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Mineralization of penguin excrements in the Admiralty Bay region (King George Island, South Shetland Islands, Antarctica)*)

ABSTRACT: Bacterial, enzymatic and chemical analyses pointed to active microbiological mineralization and transformation of penguin excrements at "maritime Antarctic". The following physiological groups of bacteria were found: proteolitic, amonifying, nitrifying, lecithin degrading, $Ca_3(PO_4)_2$ dissolving, chitin degrading and spore forming ones. The number of molds was not significant. The nitrate reducers and N_2 — fixing bacteria were not detected. About 50% of C and N were volatilized during three weeks. Some parts of $N - NH_3$ was oxidized to $N - NO_3$ in surface layer of the soil. The content of P increased during degradation of penguin excrements. About 1/3 part of total organic carbon content in bird excrements residues was derived from chitin.

Key words: Antarctic, penguin excrements, chemical composition, bacterial degradation, enzymatic activity

1. Introduction

Admiralty Bay (62 09'S, 58 28'W) of King George Island belongs to the coastal zone and off-shore islands of Antarctic (the "maritime Antarctic" of Holdgate 1964). This region has long rocky and sandy-gravel shores with variety of plants and animal life (Rakusa-Suszczewski 1980). The summer air and soil temperatures are higher and there is a great deal of precipitation (Nowosielski 1980, Zubek 1980).

The penguin rookeries play an important role in organic matter circulation between sea and lowland. In these regions the birds, and especially penguins,

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are one of main sources of organic matter for lowland ecosystems (Rakusa-Suszczewski 1980), which induces the soil formation (Syroečkovskij 1959). In the subantarctic region the role of penguin excrements was reported as a highly concentrated N and P fertilizer for plant associations (Smith 1978, 1979). The nutrients from bird rookeries could also influence the life of coastal waters (Golovkina and Pozdniakova 1964).

The rookeries of Adelie *Pygoscelis adeliae* (Hombron et Jacquinot), chinstrap *P. antarctica* (Forster) and Gentoo *P. papua* (Forster) penguins are frequent at Admiralty Bay (Jabłoński in press) and therefore they could be used for researches on penguin excrements decomposition.

2. Materials and methods

Materials for penguin excrements analyses.

The fresh Adelie penguin excrements were collected from the surface of the snow or stones. It was possible to divide it into the following natural fractions: "red" fraction containing fragments of indigested krill, "green" fraction containing probably indigested contents of the food of krill and "white" fraction of colloidal solution. 100 g of fresh mixture of penguin excrements was shaken with 500 ml volume of distilled water for 60 min. on a reciprocating shaker (40 round per minute). This suspension was filtered through the nylon sieve (60 μm) and the fraction obtained was centrifugated at $8000 \times g$ for 10 min.

Materials for laboratory experiments.

Adelie penguins excrements were collected from the surface of the snow three days after snowfall. Five grams of mineral soil was added as a bacterial inoculum for each 100 g of excrements. This soil was taken from 0—10 cm layer of mineral soil out of the rookeries. Such a mixture was assumed as two-day-old sample. A single sample of fresh penguin excrements was taken for microbiological analyses at "zero" time. This mixture of excrements was divided into two parts. One part was incubated at room temperature about +16 C. The second part was incubated in open air (shaded place) in temperature range of −1.5 C to +5.7 C (+2.3 C average). The experiment was carried out with three replications. 10 g samples were taken aseptically from each replication of experiment for analyses after 2, 6 and 21 days of incubation.

Material for field research.

Besides the laboratory experiment, several samples were collected to study the differences in character and degree of mineralization under field conditions during austral summer 1979/1980 from the following areas: Thomas Point, Llano Point and Chabrier Rock at Admiralty Bay. The 5—7 randomly selected 10—15 g samples were taken at each testing place

and brought to the laboratory in sterile glass bags. The following samples were collected:

— sample 1 — mixture of penguin excrements from inhabited rookery of Adelie penguins at Thomas Point,

— sample 2 — mixture of penguin excrements from inhabited rookery of Adelie penguins at Llano Point (Rescuers Hills),

— sample 3 — the flow of suspension of penguin excrements over the steep rocks from inhabited rookery of Antarctic penguins at Chabrier Rock,

— sample 4 — the flow of suspension of penguin excrements over the gentle slope from inhabited rookeries of the Adelie and chinstrap penguins about 10—15 m out of the nests at Llane Point,

— sample 5 — the above described flow but 50—70 m out of the nests,

— sample 6 — the organic-mineral sediment on the beach at Llano Point. This sediment was being formed in the small reservoirs of water which was flowing down together with excrements from rookeries.

Chemical analyses.

Mineral and organic compounds were determined in the samples from laboratory experiment and in the field samples. The content of carbohydrates was determined with phenolsulphuric reagent according to Dowgiałło (unpublished data). The content of chitin was determined as glucosoamine content after acid hydrolase according to Więckowska (1968). The content of uric acid was determined according to Tinsley and Nowakowski (1957).

Total nitrogen and phosphorus were determined after decomposition of sample in sulphuric acid with some drops of perhydrol according to Schillak (1966). Ammonia in water was determined by indophenol method (Solorzano 1962), N/NO_2 and N/NO_3 (after reduction to N/NO_2 on Cd column) by napthylethylene diamine method according to procedure recommended by the Smithsonian Institution (1978) in Laboratory guide for nutrient analysis of water, soil and plant material, P — by molybdenum blue method.

Rate of volatilization of N/NH_3 was measured after exposure of incubated sample for one hour in a closed small container together with a flat vessel with diluted sulfuric acid as a trap for ammonia. N/NH_4 was determined by Nessler method.

Change in weight, ash and water contents were controlled every time. Calculation of change in total content of nutrients in sample during exposition included assumption that weight and content of nutrients in added mineral soil were constant.

Enzymatic analyses.

The soil samples were dried at room temperature (+20 C), ground and

sifted through 1 mm sieve. The samples from laboratory experiments and from rookeries were suspended in saline solution. 1 gram or 1 ml from total sample was taken in three replications to measure the enzymatic activity. Some drops of toluene were added to stabilize the population of microorganisms. Activity of the following enzymes were determined:

— gelatinase activity according to Hoffman and Teicher (1959);

— asparaginase and uric acid hydrolases activity analogically to urease determination by Hoffman and Teicher (1961);

— acid and alkaline phosphatases activity according to Kramer and Erdei (1959).

Microbiological analyses.

Enumeration of different physiological groups of bacteria were estimated using dilution plate or tube methods. The following media were used:

— semi-liquid pepton yeast extract medium (PEM) for counting the total number of bacteria (Widen 1977),

— gelatin agar for counting the number of proteolitic bacteria (Harrigan and McCance 1966),

— chitin agar (2.5%) with 25% of Taylor's soil extract from rookery for counting the number of chitin degrading bacteria,

— Mienkina's lecithin agar for counting the number of organic phosphorus degrading bacteria (Paluch 1973),

— Banata and Rovina's medium for counting the number of $Ca_3(PO_4)_2$ dissolving bacteria (Paluch 1973),

— Rougieux and Girard's liquid medium with L-asparagine or uric acid for counting the number of ammonifying bacteria (Paluch 1973),

— nitrate formation medium for counting the number of nitrifying bacteria (Harrigan and McCance 1966),

— Valery and Alexander's solid medium for counting the number of nitrate reducers in anaerobic conditions (Paluch 1973),

— PEM for counting the number of spore-forming bacteria after heat treatment of the suspensions at 80°C for 15 min.,

— Burk's agar for counting the number of N_2-fixing bacteria (Paluch 1973).

Additionally, the number of moulds was determined on rose bengal agar (Harrigan and McCance 1966).

Plates and tubes were incubated at +18°C during 3—21 days. All enumerations were calculated per 1 g of dry soil without stones and gravel ($\geqslant$1 mm). Biological and chemical analyses were done at the Arctowski Station of the Polish Academy of Sciences.

3. Results and discussion

Characteristics of penguin excrements.

The krill (*Euphausia superba* Dana) are mainly food of Adelie, Antarctic

Table I

The chemical composition of krill (*Euphausia superba* Dana) and different fractions of penguin excrements (% of dry weight)

Sample	Nutrients						Organic compounds		
	C	N	P	C/N	C/P	P/N	Uric acid	Chitin	Carbo-hydrat.
Krill	34.2	10.6	1.76	3.2	18.9	0.17	0.0	5.6	1.6
Natural fractions of excrements:									
— "red"	—*)	9.55	2.45	—	—	0.26	0.0	65.8	—
— "white"	—	27.22	0.12	—	—	0.004	81.0	0.0	0.0
— "green"	—	9.90	0.86	—	—	0.09	0.0	0.0	—
Mechanical fractions of excrements:									
— ⩾ 60 μm	—	5.71	1.60	—	—	0.28	0.0	95.7	2,5
— < 60 μm	—	6.98	2.80	—	—	0.40	—	15.6	5.4
— soluble	—	25.40	4.40	—	—	0.17	18.1	0.0	—

*) not determined

and Gentoo penguins in the Admiralty Bay region (Volkman, Presler and Trivelpiece 1980). After digestion, the faeces are excreted as heterogenic material. The "white" fraction of uric acid contained the "red" fraction of krill exoskeleton with high level of chitin (65.8%) and phosphorus (2.45%) (Table I). The level of nitrogen (27.2%) in "white" fraction was similar to its content in the pure uric acid. The level of nitrogen at "red" fraction was similar to that in the chitin. The "green" fraction contained the indigested cells of algae with high level of nitrogen (9.90%) (Table I).

The 35% of total weight of penguin excrements was found in mechanical fraction with particles larger than 60 μm. The chitin (95.7%) was the predominant compound in this fraction (Table I). The second fraction was material with particles smaller than 60 μm, which constitute 45% of total weight of excrements. This fraction had some parts of mechanically desintegrated exoskeleton of krill which was showed by the presence of chitin (15.6%), high level of P (2.80%) probably as unsoluble organic or organic-mineral substances as well as carbohydrates (5.60%) probably from indigested cell walls of algae (Table I). The rest of excrements (20%) after mechanical separation was dissolved. About 50% of total nitrogen and about 30% of total phosphorus from excrements was found in this soluble fraction (Table I). This fraction was most easily washed away by water from rookeries to coastal waters. A high percentage of soluble fraction of organic and mineral compounds in excrements of Arctic birds was found by Galkina (1974).

The saprophytic, proteolitic and spore-forming bacteria were found in fresh excrements of Adelie penguins (Table II). Our results are confirmed

Table II

The number of different groups of microorganisms in penguin excrements during incubation (cells per 1 g of dry weight)

Time of incubation	Total number	Proteo-litic	Ammonifying		Nitri-fying	Chitin degrad.	Lecithin degrad.	$Ca_3/PO_4/_2$ dissol.	Spore-forming	Moulds
			L-asparag.	Uric acid						
0 day	0.4×10^6	0.1×10^6	0	0	0	0	0	0	10×10^3	0
2 days	40×10^6	12×10^6	0.1×10^6	2×10^3	4	18×10^3	12×10^3	80×10^3	35×10^3	7×10^3
6 days:										
— on open air	10×10^6	2×10^6	1.0×10^6	9×10^3	2	250×10^3	30×10^3	90×10^3	7×10^3	4×10^3
— at + 16°C	20×10^6	0.3×10^6	0.1×10^6	9×10^3	3	80×10^3	27×10^3	180×10^3	2×10^3	5×10^3
21 days:										
— on open air	14×10^6	0.2×10^6	0.1×10^6	2×10^3	12	9×10^3	6×10^3	80×10^3	10×10^3	2×10^3
— at + 16°C	16×10^6	0.1×10^6	30×10^3	300	4	113×10^3	5×10^3	18×10^3	2×10^3	11×10^3

by Margini and Castrelos (1963), who isolated spore-forming bacteria from Adelie penguins. The presence of spore-forming bacteria in excrements of Adelie penguins and absence of these bacteria in guano from rookery of Gentoo penguins (Boyd W. L., Rothenburg and Boyd J. W. 1970) is difficult to explain.

Mineralization of excrements in the laboratory experiments.

The first stage of mineralization of the tested excrements was observed in the laboratory. The liquefaction was observed at first, very intensive stage of mineralization, after 5—6 days at +16°C, but after 20 days on open air (+2.3 C average). This process was connected with bacterial and enzymatic activity. The first stage of mineralization was done by the specific proteases (determined as gelatinase activity) excreted with dejections (Table III).

Table III

Changes of the enzymatic activities during incubation of penguin excrements (per 1 g of dry weight during 24 hours at +10°C)

Time of incubation	Gelatinase mg $N\text{–}NH_2$	Hydrolases		Phosphatases	
		Asparaginase mg $N\text{–}NH_3$	Uric acid hydrolases mg $N\text{–}NH_3$	Acid mg P	Alkaline mg P
0 day	124.0	0.07	0.00	0.26	0.56
2 days	31.2	3.29	2.23	0.35	0.62
6 days:					
— in open air	24.1	22.05	9.07	5.80	37.80
— at +16°C	11.1	11.35	3.70	2.10	18.52
21 days:					
— in open air	12.7	5.17	0.00	0.28	2.95
— at +16°C	2.3	0.23	0.00	0.07	1.47

The number of proteolitic, lecithin degrading and chitin degrading bacteria (Table II) and activity of gelatinase, acid and alkaline phosphatases (Table III) were lower at +16°C than in open air, which suggested that organic macromolecules were already degradated in high percentage. These processes changed physical properties of excrements. The volatilization of $C\text{–}CO_2$ was about four times higher at +16 C than in open air (Fig. 1). It was correlated with total number of saprophytic bacteria (Table II), which utilized simple organic compounds coming from degradation of macromolecules. Mineralization of organic nitrogen to $N\text{-}NH_3$ was observed from the first days of experiment. Some part of formed ammonia was fixed by organic colloids or was dissolved in solution and the rest of it was volatilized (Fig. 2). The level of fixed ammonia was similar in both temperatures but its volatilization was about four times higher at +16 C (Fig. 2).

The microbiological mineralization of organic nitrogen was tested as the number of ammonifying bacteria (asparagine or uric acid) and as activities

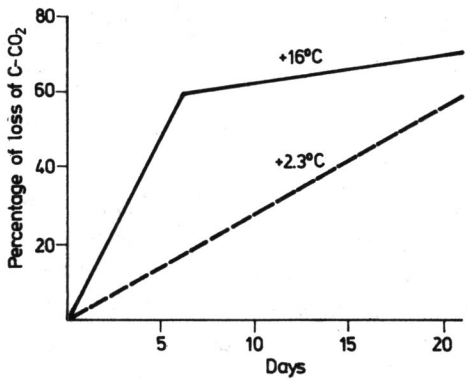

Fig. 1. The rate of loss of C–CO_2 from penguin excrements incubated in different temperatures during laboratory experiment

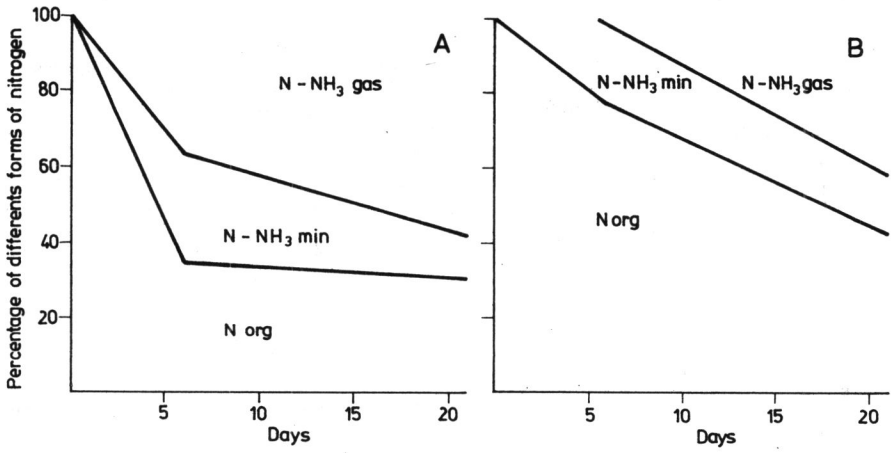

Fig. 2. Changes of proportions of nitrogen forms in the penguin excrements incubated in different temperatures during laboratory experiment

A — temperature +16°C, B — temperature +2.3°C, N–NH_3 gas — the volatilized ammonium to atmosphere, N-NH_3 min — the water soluble ammonium, N org — the organic nitrogen

of asparagine and uric acid hydrolases. The number of these bacteria and their enzymatic activities was similar or lower at +16°C than in open air (Table II and III). It was confirmed by measurement of N–NH_3 volatilization, which pointed to more than 50% of mineralization of organic nitrogen before 6 day at higher temperature (Fig. 2). Similar percentage of organic nitrogen mineralization in open air was found after 20 days. The analyses of degradation of uric acid could suggest its utilization as a second source of nitrogen for bacteria. Nevertheless, the process of mineralization was more intensive at +16°C than in open air, the level of tested parameters was similar at the end of experiment (Table II). These results

could suggest correlation between degradation of chitin and the presence of simple organic compounds, which induce this process and smaller influence of temperature. The level of C and N decreased about twofold, and P concentration increased about twofold after 21 days of penguin excrement incubation at both temperatures (Table IV). The chitin (6.9% — 8.5%) contained about 1/3 part of total organic carbon in residiues of excrements after incubation (Table IV).

Mineralization of penguin excrements under field conditions.

The excrements are fixed continually with semi-liquid organic mass of older faeces by walking penguins (samples 1 and 2). Such mixtures showed

Table IV

The chemical composition of penguin excrements from laboratory experiment (% of dry weight)

Sample	Nutrients						Organic compounds		
	C	N	P	C/N	C/P	P/N	Urid acid	Chitin	Carbo-hydrat.
Fresh excrements — "0" day	28.0	10.35	2.55	2.6	11.0	0.25	10.4	32.1	5.1
After 21 days of incubation:									
— at + 16°C	13.8	5.80	4.47	2.4	3.1	0.77	0.0	6.9	0.0
— in open air	14.2	6.63	3.40	2.1	4.2	0.51	0.0	8.5	—

Table V

The content of water extractable nutrients in differents field samples of penguin excrements (% of dry weight)

Samples	Nitrogen			$P_{orto.}$	$\frac{N_{min.}}{P_{orto.}}$	$\frac{N_{min.}}{N_{tot.}}$	$\frac{P_{orto.}}{P_{tot.}}$
	NH_3	NO_3	NO_2				
1. Mixture of excrements Thomas Point	3.25	0.001	0.001	1.07	3.0	47	23
2. Mixture of excrements Llano Point	2.65	0.001	0.001	1.16	2.3	40	28
4. Flow of suspension (10—15 m from rookery) Llano Point	0.30	0.001	0.004	0.60	0.51	6.4	11
5. Flow of suspension (50—70 m from rookery) Llano Point	0.34	0.030	0.009	0.79	0.48	13	16
6. Organic-mineral sediment Llano Point	0.01	0.017	0.001	0.09	0.30	1.6	3.5

Table VI

The chemical composition of differents field samples of penguin excrements (% of dry weight)

Samples	nutrients							organic compounds			
	C	N	P	C/N	C/P	P/N	H_2O	Ash 550°C	Uric acid	Chitin	Carbo-hydrat.
1. Mixture of excrements Thomas Point	15.1	6.88	4.56	2.2	3.3	0.66	17.0	48	10.4	3.2	3.7
2. Mixture of excrements Llano Point	18.1	5.56	4.16	2.2	4.3	0.63	70.0	32	—*	2.8	3.3
3. Flow of suspension Chabrier Rock	20.3	6.79	7.17	3.0	2.8	1.06	81.0	—	—	7.8	1.2
4. Flow of suspension (10—15 m from rookery) Llano Point	17.4	4.80	5.40	3.6	3.2	1.13	75.0	48	—	6.9	2.5
5. Flow of suspension (50—70 m from rookery) Llano Point	12.7	2.88	4.88	4.4	2.6	1.69	54.0	66	0.0	7.9	3.1
6. Org.-mineral sediment Llano Point	6.6	1.68	2.60	3.9	2.5	1.55	50.0	76	0.0	3.8	3.7

*) — not determined

significant degree of degradation because a lot of nutrients could be extracted by water (Table V). Additionally, the concentrations of C and N were lower and P content as well as chitin content were higher in comparison with fresh excrements (Table IV and VI). In these samples the level of ash was 32%—48% and indicated to the "dilution" of organic matter by mineral fraction. The level of phosphorus in the rookeries was comparable with determinations reported by several authors (Boyd W. L., Rothenburg and Boyd J. W. 1970, Smith 1978, 1979).

The high number of saprophytic bacteria — $30-42 \times 10^6$ and proteolitic bacteria — $0.7—0.8 \times 10^6$ (Table VII) as well as gelatinase and phosphatases activities (Table VIII) were also pointing to the significant stage of mineralization. The number of bacteria was smaller than that reported by Boyd W. L., Rothenburg and Boyd J. W. (1970). The high number of calcium phosphates dissolving bacteria was very characteristic in organic matter from rookeries (Table VII). The processes of calcium phosphates dissolution could give possibilities for P migration into soil profiles as well as outside rookeries with water.

The next degree of mineralization was observed after washing away the organic matter from rookeries as a flow of suspension (samples 3, 4 and 5). Chemical composition of these samples was similar to materials which were obtained after laboratory incubation (Table IV and VI). The level of P (4.88%—7.17%) and chitin (6.9%—7.9%) were significantly higher than in the samples from rookeries (Table VI). In these samples the higher number of chitin, lecithin and uric acid degrading bacteria was found (Table VII) as well as the higher activities of both tested phosphatases and uric acid hydrolases (Table VIII).

The ratios C/N and P/N were higher in amorphous flows of organic matter from rookeries (Table VI), which pointed to the dissolution of nitrogen compounds by water. These results could suggest the utilization of P-organic substances, uric acid and chitin at second stage of mineralization after partial disappearance (consumption by microorganisms, wash away by water) of simple organic compounds from excrements. The significantly higher content of nitrogen was found in a sample of suspension flow from rookery of chinstrap penguins (Chabrier Rock — sample 3) which was transported over the steep rocks without mixing with mineral fraction of the soil (Table VI). Chemical composition and biological activity of this sample was similar to samples from rookeries (Table VI and VII). However, the content of P and chitin pointed to a higher stage of mineralization. The lowest nitrogen content (2.88% and 1.68%) and the highest C/N and P/N ratios were found in material after long transport over gentle slope (sample 5) and in organic sediment on the beach, which was formed during stagnation of water flowing down from rookeries (sample 6). Materials in this degradation stage were decomposed by microorganisms slowly, which was evidenced by the small number of saprophytic bacteria (Table VII), low

Table VII

The number of different physiological groups of microorganisms in field samples of penguin excrements (cells per 1 g of dry weight)

Samples No.	Total number	Proteolitic	Ammonifying		Nitrifying	Chitin degrad.	Lecithin degrading	$Ca_3(PO_4)_2$ dissolving	Spore forming	Moulds
			L-asparag.	Uric acid						
1. Mix. of excrements Thomas Point	42×10^6	0.8×10^6	73×10^3	3×10^3	50	28×10^3	18×10^3	0.9×10^6	24×10^3	870
2. Mix. of excrements Llano Point	30×10^6	0.7×10^6	86×10^3	1×10^3	80	—	24×10^3	0.5×10^6	26×10^3	590
3. Flow of suspension Chabrier Rock	39×10^6	1.9×10^6	3×10^6	21×10^3	50	170×10^3	280×10^3	5.8×10^6	310×10^3	2×10^3
4. Flow of suspension (10—15 m from rookery) Llano Point	11×10^6	1.5×10^6	4×10^6	36×10^3	190	48×10^3	120×10^3	0.2×10^6	11×10^3	2×10^3
5. Flow of suspension (50—70 m from rookery) Llano Point	1.9×10^6	0.1×10^6	0.1×10^6	29×10^3	260	—	8×10^3	0.2×10^6	7×10^3	22×10^3
6. Org.-mineral sediment Llano Point	0.1×10^6	700	8×10^3	10×10^3	200	2×10^3	400	700	400	3×10^3

could suggest correlation between degradation of chitin and the presence of simple organic compounds, which induce this process and smaller influence of temperature. The level of C and N decreased about twofold, and P concentration increased about twofold after 21 days of penguin excrement incubation at both temperatures (Table IV). The chitin (6.9% — 8.5%) contained about 1/3 part of total organic carbon in residiues of excrements after incubation (Table IV).

Mineralization of penguin excrements under field conditions.

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Table IV

The chemical composition of penguin excrements from laboratory experiment (% of dry weight)

Sample	Nutrients						Organic compounds		
	C	N	P	C/N	C/P	P/N	Urid acid	Chitin	Carbo-hydrat.
Fresh excrements — "0" day	28.0	10.35	2.55	2.6	11.0	0.25	10.4	32.1	5.1
After 21 days of incubation:									
— at +16°C	13.8	5.80	4.47	2.4	3.1	0.77	0.0	6.9	0.0
— in open air	14.2	6.63	3.40	2.1	4.2	0.51	0.0	8.5	—

Table V

The content of water extractable nutrients in differents field samples of penguin excrements (% of dry weight)

Samples	Nitrogen			P_{orto}	$\frac{N_{min.}}{P_{orto.}}$	$\frac{N_{min.}}{N_{tot.}}$	$\frac{P_{orto.}}{P_{tot.}}$
	NH_3	NO_3	NO_2				
1. Mixture of excrements Thomas Point	3.25	0.001	0.001	1.07	3.0	47	23
2. Mixture of excrements Llano Point	2.65	0.001	0.001	1.16	2.3	40	28
4. Flow of suspension (10—15 m from rookery) Llano Point	0.30	0.001	0.004	0.60	0.51	6.4	11
5. Flow of suspension (50—70 m from rookery) Llano Point	0.34	0.030	0.009	0.79	0.48	13	16
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The chemical composition of differents field samples of penguin excrements (% of dry weight)

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	C	N	P	C/N	C/P	P/N	H_2O	Ash 550°C	Uric acid	Chitin	Carbo-hydrat.
1. Mixture of excrements Thomas Point	15.1	6.88	4.56	2.2	3.3	0.66	17.0	48	10.4	3.2	3.7
2. Mixture of excrements Llano Point	18.1	5.56	4.16	2.2	4.3	0.63	70.0	32	—*	2.8	3.3
3. Flow of suspension Chabrier Rock	20.3	6.79	7.17	3.0	2.8	1.06	81.0	—	—	7.8	1.2
4. Flow of suspension (10—15 m from rookery) Llano Point	17.4	4.80	5.40	3.6	3.2	1.13	75.0	48	—	6.9	2.5
5. Flow of suspension (50—70 m from rookery) Llano Point	12.7	2.88	4.88	4.4	2.6	1.69	54.0	66	0.0	7.9	3.1
6. Org.-mineral sediment Llano Point	6.6	1.68	2.60	3.9	2.5	1.55	50.0	76	0.0	3.8	3.7

*) — not determined

significant degree of degradation because a lot of nutrients could be extracted by water (Table V). Additionally, the concentrations of C and N were lower and P content as well as chitin content were higher in comparison with fresh excrements (Table IV and VI). In these samples the level of ash was 32%—48% and indicated to the "dilution" of organic matter by mineral fraction. The level of phosphorus in the rookeries was comparable with determinations reported by several authors (Boyd W. L., Rothenburg and Boyd J. W. 1970, Smith 1978, 1979).

The high number of saprophytic bacteria — $30-42 \times 10^6$ and proteolitic bacteria — $0.7—0.8 \times 10^6$ (Table VII) as well as gelatinase and phosphatases activities (Table VIII) were also pointing to the significant stage of mineralization. The number of bacteria was smaller than that reported by Boyd W. L., Rothenburg and Boyd J. W. (1970). The high number of calcium phosphates dissolving bacteria was very characteristic in organic matter from rookeries (Table VII). The processes of calcium phosphates dissolution could give possibilities for P migration into soil profiles as well as outside rookeries with water.

The next degree of mineralization was observed after washing away the organic matter from rookeries as a flow of suspension (samples 3, 4 and 5). Chemical composition of these samples was similar to materials which were obtained after laboratory incubation (Table IV and VI). The level of P (4.88%—7.17%) and chitin (6.9%—7.9%) were significantly higher than in the samples from rookeries (Table VI). In these samples the higher number of chitin, lecithin and uric acid degrading bacteria was found (Table VII) as well as the higher activities of both tested phosphatases and uric acid hydrolases (Table VIII).

The ratios C/N and P/N were higher in amorphous flows of organic matter from rookeries (Table VI), which pointed to the dissolution of nitrogen compounds by water. These results could suggest the utilization of P-organic substances, uric acid and chitin at second stage of mineralization after partial disappearance (consumption by microorganisms, wash away by water) of simple organic compounds from excrements. The significantly higher content of nitrogen was found in a sample of suspension flow from rookery of chinstrap penguins (Chabrier Rock — sample 3) which was transported over the steep rocks without mixing with mineral fraction of the soil (Table VI). Chemical composition and biological activity of this sample was similar to samples from rookeries (Table VI and VII). However, the content of P and chitin pointed to a higher stage of mineralization. The lowest nitrogen content (2.88% and 1.68%) and the highest C/N and P/N ratios were found in material after long transport over gentle slope (sample 5) and in organic sediment on the beach, which was formed during stagnation of water flowing down from rookeries (sample 6). Materials in this degradation stage were decomposed by microorganisms slowly, which was evidenced by the small number of saprophytic bacteria (Table VII), low

Table VII

The number of different physiological groups of microorganisms in field samples of penguin excrements (cells per 1 g of dry weight)

Samples No.	Total number	Proteolitic	Ammonifying		Nitrifying	Chitin degrad.	Lecithin degrading	$Ca_3(PO_4)_2$ dissolving	Spore forming	Moulds
			L-asparag.	Uric acid						
1. Mix. of excrements Thomas Point	42×10^6	0.8×10^6	73×10^3	3×10^3	50	28×10^3	18×10^3	0.9×10^6	24×10^3	870
2. Mix. of excrements Llano Point	30×10^6	0.7×10^6	86×10^3	1×10^3	80	—	24×10^3	0.5×10^6	26×10^3	590
3. Flow of suspension Chabrier Rock	39×10^6	1.9×10^6	3×10^6	21×10^3	50	170×10^3	280×10^3	5.8×10^6	310×10^3	2×10^3
4. Flow of suspension (10—15 m from rookery) Llano Point	11×10^6	1.5×10^6	4×10^6	36×10^3	190	48×10^3	120×10^3	0.2×10^6	11×10^3	2×10^3
5. Flow of suspension (50—70 m from rookery) Llano Point	1.9×10^6	0.1×10^6	0.1×10^6	29×10^3	260	—	8×10^3	0.2×10^6	7×10^3	22×10^3
6. Org.-mineral sediment Llano Point	0.1×10^6	700	8×10^3	10×10^3	200	2×10^3	400	700	400	3×10^3

Table VIII

The enzymatic activities of field samples of penguin excrements from Llano Point (per 1 g of dry weight during 24 hours at + 10°C)

No. samples	Hydrolases			Phosphatases	
	Gelatinase mg $N-NH_2$	asparaginase mg $N-NH_3$	uric acid hydrolases mg $N-NH_3$	Acid mg P	Alkaline mg P
2. Mixture of excrements	4.8	5.46	trace	0.26	0.56
5. Flow of suspension (50—70 m from rookery)	2.9	2.06	0.03	3.50	6.15
6. Organic-mineral sediment	0.5	0.22	0.23	0.17	0.31

enzymatic activities (Table VIII) and low level of extractable nutrients (Table V). Only the number of chitin degrading bacteria and uric acid ammonifying bacteria as well as uric acid hydrolases activity were significant (Table VII and VIII). The 3.8% of chitin in organic sediment contained about 1/3 part of total organic carbon (Table VI).

The number of nitrifying bacteria (200—250) and the content of $N-NO_3$ (0.030% and 0.017%) determined in samples 5 and 6 showed the possibilities of ammonium oxidation after mineralization of simple organic substances. The level of $N-NO_3$ was lower than reported by Boyd W. L., Rothenburg and Boyd J. W. (1970) in Gentoo penguins guano. In any samples the N_2-fixing and nitrate reducing bacteria were not found. The number of moulds increased during degradation of excrements.

4. Conclusion

The excrements of penguins in "maritime" Antarctic were degradated microbiologically. About 50% of C and N was volatilized during three weeks. The mineralization of nitrogen compounds were observed as effect of activity of enzymes from digestive system of penguins (proteases) and under influence of bacteria (proteolitic and ammonifying). The uric acid could be degradated totally in the second stage of mineralization. The chitin was found to be partially stable under natural conditions on the field and at laboratory experiment. About 1/3 part of total organic carbon in birds excrements residues consisted of chitin. Ammonium was partially oxidized to nitrates at the organic horizon of soils. Mineralization of P-organic substances was intensive in rookeries and in the first stage of transport of organic matter to the sea. The calcium phosphates dissolving bacteria were more frequent in the materials with high content of organic compounds.

5. Резюме

Были проведены исследования химического состава, энзиматической активности, а также численности разных физиологических групп бактерий в процессе разложения экскрементов пингвинов из рода *Pygoscelis* в районе залива Адмиралти на острове Кинг Джордж. Химический анализ как собранных в окружающей местности естественных фракций, так и фракций механически выделенных из смеси экскрементов пингвинов Алели, показал значительную концентрацию фосфора и хитины по сравнению с употребляемой пищей (таблица I). Основную массу твердой т. наз. „красной" фракции экскрементов, содержащей фрагменты больше чем 60 μм, составляет хитина (таблица I). Около 50% общего количества азота и около 30% общего количества фосфора содержится в 20% общей растворимой в воде массы (таблица I).

В результате исследования минерализации экскрементов в лабораторных условиях, а также на основании проб, собранных в окружающей местности, было установлено значительное участие сапрофитических бактерий активных в деградации разных компонентов пингвиньих экскрементов: протеолитические, разлагающие хитину, разлагающие лецитин, аммонификаторы (использующие аспарагин или мочевую кислоту), а также бактерии, растворяющие $Ca_3(PO_4)_2$. Кроме того было обнаружено присутствие нитрификаторов и плесневых грибов (таблицы II и VII). Денитрификаторы и бактерии, соединяющие свободный азот, отсутствовали.

Исследовалась также энзиматическая активность: аспарагиназы, гидролаз мочевой кислоты, кислой и щелочной фосфатаз (таблицы III и VIII).

Разложение белков происходило на первом этапе под влиянием энзимов, выделяемых из пищевого тракта вместе с экскрементами (протеазы), а также под влиянием протеолитических бактерий (таблицы II и III). Вторым этапом минерализации органических соединений азота (аминокислот и мочевой кислоты) является бактерийная аммонификация, ведущая к возникновению свободного аммиака, который в количестве, превышающем 50% улетучивался в атмосферу в течение первых трех недель (рис. 2). Одновременно в то же самое время улетучивалось ок. 50% общего количества углерода (рис. 1). Была обнаружена возможность окисления аммиака до формы нитратов в поверхностных слоях грунта после минерализации и выщелачивания простых органических соединений (таблицы V и VII).

В остальной части пингвиньих экскрементов установлено повышенное содержание фосфора и хитины как результат микробиологической минерализации и выщелачивания простых органических соединений, растворимых в воде (таблицы IV, V, VI). Органический углерод хитины составлял 1/3 оставшегося количества углерода в остатках экскрементов как в полевых, так и в лабораторных условиях.

6. Streszczenie

Przeprowadzono badania składu chemicznego, aktywności enzymatycznej oraz liczebności różnych grup fizjologicznych bakterii w trakcie procesów rozkładu odchodów pingwinów z rodzaju *Pygoscelis* w rejonie Zatoki Admiralicji na wyspie Króla Jerzego. Analiza chemiczna zebranych w terenie frakcji naturalnych jak i rozdzielonych mechanicznie z mieszaniny odchodów pingwinów Adeli wykazała znaczne zagęszczenie fosforu oraz chityny w porównaniu do pobieranego pokarmu (tabela I). Główną masę stałej, tzw. „czerwonej", frakcji odchodów, która pod względem mechanicznym zawiera fragmenty większe od 60 μm, stanowi chityna (tabela I). Około 50% całkowitej ilości azotu i około 30% całkowitej ilości fosforu zawarte jest w 20% ogólnej masy rozpuszczalnej w wodzie (tabela I).

W trakcie badań nad mineralizacją odchodów w warunkach laboratoryjnych jak i na podstawie próbek pobranych w terenie stwierdzono znaczny udział bakterii saprofitycznych

degradujących różne składniki odchodów: proteolityczne, degradujące chitynę, degradujące lecytynę, amonifikatory (wykorzystujące asparaginę lub kwas moczowy) oraz rozpuszczające $Ca_3(PO_4)_2$. Ponadto stwierdzono występowanie nitryfikatorów oraz grzybów pleśniowych (tabela II i VII). Nie stwierdzono obecności denitryfikatorów oraz bakterii wiążących wolny azot.

Badano również aktywność enzymatyczną: żelatynazy, asparaginazy, hydrolaz kwasu moczowego, fosfatazy kwaśnej i zasadowej (tabela III i VIII).

Rozkład białek następował w pierwszym etapie pod wpływem enzymów wydalanych z układu pokarmowego wraz z odchodami (proteazy) jak i pod wpływem bakterii proteolitycznych (tabela II i III). Drugim etapem mineralizacji organicznych związków azotu (aminokwasów i kwasu moczowego) jest amonifikacja bakteryjna powodująca powstawanie wolnego amoniaku, który w ilości przekraczającej 50% ulatniał się do atmosfery w okresie pierwszych trzech tygodni (rys. 2). Równolegle około 50% ogólnej ilości węgla ulatniało się w tym samym czasie (rys. 1). Stwierdzono możliwość utleniania amoniaku do azotanów w powierzchniowych warstwach gleb po zmineralizowaniu i wymyciu prostych związków organicznych tabela V i VII).

W pozostałych resztkach odchodów pingwinich stwierdzono zagęszczenie fosforu i chityny jako rezultat mikrobiologicznej mineralizacji i wymywania prostych związków organicznych rozpuszczalnych w wodzie (tabela IV, V i VI). Węgiel organiczny chityny stanowił aż 1/3 część pozostałej ilości węgla w resztkach odchodów zarówno w warunkach terenowych jak i w doświadczeniach laboratoryjnych.

7. References

1. Boyd W. L., Rothenburg I., Boyd J. W. 1970 — Soil microorganisms at Paradise Harbour, Antarctic — Ecology, 51: 1040—1045.
2. Galkina W. N. 1974 — O himičeskom sostave rastvorimych veščestv ekskrementov morskih rybojadnych ptic — Ekologija, 5: 23—28.
3. Golovkina A. N., Pozdniakova L. E. 1964 — Vlijanie morskih kolonialnyh ptic na režim biogennyh elementov v priberežnyh vodah vostočnogo Murmana — Tr. Murmanskogo Morsk. Biol. In-ta, 6, 10: 42—55.
4. Harrigan W. F., Mc Cance M. E. 1966 — Laboratory methods in microbiology — Academic Press, London — New York.
5. Hoffman G., Teicher K. 1959 — Das Enzymsystem unserer Kulturboden. VII. Proteasen — Z. Pflanzenernähr. Düng. Bodenkunde, 85: 193—197.
6. Hoffman G., Teicher K. 1961 — Ein Kolorimetrischer Verfahren zur Bestimmung der Urease-Aktivität im Boden — Z. Pflanzenernähr. Düng. Bodenkunde, 95: 55—61.
7. Holdgate M. W. 1964 — Terrestrial ecology in the maritime Antarctic — In: Biologie Antarctique, Eds. R. Carrick, M. W. Holdgate, J. Prevost Hermann, Paris: 181—194.
8. Jabłoński B. 1984 — Distribution, members and breeding preferences of penguins in the region of the Admiralty Bay (King George Island South Shetland Islands) in the season 1979/1980 — Pol. Polar Res., 5:
9. Kramer M., Erdei S. 1959 — Primenenie metoda opredelenia aktivnosti fosfatazy v agrotehničeskih issledovanijah — Počvovedenie, 9: 99—104.
10. Margini R. A., Castreolos O. D. 1963 — Exameenes bacteriologicos de aire, nieve y suelo de Cabo Primavera y Estacion Cientifica Ellsworth — Contrib. Inst. Antartico Argentino, no. 76: 1—15.
11. Nowosielski L. 1980 — Meteorological conditions at Arctowski Station in 1978 (South Shetland Islands) — Pol. Polar Res., 1: 83—93.
12. Paluch J. 1973 — Mikrobiologia wód — PWRiL, Warszawa.
13. Rakusa-Suszczewski S. 1980 — Environmental conditions and the functioning of

Admiralty Bay (South Shetland Islands) as a part of near shore antarctic ecosystem — Pol. Polar Res., 1: 11—27.
14. Schillak R. 1966 — Oznaczanie składników mineralnych w materiałach roślinnych. I. Mineralizowanie materiału roślinnego i roztwór podstawowy — Roczn. Nauk roln. A, 92, 265—283.
15. Smith V. R. 1978 — Animal — Plant — Soil nutrient relationships on Marion Island (Subantarctic) — Oecologia, 32: 239—253.
16. Smith V. R. 1979 — The influence of seabird manuring on the phosphorus status of Marion Island (Subantarctic) soils — Oecologia, 41: 123—126.
17. Solorzano L. 1962 — Determination of ammonia in natural water by the phenol-hypochlorite method — Limnol. Oceanogr., 14: 799—801.
18. Syroečkovskij E. E. 1959 — Rol životnyh v obrazovanii piervičnyh počv v usloviah pri-polarnych oblasti ziemnogo šara (na primere Antarktidy) — Zool. Ž., 38: 1770—1791.
19. Tinsley J., Nowakowski T. Z. 1957 — The determination of uric acid, particularlly in avian excrements — Analyst, 82: 110—116.
20. Volkman N. J., Presler P., Trivelpiece W. 1980 — Diets of pygoscelid penguins at King George Island, Antarctic — Condor, 82: 373—378.
21. Więckowska E. 1968 — Oznaczanie chityny na podstawie zawartości glukozoaminy — Chemia analit., 13: 1311—1317.
22. Widen P. 1977 — Microbial decomposition on Truelove Lowland. In: Truelove Lowland, Devon Island, Canada: A High Arctic Ecosystem, Ed I. C. Bliss — Univ. Alberta Press, 505—530.
23. Zubek K. 1980 — Climatic conditions at the Arctowski Station (King George Island, South Shetland Islands) in 1977 — Pol. Arch. Hydrobiol., 27: 235—244.

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