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Chemical relationships in earthworm casts of two urban green spaces indicate the earthworm contribution to urban nutrient cycles

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Abstract: Due to the earthworms' implications in nutrient cycles through their burrowing and casting activity, earthworms are worth considering when urban biogeochemical cycles are analysed. Several chemical parameters and their relationships were analysed in earthworm casts of two urban parks, namely the pH, total organic carbon (TOC), total nitrogen (Nt), plant available phosphorus (P), plant available potassium (K), and calcium water soluble (Ca). It was statistically significantly found that the TOC, Nt, P and K are reciprocally determined in the earthworm casts: 74.4% of the pH variability is co-determined by the N, P, K, TOC, and Ca contents; 95.9% of the Nt variability is co-determined by the pH, P, K, TOC, and Ca contents; 95.4% of the P variability is co-determined by the pH, N, K, TOC, and Ca contents; 94.5% of the K variability is co-determined by the pH, N, P, TOC, and Ca contents; 86.6% of the TOC variability is co-determined by the pH, N, P, K, and Ca contents. This study revealed the complexity of the chemical relationships inside earthworm casts, their reciprocal dependencies, and highlighted the complexity of the earthworms' contribution to biogeochemical cycles in urban areas. Our findings propose earthworms as indicators of the integrative conservation management of urban ecosystems.

Keywords: biogeochemical; indicator; Oligochaeta: Lumbricidae; urban park; urban sustainability

Earthworms are key indicators of soil sustainability in urban ecosystems (Paoletti 1999; Phillips et al. 2019). However, in cities, they are little studied. Earthworms are sustainability indicators of ecosystems either directly, through their abundance, biomass or species composition, or indirectly through their casts, which are excretion products after digestion transit. Although it is already known that earthworms casts contain higher amounts of nutrients in readily available forms per their chemical composition for plants than the adjacent soil (Teng et al. 2012), and several studies have investigated the correlations occurring between the chemical factors of the casts and those of the surrounding soil (Abail et al. 2017), it still remains insufficiently studied the chemical relationships occuring inside the cast, between the

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chemical factors of the cast composition. To accomplish their habitat role for human beings, cities must accomplish their sustainability goals and provide all services that humanity requires. Biodiversity is a tool in achieving these goals. Urbanisation is projected to appear in biodiversity hotspot areas (Seto et al. 2012) and it is expected that urban areas will triple by 2030 according to several projections (Seto et al. 2012). Considering all the present biodiversity achievements, future cities need to be bio-diverse to be both sustainable and liveable in and it cannot fulfil these conditions as apart from all other systems of the ecosphere.

In Romania, urban soil is insufficiently and poorly studied, although other researches in other parts of the world have clearly shown the specific functions and services of urban soils for the urban ecosystem, as revegetation in urban green spaces or parks show benefits to human health with regards to urban design and environmental buildings (Mills et al. 2020), and also acts as a pollution buffer, provides water storage and regulation, protection against flooding (Pennino et al. 2016), carbon storage, micro climate modulation (Bonilla & Bedoya 2022), recreation and mental health (Li et al. 2018). Few studies have been conducted on earthworms of urban soils in Romania (Iordache and Gaica 2021), and even fewer have appeared on earthworm casts, which are the reasons and the motivation of this research, as a contribution to rethinking the approach of the concept of urban soil, through its biodiversity contribution, as an essential part of urban sustainability.

MATERIAL AND METHODS

The study was conducted in two urban green spaces of Timișoara (45°44'58''N, 21°13'38''E), Timiș County, Romania, located at a 2 km walking distance one from another, noted as Urban Park 1 – UP1 (45°44'58''N, 21°13'38''E) and Urban Park 2 – UP2 (45°45'18''N, 21°13'28''E). The ecopedological conditions of the researched area (Timiș County) are closely related to its great diversity of the relief forms and to its specific climate which emerged in 18 types of soil according to the Food and Agriculture Organization of the United Nations (FAO) System (Borza et al. 2007). However, the soil of the studied green parks is a technosol, due to the multiple anthropic interventions in their set up and subsequent management (Burghardt et al. 2015).

The casts were collected directly by hand and removed from the soil surface. The casts that were collected were selected based on their shape and size, so that only those that were collected have been established to belong to the anecic, endogeic or at most epi-endogeic earthworm species. The collection was conducted in the sample surfaces over a 2 m^2 plot. Six samples and eight samples of earthworm casts were collected from Urban Park 1 and Urban Park 2, respectively. The earthworm casts were aged afterwards in the laboratory for three weeks in conditions of natural lighting and an air temperature of 20 ± 2 °C before be chemically analysed.

The chemical parameters to be analysed were chosen due to their significant importance in soil quality and plant nutrition, and because several studies (Rawlins et al. 2008; Jia et al. 2012) recommend these parameters as indicators in assessing the evolution of natural ecosystems: pH, total organic carbon (TOC), total nitrogen (Nt), plant available phosphorus (P), plant available potassium (K), and calcium water soluble (Ca).

The chemical analyses of the casts were made on old casts using the following methods: pH – the potentiometric method in an aqueous suspension (pH_{H_2O}, a ratio of 1 : 2.5) according to the standard SR 7184-13: 2001 PS-03; TOC – the Walkley procedure through rapid dichromate oxidation (Walkley 1947); total N – the Kjeldahl method (Kjeldahl 1883); plant-available P – the Egner-Riehm-Domingo method (Egner et al. 1960) and spectrophotometry; plant-available K – the Egner-Riehm-Domingo method (Egner et al. 1960) and flame spectrometry; Ca water soluble – ethylenediaminetetraacetic acid (EDTA) complexometric titration in a water extract – a ratio of 1 : 5, according to STAS 7184/7-87 PL-06. The water soluble Ca in earthworm casts was chosen to be investigated because earthworms have oesophageal calciferous glands (Hopfensperger et al. 2011), and because, generally, in a majority of soils, Ca is strongly adsorbed on the soil exchange complex (not readily plant available) or with carbonates (plant unavailable) which necessitates a complex mechanisms to be displaced by plants, such as root proton pumps (https://www.fertilesoilsolutions.com/agricultural-news/soluble-vs-exchangeable-calcium/), while the soluble Ca is free to the plant and readily available. The statistical processing of the data was performed using IBM SPSS Statistics (Ver. 28.0.0.0).

RESULTS

The study aimed to test the hypothesis that regardless of the collecting location across the analysed

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city (Urban Park 1 or Urban Park 2), the samples of earthworm casts will show similar chemical relationships and correlations between the chemical parameters in order to assume the generality of our findings for the study area. A one-sample Kolmogorov-Smirnov test was performed to test if the samples were normally distributed in both sample locations (Table 1). The percentages of the variables (physical-chemical and chemical properties of the earthworm casts) of the two groups (UP1 and UP2) were both normal, indicating that the data (the values of the studied variables) were normally distributed in both groups with one exception: the values recorded for the parameter pH in Urban Park 2, which was not considered as violating the assumption due to the high variability in the pH values in the earthworms casts due to soil bioturbation (burrowing behaviour across the soil layers) associated factors: the removal and transportation of organic layers of the soil, the transportation of base cations from deeper layers in the upper part of the mineral soil rich (Ferlian et al. 2020), the calcium carbonate secretion as spherulites produced by the oesophageal calciferous glands (Hopfensperger et al. 2011), the species richness, and the richness of the ecological group (Ferlian et al. 2020). The soil pH is reported to be generally increased through the earthworms' activity as result of their burrowing. The means of the analysed parameters of the earthworm casts are presented in Table 1.

Linear regressions were run to find the dependency relationships of each chemical parameter of the earthworm casts with the rest of the parameters. It was found that the TOC is statistically significantly determined by the Nt: $F(1,12) = 33.857$, $P < 0.001$, $R^2 = 0.738$, by P: $F(1,12) = 21.078$, $P < 0.001$, $R^2 = 0.637$, and by K: $F(1,12) = 18.453$, $P < 0.001$, $R^2 = 0.606$. However, it was also found that the TOC content of the earthworm casts determines the Nt: $F(1,12) = 33.857$, $P < 0.001$, $R^2 = 0.738$, the P: $F(1,12) = 21.078$, $P < 0.001$, $R^2 = 0.637$, and the K: $F(1,12) = 18.453$, $P = 0.001$, $R^2 = 0.606$. The linear regression did not show that the pH determines the TOC or that the TOC determines the pH in this study. The linear regression coefficients, which support these relations between the analysed chemical parameters of the earthworm casts, are listed in Table 2: the β coefficients and the *P*-values confirm the identified relationships.

A multiple regression analysis was run to assess the relationships between each studied chemical

Table 1. Test of normality distribution (one-sample Kolmogorov-Smirnov test) of the studied variables in two urban parks from Romania (physical-chemical and chemical properties of earthworm casts)

		Urban Park 1						Urban Park 2					
		pH	TOC	Nt	P	K	Ca	pH	TOC	Nt	P	K	Ca
			(%)		(ppm)		(%)		(%)		(ppm)		(%)
Normal parameters[a,b]	mean	7.4650	3.1400	0.1950	35.9267	317.0000	18.7033	7.5262	4.8638	0.2963	149.5100	1 161.8750	17.8113
	SD	0.11811	0.76032	0.01871	8.36511	82.19732	2.93721	0.35404	1.74749	0.06140	73.80466	430.73473	4.34738
Test statistic		0.217	0.265	0.122	0.218	0.225	0.141	0.312	0.189	0.209	0.161	0.205	0.182
Asymptotic significance (2-tailed, significance level 0.050)		0.200	0.200	0.200	0.200	0.200	0.200	0.021	0.200	0.200	0.200	0.200	0.200
Interpretation	Z^*	0.217	0.265	0.122	0.218	0.225	0.141	0.312	0.189	0.209	0.161	0.205	0.182
	P	> 0.05[a]	> 0.05[a]	> 0.05[a]	> 0.05[a]	> 0.05[a]	> 0.05[a]	< 0.05	> 0.05[a]	> 0.05[a]	> 0.05[a]	> 0.05[a]	> 0.05[a]

TOC – total organic carbon; Nt – total nitrogen; P – plant available phosphorus; K – plant available potassium; Ca – calcium water soluble; *Z*, *P* – statistics of Kolmogorov-Smirnov test which indicates the significance level of distribution; SD – standard deviation; [a]normal distribution; [b]calculated from data; *$Z(6)$ for Urban Park 1 and $Z(8)$ for Urban Park 2

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Table 2. Relationships between the chemical parameters of the earthworm casts (linear regression)

Dependent factor	Determinant factors	B coefficient	Standard error of B coefficient	β coefficient	*t*-value	*P*-value
	Nt	20.049	3.446	0.859	5.819	< 0.001
TOC	P	0.016	0.004	0.798	4.591	< 0.001
	K	0.002	0.001	0.778	4.296	0.001
Nt		0.037	0.006	0.859	5.819	< 0.001
P	TOC	39.112	8.519	0.798	4.591	< 0.001
K		257.832	60.022	0.778	4.296	0.001

TOC – total organic carbon; Nt – total nitrogen; P – plant available phosphorus; K – plant available potassium; B coefficient, standard error of B coefficient, β coefficient, *t*-value and *P*-value – statistics of linear regression test

parameter (as a dependent variable) to the rest of the parameters (as the predictor variables) and to predict the models of co-influence of multiple parameters upon a certain parameter in the earthworm casts. It was statistically significantly found (analysis of variance (ANOVA), $P < 0.05$, 0.01) that 74.4% of the pH variability is co-determined by the N, P, K, TOC, and Ca contents; 95.9% of the Nt variability is co-determined by the pH, P, K, TOC, and Ca contents; 95.4% of the P variability is co-determined by the pH, N, K, TOC, and Ca contents; 94.5% of the K variability is co-determined by the pH, N, P, TOC, and Ca contents; 86.6% of the TOC variability is determined by the pH, N, P, K, and Ca contents (Table 3). The Ca is not determined by the combined action of all the other chemical elements in the earthworm casts.

Pearson's, Kendall's, Spearman's correlations were all performed to identify the interrelations of the chemical elements of the earthworm cast composition (Table 4). Positive correlations between the pH and K, and respectively between the pH and TOC, and also between the chemical parameters with the main contribution to soil fertility: Nt, P and K (Table 4) were found to be statistically significant ($P < 0.05$, 0.01).

DISCUSSION

The chemical relationships found in the earthworm casts have been discussed within this study as being related to those encountered in the soil in order to explain them, because, based on the shape and size of the collected earthworm casts, these have been assigned to the anecic and endogeic earthworms or at most to the epi-endogeic species of earthworms, and thus the hypothesis of interpreting the results as being related to the chemical relationships appearing in vermicast during vermicomposting has been eliminated when epigeic species are involved.

The findings of the study showed that there are four chemical elements in the earthworm casts, which establish reciprocal positive correlations: N, P, K, and TOC. Also, it was found that the TOC determines the N, P and K in the earthworm casts, while the reciprocal is valid too. These results indicate the close interdependency of these four main chemical elements and nutrients in realising the biogeochemical cycles of the soil.

A close positive correlation between N and the organic C in soil fractions has been already found in other studies (Li et al. 2014; Sheehy et al. 2019).

Table 3. Relationships between the chemical parameters of the earthworm casts (multiple regression, ANOVA)

Dependent factor	Co-determinant factors	R^2	ANOVA	
			F(5,8)	P
pH	N, P, K, TOC, Ca	0.744	4.657	< 0.05
Nt	pH, P, K, TOC, Ca	0.959	37.133	< 0.01
P	pH, N, K, TOC, Ca	0.954	32.984	< 0.01
K	pH, N, P, TOC, Ca	0.945	27.503	< 0.01
TOC	pH, N, P, K, Ca	0.866	10.296	< 0.01

Nt – total nitrogen; P – plant available phosphorus; K – plant available potassium; TOC – total organic carbon; R^2, F – statistics of multiple regression, ANOVA test

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Also, this positive correlation was confirmed by previous studies in an earthworm-mediated C and N cycle and was attributed to the soil microorganisms which need a good balance of carbon and nitrogen to remain active in the mineralisation process (Barrington et al. 1997). The positive correlations found within this study in the earthworm casts between the TOC and N, as well as their reciprocal determination were explained in other studies through the interactions occurring between C and N as a result of the microbiological activity expressed as the enzymatic activity was enhanced through earthworm locomotion and casting as the ending process of earthworm feeding (Lu et al. 2021). Another factor explaining the positive TOC-N correlation in the earthworm casts is the ability of the earthworms to stabilise the soil organic carbon and to redistribute the soil aggregates alongside the soil profile through their casts (Arai et al. 2018), which engages a redistribution in the soil organic carbon and N (Sheehy et al. 2019), which is very important since it has been demonstrated that C and N mineralisation are strongly dependant and positively correlated in soils (Li et al.2014).

In this study, it was found, in the earthworm casts, that the P is determined by the TOC and vice versa, and co-determined by all the other studied factors (Table 3). An ecological group of earthworms has been demonstrated to determine the earthworm contribution to P cycling (Bohlen et al. 2004). The epigeic, endogeic and geophagus species of earthworms differently rely on the TOC content from soil in their feeding, which is present in various amounts in soils. Several studies (Spohn 2020) have shown that different types of P fractions in the soil (organic and inorganic) play different roles in the stabilisation of organic matter, which influences the organic carbon content of the soil. It is possible that earthworms mediate this type of relationship because earthworms are able to shift between inorganic and organic forms of P after soil ingestion and contribute to the P turnover in the soil (Nahidan & Ghasemzadeh 2022). Another explanation of the TOC-P relationship is given by the earthworm cast capacity to form water stable aggregates, which is a property depending on the ecological group of earthworms and is associated to the P exchangeability (Suarez et al. 2004). The positive correlation between the TOC-K and the

Table 4. Pearson's, Kendall's, Spearman's correlations between the chemical parameters of the earthworm casts

Correlations		pH	TOC	Nt	P	K	Ca
			(%)		(ppm)		(%)
pH	Pearson	1	0.439	0.202	0.291	0.452	0.407
	Kendall	1.000	0.376	0.294	0.354	0.552**	0.078
	Spearman	1.000	0.535*	0.415	0.493	0.724**	0.137
TOC	Pearson	0.439	1	0.859**	0.798**	0.778**	–0.304
	Kendall	0.376	1.000	0.753**	0.538**	0.604**	–0.199
	Spearman	0.535*	1.000	0.906**	0.723**	0.776**	–0.262
Nt	Pearson	0.202	0.859**	1	0.947**	0.901**	–0.413
	Kendall	0.294	0.753**	1.000	0.708**	0.708**	–0.203
	Spearman	0.415	0.906**	1.000	0.857**	0.824**	–0.319
P	Pearson	0.291	0.798**	0.947**	1	0.945**	–0.392
	Kendall	0.354	0.538**	0.708**	1.000	0.670**	–0.133
	Spearman	0.493	0.723**	0.857**	1.000	0.868**	–0.282
K	Pearson	0.452	0.778**	0.901**	0.945**	1	–0.199
	Kendall	0.552**	0.604**	0.708**	0.670**	1.000	–0.022
	Spearman	0.724**	0.776**	0.824**	0.868**	1.000	–0.020
Ca	Pearson	0.407	–0.304	–0.413	–0.392	–0.199	1
	Kendall tau_b	0.078	–0.199	–0.203	–0.133	–0.022	1.000
	Spearman	0.137	–0.262	–0.319	–0.282	–0.020	1.000

TOC – total organic carbon; Nt – total nitrogen; P – plant available phosphorus; K – plant available potassium; Ca – water soluble calcium; **,* $P < 0.01, 0.05$

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interdependency relationship between these two factors found within this study in the earthworm casts are attributed to two factors previously mentioned by other studies: the soil properties (Linquist et al. 2022) and the microbiological transformations from the earthworm gut which make, from their casts, excellent instruments of shifting between different types of K pools in the soil (Wang et al. 2021).

The other positive correlations and reciprocal dependency relationships found within this study between the N, P and K factors of the earthworm casts show the complexity of nutrient cycles in earthworm casts which emphasises the earthworm contribution to soil biogeochemistry. For example, it was found that earthworm casts influence the P solubility which increases the nitrification (Kawakami & Makoto 2017). The relationships between the earthworm digestion processes and the C, N and P cycling were also found by Bi et al. (2021).

No significant correlations have been found between the Ca and the other chemical parameters of casts in this study, but other studies indicated the implications of Ca in the earthworms' contribution to the biogeochemical cycles which motivated the inclusion of this element in the study. An investigation of Thomas et al. (2020), regarding the role of calcite granules in earthworm casts, showed that the granules are structures involved in the C storage through the following mechanisms: soil – organic matter aggregation in casts, hydrophobicity of casts, organo-mineral complexes of casts.

In this study, the pH established reciprocal positive correlations with K and TOC only (Table 4) and its interrelations with the other chemical elements in the earthworm casts were highlighted only in the co-dependence models of interaction (Table 3). These positive correlations in earthworm casts could be explained through the increased availability of K and TOC in the earthworm casts as compared to the adjacent soil due to the pH modulation determined by the microbiological processes initiated during the gut transition (Basker et al. 1994). No correlation between the pH and N was found in earthworm casts, although several studies indicated that earthworms contribute to the N cycle in the pH related mechanisms, such as nitrate formation and leachability, both mediated by the nitrifying bacteria, for which earthworms also create appropriate aerobiosis conditions through their feeding, locomotion and casting activity (Ferlian et al. 2020). A correlation between the pH and Ca was expected to be found in earthworm casts due to $CaCO_3$ production of oesophageal glands of lumbricid earthworms, known as pH modulator, but this was not identified in this study.

The findings of this study highlighted the complex relationships of the nutrients in earthworm casts which recommend them as analysis tool of urban sustainability because the earthworms' casting activity is the main expression their activity.

CONCLUSION

This study revealed the complexity of the chemical relationships occurring inside earthworm casts collected from two urban green spaces and showed the reciprocal dependencies between the chemical elements of earthworm casts and, thus, highlighted the complexity of the biogeochemical cycles and the earthworms' contribution to these cycles in urban areas towards a better understanding of the urban sustainability through their soil biodiversity contribution. This study contributes to better understanding the ecological role of earthworms in urban green spaces and in urban ecosystems, which can provide insights into understanding urban sustainability as given by every piece of the whole urban ecosystem, thus understanding urban sustainability through its defining components as a part of the whole ecosphere. Our findings indicate that earthworm casts should be included as an indicator of urban sustainability in the integrative assessment of urban ecosystems.

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