Zbigniew Jóźwik

7.2 Heavy metals in plants and soil bacteria

The first studies on on the concentration of copper (Cu), manganese (Mn), zinc (Zn), lead (Pb) and cadmium (Cd) in the plants and soil of the area of Bellsund (Spitsbergen) were carried out in years 1987-1995. Studies focused on the dominant species of vascular plants, bryophytes and lichens collected in 1987, 1989, 1991, 1993 and 1995 from the following areas: coastal plains – Calypsostranda, Lyellstranda, Dyrstadflya, Lognedalsflya and Tomtodden; valleys – Chamberlin, Tjørn, Blomli and Dunder; as well as slopes and mountain summits: Wijkanderberget, Bohlinryggen, Observatoriefjellet and Magnethøgda (Fig. 7.2.1). In 2000 the research related to the content of these elements in the plants and the soil was continued, this time taking place along the cross section of the Calypsostranda, on nine selected geo-ecosystems: terrace under a dead-cliff, base of the slope, area cover with mottled tundra, area covered with dry lichen tundra, the cores of patterned ground, rock outcrops (Photos 7.2.1 and 7.2.2). In order to separate the soil bacteria soil samples were also collected in places of plant collection. The results of the research were published both in specialist magazines, as well as in collective studies (Jóźwik 1988, 1990ab, 1991, 1992ab, 1993, 1994ab, 1996, 1998, 2000; Jóźwik & Magierski 1991, 1992, 1993, 1994, 1995, 1996; Janowiec *et al.* 1993).

This study constitutes a summary of the research carried out by the Faculty of Biology and Earth Sciences of the Maria Curie-Skłodowska University in Lublin on the metal content in plants and the isolation of soil bacteria on Spitsbergen.

Research focus and methodology

The research focused on 21 species of vascular plants: *Carex misandra, Cerastium arcticum, Cochlearia groelandica, Deschampsia alpina, Draba corymbosa, Dryas octopetala, Equisetum arvense, Luzala arctica, Oxyria digina, Papaver dahlianum, Poa alpina, Polygonum viviparum, Salix polaris, S. reticulata, Saxifraga aizoides, S. cespifosa, S. cernua, S. flagellaris, S. hirculus, S. oppositifolia* and *Silene acaulis*; 25 species of bryophytes: *Aulacomnium palustre, A. turgidum, Bryum crithatum, B. cryophilum, B. elegans, B. schleicheri var. Latifolium, Camphylium polygamum, Catoscopium nigritum,* *Dicranoweissia crispula, Dicranum bonjeanii, D. elongatum, D. fuscescens, Distichium capillaceum, Drepanocladus fluitans, D. revolvens, D. uncinatus, Hylocomium splendens, Oncophorus wahlenbergii, Pohlia cruda, P. polymorpha, Ptilidium ciliare, Raconitrium lanuginosum, Schistidium apocarpum, Sphagnum aquarrosum* and *Tetralophosia setiformis*; as well as 13 species of lichens: *Cetraria crispa, C. hiascens, C. nivalis, Cladonia gracilis, C. mitis, Ochrolechia frigida, Parmelia sp., Peltigera sp., Stereocaulon sp., Thamnolia vernicularis, Umbilicaria sp.* and *Xantoria elegans.* Up to a dozen specimens of every species found in different tundra covers were collected. The plants were dried, milled in agate mortars and unified by mixing; and then the dry weight was determined. The plants were collected in July and August during successive research trips. In the case of isolating bacteria, the soil samples were collected in a sterile manner from various sections of the tundra, as well as from Longyearbyen and Barentsburg towns (Fig. 7.2.1).

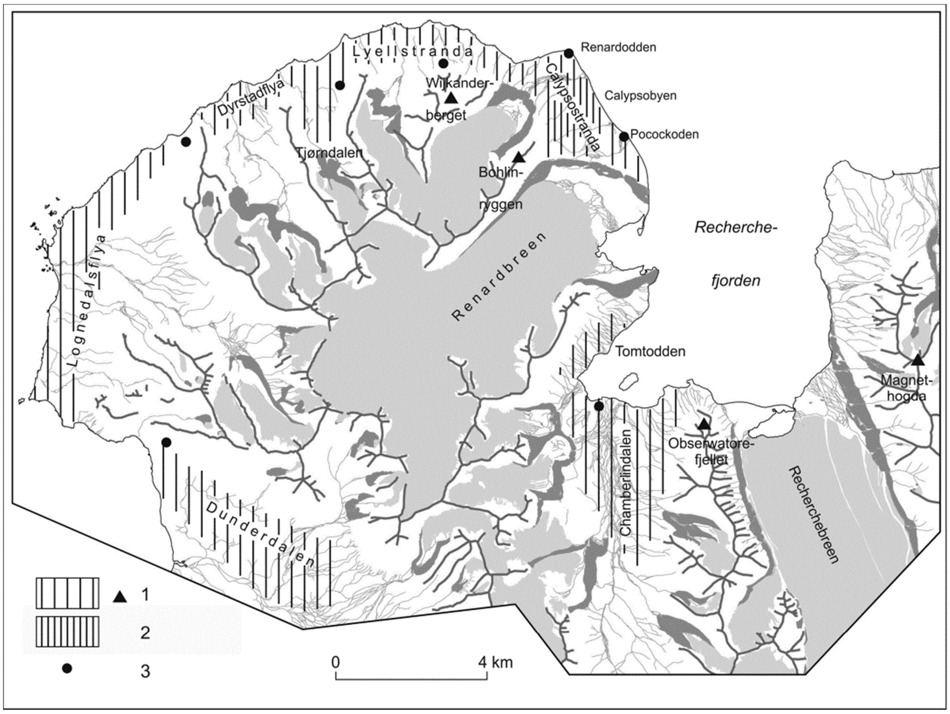

Fig. 7.2.1. Location of sampling for research: 1- in the years 1987-1995, 2- in the year 2000 (Calypsostranda), 3- location of soil sampling for isolation of bacteria.

Content of Cu, Mn, Zn, Pb, and Cd in plant samples was determined using an atomic absorption spectrophotometer in a Pye Unicam SP9 apparatus. The plant material was burned in a mixture of nitric and tetraoxochloric acid in the proportion of 7:1 /v/v/. Bacteria were isolated according to standard methods on selected media: Bunt and Rovixy, universal medium, medium for actinomycetes, nutritious agar and Burke’s medium.

Photo 7.2.1. A- beach – terrace (Plants: *Saxifraga oppositifolia*, *Silene acaulis*), B- Calypsobyen – slope (Plants: *Saxifraga oppositifolia*, *Drepanocladus unicinatus*), A- Calypsostranda – mottled tundra (Plants: *Silene acaulis*, *Saxifraga oppositifolia*, *Cerastia hiascens*, *Salix polaris*) (Photos Z. Jóźwik 2000)

Photo 7.2.2. A- Calypsostranda – patterned ground (Plants: *Saxifraga oppositifolia*, *Drepanocladus revolvens*, *Silene acaulis*) (Photos Z. Jóźwik 2000), B- Calypsostranda – ground near rock outcrop (Plants: *Saxifraga oppositifolia*, *Salix polaris*, *Dicranoweissia crispula*), C- Calypsostranda – ground between rock outcrops (Plants: *Dryas octopetala*, *Salix polaris*, *Dicranoweissia crispula*) (Photos Z. Jóźwik 2000).

Results

Metals*.* The research interest in heavy metals that has been growing in recent decades is associated with the threat they pose to plants, animals, as well as to humans. Therefore the research was also carried out in Svalbard – a place on Earth which seemingly should be free of contamination by such metals. Based on the presented results it may be concluded that some of the studied plants show high tolerance to heavy metals, and accumulate them. Such metals include Pb, Zn, and Cd. Concentration of these elements that is above the average level in the case of Pb or above the physiological level in the case of Zn, may indicate that the natural environment in the study area is under risk of contamination by heavy metals In the case of Cu, there is a deficiency of this metal in vascular plants as well as bryophytes and lichens. This may be caused by the low content of Cu in local soils, (Jóźwik & Magierski 1993). It is also possible that the studied species have a low demand of copper or lack the ability to accumulate Cu.

Copper (Cu). The average concentration of Cu in plants ranges between 5-20 ppm. In the case of studied plants, some species demonstrate Cu deficiency. This group includes the following vascular plants: *Cochlearia groelandica* – 3.7ppm, *Luzula arctica* – 4.8 ppm, *Oxyria digina* – 4.8 ppm, *Polygonum viviparum* – 2.7 ppm, *Salix reticulata* – 4.9 ppm, *Saxifraga aizoides* – 3.5 ppm, *S. cernua* – 4.1 ppm, *S. flagellaris* – 4.1 ppm, *S. oppositifolia* – 4.0 ppm and *Silene acaulis* – 4.5 ppm. In the bryophyte group, Cu deficiency has been found in: *Dicranum elongatum* – 4.2 ppm*, D. fuscescens* – 3.5 ppm, *Distichium capillaceum* – 4.1 ppm, *Racomitrium lanuginosum* – 3.9 ppm and *Tetralophozia setiformis* – 3.6 ppm. Lichens with this characteristic include the following: *Cetraria crispa* – 3.1 ppm, *C. hiascens* – 3.1 ppm, *C. gracilis* – 4.4 ppm, *Cladonia mitis* – 3.9 ppm, *Stereocaulon sp.* – 3.5 ppm and *Thamnolia vernicularis* – 2.9 ppm. Copper deficiency has been found in 24 species, which constitutes 40% of all studied plants. The remaining plants demonstrate physiological levels of Cu concentration (Jóźwik & Magierski 1993).

Manganese (Mn). The concentration of manganese in plants ranges between 9-900 ppm. The concentration of this element was at the physiological level in all analysed plants.

Zinc (Zn). The concentration of zinc in the plants ranges between 15-80 ppm. Many of the studied plants demonstrated increased concentrations of this element, exceeding physiological levels, despite the relatively low concentration of Zn in the soil (Jóźwik & Magierski 1993). It might be assumed that plants accumulate this element. Increased concentration of Zn was found, mainly, in *Salix polaris*, one of the dominant plants in Spitsbergen. The average Zn concentration in this plant was 160 ppm. This is most probably a species-dependent pattern.

Zinc (Zn). The content of zinc in the plants ranges between 15-80 ppm. Many of the studies plants demonstrated increased concentrations of this element, exceeding physiological levels, despite the relatively low concentration of Zn in the soil (Jóźwik &

Magierski 1993). It might be surmised that plants accumulate this element. Increased concentration of Zn was found, first and foremost, in *Salix polaris*, one of the dominant plants of the Spitsbergen area. The average Zn content in this plant was 160 ppm. This is most probably a species-dependent pattern.

Other plants with increased values of Zn concentration include: *Salix reticulata* – 174.4 ppm, *Cerastium arcticum* – 97.4 ppm. Among bryophytes: *Bryum crithatum* – 157.0 ppm and *Pohlia polymorpha* – 101.0 ppm. In the case of lichens the Zn concentration values were at the physiological level in all specimens.

Lead (Pb). In terms of heavily toxic elements, which find their way into the human organism via food, the following metals are usually considered: Cd, Pb, arsenic (As) and mercury (Hg). However, only Pb is accumulated by humans in amounts that are similar to clinically toxic levels. The threat of presence of Pb stems from the fact, that our organisms balance the levels of this element similarly to the way they balance the levels of calcium. Pb poses a unique threat to the natural environment. Any amount of this element is undesirable. It is assumed that the average concentration in plants ranges from 0.9 to 9 ppm.

During the research period, increased concentrations of lead were found in numerous plants, mainly bryophytes and lichens – these values exceeded the average values listed in the literature on the subject (Kabata-Pendias & Pendias 1993). In the case of many plants the Pb content was two or even three times higher than the average. Bryophytes and lichens take up this element straight from the air. Presence of lead in these plants could most likely be explained by the impact of human pressure on the environment in question. But increased amounts of Pb were also found in vascular plants. This may suggest that the element is accumulated through the root system. Out of all the studied plants, the following vascular plants demonstrated a higher-than-average content of Pb: *Cerastium arcticum* – 15.2 ppm, *C. officinalis* – 13.5 ppm, *Draba corymbosa* – 13.9 ppm, *Dryas octopetala* – 12.4 ppm, *Luzula arctica* – 13.9 ppm, *Polygonum viviparum* – 10.6 ppm, *Salix polaris* – 10.4 ppm, *S. reticulata* – 16.1 ppm, *Saxifraga aizoides* – 20.6 ppm, *S. cernua* – 15.1 ppm, *S. oppositifolia* – 10.0 ppm and *Silene acaulis* – 115.5 ppm.

In the case of bryophytes, only 4 of the 25 analysed species had a lead concentration that did not exceed the average level, and in the case of lichens, out of 13 analysed species 11 contained increased amounts of lead. Out of a total of 60 studied plant species, 44 were found to contain increased concentrations of Pb, which is as much as 73 %. Thus, we may conclude that the natural environment in the study area is under risk of contamination by lead.

Cadmium (Cd). Despite the fact that cadmium is easily accumulated by plants, proportionally to its concentration in the soil, it is not actually needed for the plants to grow (Kabata-Pendias & Pendias 1993). Most of plant species are characterised by a high tolerance to this metal and do not exhibit toxicity symptoms even in case of relatively high concentrations of the element. The content of Cd in the soil is between

0.15-0.2 ppm, and in plants this value may be as high as 1 ppm. Cadmium concentrations of 4-13 ppm are considered toxic for plants (Kabata-Pendias & Pendias 1993).

In most of the studied plants the Cd concentrations were equal to average values listed in the literature on the subject. One exceptional case was *Salix polaris* – a species which, regardless of where the samples were taken, contained increased concentrations of Cd (1.7 ppm on average). It may be that this particular plant has the ability to accumulate cadmium in large concentrations. Significantly higher levels of Cd concentrations were also found in some lichens: *Cladonia gracilis* – 1.4 ppm, *Ochrolechia frigida* – 1.4 ppm, *Stereocaulon denudatum* – 3.2 ppm and *Xantoria elegans* – 1.7 ppm. The concentrations of Cu, Mn, Zn, Pb and Cd in samples collected in the year 2000 along the cross-section of the Calypso plain are consistent with the results of research carried out in the years 1987-1995.

In the plants selected for research the diversity of heavy metal conconctrations is non-uniform and only in few cases depends in the area, where the samples were collected. One notable case is undoubtedly *Salix polaris* which, regardless of the place of sample collection, accumulates zinc in amounts exceeding the physiological levels and Cd in amounts exceeding the average values. Thus, in the case of this plant the species-related pattern can be considered. Other examples include *Cetraria hiascens* and *Racomitrium lanuginosum* which, regardless of where the samples were taken, demonstrated Cu deficiency. These characteristics are most probably also species-dependent.

In recent years, other researchers, e.g. Drbal *et al.* (1992) and Grodzieńska *et al.* (1993), carried out studies concerning the content of heavy metals in the plants of the western and southern Spitsbergen. The results of these studies with regard to certain metals, especially in the case of lichens, are quite different than those presented in this work. It seems that this might be due to differences in geological, hydrological and soil conditions, as well as the fact that the impact of human pressure is different in respective areas. In particular, the human pressure is a factor controlling the concentrations of heavy metal in the environment (Jaworowski 1989; Heintzenberg 1989).

Bacteria. The isolation of soil bacteria in the NW part of Wedel Jarlsberg Land in Svalbard was attempted due to two main scientific reasons. The first one was to find out whether any bacteria occur in the soil of Svalbard that produce antibiotics against tubercle bacilli; and the second one – to find out if tubercle bacilli occur in the Svalbard soil. The research area (presented on the map) had not yet been studied in this respect.

Bacteria were isolated from 69 soil samples collected in the years 1991-2000 from: Tomtodden (where the Pomors lived), Pocockodden and Renardodden (where the whalers lived), Chamberlindalen and Dunderbukta (from peaty soil), Barentsburg and Longyearbyen (where people currently dwell) and several locations of the tundra in the area of Bellsund (Fig. 7.2.1).

Differentiation of isolated bacteria strains was based on morphological tests, physiological tests and the Gram staining method. Biochemical tests were only conducted in the case of antibiotic-producing strains. Antibiosis was determined by employing the double agar layer method developed by Gratia (Fredericq 1957) in relation to acid-resistant saprophyte, *Mycobacterium 279*, with the use of isolating media. The culture of the strain *M. 279* was developed on Sauton's medium. Bacteria isolation took place on selected media in temperatures of 4°C, 18°C and 37°C. Thousands of strains were isolated and, after a preliminary selection, 931 of these became exhibits in the Strain Museum of the Institute of Plant Physiology at Maria Curie-Skłodowska University in Lublin. This Institute carried out research dealing with bacteria characteristics and finding strains that produce antibiotics inhibiting the development of tubercle bacilli. Out of a total number of 931 strains, the following were determined: 194 strains were isolated on nutritious agar 1.4%, and among these: 6 strains isolated in the temperature of 4°C, 125 strains isolated in the temperature of 18°C and 63 strains isolated in the temperature of 37°C; 218 strains were isolated on Bunt and Rovixy medium, out of which 20 strains isolated in the temperature of 4°C, 150 strains isolated in the temperature of 18°C and 47 strains isolated in the temperature of 37°C; 25 strains were isolated on Burke's medium, and among these: in the temperature of 4°C no strains were isolated, in the temperature of 18°C – 15 strains, and in the temperature of 37°C – 10 strains. When using the universal medium, 224 strains were isolated, out of which: 20 strains were isolated in the temperature of 4°C, 157 strains were isolated in the temperature of 18°C, and 47 strains were isolated in the temperature of 37°C. In the case of the medium for actinomycetes, 270 strains were isolated, and these included: 11 strains in the temperature of 4°C, 188 strains in the temperature of 18°C, and 7 strains in the temperature of 37°C. When using the Gram staining method, 372 coccus strains were identified as gram positive, and 452 coccus strains were identified as gram negative. Out of a total of 55 bacilli, 16 were gram positive and 39 were gram negative. In the case of 52 enteric bacteria, 23 strains were found to be gram positive and 29 – gram negative. Other organisms that were isolated in large numbers were the fungi.

All of the isolated strains, characterised morphologically, were studied in terms of growth on bouillon, bouillon with 7% NaCl, on agar slopes and isolating media. The Gratia method was also employed, as it allows to identify antibiotic-producing bacteria. As a result, within the group of 931 studied strains 37 were found to show antagonistic activity towards *Mycobacterium 279*. This constituted approximately 4% of all studied bacteria. It might seem a small percentage. However, we should remember that a relatively small number of soil bacteria produce antibiotics in the first place. In this group of 37 strains that do create these compounds, 6 strains did so on a bouillon medium.

Acid-resistant saprophytes were also isolated in places, where humans dwelled in the past or still dwell today. One of these strains was further analysed to determine its species. It turned out to be *Mycobacterium friburgensis*, a relative pathogen for tortoises and snakes. This strain was isolated from the archeological site 'Renardbreen-2' ('Viking'), located under the Renardbreen moraine (Janowiec *et al.* 1993). The soil sample in which this strain was found is believed to originate from the second half of the 17th century (Dzierżek *et al.* 1990ab).

The results of research allow us to conclude that the soil of western Spitsbergen in the area of Bellsund is rich in microorganisms belonging to various morphological groups. Coccuses are the dominant group among the isolated bacteria.

Relatively few strains were isolated in the temperature of 4°C in comparison to the number of strains isolated in the temperature of 18°C and 37°C, which seems intriguing, particularly that 4°C is the average temperature level during the Arctic summer in the area. It is possible that using different isolation media would allow us to isolate different bacteria species. Most strains were isolated in the temperature of 18°C. In the summer season the soil temperature in the root area reaches a dozen or so Celsius degrees. This is most probably the optimal growth temperature for these strains.

Conclusion

The presented results of the investigations of concentration of Cu, Mn, Zn, Pb and Cd in the tundra plants lead to the following conclusions: (1) many plant species show a deficiency of Cu; (2) some plant species demonstrate increased concentrations of Zn, exceeding the physiological levels, and others contain increased amounts of Pb and Cd, which exceed the average values found in plants; (3) the concentrations of Mn in all studies plants are within the physiological levels.

One alarming fact is the accumulation of Pb by bryophytes and lichens, as well as certain species of vascular plants. Although plants develop special protective mechanisms against high concentrations of trace elements, and demonstrate high tolerance to certain metals; the increased content of Pb and Zn may indicate a threat to the natural environment. In the majority of cases, actinomycetes were the dominant strain of isolated bacteria. This confirms some of the previous research (Jóźwik 1996). It is important to emphasise that out of all the analysed bacteria strains, 37 were found to show antagonistic activity towards *Mycobacterium 279.* This is an important piece of information, because the number of tubercle bacilli that are immune to the currently-used antibiotics, as well as the number of so-called 'atypical bacilli', is constantly growing. Every new antibiotic-producing strain gives hope for new antituberculous medicines.

Streszczenie

Metale ciężkie w roślinach i bakterie glebowe

Badania nad zawartością metali ciężkich (miedzi, manganu, cynku, ołowiu i kadmu) w roślinach prowadzono w północno-zachodniej części Wedel Jarlsberg Land w latach 1987-1995. W roku 2000 kontynuowano te badania w dziewięciu geoekosystemach, rozmieszczonych wzdłuż przekroju poprzecznego Calypsostrandy. Z miejsc, z których zbierano rośliny, pobierano także próbki glebowe w celu izolacji bakterii.

Bakterie izolowano także z próbek, pobranych w miejscach dawnych, sezonowych osiedli (myśliwych i łowców wielorybów) oraz współczesnych osad stałych (Longyearbyen i Barentsburg). W roślinach tundry spitsbergeńskiej stwierdzono zwiększone ilości Zn ponad stężenia fizjologiczne: w *Salix polaris* – 160,0 ppm, w *Salix reticulata* – 174,4 ppm, w *Cerastium arcticum* – 97,4 ppm, w *Bryum trithatum* (mszaki) – 157,0 ppm. Stwierdzono także zwiększone ilości Pb i Cd. W roślinach naczyniowych zawartość Pb wynosiła: w *Cerastium arcticum* – 15,2 ppm, w *Cerastium officinalis* – 13,5 ppm, w *Draba corymbosa* – 13,9 ppm, w *Dryas octopetala* – 12,4 ppm, w *Salix polaris* – 10,4 ppm. Natomiast u mszaków średnia zawartość ołowiu waha się w granicach 0,9-9,0 ppm, zaś na 25 zbadanych gatunków, przekroczenia stwierdzono u 11 gatunków. Kadm w badanych roślinach występował w zakresie średnich opisanych w literaturze (do 1 ppm). Wyjątkami, w których stwierdzono nieco większe stężenie, są: *Salix polaris* – 1,7 ppm, *Cladonia gracilis* – 1,4 ppm, *Ochrolechia frygida* – 1,4 ppm, *Stereocaulon denudatum* – 3,2 ppm i *Xantoria elegant* – 1,7 ppm. Z kolei u niektórych badanych roślin stwierdzono niedobór miedzi, przy średniej zawartości 5-20 ppm. Przykładowo, w roślinach naczyniowych stwierdzono następujące ilości tego pierwiastka: w *Luzula arctica* – 4,8 ppm, w *Oxyria dygina* – 4,8 ppm, w *Salix reticulata* – 4,9 ppm, w *Saxifraga flagellaris* – 4,1 ppm i w *Silene acaulis* – 4,5 ppm. W grupie mszaków wystąpiły stężenia: w *Dicranum elongatun* – 4,2 ppm, w *Lacomitriun lanoginosum* – 3,9 ppm, zaś w porostach: *Cetraria hiascens* – 3,1 ppm, *C.gracilis* – 4,4 ppm, *Cladonia mitis* – 3,9 ppm. Niedobór Cu stwierdzono u 24 badanych gatunków, co stanowi 40% zbadanych roślin, natomiast mangan występuje u wszystkich analizowanych roślin w granicach stężeń fizjologicznych.

W badaniach laboratoryjnych nad izolacją bakterii glebowych wydzielono 931 szczepów z 69 próbek gleb tundrowych, pobranych w różnych miejscach. Do analiz wytypowano: 194 szczepy izolowane na agarze odżywczym, 218 szczepów na podłożu Bunta i Rowixy, 224 na podłożu uniwersalnym oraz 270 na podłożu dla promieniowców. Spośród 931 izolowanych szczepów 37 wykazywało antagonistyczne działanie wobec *Mycobacterium 279*. Izolowano także saprofity kwasoodporne z dawnych i współczesnych osad. Jeden z tych szczepów, oznaczony jako *Mycobacterium friburgensis*, jest względnym patogenem dla węży i żółwi. Na podstawie prowadzonych badań gleby Spitsbergenu można uznać za źródło szczepów bakterii, charakteryzujących się właściwościami antagonistycznymi wobec prątków gruźlicy.

Objaśnienia

Ryciny

Ryc. 7.2.1. Lokalizacja poboru prób do badań: 1- w latach 1987-1995, 2- w roku 2000 (Calypsostranda), 3- lokalizacja próbek glebowych do izolacji bakterii.

Fotografie

Fot. 7.2.1. A- plaża - terasa (*Saxifraga oppositifolia*, *Silene acaulis*), B- Calypsobyen – stok (roślinność: *Saxifraga oppositifolia*, *Drepanocladus unicinatus*), C- Calypsostranda –tundra plamista (roślinność: *Silene acaulis*, *Saxifraga oppositifolia*, *Cerastia hiascens*, *Salix polaris*) (fot. Z. Jóźwik 2000).

Fot. 7.2.2. A- Calypsostranda – wieńce kamieniste (rośliność: *Saxifraga oppositifolia*, *Drepanocladus revolvens*, *Silene acaulis*), B- Calypsostranda – grunty w podliżu skałek strukturalnych (roślinność: *Saxifraga oppositifolia*, *Salix polaris*, *Dicranoweissia crispula*), C- Calypsostranda – grunty między skałkami strukturalnymi (roślinność: *Dryas octopetala*, *Salix polaris*, *Dicranoweissia crispula*) (fot. Z. Jóźwik 2000).