (surface or subsurface) of earthworms, and their feeding behavior, habitat behavior and casting behavior are not always clearly separated traits, and because until now it is insufficiently known how the environmental conditions affect the surface or subsurface casts production in earthworms, or how these conditions determine more than a single casting behavior for one species, there had been difficult to conclude which of the found species within the studied urban park is responsible for the surface casting. However, the size of the coprolites and their shape related to features of habitat ecology and feeding ecology of the sampled earthworm species could indicate three species possibly responsible for surface castings: *Lumbricus terrestris, Aporrectodea longa* and *Aporrectodea caliginosa.*

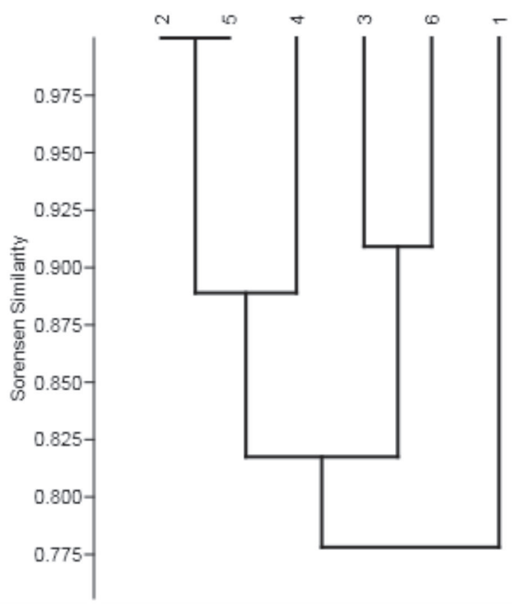

Fig. 2. The dendrogram of Sørensen similarity (%) of sampling points (ranging from 0 to 1, meaning 0 to 100% similarity).

Because of the high Sørensen similarity (72-100%) of sampling points as composition in earthworm species, there was considered that the selected six points were relevant and sufficient to analyze the chemical composition of earthworm castings. These have been evaluated for pH, for three main elements with major implication in soil fertility and plant nutrition (plant available N, P, K), and for calcium water soluble (Ca), considering the role of calciferous glands of earthworms [49] located in their esophagus (Table 4).

Several statistical processings (Pearson, Kendall and Spearman Correlations, $p<0.05$) have been made to establish whether there are correlations between the determined chemical parameters of earthworm castings. There have been found positive significant statistical correlations ($p<0.05$) between four chemical parameters: pH, the content of total organic carbon (TOC), the available potassium (K), and the content of Calcium soluble in water (Ca) (Table 5).

The production of surface castings depends on several factors like food type [50-51] or cropping intensity or several chemical elements [52]. Asawalam 2006 [52] found a strong linear association between the amount of TOC from earthworm castings and the quantity of produced castings. The chemical composition of earthworm castings depends as well on food type and quality [50], species [50-51, 53] and also on selective feeding behavior [54].

A recent study regarding the chemical composition on earthworm castings developed in a greenhouse pot experiment [53] for eight earthworm species, (including *L. terrestris, A. rosea, A. longa, A. caliginosa, A. chlorotica, D. veneta*) revealed pH values (ranging between 7.4 and 8.2).

A positive significant correlation has been found in this study between pH and K in earthworm castings and can be explained since the pH of soils influences the availability of K, which becomes more available in soils with pH between 6.5 to 8.0 [55]. Other authors [13, 17, 47] showed that the availability of K in earthworm casts is the result of the processes (physico-chemical, microbial) from earthworms gut.

Table 4. The chemical composition of surface earthworm castings.

Chemical parameter	N	Minimum value	Maximum value	Mean
pH (pH units)	6	7.27	7.57	7.46±0.11
Total Organic Carbon (%)	6	1.73	3.97	3.14±0.76
Total nitrogen (%)	6	0.17	0.22	0.19±1.87
Plant available phosphorus (ppm)	6	24.55	46.32	35.92±8.36
Plant available potassium (ppm)	6	237.00	465.00	317.00±82.19
Calcium water soluble (mg/100 g soil)	6	14.03	22.64	18.70±2.93

The earthworm castings play a major role in carbon turnover [56] and contribute to carbon cycling [20]. There was found within this study a positive correlation between pH and TOC in earthworm castings, that can be explained as related to several facts already known about earthworms: the calciferous glands of earthworms contain carbonic anhydrase that catalyzes the fixation of CO_2 as $CaCO_3$ reducing in the same time the decrease of pH, along with contribution of earthworm secretion of NH_4^+ ions which reduce the pool of H^+ ions responsible for acidity [17]. The secretion of the calciferous glands of the Lumbricidae is 95-97% calcium carbonate [49]. Its physiological role is insufficiently known [57], but is associated with digestion function [49]. The results of Bossuyt (2005) [14] clearly indicated that earthworms are directly involved in the protection of soil C, enouncing as well a possible long-term stabilization of soil C, due to earthworm feeding behavior to ingest the organic matter and mix it with inorganic material of soil, excreting then this mixture as castings. Additionally, Lee et al. (2008) [58] reported that earthworms produce sufficient $CaCO_3$ to have, through their casts, a measurable impact on the C cycling in soil, whose quantity is conditioned by factors like biological variations between individuals. These findings and others [59] may also explain the positive correlation found between TOC and Ca in the earthworm castings within this study. Positive correlations between the

Table 5. Correlations between several chemical parameters of earthworm castings (Pearson, Kendall and Spearman Correlations, $p<0.05$).

Parameters	Statistical correlations		
pH - K	Kendall's tau_b	Correlation Coefficient	0.600
		Sig. (1-tailed)	0.045*
		N	6
	Spearman's rho	Correlation Coefficient	0.771
		Sig. (1-tailed)	0.036*
		N	6
pH - TOC	Pearson Correlation	Correlation Coefficient	0.740
		Sig. (1-tailed)	0.046*
		N	6
TOC - Ca	Kendall's tau_b	Correlation Coefficient	0.600
		Sig. (1-tailed)	0.045*
		N	6
	Spearman's rho	Correlation Coefficient	0.771
		Sig. (1-tailed)	0.036*
		N	6
	Pearson Correlation	Correlation Coefficient	0.780
		Sig. (1-tailed)	0.034*
		N	6

*Correlation is significant at the 0.05 level (1-tailed) content of organic carbon and pH and Ca^{2+} in earthworm castings have been as well previously recorded [60]. The positive correlation found between TOC and Ca in the coprolites of lumbricid earthworms might contribute to understand issues like the formation of $CaCO_3$ granules and the origin of C from the $CaCO_3$ granules in this group of earthworms (all species of *Lumbricidae* family have calciferous glands [61, 62]), launched by several authors: Garcia-Montero et al. (2013) [62] have enounced a relation between TOC and formation of $CaCO_3$ granules and hypothesized that the mean C source of the $CaCO_3$ granules in lumbricid earthworms derives from the soil TOC and atmospheric CO_2. Other findings [63] showed in laboratory experiments that the origin of the C from $CaCO_3$ granules produced by the calciferous glands of *L. terrestris* is the dietary intake (litter) and only partially the atmospheric CO_2 and the ingested soil. Regardless the origin source of C, these authors stated that lumbricid earthworms really synthesize calcium carbonate and not just recycle the ingested material [61, 63].

Because relations of dependency between chemical elements constituting earthworm casts in a natural phase have been rarely studied, it is hard, without subsequent researches, to explain the correlations pH-TOC and TOC-Ca found in this study, and particularly if these correlations respect findings widely acknowledged in the case of soils, like the significant influences of soil pH on TOC contents as regulator, among others, of the nutrient bioavailability and organic matter turnover, cation exchange capacity, biological processes, or newly discovered (for example, the high concentration of SiO_2 in agricultural soils of North-Central Europe is highly correlated to co-existence of low TOC and low pH values in these soils [64])

The nutrient availability in earthworm castings is in great measure the result of biochemical processes from the earthworm gut [22] and depends on more factors. Ganeshamurthy (1998) [54] found that the increased amounts of organic C and total N from earthworm castings may be the result of selective feeding behavior and repeated feeding on the castings, while the increased amounts of extractable P in casts could be a result of the increased enzymatic activity. The contribution of earthworms on phosphorus cycle is essential, because in soil P is mainly bound by the solid phase of it [65] and not always its release in soil solution is satisfactory for plants requirements. Considering the decreasing reserves of P in soils, becomes important to valuate this nutrient through earthworm casting activity. Although the implications of earthworms in the availability of not only the P but also the N and K in the soil remain unsolved yet, certain factors have been however clearly indicated [66-70]: species, feeding ecology (castings are richer in nutrients in detritivore than in geophageous), biochemical processes during the gut, the location into the soil (near or far the drilosphere), vegetation cover, rhizosphere, soil factors, climate factors.

Conclusions

Within the Plevnei Park (45°44'58''N, 21°13'38''E) from Timişoara, Romania, eight lumbricid earthworm species have been identified: *L. terrestris, A. rosea, A. longa, A. caliginosa, A. chlorotica, D. veneta, D. octaedra, D. rubidus*. As habitat ecology, three of these species are epigeic, three are endogeic, and two are anecic. As feeding ecology, five of these species are detritivore and three species are geophage.

The Jaccard similarity (coenotic affinity) of earthworm species was 100% between six pairs of earthworm species: *L. terrestris – A. rosea, L. terrestris – A. longa, L. terrestris – A. chlorotica, A. rosea – A. longa, A. rosea – A. chlorotica, A. longa – A. chlorotica.* Nule Jaccard coenotic affinity was found between two pairs of earthworm species: *D. veneta – D. rubidus* and *D. octaedra – D. rubidus*. The cluster dendrogram of Jaccard coenotic affinity showed four species highly closed: *L. terrestris, A. rosea, A. longa* and *A. chlorotica.* One species – *D. rubidus* was found entirely separated from all the others.

The Sørensen similarity of sampling points as species composition showed for all pairs of sampling points a high similarity, ranging between 72% and 100%. The dendrogram of Sørensen similarity showed a high similarity – over 72% for all hierarchical clusters of sampling points.

It has been difficult to conclude which of the earthworm species found within the studied urban park was responsible for the surface casting, due to limited information regarding the how clearly separated traits are the casting behavior (surface or subsurface) of earthworms, their feeding and habitat behavior, or how the environmental conditions determine more than a single casting behavior for one species. The statistical processings (Pearson, Kendall and Spearman Correlations, $p<0.05$) showed positive significant statistical correlations ($p<0.05$) between four chemical parameters paired as following: pH-K, pH-TOC, and TOC-Ca.

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