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Objectives, scope and organisation of research in the Olkusz Ore-bearing Region

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The main objective of the research done for the "Vegetation of calamine soils and its importance for biodiversity and landscape conservation in post-mining areas" project (EEA FM PL0265) was to describe in detail the botanical and ecological characteristics of vegetation of the Olkusz Ore-bearing Region (OOR), and in particular the composition, diversity, distribution and abundance of species, and the degree to which the vegetation cover follows a mosaic pattern. The project addressed the following issues: (1) the dependence of the above features on spatial scale, on variation of environmental factors (soil physicochemical properties, geology, topography, land use type, relief) and on interspecific relations; (2) the impact of vegetation on soil biological activity and on the stability of substrate geochemistry; and (3) the significance of vegetation for rehabilitation of the degraded landscape.

The data gathered were used to assess the value and condition of the vegetation as well as the effectiveness and long-term effects of remediation (afforestation) in post-mining areas. Some of the results have been published in numerous original papers in scientific journals, popular-science articles, monographs, and a dozen or so conference communiques (Grodzińska and Godzik – Chapter 5, this volume).

The project was split into four kinds of tasks:

- identification of vegetation types and mapping of their distribution;
- studies of vascular plant, bryophyte and lichen species diversity;
- studies of soil physicochemical properties and soil organism activity;
- studies of selected characteristics of pine stands.

The particular aims of research tasks are presented, with descriptions of each of the tasks.

The research was done on different spatial scales:

- over the whole area of the OOR (48 km^2; Fig. 1);
- at 49 permanent sites (400 m^2 each) in selected vegetation patches representing different types of OOR communities (Fig. 2);
- in 441 plots (4 m^2 each) on the 49 permanent sites (Fig. 3).

Detailed description of the tasks and results

Identification of vegetation types and mapping of their distribution

The aims of this task were to:

1. map the spatial variability of vegetation types in the OOR;
2. evaluate the OOR's vegetation based on the presence and extent of particularly valuable plant communities;
3. determine the relations between the intensity of anthropopression and vegetation diversity, on species level and on flora biocoenosis level.

Over the whole study area (48 km^2), all homogeneous patches of vegetation covering at least 500 m^2 were mapped in the field at 1:5000 scale using aerial photographs. Patch location was determined with GPS measurements when necessary.

For each of the distinguished and mapped vegetation patches, a list of the dominant species, and species of phytosociological units considered diagnostic in terms of class, order or association, was drawn up in the field. The list usually contained about a dozen plant species. Based on this information, the majority of the vegetation patches were assigned to vegetation units at least at the rank of association, already in the field. The remaining patches were classified after the whole area was mapped and the inventories were compared. Patches associated with calamine substrate were identified on the basis of the presence of indicator species given by Grodzińska and Szarek-Łukaszewska (2009). The classification of forest communities also considered forest stand age, estimated by counting the whorl branches of Scots pine (age classes: up to 20 years, 21–40 years, over 40 years).

After scanning the first drafts of the field vegetation maps, the collected data were digitised in a GIS environment and individual vegetation patches were coded according to the classification developed for the OOR.

For statistical processing of the data, the study area was divided into ATPOL squares, each covering 1 km^2. In each square the number of vegetation types, the area they occupied, and the number and size of homogeneous plant patches were determined.

The results of the work on this task are presented in Chapter 7 of this volume (Holeksa *et al.*).

Studies of vascular plant, bryophyte and lichen species diversity

The first part of the work on this task was a **floristic inventory** of the entire OOR (48 km^2). The aims were to:

1. make a complete floristic inventory;
2. evaluate the plant cover of the OOR by analysing its species richness and the distribution and frequency of species in 48 ATPOL squares, with particular attention to protected species, species designated rare in the Polish flora, and alien and invasive species;
3. determine the contribution of geohistorical groups and the syntaxonomic affiliation of flora components;
4. determine the relationship between the occurrence of selected species and the character of land use;
5. compare the species composition of the calamine grassland vegetation of the OOR with the species composition of similar communities occurring in other parts of the Garb Tarnogórski mesoregion;
6. describe the changes in the species composition of grassland vegetation and the entire flora of the OOR over the last 15 years.

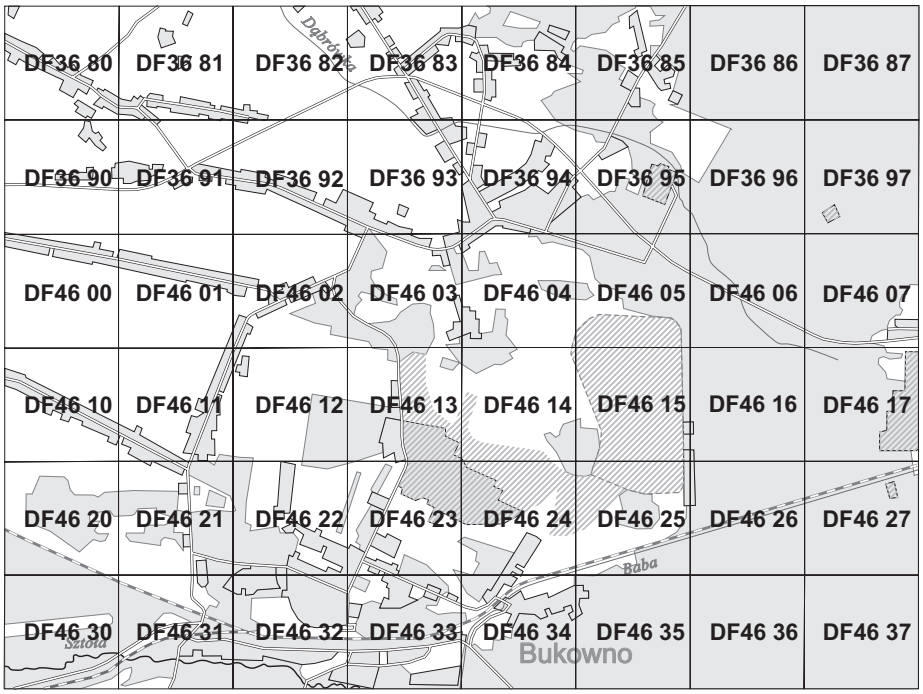

Fig. 1. Study area of project EEA FM PL0265. Grid squares in the ATPOL system

Ryc. 1. Teren objęty badaniami w ramach projektu MF EOG PL0265. Oznaczenie kwadratów w systemie ATPOL

As mentioned above, the basic research unit of the floristic inventory of the 48 km^2 area was a 1 km^2 square (Fig. 1), but results were also reported for larger (2 × 2 km) squares, for comparison with results from floristic studies done on a larger scale (Garb Tarnowski mesoregion) in 1990–1996. In both cases the method of subdividing the land into research fields was consistent with the ATPOL grid methodology employed for the *Atlas of vascular plant distribution in Poland* (Zając 1978; Zając and Zając 2001). The study area straddles two 100 km^2 ATPOL grid squares (DF36, DF46). Sixteen 1 km^2 squares in DF36 were studied, and 32 squares in DF46 (Fig. 1; Nowak *et al*. 2011).

The field research was done in the growing seasons of 2008 and 2009, and some follow-up work was done in the spring of 2010. In the field, 1:10,000-scale topographic maps and 1:5000-scale aerial photographs were used. Each square was explored once or twice during different periods of the growing season. The presence of a given taxon in the square was noted on prepared forms. All taxa (species, subspecies, taxa of hybrid origin) occurring in the area spontaneously were reported, as well as introduced taxa and escapees from cultivation. More than 8100 original floristic data were collected, together with herbarium documentation comprising approximately 400 specimens, which were deposited in the herbarium of the W. Szafer Institute of Botany of the Polish Academy of Sciences (KRAM).

The results of the work on this task are presented mainly in a monograph by Nowak *et al*. (2011) and in Chapter 8 (Nowak *et al*.), Chapter 9 (Ochyra and Godzik) and Chapter 10 (Bielczyk) of this volume.

The second part of this research task was to **characterise selected plant communities** in terms of the species composition and diversity of flowering plants, ferns, mosses and lichens occurring on the 49 research sites. The aims were to:

1. prepare a detailed floristic characterisation of the plant communities prevailing in the area, including grassland and pine forest on dolomite waste or sandy soil variously contaminated with heavy metals, determining the frequency and abundance of flowering plants, ferns, mosses and lichens;
2. analyse the differences in the species composition and richness of herbaceous vegetation between spontaneously formed communities (grasslands) and artificial communities (pine forests) on the same type of substrate;
3. determine the role of substrate in the formation of plant cover by comparing grassland growing on dolomite waste, psammophilous grassland, managed pine forest on podzols, and pine forest planted on dolomite waste for remediation;
4. evaluate the role of the studied plant communities in maintaining high biodiversity of post-mining areas;
5. determine the syntaxonomic position of OOR calamine grassland (occurring on zinc- and lead-enriched substrate) based on literature data profiling similar communities in other parts of Europe.

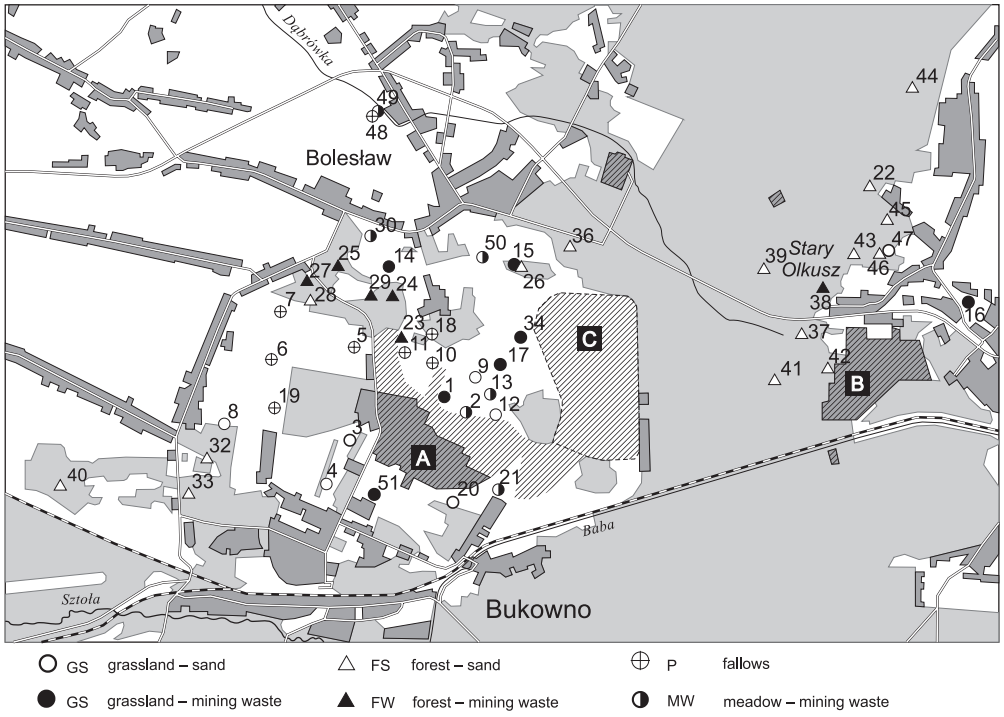

Fig. 2. Distribution of research sites belonging to the six dominant communities in the OOR. A – ZGH Bolesław zinc smelter, B – flotation works, C – flotation tailings heap

Ryc. 2. Rozmieszczenie powierzchni badawczych należących do 6 dominujących w OOR zbiorowisk roślinnych. A – huta cynku ZGH Bolesław, B – zakład flotacji, C – hałda odpadów poflotacyjnych

The study covered 49 uniform research sites (vegetation patches) belonging to the six communities dominant in the OOR (Fig. 2):

- thermophilous grassland on dolomite waste (abbreviated GW, N = 7);
- grassland dominated by *Molinia caerulea* on dolomite waste (MW, N = 6);
- pine forest on dolomite waste (FW, N = 6);
- thermophilous grassland dominated by *Festuca ovina* on sandy substrate (GS; N = 7);
- mesophilous grassland on old fields (P, N = 8);
- pine forest on sandy substrate (FS, N = 15).

Affiliation to a given group was determined from a cursory assessment of the vegetation structure and substrate type (soil was locally uncovered to a depth of 2 mineral horizons). The choice of permanent sites was guided by the need to reflect the habitat variability of the OOR and the need for reliable, representative sampling (sites of the same category were scattered over the largest possible area or, at minimum, they were not adjacent to each other; Fig. 2).

At each of the 49 sites, 9 permanent plots (circular, ca. 4 m^2) were laid out at intervals of 100 m in a regular 20 × 20 m grid, as shown in Figure 3, giving a total 441 plots. The position of the central plot was determined with a GPS of submetric accuracy.

For each plot we listed the flowering plants, ferns, mosses and lichens occurring there and recorded their cover on a 6-point Braun-Blanquet scale. The species occurring in the entire area of the grid (400 m^2) were also listed. Total vegetation cover was determined for the ground layer, shrub layer and tree layer. The main part of the field research for this task was done in 2008, with some additional follow-up in 2009.

The main sources of variation in the species composition of plants and lichens were identified on the basis of a DCA (detrended correspondence analysis) for all 441 plots. In this case the results were interpreted based on knowledge of the habitat preferences of individual species. After entering the variables describing soil physicochemical properties, RDA (redundancy analysis) was performed to determine the actual relationship between community structure and habitat factors. The statistical significance of differences in species richness and total plant cover between the community categories was assessed by analysis of variance.

The results of the work on this task are presented in Chapter 13 of this volume (Kapusta *et al.*).

Studies of soil physicochemical properties and soil organism activity

The first part of this task consisted of **assessing the variability of soil physicochemical properties and other environmental factors** at the permanent sites. The aims were to:

1. identify the source, level and variability of soil pollution by heavy metals in the OOR;
2. determine the physicochemical properties of the soils in the studied communities;
3. evaluate the impact of Scots pine on soil physicochemical properties by comparing remediated (pine forest) and non-remediated (grassland) areas having the same substrate type.

The study covered 49 research sites representing the six communities dominant in the OOR (GW, GS, P, MW, FW, FS; Fig. 2). During the first year of this task (2008), at each site the soil profile was opened to a depth of 2–4 horizons (depending on soil type) in order to determine the affiliation of a given

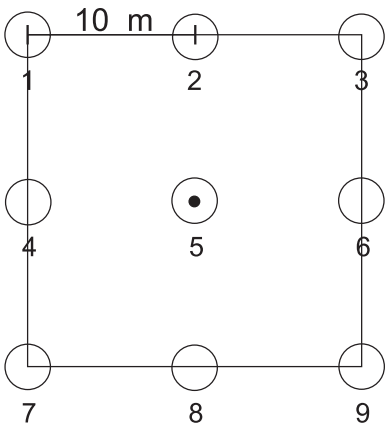

Fig. 3. Circular plots (ca 4 m^2) established at the research sites in the OOR

Ryc. 3. Kołowe poletka (o powierzchni 4 m^2) na wybranych powierzchniach badawczych w OOR

site to one of the six habitat categories and to develop methods for soil sample collection and analysis.

In the following season, soil samples were collected for laboratory analysis. Three subsamples from two or three mineral horizons (down to ca. 50 cm depth) were collected from the central plot of each research site. From the remaining 8 plots, three subsamples were taken only from the upper mineral horizon (usually from horizon A). The samples for particular horizons were combined. The samples for the central plots were divided into two parts, for physicochemical and for microbiological analyses. The following characteristics were determined for the soil samples: water content, granulometric composition, pH, organic matter content, total concentrations of C, N and S, total content of P, and the content of its available fraction. The concentrations of Al, Fe, Mn, K, Ca, Mg, Zn, Cd and Pb were measured as total, exchangeable (in extract of $BaCl_2$) and water-soluble. Cation exchange capacity was also determined.

The general soil characteristics were described for all 411 plots: basic soil properties such as origin, permeability, moisture, thickness, number and size of plant roots growing through the soil, separately for different horizons. For each site, within the central plot, continuous temperature measurements at the soil surface and at ca. 5 cm depth were made using autonomous temperature loggers. Temperature was recorded every 15 minutes over a 3-month period (July-September 2009).

Factor analysis was used to extract the main components of variation (i.e. variables not correlated with each other, used in subsequent analyses). This method was also used to identify relationships between different soil properties. Analysis of variance was used to test the significance of differences in soil properties between habitat categories.

The results of the work on this part of the task are presented in Chapter 13 of this volume (Kapusta *et al.*).

The second part of this task was aimed at **assessing the microbiological status of the studied soils** of all permanent sites. The aims were to:

1. analyse microbial activity and biomass, and assess the ability of soil bacteria and fungi to utilise organic compounds in two soil horizons;
2. compare the effects of heavy metals on soil microorganisms between forest and non-forest (grassland, fallows) ecosystems;
3. compare soil microbiological parameters between habitat types dominating the OOR – forest and grassland on sand and on mining waste, as well as fallows;
4. assess the influence of soil physicochemical properties and of herbaceous species diversity and composition on soil microorganisms.

The 49 sites representing the six dominant habitat categories (GW, GS, P, MW, FW, FS; Fig. 2) were studied. Soil samples for analysis of microbiological parameters were

taken from the central plots, from two soil horizons: (1) A, Ap, or AE, and (2) B. Soil basal respiration was measured in order to assess overall microbial activity. Soil samples with standardised moisture were incubated at constant temperature and the emitted CO_2 was absorbed in NaOH. The excess hydroxide was titrated with HCl after incubation. The amount of emitted CO_2 was calculated from the amount of HCl used for titration. After the basal respiration measurements, glucose was added to the soil samples in order to estimate substrate-induced respiration. After incubation of the glucose-amended samples at constant temperature, respiration was measured as described above. On this basis, the amount of microbial biomass, or more accurately the carbon of the microbial biomass in soil, was calculated. The metabolic quotient was calculated as the ratio of respiration to microbial biomass. The qCO_2 quotient can be helpful in assessing the impact of stress factors such as heavy metals on soil microbial communities.

To analyse the ability of soil microorganisms to degrade organic substrates, 96-well Biolog plates were used: GN2 for bacteria and SFN2 for fungi. These plates contain 95 different carbon compounds, including carbohydrates, amino acids and carboxylic acids, which are nutrients for heterotrophic microorganisms. The Biolog method is based on measuring the activity of the soil bacteria or fungi on each substrate. On this basis, it is possible to calculate the number of compounds consumed by the microorganisms, assess the level of substrate consumption, and compare the substrate utilisation patterns (physiological profiles) of bacterial and fungal communities from different soils.

Soil samples were shaken in physiological saline and the obtained extracts were diluted and inoculated onto plates, which were then incubated at constant temperature. Absorbance, reflecting microorganism activity, was measured spectrophotometrically twice a day during the incubation period. The number of substrates used, reflecting the functional richness of the microbial community, and also average bacterial and fungal activity on the Biolog plates, were calculated.

Factor analysis incorporated variables describing soil physicochemical properties as well as the plant communities. The resulting factors were further used in multiple regression analysis as independent variables to assess the impact of habitat characteristics on microbial community parameters of the upper soil horizon.

The nonparametric Mann-Whitney U test was used to compare microbial activity between the two soil horizons. Thirty-four of the 49 study sites were chosen, where two horizons were distinguishable. To assess the effect of metals on microorganisms of the upper horizon, correlation analyses were done separately for forest and nonforest (grassland and fallows) habitats. Analysis of variance was performed to compare microbial parameters between the six habitat categories.

The results of the work on this part of the task are presented in Chapter 14 of this volume (Stefanowicz).

The third part of the task consisted of **assessing the variability of soil mesofauna abundance**. The aims were to:

1. compare the density of soil mesofauna (with special emphasis on Enchytraeidae) in the studied communities, the soils of the OOR, and soils of other regions;
2. determine the impact of heavy metals on soil mesofauna density;
3. determine the influence of soil physicochemical properties and herbaceous species diversity and composition on soil mesofauna density;

4. examine the relations between soil mesofauna activity and microbiological parameters.

The study covered 49 sites representing the six communities prevailing in the OOR (GW, GS, P, MW, FW, FS; Fig. 2). The density of enchytraeids (Enchytraeidae), nematodes (Nematoda) and tardigrades (Tardigrada) was determined in soil collected from 13 cm depth using a soil corer 3.5 cm in diameter. One sample from each plot (N = 441) was collected over several consecutive days in late June and July 2009 after rainy periods.

Mesofauna were extracted with wet O'Connor funnels; lamps were used as a heat source to speed up extraction. Mesofauna were identified and counted under a binocular microscope.

Analysis of variance was performed to compare soil mesofauna activity between the studied communities. The effect of soil physicochemical properties and plant community parameters on mesofauna density was estimated by multiple regression. The dependence of soil microorganism activity on the density of enchytraeids, nematodes and tardigrades was estimated the same way.

The results of the work on this part of the task are presented in Chapter 13 of this volume (Kapusta *et al.*).

Studies of selected characteristics of pine stands

The first part of the study consisted of an **evaluation of pine (*Pinus sylvestris*) ectomycorrhizal species diversity and abundance** at the forest research sites. This part was extended to include analyses of fruiting bodies of mycorrhizal fungi and, for comparative purposes, of saprobiontic fungi. The aims were to:

1. compare the species composition and quantitative parameters of saprobiontic and mycorrhizal macrofungi communities and pine ectomycorrhizae in pine forests on dolomite waste and on sandy substrate;
2. determine the impact of soil physicochemical properties (including heavy metal content) and quantitative parameters of herbaceous vegetation on the composition and species richness of the fungal groups mentioned above.

The study covered 21 permanent forest sites, some on dolomite waste and the others on sandy substrate (FW, FS; Fig. 2). In 2008 and 2009, the sites were examined for the occurrence of fruiting bodies of macrofungi, saprobiontic and mycorrhizal fungi. Species appearing on the surface were recorded and counted.

The results of the work on this part of the task are presented in Chapter 11 of this volume (Mleczko and Beszczyńska).

In 2009, soil samples with roots (5 cm diameter, 20 cm depth) were collected from all 9 plots of each site (400 m^2). The samples were protected from drying and transported to the laboratory. Roots were isolated from the soil (separately from the organic and mineral horizons) by washing with running water and then separated with the aid of binocular magnifying glasses. Pine roots were separated from the roots of herbaceous plants and other trees according to their macromorphology. Living roots were separated from dead ones. They were scanned and the scans were analysed using WinRhizo. The parameters analysed included diameter, length, area and volume of thin roots, and density of side branches.

Pine ectomycorrhizae were separated into morphotypes and counted. These data were used to calculate the density of ectomycorrhizae on roots and in particular soil horizons. The ectomycorrhizae of specific morphotypes were preserved for comparison and for molecular analysis.

The ectomycorrhizae were identified by amplifying and analysing the ITS rnDNA region. Both the ectomycorrhizae and fruiting bodies of ectomycorrhizal fungi collected in the research area underwent molecular analysis. The ITS rnDNA sequences of ectomycorrhizae were compared with those of reference fruiting bodies and those in the NCBI (GenBank) and UNITE databases.

Analysis of variance was used to determine the significance of differences in quantitative features of the ectomycorrhizae and macrofungi communities of the two habitat types. PCA was used to identify differences in the macrofungal species composition between habitat types, and RDA was used to assess the dependence of species composition of fungi on habitat characteristics (soil physicochemical properties, quantitative parameters of undergrowth). This part of the task requires further work and will be reported in a separate paper.

The second part of the task dealt with **determining the characteristics of pine (*Pinus sylvestris*) dynamics** at the forest research sites. The aims were to:

1. determine the incremental reaction of pine trees to the temporal variability of pollutant inflow;
2. compare the parameters of pine stands growing on dolomite waste and on sandy substrate;
3. determine the effect of heavy metals on pine tree growth.

The study covered 21 permanent sites of forest in two habitat categories (FW, FS; Fig. 2). Diameter at breast height (DBH) and the height of all trees (living and dead) at a given site were measured, and their species was determined. Cores were collected from the 10 trees with the highest DBH (130 cm) using a Pressler borer.

Incremental change was calculated on the basis of the ratio of average growth over a decade to average growth over the preceding decade. The parameters of the tree stands on the two types of substrate were compared by ANOVA.

The results of the work on this task are presented in Chapter 12 of this volume (Zielonka *et al*.).

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