

# *Cladonia* lichens as the most effective and essential pioneers in strongly contaminated slag dumps

## Research Article

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**Abstract:** The epigeic lichen biota of ten post-smelting slag dumps were studied to identify the ecological importance of *Cladonia* species in spontaneous succession within artificial areas. Species diversity, abundance, constancy and cover indices were determined at the initial stages of vegetation establishment. Substrates under lichen vegetation were characterised in terms of the pH, organic C and total N content, C/N ratio and Zn, Pb, Cd and As concentration. Species occurrence and abundance indices were related to habitat type, enabling the ability to identify species-specific responses to environmental factors. Despite the unfavourable and artificial habitat conditions at dump sites, some *Cladonia* appear abundant and form swards, with *Cladonia rei* as a dominant species. They appear to be well-adapted for slag substrate and their special arrangement illustrative of site contamination. Certain sward-forming *Cladonia* lichens play a fundamental role as effective pioneers in the initial stage of succession and provide stable plant cover development.

**Keywords:** Slag waste • Metal contamination • Lichen succession • Metal resistance • Industrial area

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## 1. Introduction

Mining and heavy industry have substantially transformed the landscape and contaminated the environment. Large quantities of wastes have been deposited in post-industrial areas as dumps, and the amount of land base taken up by these artificial deposits is extensive. The dumps associated with the extraction of zinc and lead ores have different physical and chemical properties, which are the result of different processing methods. They can be generally divided into three types [1,2]: (1) post-mining dumps - a mixture of dolomites, limestone, clays, sands and gravels; (2) post-flotation dumps - a flotation sludge mixed with finely ground dolomite and (3) post-smelting dumps - slag from blast and muffle furnaces consisting of crumbs of dolomite, sinter slag, coke residue, Pb-rich and Zn-rich wastes. The latter dumps constitute completely artificial environment ecosystems characterised by extremely

high concentrations of toxic elements [3] and the most unfavourable habitat conditions [4].

Due to the necessity of reducing the negative impacts on the local environment and indigenous populations and the need for reclamation interventions, post-industrial areas have become interesting opportunities for biological and ecological investigations (e.g. [5-7]). Some lichens are known as effective and rapid colonisers of bare ground and their pioneer nature is not only associated with a primary succession of natural sites, but also concerns anthropogenic and artificial habitats (e.g. [8,9]). Anthropogenic disturbances frequently contribute to the formation of lichen dominated communities (e.g. [10]), and many species are important colonisers of degraded soil [11]. Since different lichen taxa display different levels of sensitivity to heavy metals, the presence of particular elements in the environment may produce changes at the community level [12]. Lichens from various genera, including *Cladonia*, are known to

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tolerate heavy metal contamination and grow in both polluted and unpolluted areas (see [13,14]). There are also examples of lichens that appear to be limited to heavy metal-rich substrates, and thus, these lichens are often used as good indicators of the presence of certain metal elements in the substrate (see [14]). Moreover, several new species have been recognised and described from areas enriched with metals, such as Cd, Cu and Zn [15-17].

The data on the lichen biota existing in extremely contaminated sites and especially the knowledge about the response of particular species to highly unfavourable habitat conditions are very limited [e.g. 18-23]. Despite adverse factors, there are lichens that effectively colonise such areas, and interestingly, some of them are abundant and form compact swards. This study focuses on epigeic lichens, primarily those of *Cladonia* species, as they constitute the largest proportion of biomass in the early stages of succession at man-made dump sites. Specifically, the research (1) determines the diversity of lichen species and their abundance and (2) estimates the relationships between the abundances of particular species and specific habitat factors.

## 2. Experimental Procedures

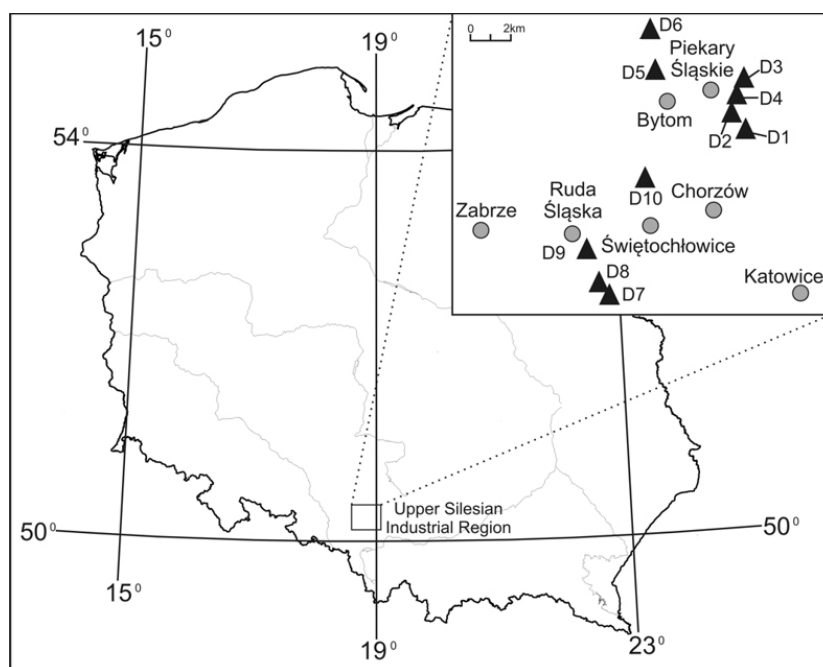
### 2.1 Studied post-smelting dumps

The study concerns areas directly associated with the processing of lead and zinc ores in the central

part of the Upper Silesian Industrial Region, S Poland (Figure 1). The primitive metal smelting technology of the past has resulted in large quantities of wastes, which were deposited in post-industrial areas as dumps. The locations and size of the dumps are provided in Table 1. The matter of dumps primarily constituted post-smelting slag, which has weathered over time into a form of friable substrate or partially moulding sinters. Despite the fact that generally the main deposition of slag wastes was finished, the dumps are still partially exploited and notoriously affected by human activities (e.g. extraction of material for roads construction, crushing by heavy vehicles, trampling, searching for scrap-iron by local residents). The extent of the area, an extreme degree of contamination and difficulties in reclamation cause the dumps a large and still unsolved environmental problem in the Silesian region [24].

### 2.2 Field studies and sampling

The dumps were studied in 2010 and 2011 during a dry spring seasons. Lichens overgrowing friable slag substrate were examined and as the examples of typical apophytes (see [25,26]) they were treated as epigeic species. At each dump, three study plots of 100×100 cm representing the initial stage of succession were randomly selected to determine the abundance of lichen species. The following cover-abundance scale was used (modified after [27]): r: <5% cover and 1–5 individuals; 1: <5% cover and several individuals; 2a: cover 5–12.5%; 2b: cover 12.5–25%; 3: cover



**Figure 1.** Locations of study dumps in the Upper Silesian Industrial Region (▲D1–D10 – dumps, see Table 1; ● main towns in the study area).

Dump	Locality (town)	Coordinates (centre of the dump)	Approx. area (m <sup>2</sup> )
D1	Piekary Śląskie	50° 20' 34"N, 18° 58' 28"E	180000
D2	Piekary Śląskie	50° 21' 11"N, 18° 58' 00"E	255000
D3	Piekary Śląskie	50° 22' 04"N, 18° 58' 07"E	62500
D4	Piekary Śląskie	50° 21' 53"N, 18° 58' 03"E	75000
D5	Bytom	50° 22' 09"N, 18° 54' 31"E	9581
D6	Radzionków	50° 22' 57"N, 18° 54' 15"E	110000
D7	Ruda Śląska	50° 15' 58"N, 18° 52' 11"E	105000
D8	Ruda Śląska	50° 16' 09"N, 18° 52' 02"E	50000
D9	Ruda Śląska	50° 16' 42"N, 18° 51' 51"E	39200
D10	Świętochłowice	50° 19' 05"N, 18° 54' 13"E	107600

**Table 1.** Locations and general size of the examined dumps.

25–50%; 4: cover 50–75% and 5: cover 75–100%. Phytosociological constancy was determined [28] and cover index was calculated for each species according to the formula: the sum of average percent coverage of the species in each plot/total number of plots × 100%. Besides epigeic lichen biota, the presence of epilithic lichens was noted or individuals were collected for detailed analysis. Total coverage of bryophytes and vascular plants was estimated. The top layers of substrate under lichen vegetation were collected to a depth of 5 cm and used in the chemical analyses.

The epigeic lichen biota occurring within grassland community (marked here as GSL) situated near dumps D5 and D6 was studied at three plots for comparative purposes and in order to check the general species diversity in the vicinity of the examined dumps. This community is characterized by a large proportion of the plant species from the class *Festuco-Brometea*. The distance between this site and D5, D6 was five kilometres. The samples of substrate from these plots were also used for chemical analyses.

The collected specimens were determined under stereomicroscope and/or light microscope according to appropriate key books (e.g. [29,30]). The lichen secondary substances were determined by means of thin layer chromatography (TLC), in solvent systems C and G, in accordance with the standardised method summarised by Orange *et al.* [31]. All specimens are housed in KRA-L herbarium (Institute of Botany, Jagiellonian University, Kraków).

### 2.3 Substrate analysis

The substrate samples were dried and passed through a 1 mm sieve. Acidity (pH) was determined by suspending the matter in KCl, C organic content using Tiurin's method modified by Oleksynowa [32] and total N using

Kjeldahl's method with a Kjeltac 8100 (Foss Tecator) apparatus. Subsequently, the C/N ratio was calculated. The total concentrations of Zn, Pb and Cd were determined after the digestion of 2 g dw in a solution of 65% HNO<sub>3</sub> (Merck, Suprapur) and 70% HClO<sub>4</sub> (Merck, Suprapur) (2:1) and were measured by atomic absorption spectrometry (AAS, Thermo Elemental Solaar). As content was made using the method of extraction of the matter in an HCl:HNO<sub>3</sub> (3:1) solution (*aqua regia*); afterwards, inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 7300DV) was used. Certified standard solutions (Merck-Titrisol, Merck-CertiPUR, Fluka-Trace CERT) were used to prepare the elemental calibration standards and quality assurance. Appropriate solutions without samples were used as reagent blanks. The analyses of elements were repeated at least three times, and the mean values were used as one observation. The values of relative standard deviation (RSD) for Zn, Pb, Cd and As were within the ranges 0.1–4.1%, 0.2–7.8%, 0.2–5.2% and 0.4–6.7%, respectively. These values showed that there was good homogeneity within a given sample.

### 2.4 Statistical analysis

A one-way analysis of variance (ANOVA) was used (after using the Shapiro-Wilk and Lilliefors tests to verify whether the data were normally distributed and Levene's test to assess the equality of variances) to compare differences in habitat parameters between particular dumps. Prior to the analysis all data were transformed into their logarithms. Next, the differences between groups were evaluated with Tukey's (HSD) test. Factor analysis, using PCA for factor extraction, was used to investigate relationships among examined habitat parameters. This technique enable reducing the number of variables and detect structure in the

relationships between them. Prior to analysis, the data were standardised to avoid the effect of different scales of measurement. Multivariate data analysis techniques, such as indirect and direct gradient analyses, were applied to estimate the relationships between the species abundance at particular dumps and the considered habitat parameters. To account for the differing percentages of total lichen coverage in each of the surveyed plots and to show the actual relationship between species abundance proportions and habitat conditions, numerical values were transformed into a percentage scale, assuming the sum of all species abundances to be 100%. A detrended correspondence analysis (DCA) was first conducted to determine whether the species matrix had a linear or a unimodal response. The length of the species abundance gradient was relatively short (*i.e.* 1.71), and as a consequence, the linear ordination method (RDA - redundancy analysis) was used [33]. Subsequently, a Monte Carlo permutation test was used to assess the statistical significance of the relationships between species, particular environmental factors and axes. Before the analyses, the Pearson correlation coefficients were calculated in order to reveal the correlation matrix among the examined habitat characteristics and to check if any strong correlation ( $r > 0.90$ ) exists between variables that could potentially affect the results of multivariate analyses. Redundancy analysis was conducted on the basis of the species abundance and habitat factor results obtained from three plots per dump. Statistical calculations were performed using the STATISTICA (version 9.1; Stat-Soft Inc., <http://www.statsoft.com>) and CANOCO software [33].

### 3. Results

#### 3.1 Substrate chemical properties of the dumps

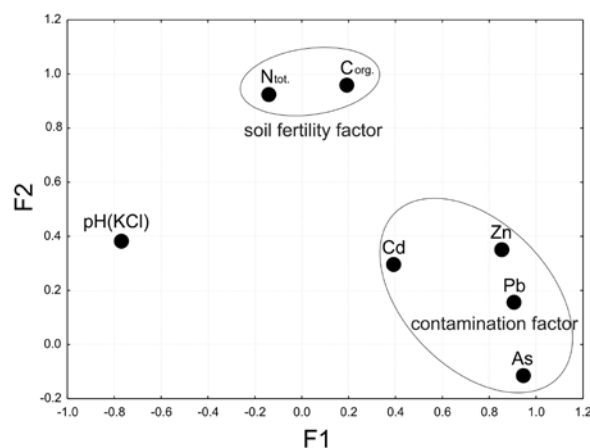
Each dump can be characterized by highly unfavorable habitat conditions and extreme heavy metal contamination. Selected chemical characteristics of the substrates obtained from the plots are presented in Table 2. One-way ANOVA ( $P < 0.05$ ) revealed significant differences in all characteristics of the examined dumps (for F-values see Table 2). The substrates proved to be slightly acidic to alkaline and were characterized by low organic carbon and total nitrogen contents. The analyzed metal elements were recorded as a rule at much elevated concentrations even compared to the acceptable levels for post-industrial wastes (see Table 2). Figure 2 shows a plot of factor loadings of the first two factors of selected chemical properties. Three factors explained 95.0% of the total variation in the chemistry of the substrates that had developed on post-smelting dumps (Table 3). In

Factor 1, the most important variables were Zn, Pb and As; they comprised a positively correlated group, which represent heavy metal elements associated directly with lead and zinc smelting activities. Factor 2 includes C and N, which represent soil organic matter, indirectly related to soil fertility. Whereas, Factor 3 consists only of Cd.

#### 3.2 Epigeic lichen biota

The list of epigeic lichens with their abundances, phytosociological constancies and cover indices are provided in Table 4. The most abundant was *C. rei*, which was frequently up to 75% coverage in the plots. Moreover, its constancy and cover index are the highest. The second most common species is *C. cariosa*, which accompanies and often coincides with *C. rei*. The other species are less frequent and have considerably lower cover indices. Nevertheless, they are usually represented by several individuals on each dump. The exception is *C. cryptochlorophaea*, which was recorded only on the dumps strongly contaminated with Zn. Apart from *Cladonia*, representatives of two other lichen genera were present on friable slag substrate, *i.e.* *Baeomyces rufus* and *Diploschistes muscorum*. Both species have less than five per cent coverage in the plots. The latter species was sometimes observed as abundant but only as a parasite of *C. rei*.

Habitat factors in the vicinity of the dumps are more favourable for vegetation (Table 2). Consequently, the diversity of epigeic lichen biota at the plots of neighbouring grassland community is apparently higher than at the examined dumps. Twenty species representing ten genera were recorded: *Baeomyces rufus* (Huds.) Rebert., *Bacidia bagliettoana* (A. Massal. & De Not.) Jatta, *Cladonia cariosa* (Ach.) Spreng., *C. cervicornis* subsp. *verticillata* (Hoffm.) Ahti, *C. chlorophaea* (Flörke ex Sommerf.) Spreng., *C. fimbriata*



**Figure 2.** Scatterplot of the factor loadings (F1, F2) of substrate chemical properties for examined post-smelting dumps.

Dump	pH (KCl)	C <sub>org</sub> (%)	N <sub>tot</sub> (%)	C/N	Zn (mg g <sup>-1</sup> )	Pb (mg g <sup>-1</sup> )	Cd (mg g <sup>-1</sup> )	As (mg g <sup>-1</sup> )
D1	7.45±0.30 D: 2, 3, 4	2.71±1.47 -	0.15±0.01 D: 5, 9	17.83±9.01 D: 2, 4, 7	22356±1776 D: 3, 4, 5, 6, 7, 10	3029±1314 -	35±21 D: 5, 8	243±198 -
D2	7.14±0.04 D: 1, 3, 5	1.47±0.21 D: 6, 7	0.08±0.02 D: 6, 8	20.16±3.75 D: 1, 4, 5, 6, 7	1446±1114 -	528±283 -	5±3 -	61±46 -
D3	7.46±0.06 D: 1, 2, 4	6.29±0.65 D: 4, 5, 9, 10	0.21±0.02 D: 4, 5	29.92±0.96 D: 4, 5, 6, 10	40082±16820 D: 1, 4, 5, 6, 7, 8, 9, 10	14797±2562 D: 4, 5, 6, 7, 8, 9, 10	333±17 D: 4, 9	807±684 D: 4
D4	7.86±0.02 D: 1, 3	5.84±0.23 D: 3, 5, 8, 9	0.27±0.01 D: 3, 10	21.75±1.27 D: 1, 2, 4, 5, 6, 7	31387±351 D: 1, 3, 5, 6, 7, 8, 9, 10	11789±154 D: 3, 5, 10	362±6 D: 3	782±82 D: 3, 5
D5	7.00±0.19 D: 2, 10	5.13±0.35 D: 3, 4, 8, 9	0.20±0.02 D: 1, 3, 9	26.25±0.67 D: 2, 3, 4, 6, 7, 10	28529±34 D: 1, 3, 4, 6, 7, 9, 10	11720±258 D: 3, 4, 10	56±1 D: 1, 6, 7, 8, 9, 10	2265±118 D: 4, 6, 8, 9, 10
D6	6.47±0.33 D: 7, 8, 9, 10	1.67±0.10 D: 2	0.07±0.01 D: 2, 7, 8	25.62±2.77 D: 2, 3, 4, 5, 7, 10	47849±6349 D: 1, 3, 4, 5, 7, 8, 9, 10	24211±947 D: 3, 7, 8, 9, 10	91±1 D: 5, 7, 9, 10	3836±513 D: 5, 6, 8, 9, 10
D7	6.18±0.03 D: 6, 8, 9	1.03±0.04 D: 2	0.05±0.01 D: 6	20.48±1.81 D: 1, 2, 4, 5, 6	28360±1686 D: 1, 3, 4, 5, 6, 9, 10	22433±3388 D: 3, 6, 8, 9, 10	104±27 D: 5, 6, 9, 10	12478±3306 D: 8
D8	6.44±0.06 D: 6, 7, 9, 10	3.99±0.34 D: 4, 5	0.08±0.00 D: 2, 6	51.17±2.35 D: 9	97831±2672 D: 3, 4, 6, 9, 10	20305±361 D: 3, 6, 7, 9, 10	22±1 D: 1, 5	4590±106 D: 5, 6, 7, 9, 10
D9	6.31±0.03 D: 6, 7, 8	7.42±0.34 D: 3, 4, 5, 10	0.16±0.00 D: 1, 5	46.96±2.54 D: 8, 10	74618±235 D: 3, 4, 5, 6, 7, 8, 10	21880±111 D: 3, 6, 7, 8, 10	114±2 D: 3, 5, 6, 7, 10	2465±35 D: 5, 6, 8, 10
D10	6.67±0.30 D: 5, 6, 8	9.18±1.10 D: 3, 9	0.28±0.03 D: 4	32.88±0.97 D: 3, 5, 6, 9	54581±17869 D: 1, 3, 4, 5, 6, 7, 8, 9	18714±6333 D: 3, 4, 5, 6, 7, 8, 9	82±5 D: 5, 6, 7, 9	3357±1508 D: 5, 6, 8, 9
F value	54.68	86.73	131.83	22.76	67.48	141.26	36.45	53.03
GSL	6.17±0.15	12.23±0.59	0.58±0.10	21.56±3.09	403.45±143.75	2188.52±240.05	1.4±0.60	6.00±1.08
		Max acceptable level for post-industrial areas in Poland <sup>1</sup>			1000	600	15	60
		Max concentrations for uncontaminated soils <sup>2</sup>			100	50	1	20

**Table 2.** Chemical properties of the substrate for particular dumps (n=3, mean values ±SD). The dumps listed after mean values indicate that the particular dump is not significantly different from them at the P<0.05 level according to the Tukey (HSD) test.

<sup>1</sup>Source: Regulation of the Minister of Environment (9 Sep 2002) [62]

<sup>2</sup>Source: [63]

(L.) Fr., *C. furcata* (Huds.) Schrad., *C. conista* A. Evans, *C. pocillum* (Ach.) O.J. Rich., *C. pyxidata* (L.) Hoffm., *C. rei* Schaer., *C. subulata* (L.) Weber ex F.H. Wigg., *C. symphyarpa* (Flörke) Fr., *Diploschistes muscorum* (Scop.) R. Sant., *Leptogium biatorinum* (Nyl.) Leight., *Mycobilimbia tetramera* (De Not.) Vitik., Ahti, Kuusinen, Lommi & T. Ulvinen ex Hafellner & Türk, *Peltigera didactyla* (With.) J.R. Laundon, *Sarcosagium campestre* (Fr.) Poetsch & Schied., *Stereocaulon incrustatum* Flörke, *Vezdea leprosa* (P. James) Vezda.

### 3.3 Epigeic lichen responses to habitat factors

The relationships between lichen species distribution and habitat conditions were determined by means of RDA (Figure 3). The eigenvalues of axes 1 and 2 were 0.322 and 0.152, respectively. This implies fairly strong gradient for the first axis and slightly weaker for the second one. The first two axes of RDA explained 57.4% of species variability (32.2% by axis 1, 25.2% by axis

Parameter	Factor 1	Factor 2	Factor 3
Explained variance (%)	51.7	34.7	8.5
C <sub>org</sub> (%)	0.190	<b>0.960</b>	0.104
N <sub>tot</sub> (%)	-0.146	<b>0.926</b>	0.278
pH (KCl)	<b>-0.770</b>	0.385	0.442
Zn (mg g <sup>-1</sup> )	<b>0.850</b>	0.352	0.237
Pb (mg g <sup>-1</sup> )	<b>0.905</b>	0.157	0.382
Cd (mg g <sup>-1</sup> )	0.384	0.300	<b>0.849</b>
As (mg g <sup>-1</sup> )	<b>0.943</b>	-0.113	0.183

**Table 3.** Factor loadings (varimax-rotated) of substrate chemical properties determined by factor analysis for the ten studied dumps.

Loadings greater than 0.5 are shown in bold.

2). Whereas, the cumulative percentage of variance in the species-environment relationships accounted by

Species	Post-smelting dumps										Constancy	Cover index
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10		
<i>Cladonia cariosa</i> (Ach.) Spreng. chemotype I <sup>1</sup>	2b	3	r	-	-	2a-2b	1	1	r	1	IV	733
<i>C. chlorophaea</i> (Flörke ex Sommerf.) Spreng.	r	r-1	r	r	1-2a	1	r	r	1	r-1	IV	175
<i>C. cryptochlorophaea</i> Asahina <sup>2</sup>	-	-	-	-	-	-	r	1	r	r	II	54
<i>C. fimbriata</i> (L.) Fr.	1	1	r	r	2a	r	r	-	r	r	IV	179
<i>C. conista</i> A. Evans <sup>3</sup>	1	r-1	r-1	r-1	-	2a	r	1	r	r	IV	200
<i>C. pyxidata</i> agg. (including <i>C. pyxidata</i> (L.) Hoffm. and <i>C. monomorpha</i> Aptroot, Sipman & van Herk <sup>4</sup> )	r	r	2b	2a-2b	r	r	1	r	1	r	IV	392
<i>C. rei</i> Schaer. chemotypes I, II <sup>5</sup>	4	4-5	4	4	2b	3-4	3-4	3-4	3-4	3-4	V	4792
<i>Baeomyces rufus</i> (Huds.) Rebert.	r	r	r	r	r	-	r	r	-	r	III	67
<i>Diploschistes muscorum</i> (Scop.) R. Sant. <sup>6</sup>	r	r	-	-	-	r	r	r	r	-	II	50

**Table 4.** List of epigeic lichens on particular dumps together with their abundances (averaged on the basis of the data obtained from the plots), phytosociological constancies and cover indices.

<sup>1</sup>after Osyczka and Skubala [64];

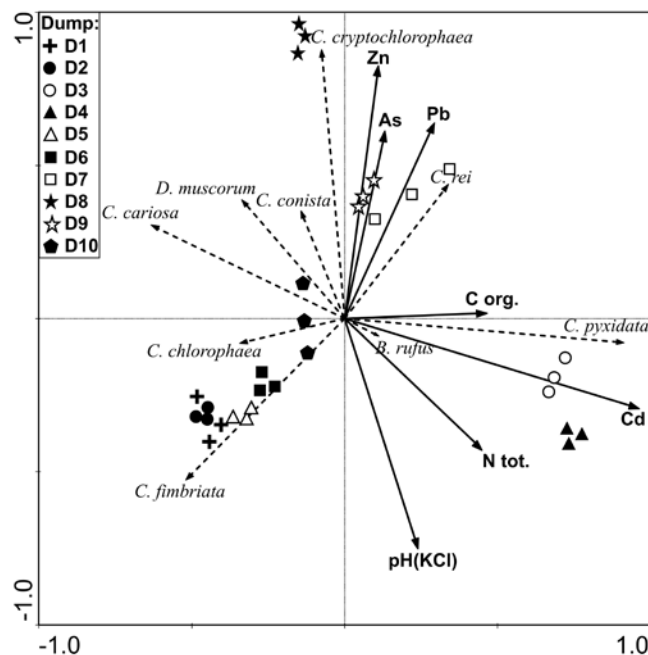
<sup>2</sup>taxon of uncertain status; frequently treated only as the chemical variant of *C. chlorophaea* (see [30]);

<sup>3</sup>previously often treated only as the chemical variant of *C. humilis* (see [29]), currently considered as the separate species [65]; only two samples with atranorin instead of bourgeanic acid were encountered on the dump D6;

<sup>4</sup>taxon of uncertain status [30,66]; both forms/taxa were found in tight association;

<sup>5</sup>after Dolnik et al. [40]; both chemical races occurred jointly at the study plots;

<sup>6</sup>abundances are related to thalli growing directly on substrates without *C. rei* host.



**Figure 3.** Redundancy analysis (RDA) ordination diagram (first two axes) for the lichen species abundance and primary substrate characteristics (pH, C<sub>org.</sub>, N<sub>tot.</sub>, Zn, Pb, Cd, As) of the studied plots located on post-smelting dumps (D1–D10). The cumulative percentage of variance explained by the first axis was 51.0% and the second explained 24.1% (D1–D10 – dumps; see Table 1).

the first two axes equalled 75.1% (51.0% and 24.1%, respectively, for the first and second axes). Species-environmental correlations in RDA for the first two axes were high ( $r_{ax1}=0.98$ ,  $r_{ax2}=0.88$ ). This indicates that the variables included in the analysis really captured much of the variation in the species data. The Monte Carlo permutation test showed that both the first axis and all canonical axes taken together are statistically important (F-ratio=10.43, P=0.001; F-ratio=5.37, P=0.001, respectively). RDA ordination distinguished the highly polluted and moderately polluted dumps (the upper side of the graph) from the less polluted ones (the lower side). According to the correlation of habitat factors with the axes, the variables determining the gradients in the RDA diagrams were, successively, Cd,  $N_{tot}$  and  $C_{org}$  for axis 1 and Zn, pH (KCl), Pb and As for axis 2. Moving from the left to the right side of the diagram, substrate fertility and Cd concentration increase. The second axis determines the gradient of increasing Pb, Zn and As contents and simultaneously decreasing pH values. The scatter of species and sites may be divided into several branches. In the upper right portion of the chart, the species resistant to high concentrations of heavy metals in the substrate are clustered. These unfavourable factors are characteristic of the most contaminated D7, D8 and D9 dumps. *Cladonia rei* and *C. cryptochlorophaea* could be included in this group. *Diploschistes muscorum*, *C. cariosa* and *C. conista* are associated with slightly less contaminated dumps, which are also characterized by adverse soil conditions, such as low nitrogen and organic carbon content and low pH. These five lichens appeared to be the earliest and most effective colonisers of the dumps. *Cladonia pyxidata* appears to be associated mainly with dumps D3 and D4, which are characterised by high concentrations of Cd and relatively high organic carbon contents. The occurrence of *C. fimbriata* and *C. chlorophaea* is primarily associated with less contaminated dumps with a lower content of heavy metals in the substrate. Finally, *Baeomyces rufus* appears to be directed somewhat towards a higher pH value, organic carbon and nitrogen contents.

### 3.4 Other vegetation

Several epilithic lichens, mainly crustose, were recorded within examined plots: *Candelariella aurella* (Hoffm.) Zahlbr., *C. vitellina* (Ehrh.) Müll. Arg., *Cladonia rei* (directly on sinter slag), *Lecanora dispersa* s.lat., *L. muralis* (Schreb.) Rabenh., *Lecidea lithophila* (Ach.) Ach., *Stereocaulon nanodes* Tuck., *S. cf. tomentosum* Th. Fr., *Xanthoria elegans* (Link) Th. Fr. Their occurrence was restricted to small conglomerates of sintered slag and industrial rubble. The total coverage of bryophytes and vascular plants was low and usually oscillated between five and ten percent.

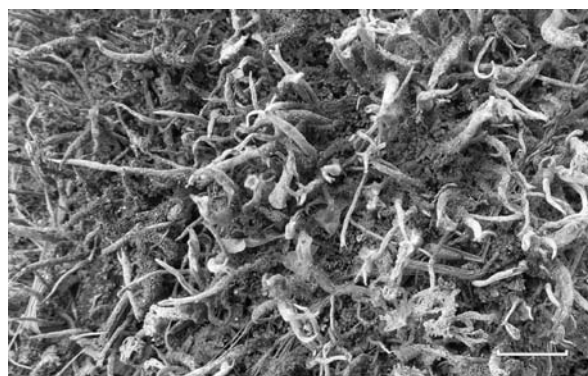
## 4. Discussion

### 4.1 Habitat conditions at the dumps

A slag dump is an example of an entirely artificial industrial habitat and constitutes an unfavourable environmental conditions for establishing vegetation. Dump sites are characterized by, high toxicity of the substrate, unstable and desiccated ground, immediate water flow, very high ground permeability, strong insolation and the dark colour of the substrate, which causes an intense accumulation of heat as well as daily thermal fluctuations [4]. Such disturbed habitat conditions have led to the emergence of ecosystems that are functioning under stress conditions, and new competitive systems between pioneer organisms have generated a specific species composition [34]. The total N content in the substrate samples was much lower than the ranges of average values given by Markert [35] for natural soils. The low total N content of the substrate and the simultaneous high C/N ratio (see Table 2) creates a very unfavourable habitat for vegetation. The high C/N ratio limits the availability of nitrate for plant growth, and the nitrogen that is available for plants is taken up by microorganisms and therefore temporarily immobilised [36]. The most alarming and rather startling results are the concentrations of the selected heavy metals (Table 2). As it can be seen, the concentrations of heavy metals are measured on a percentage scale rather than at a trace level.

### 4.2 Early epigeic colonisers

The lichens, primarily *Cladonia* sp. div., dominate and occur in large masses in the early stage of vegetation on the examined dumps. Undoubtedly, *Cladonia rei* (Figure 4) is the most abundant among all of the recorded lichens. Typically, it covers open areas and frequently forms large swards with *C. cariosa* and



**Figure 4.** Sward with *Cladonia rei* as a dominant species in the initial stage of vegetation of post-smelting dump (D4). Scale = 2 cm.

representatives of the *C. pyxidata-chlorophaea* group as accompanying species. The occurrence of some *Cladonia* species within metal-enriched areas was previously documented in other parts of the world (e.g. [13,18,37,38]); however, the abundance, ecological value and upper limit of tolerance for metal enrichment in the environment of these species have not been resolved thus far. Apart from *Cladonia*, individuals of only two other epigeic lichen genera were observed on the examined dumps (see Table 4). This fact underlines the great ecological importance of *Cladonia* as early hardy epigeic colonisers of friable slag substrate. Moreover, *Diploschistes muscorum* is fairly common in some areas, but its abundance is solely correlated with *C. rei* presence, on which it is frequently parasitic. Without the host, it was only sporadically encountered (Table 4). The sparse appearance of *B. rufus* is restricted to local cavings and cracks, where the moisture and fertility conditions are more favourable. The similar aggregates of epigeic species, mostly *Cladonia*, appearing at the dumps indicate that these species are well-adapted for slag substrate and their occurrence is not accidental. It concerns also the dumps D1 and D2 which are somewhat less contaminated compared to the others. The mass occurrence of *Cladonia* species in the initial stage of succession is additionally favoured by low competition from other organisms (see also [34]).

Generally, a majority of *Cladonia* individuals were observed in relatively good condition and, importantly, frequently in the fertile stage. However, some abnormal forms of growth were also found and can be regarded as specific ecotypes occurring in such disturbed sites. We observed a full range of known *C. rei* morphotypes [39,40] with a new distinct form: primary squamules conspicuous and densely packed, frequently forming compact swards, podetia robust, augmentative and decumbent, surface of podetia rough and partly densely covered by microsquamules and squamules. Atypical forms *C. fimbriata* were also frequently noticed. These forms can be characterised by elongated, almost subulate stalks of podetia ending with narrow and inconspicuous cups. Two morphological forms of *Cladonia pyxidata*, typical for the species and 'lush', were recorded on the dumps; the latter form has been sometimes treated as the separate species *C. monomorpha* [41,42]. Both forms/taxa were usually encountered in tight association; thus, we consider them jointly. Aside from morphological modifications, the colour change of thalli to a brownish or even dark brown tint was sometimes observed, especially in *C. rei* and *C. conista*. Such pigmentation is frequently explained

by the increased content of heavy metals in the thalli and can also occur in other epigeic lichens [43,44].

### 4.3 Heavy metal-resistant lichens

Lichens are usually considered a group of organisms that are sensitive to the presence of various pollutants in the environment, and therefore, they have been widely treated as good indicators of anthropogenic changes (e.g., [45-50]). However, there are some lichens known to have the unique ability to grow in heavily contaminated areas due to adaptive mechanisms allowing for a high tolerance to metals. Physiological processes enabling resistance to heavy metals have been observed in lichens with various ecological patterns, for example, in *Xanthoria parietina* (L.) Beltr., *Acarospora smaragdula* (Wahlenb.) A. Massal. and *Diploschistes muscorum* [51-53]. Many epigeic lichens, including *Cladonia* spp., have been reported in Zn/Pb/Cu enriched areas [38,54-58]; however, previously published records are not associated with sites as strongly affected as the examined dumps.

Our list of epigeic lichens is restricted mostly to *Cladonia* species. The relatively high diversity of *Cladonia* species combined with the deficiency of other lichen genera suggest the low sensitivity of some species of the genus to heavy metals in the environment. Although many epigeic lichen species were recorded in the vicinity of the dumps, only a few of them had colonised the examined objects and can be considered as effective pioneers. Species-specific differences in heavy metal susceptibility are reported here, which result in increasing abundances of certain species in strongly contaminated areas, whereas other species decrease their abundances along increasing pollution gradients. According to our results, the presence of *C. cryptochlorophaea* and *C. rei* is associated with the most contaminated dumps. Currently described specific restrained heavy metal accumulation pattern of *C. rei* reflected by a power function [23] may be one of the important attributes that facilitates its colonization of extremely contaminated dumps. *Cladonia cariosa*, *C. conista* and *D. muscorum* are primarily associated with less contaminated dumps and appear frequently at sites with low substrate fertility. *Cladonia pyxidata* grew more abundantly on dumps with a high Cd concentration. On the other hand, *C. chlorophaea* and *C. fimbriata* appear to be somewhat more sensitive to heavy metals compared to the others (Figure 3). Cuny *et al.* [14] also considered *C. conista/humilis*, *C. rei* and *D. muscorum* to be strongly linked to heavy metal contamination, whereas *C. chlorophaea* and *C. fimbriata* are considered to have intermediate stress tolerance.



#### 4.4 Role of *Cladonia* in spontaneous succession

Due to their pioneer nature, cryptogams, mainly lichens and bryophytes, are known as effective and rapid colonizers of bare ground not only natural sites, but also anthropogenic and artificial habitats [e.g. 8,18,20,23]. The epigeic lichens play a fundamental role in the natural soil formation process, the consolidation of the upper substrate layer and slope stabilisation. Their thalli accumulate water droplets and atmospheric vapour, which improves local moisture conditions. Lichens provide organic matter and fixed nitrogen, and therefore, they contribute to the fertility of the substrate [59]. The colonisation and natural regeneration of the considered dumps is extremely sluggish. The process of spontaneous succession begins from a sterilised habitat and progresses towards the formation of xerothermic grasslands resembling poorly developed *Festuco-Brometea* or *Koelerio glaucae-Corynepheretea canescentis* associations [4,60]. Taking into consideration fruticose growth form and relatively high biomass of *Cladonia*, this genus proves to be the most important for the effective natural colonisation of post-smelting dumps. Although several epilithic lichens were recorded in the study plots, their occurrence was limited to small pieces of sintered slag, and thus they make a rather negligible contribution. Consequently, *Cladonia* swards should be considered an essential stage of succession, without which the development of stable plant cover would not be possible. This can be confirmed by earlier reclamation attempts. The support of plant cover development on such dumps by means only of 'rapid greening' methods (sowing and planting mixtures of taxa, including grasses, legumes and trees) either failed or produced poor results [4,61].

Finally, in order to better understand lichen vegetation on slag dumps and the function of particular

species, we plan to perform further studies: intracellular trace element accumulation by *Cladonia* thalli as a response to habitat contamination, relationship between specific *C. rei* morphotype and the molecular pattern of the species, transplantation of a small cluster with *C. rei* to bare areas of dumps in order to initiate and accelerate the process of succession.

## 5. Conclusions

Lichen species of *Cladonia* occur frequently and form dense swards on post-smelting dumps. In particular, *C. rei* proved to be the most tolerant and abundant epigeic species. The relatively great similarity of lichen species composition on post-smelting dumps indicates that those species are well-adapted for slag substrate and that their occurrence is not accidental. The spontaneous and rapid appearance of *Cladonia* lichens on slag dumps should be regarded as a positive phenomenon, crucial for the gradual and stable plant colonisation. The awareness of these facts can be helpful for planning alternative reclamation interventions. The first action we suggest to implement is protection of *Cladonia* swards, which could be easily achieved by restricting public access to the dumps.

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