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Tytuł rozprawy w języku angielskim: **Biological samples derived from seabirds and Svalbard reindeer as a source of information on polar environment contamination**

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Spis treści

1. Wykaz publikacji stanowiących podstawę dysertacji

I. **Aneta Dorota Pacyna**, Marek Ruman, Jan Mazerski, Żaneta Polkowska, 2018. Biological responses into environmental contamination. How metal pollution may impact signal honesty in avian species? Ecology and Evolution 8, 7733-7739, DOI:10.1002/ece3.4192; IF:2,415; Q1

II. **Aneta Dorota Pacyna,** Katarzyna Koziorowska, Stanisław Chmiel, Jan Mazerski, Żaneta Polkowska, 2018. Svalbard reindeer as an indicator of ecosystem changes in the Arctic terrestrial ecosystem, Chemosphere 203, 209-218, DOI: 10.1016/j.chemosphere.2018.03.158; IF: 5,108; Q1

III. **Aneta Dorota Pacyna***, Marcin Frankowski, Krystyna Kozioł, Michał Hubert Węgrzyn, Paulina Wietrzyk-Pełka, Sara Lehmann-Konera, Żaneta Polkowska, 2019. Evaluation of the use of reindeer droppings for monitoring essential and non-essential elements in the polar terrestrial environment, Science of the Total Environment 658, 1209-1218, DOI: 10.1016/j.scitotenv.2018.12.232; IF: 5,589; Q1

IV. **Aneta Dorota Pacyna-Kuchta*,** Paulina Wietrzyk-Pełka, Michał Hubert Węgrzyn, Marcin Frankowski, Żaneta Polkowska, A screening of select toxic and essential elements and persistent organic pollutants in the fur of Svalbard reindeer, Chemosphere [przyjęty do druku]; IF: 5,108; Q1

V. **Aneta Dorota Pacyna***, Dariusz Jakubas, Anne N.M.A. Ausems, Marcin Frankowski, Żaneta Polkowska, Katarzyna Wojczulanis-Jakubas, 2019. Storm petrels as indicators of pelagic seabird exposure to chemical elements in the Antarctic marine ecosystem, Science of The Total Environment 692, 382-392; DOI: doi.org/10.1016/j.scitotenv.2019.07.137; IF: 5,589; Q1

VI. **Aneta Dorota Pacyna-Kuchta***, Dariusz Jakubas, Marcin Frankowski, Żaneta Polkowska, K. Wojczulanis-Jakubas, Exposure of a small Arctic seabird, the little auk

(*Alle alle*), to selected elements throughout the course of a year (w recenzji czasopisma Science of the Total Environment)

***autor korespondencyjny**

Udział w przygotowaniu publikacji:

Aneta Dorota Pacyna-Kuchta (autor główny oraz korespondencyjny)

o Koncepcja publikacji;

o Opracowanie części eksperymentalnej;

o Opracowanie danych;

o Udział w dyskusji;

o Przygotowanie wniosków i pierwszej wersji publikacji;

o Przygotowanie odpowiedzi po recenzjach;

o Podsumowanie.

Zaproszeni współautorzy do interpretacji międzydziedzinowej

- Wykonanie analizy statystycznej;
- Udział w tworzeniu koncepcji pracy i dyskusji wyników.

2. Wykaz skrótów używanych w pracy

Alfa-HCH- alfa-heksachlorocykloheksan (ang. α-Hexachlorocyclohexane)

AMAP- Programu Monitorowania i Oceny Arktyki (Arctic Monitoring and Assessment Programme)

CV – współczynnik zmienności, (ang. coefficient of variation)

CV-AAS- technika zimnych par z atomową spektrometrią absorpcyjną (ang. cold vapor atomic absorption spectrometry)

DCM – dichlorometan, (ang. dichloromethane)

DDD- dichlorodifenylodichloroetan (ang. dichlorodiphenyldichloroethane)

DDE- dichlorodifenylodichloroetylen (ang. dichlorodiphenyldichloroethylene)

DDT- dichlorodifenylotrichloroetan (ang. dichlorodiphenyltrichloroethane)

GC-MS/MS - chromatografia gazowa sprzężona z tandemowym spektrometrem mas (ang. gas chromatography – Triple Quadrupole Mass Spectrometer)

HCA- hierarchiczną analizą klastrów (ang. hierarchical cluster analysis)

HCB- heksachlorobenzen (ang. hexachlorobenzene)

HCl- kwas solny (ang. hydrochloric acid)

ICP-MS – spektrometria mas ze wzbudzeniem w plazmie indukcyjnie sprzężonej (ang. inductively coupled plasma – mass spectrometry)

ICP-OES - technika atomowej spektrometrii emisyjnej ze wzbudzeniem w plazmie indukowanej (ang. inductively coupled plasma optical emission spectrometry)

LOD – granica wykrywalności, (ang. limit of detection)

LOQ – granica oznaczalności, (ang. limit of quantification)

MRM- monitorowanie reakcji wielokrotnych (ang. Multiple Reaction Monitoring)

OCP- pestycydy chloroorganiczne (ang. organochlorine pesticides)

PCA – analiza głównych składowych, (ang. principal component analysis)

PCB – związki z grupy polichlorowanych bifenyli, (ang. polichlorinated biphenyls)

SPE- ekstrakcja do fazy stałej (ang. solid phase extraction)

WWA – związki z grupy wielopierścieniowych węglowodorów aromatycznych (ang. polycyclic aromatic hydrocarbons, PAHs)

3. Streszczenie

Zanieczyszczenie środowiska, może prowadzić do poważnych zmian ekosystemowych, wpływając na faunę oraz florę. Obszary polarne Arktyki oraz Antarktyki są cennym źródłem informacji o globalnym wpływie transgranicznego transportu zanieczyszczeń na środowisko. W ekosystemie polarnym mogą być akumulowane pierwiastki pochodzenia naturalnego i antropogenicznego, a także wiele zanieczyszczeń organicznych wytwarzanych na obszarach bardziej zurbanizowanych i transportowanych przez prądy powietrzne oraz morskie. W badaniach ekotoksykologicznych poziomy zanieczyszczeń są często analizowane w tkankach wewnętrznych różnych gatunków zwierząt. Jednak procedura pobrania próbek niesie ze sobą wątpliwości etyczne oraz problemy praktyczne takie jak odpowiednie warunki przechowania i transportu. Osobniki wykorzystywane w badaniach mogą nie być odpowiednio reprezentatywne dla losowego przekroju całej populacji.

Wykorzystanie nie-destrukcyjnie pobranych tkanek takich jak sierść oraz pióra umożliwia pobieranie próbek od żywych osobników, nie naruszając struktury populacji oraz kwestii moralnych związanych z odłowem. W związku z tym możliwe jest śledzenie zmian spowodowanych podwyższonym poziomem zanieczyszczeń. W ostatnich latach, wykorzystanie próbek pobranych nie-destrukcyjnie staje się istotną częścią badań środowiskowych. Poziomy zanieczyszczeń różnią się w zależności od rodzaju tkanki i ich zdolności do akumulacji związków, co ma wpływ na wiarygodny monitoring. Przykładowo tkanka skeratynizowana, taka jak pióro stanowi źródło informacji o elementach obecnych we krwi podczas jego wzrostu. Kiedy krew jest dostarczana do pióra, związki o wysokim powinowactwie do grup funkcyjnych keratyny wiążą się z wiązaniami disiarczkowymi zawartymi w strukturze pióra. Kiedy piórko jest w pełni uformowane, naczynie krwionośne zanika, i pióro zostaje odizolowane od dalszego pobierania związków chemicznych.

W badaniach będących podstawą rozprawy doktorskiej wykorzystane zostały próbki pobrane nie-destrukcyjnie od kluczowych gatunków polarnego ekosystemu morskiego oraz lądowego. Ekosystem morski jest reprezentowany przez trzy gatunki ptaków morskich: oceannika czarnobrzuchego i żółtopłetwego oraz alczyka. Ptaki morskie odgrywają szczególną role w środowisku, i są jednym z bardziej charakterystycznych części polarnego środowiska morskiego. Gatunki lądowe są rzadziej badaną, jednak szczególnie ważną częścią ekosystemu

polarnego. Renifer Svalbardzki jest jedynym dużym ssakiem roślinożernym zamieszkującym obszar Arktyki Europejskiej. Analizy wykonane zostały pod kątem obecności wybranych metali, nie-metali oraz metalloidów (wykonane przy zastosowaniu ICP-MS, ICP-OES, Bezpośredniego Analizatora Rtęci) głównie w tkankach skeratynizowanych (pióra oraz sierść) oraz dodatkowo w odchodach renifera oraz skorupkach powylęgowych alczyka. Próbki sierści dodatkowo przeanalizowano pod kątem obecności polichlorowanych bifenyli, wielopierścieniowych węglowodorów aromatycznych oraz pestycydów chloroorganicznych (przy zastosowaniu GC-MS/MS). Badania są dowodem, iż materiały biologiczne pobrane w sposób nie-destrukcyjny stanowią cenne źródło informacji w badaniach ekotoksykologicznych, i mogą być szczególnie użyteczne w przypadku chronionych gatunków polarnych.

4. Abstract

The constant emission of pollutants is undeniably a serious global problem and is considered a grave threat to ecosystem stability. Arctic and Antarctic areas are both invaluable sources of information on the global impact of the long-range transport of contaminants. The polar ecosystem may be exposed to an increased level of metals and metalloids of natural and anthropogenic origin, as well as a multitude of organic pollutants produced in more urbanised areas and transported on sea and air currents.

Contaminant levels are often reported as concentrations in internal tissues from randomly selected individuals that were either collected dead or sacrificed. Apart from ethical implications such a sample collection possibly carrying potentially unquantified biases. Such individuals may also not be representative of a random cross-section of the whole population. Non-lethally obtained samples allow individuals to be released after sample collection. Consequently, it is possible to collect samples from the same individuals over several seasons and thus to track changes caused by elevated contaminants levels. Therefore, the use of non-lethally collected samples is becoming a highly significant aspect of environmental studies.

The levels of contaminants vary according to tissue type and those tissues' propensities to bind or deposit compounds, which has implications for reliable monitoring. For example, feather represents a source of information about elements present in the blood during the time of the feather's growth. When blood is delivered to the feather, compounds with high affinity to sulfhydryl groups bond to disulfide bonds and are thus included in the structure. When the feather is fully grown the blood vessel disappears, elements are no longer deposited inside and the feather becomes isolated from any further element uptake.

In my thesis I am focusing on contaminant determination in several non-lethally collected samples from keystone species of the marine and terrestrial polar ecosystems. The marine environment is represented by seabirds: the little auk, the black-bellied storm petrel and Wilson's storm petrel. Seabirds play an important role in polar ecosystems, and are one of the most characteristic parts of polar marine life. Also, being high up in the trophic network, they are exposed to a broad array of environmental contaminants. Terrestrial species are often under-investigated and yet are crucial parts of any polar ecosystem. Reindeers are a key component of the Arctic terrestrial ecosystem, and Svalbard reindeer is the only large grazing mammal in the European High Arctic. Exposure assessment includes analysis of several

elements (analysed on ICP-MS, ICP-OES and Direct Mercury Analyser apparatus, mostly in keratinised tissues (feathers and reindeer fur) and additionally in reindeer dung and seabirds' residual eggshells. Additionally reindeer fur was analysed for polychlorinated biphenyls, polycyclic aromatic hydrocarbons and organochlorine pesticides (analysed on GC-MS/MS). The research presented so far provides evidence that non-lethally collected tissues can be a valuable source of information in ecotoxicological studies, and can be particularly useful for sampling tissues from protected polar species.

5. Wstęp

Ze względu na izolację geograficzną, surowy klimat, oraz ograniczoną lokalną działalność człowieka, obszary polarne przez długi czas były postrzegane jako miejsca niezanieczyszczone substancjami powstającymi w skutek procesów utożsamianych z rozwojem gospodarczym. Obecnie nasza wiedza na temat mechanizmów transgranicznego napływu zanieczyszczeń do środowiska polarnego jest dużo większa, o czym świadczą między innymi raporty środowiskowe Programu Monitorowania i Oceny Arktyki (AMAP, ang. Arctic Monitoring and Assessment Programme), podejmujące kwestie wpływu zanieczyszczeń dostających się do arktycznego łańcucha pokarmowego na organizmy morskie oraz lądowe [1-3].

Zanieczyszczenie środowiska, może prowadzić do poważnych zmian ekosystemowych. Zarówno raporty AMAP, jak i indywidualne badania naukowców sugerują znaczący wpływ transportu zanieczyszczeń z niższych szerokości geograficznych na poziomy pierwiastków o działaniu toksycznym oraz związków z grupy TZO wykrywanych na obszarach polarnych [1-6]. Problem ten dotyczy zarówno półkuli północnej (Arktyka Europejska, Alaska, Syberia), jak i południowej (Antarktyka). Zanieczyszczenia te stanowią dodatkowy czynnik stresowy, potencjalnie wpływający na zdrowie oraz szanse przetrwania zwierząt i w konsekwencji mogą wpłynąć na liczebność i kondycję populacji [7,8].

Informacji o stanie ekosystemu i zmianach zachodzących w środowisku pod wpływem związków toksycznych mogą dostarczyć organizmy biowskaźnikowe. Reprezentatywny organizm biowskaźnikowy powinien cechować się dużą liczebnością w badanym środowisku, mieć relatywnie długi okres życia oraz być istotnym ogniwem łańcucha pokarmowego. Ptaki morskie oraz duże ssaki roślinożerne spełniają podane kryteria i mogą być wykorzystane w celu oceny stanu środowiska w którym żyją. W badaniach ekotoksykologicznych nastawionych na ocenę ekspozycji osobników danego gatunku na związki toksyczne dominuje użycie tkanek wewnętrznych [9]. Zwłaszcza wysoka zawartość tłuszczu w wątrobie oraz nerkach oraz ich detoksykacyjna funkcja w organizmie, może skutkować wysoką zawartością związków uznawanych za toksyczne [10]. Jednak wykorzystanie tkanek wewnętrznych jest dyskusyjne etycznie (ze względu na pozyskiwanie tkanek z martwych zwierząt), jak i praktycznie (ze względu na konieczność przechowania oraz transportu próbek w chłodniczych warunkach). Próbki nie mogą być pozyskiwane od gatunków zagrożonych oraz nie powinny być pobierane w środku sezonu lęgowego, gdyż w przypadku gatunków podejmujących opiekę rodzicielską

wyeliminowanie jednego z rodziców może wpłynąć negatywnie na szanse przeżycia potomstwa. Badane osobniki są eliminowane z populacji, bez możliwości dalszego ich monitoringu. Z kolei osobniki znalezione już martwe, zwykle nie stanowią reprezentatywnej grupy dla oceny całej populacji.

W ostatnich latach popularność zyskuje użycie tkanek pozyskanych od osobników żywych, bez konieczności ich uśmiercania. Wykorzystanie tkanek skeratynizowanych umożliwia pobieranie próbek bezpośrednio od wytypowanych osobników, nie naruszając struktury populacji oraz kwestii moralnych związanych z odłowem. Tkanki skeratynizowane, takie jak pióra ptaków oraz sierść ssaków, mogą stanowić źródło informacji o zanieczyszczeniach przyjętych głównie z diety w okresie wzrostu tkanki [10,11]. Dodatkowo dzięki badaniu parametrów takich jak stałe izotopy azotu oraz węgla, zarówno pióra jak i sierść mogą być dodatkowo źródłem informacji o czasowych i przestrzennych zmianach w diecie, mogą pomóc w ustaleniu ścieżek migracji oraz wyboru siedliska [12-14]. Przechowywanie oraz transport tkanek skeratynizowanych nie wymaga warunków chłodniczych, co ułatwia procedurę analityczną [15]. Inne materiały biologiczne pobrane w sposób nie wymagający uśmiercenia obiektu badań stanowią między innymi krew oraz osocze, skorupki powylęgowe, wydzielinę z gruczołu kuprowego ptaków oraz odchody.

Wykorzystanie tkanek pobranych w sposób nie-destrukcyjny, jest coraz częściej wybieraną metodą analizy zawartości pierwiastków śladowych oraz trwałych związków organicznych [10,11,15]. Jednakże, w przypadku wielu gatunków zamieszkujących obszary polarne, aktualne wykorzystanie jest minimalne lub zerowe. W przypadku braku wiedzy na temat odpowiedzi fizjologicznej organizmu na zanieczyszczenia obecne w środowisku niemożliwe jest modelowanie dalszego losu populacji, która będzie narażona na coraz szybciej postępujące zmiany w ekosystemie polarnym. Poznanie aktualnych poziomów związków toksycznych w tkankach mogą posłużyć lepszemu zrozumienia odpowiedzi fizjologicznej organizmów polarnych na zwiększające się zanieczyszczenie środowiska.

6. Czynniki mające wpływ na depozycję zanieczyszczeń na obszarach polarnych

Intensyfikacja działalności człowieka oraz w konsekwencji emisja zanieczyszczeń na obszarach zurbanizowanych średnich oraz niskich szerokości geograficznych jest jednym z najważniejszych czynników wpływających na rodzaj oraz ilość zanieczyszczeń dostarczanych w

rejony polarne [2, 5, 16, 17]. Związki wykazujące właściwości lotne (np. związki rtęci oraz związki z grupy PCB lub WWA), mogą przemieszczać się wraz z masami powietrza z obszarów o klimacie umiarkowanym na północ, gdzie ulegają skropleniu w niższej temperaturze i przedostają się na ląd oraz obszary morskie. Proces ten nazywany jest ‘efektem konika polnego’ (ang. ‘grasshopper effect’) [18]. Substancje rozpuszczalne w wodzie mogą być transportowane na obszary arktyczne wraz z prądami morskimi, podobną drogę pokonuje tzw. brudny śnieg- dryfujące kry zawierające cząstki zdeponowane na lodzie podczas jego formacji [18].

Chociaż surowy klimat oraz izolacja geograficzna ograniczyły możliwości rozwoju działalności antropogenicznej na obszarach polarnych, może ona być źródłem zanieczyszczeń lokalnych. Dla rejonów arktycznych będą to głównie osady ludzkie (np. okolica stolicy Svalbardu, Longyearbyen), aktywne oraz opuszczone kopalnie węgla [19-21] oraz platformy do wydobycia ropy naftowej. Na obszarach polarnych półkuli południowej, działalność tego typu jest ograniczona prawnie. Na podstawie Protokołu o Ochronie Środowiska do Układu Antarktycznego sporządzonego w Madrycie 4 października 1991 roku, Antarktyka została uznana za rezerwat przeznaczony dla nauki i pokoju, w szczególności Artykuł 2 Protokołu, precyzuje : ”działalność na obszarze Układu Antarktycznego jest planowana i prowadzona w ten sposób, aby ograniczyć jej szkodliwy wpływ na środowisko Antarktyki oraz zależne od niej i powiązane z nią ekosystemy” [22]. Jednakże, zarówno dla obszarów polarnych znajdujących się na północnej jak i na południowej półkuli, dodatkowym źródłem zanieczyszczeń może być rosnąca aktywność turystyczna i badawcza oraz emisje ze statków transportowych.

Pomijając źródła antropogeniczne, obszary polarne są również miejscem depozycji zanieczyszczeń powstałych w wyniku procesów naturalnych takich jak aktywność wulkaniczna i pożary lasów (związki przenoszone wraz w prądami powietrza), wietrzenie skał oraz aerozole morskie. W ostatnich latach pojawiło się również większe zainteresowanie udziałem bio-wektorów w przenoszeniu związków głównie ze środowiska morskiego na lądowe np. przez ptaki, [23], oraz re-depozycją zanieczyszczeń z lodowców oraz gleby [24].

6.1. Charakterystyka miejsc prowadzenia badań

Chociaż rozróżnienie źródła emisji zanieczyszczeń przenoszonych transgranicznie może być problematyczne, nie ulega wątpliwości iż zanieczyszczenia pochodzenia

antropogenicznego emitowane do środowiska znacząco wpływają na równowagę ekologiczną oraz stan ekosystemów [1,2,3]. Przykładowo od czasu rewolucji przemysłowej (~około 1850 roku), depozycja metali w środowisku powstałych w wyniku procesów powiązanych z działalnością człowieka wzrosła 10-krotnie [47].

Próbki do badań wykorzystanych w celu realizacji rozprawy doktorskiej pobierane były od zwierząt zamieszkujących rejony polarne półkuli północnej (Arktyka Europejska) oraz południowej (Antarktyka). Obszary polarne arktyczne i antarktyczne znajdujące się na przeciwnych biegunach Ziemi mają zarówno cechy wspólne (surowy klimat, odizolowanie geograficzne, rozległy obszar), jak i cechy różnicujące (m.in. rodzaj pokrywającej je roślinności oraz gatunki zwierząt, rodzaj występujących gleb i rzeźba terenu).

6.1.1. Arktyka Europejska- Spitzbergen

Archipelag Svalbard to odizolowany geograficznie obszar, stanowiący część Arktyki Europejskiej (71°-81° N, 10° -35° E), będący terytorium zależnym Norwegii . Największą wyspą archipelagu jest Spitzbergen, zajmujący powierzchnię około 39 tysięcy km^2. Znaczny obszar Spitzbergenu zajmują lodowce oraz góry. Roślinność pokrywa około 6-7% powierzchni Svalbardu, a dominujące gatunki to głównie mchy, porosty i rośliny naczyniowe. Okres wegetacji trwa około 90 dni [25]. Ze względu na brak systemu korzeniowego, powolny, długoletni wzrost i rozbudowaną powierzchnię organizmy takie jak mszaki oraz porosty są podatne na akumulowanie związków toksycznych z atmosfery [26-28], z topniejących lodowców oraz w przypadku tych rosnących w bliskiej odległości od morza, z cząsteczek przeniesionych z aerozolem morskim [28,29].

Najważniejsze czynniki wpływające na ułatwioną depozycję zanieczyszczeń na Svalbardzie to:

a) relatywnie mała odległość od kontynentalnej Europy i obszarów wysoko zurbanizowanych (około 800 km do Półwyspu Skandynawskiego);

b) kierunki cyrkulacji atmosferycznej zmieniające się w ciągu roku; zimą przepływ mas powietrza znad Centralnej Europy, Skandynawii o północnozachodniej Rosji [30];

c) krajobraz zdominowany przez lodowce i góry, stanowiące naturalną barierę dla mas powietrza napływających znad Europy oraz północno-zachodniej Azji;

d) niska temperatura zwiększająca prawdopodobieństwo zanieczyszczeń do kondensacji i depozycji w śniegu, opadach atmosferycznych, wodzie oraz glebie;

e) relatywnie mała ilość formującego się lodu morskiego, w porównaniu do reszty Arktyki umożliwiająca rozwój turystyki i transportu wodnego;
f) układ oraz kierunek prądów morskich [31].

Faunę zamieszkującą Svalbard stanowią zarówno gatunki migrujące przebywające na wyspie wyłącznie w sezonie letnim, jak i mniej liczna grupa gatunków pozostających cały rok. Około 70 gatunków ptaków morskich gniazduje regularnie w Arktyce, z czego około 36 na Spitzbergenie. Mniejsza bioróżnorodność równoważona jest dużą liczebnością (ptaki arktyczne tworzą jedne z największych kolonii lęgowych) [18]. Poniżej przedstawiono przykładowe gatunki charakterystyczne dla krajobrazu arktycznego (Rysunek 1.)

Rysunek 1. Przykłady ptaków morskich zamieszkujących rejony polarne i sub-polarne; od góry fulmar *Fulmarus glacialis*, mewa trójpalczasta *Risa tridactyla*, maskonur *Fratercula arctica*, rybitwa popielata *Sterna paradisaea* (fot. Aneta Pacyna-Kuchta)

Ze względu na surowy klimat oraz trudności w zdobyciu pokarmu w okresie zimowym, relatywnie mała grupa zwierząt przebywa na wyspie przez cały rok. Należą do niej m.in. niedźwiedź polarny *Ursus maritimus,* lis polarny *Vulpes lagopus*, pardwa *Lagopus lagopus* oraz

renifer Svalbardzki *Rangifer tarandus platyrhynchus*. Gatunki te przystosowały się do trudnych warunków klimatycznych i pogodowych, jednak zmiany klimatyczne mogą negatywnie wpływać na ich szanse przetrwania. Przykładowo niedźwiedź polarny ze względu na coraz dłuższe okresy dodatniej temperatury i związane z tym późniejsze tworzenie się lodu morskiego skutkujące niewystarczającą do polowania na foki ilością lodu na morzu, może mieć trudności ze zdobyciem wystarczającej ilości pokarmu. Prawdopodobnie jest to przyczyną odnotowania w ostatnich latach prób polowań niedźwiedzi na renifery, które standardowo nie stanowią składnika diety niedźwiedzi [97]. Renifer Svalbardzki jest lokalnym roślinożercą, którego granice terytorium ustalone są przez naturalne bariery terenowe takie jak lodowce, pas fjordów oraz pasma górskie [99]. Obecnie, poza lokalnymi polowaniami oraz okazjonalnymi próbami polowania przez niedźwiedzie, główną przyczyną śmiertelności wśród reniferów są zmieniające się warunki klimatyczne na Svalbardzie [98,99]. Krajobraz Spitzbergenu w okolicy Polskiej Stacji Polarnej, Hornsund oraz renifera Svalbardzkiego przedstawiono na Rysunku 2.

Rysunek 2. Renifer Svalbardzki *Rangifer tarandus platyrhynchus*

6.1.2. Antarktyka- Wyspa Króla Jerzego

Antarktyka to obszar znajdujący się na półkuli południowej obejmujący Antarktydę oraz otaczający ją Ocean Południowy, wraz z położonymi na nim wyspami. Status prawny Antarktyki określają traktaty międzynarodowe, z czego najważniejszy jest Układ Antarktyczny z roku 1959, nadający obszarowi status rezerwatu przyrody [22]. Surowe warunki klimatyczne panujące na Antarktyce stanowią wyzwanie dla przebywających tam organizmów, które również muszą zmierzyć się wysoką konkurencja o pokarm, drapieżnikami i zanieczyszczeniem środowiska [32,33]. Zwierzęta przebywające na terenie Antarktyki korzystają głównie z zasobów morskich, na obszarze tym nie ma naturalnie występujących ssaków ani ptaków korzystających wyłącznie z pokarmu rosnącego na lądzie [34].

Zanieczyszczenia trafiają na obszar Antarktyki w wyniku procesów naturalnych, z czego jednym z najważniejszych jest aktywność wulkaniczna, będąca źródłem m.in. metali ciężkich [35, 36]. Gleba na Wyspie Króla Jerzego składa się głównie z minerałów, kawałków skał oraz pyłów wulkanicznych, które trafiły tam po erupcji wulkanu, znajdującego się w odległości około 130 km na południowy zachód w późnych latach 60' [37-39]. Źródłem zanieczyszczeń emitowanych do środowiska mogą być również chemiczne i mechaniczne wietrzenie skał, aerozole morskie oraz kolonie ptaków i ssaków morskich, przenoszące zanieczyszczenia z morza na obszar lądowy [40, 41]. Źródła antropogeniczne obejmują transport transgraniczny z obszaru Ameryki Południowej [42, 43, 44], lokalną działalność badawczą i turystyczna oraz związany z tym transport morski (m.in. spalanie paliwa, wycieki paliwa, miejsca składowania odpadów [44, 45, 46].

7. Akumulacja zanieczyszczeń w organizmach zwierzęcych na przykładzie tkanek skeratynizowanych

Po przedostaniu się do ekosystemu związki toksyczne mogą podlegać procesom bioakumulacji (akumulacji w tkankach), biowzmacnienia (zjawisko, w którym poziom danego związku zwiększa się wraz ze wzrostem poziomu troficznego) oraz biotransformacji (zmiany stężenia lub chemicznej formy związku) [100]. Zarówno toksyczność jak i biodostępność metali zależy w dużym stopniu od formy chemicznej w jakiej występują w środowisku [48], stężenia,

drogi ekspozycji, a także wieku, płci oraz kondycji narażonego osobnika. Ze względu na wysoką toksyczność nawet w małej dawce pierwiastki takie jak As, Cd, Cr, Pb oraz Hg są często uznawane za priorytetowe w badaniach ekotoksykologicznych [49]. W przypadku większości gatunków ptaków oraz ssaków głównym źródłem dostania się związków toksycznych do organizmu jest droga pokarmowa. Stopień akumulacji związków różni się w zależności od rodzaju tkanki i jej struktury (m.in. procentowej zawartości tłuszczu) [101]. Poniżej opisano główne czynniki wpływające na akumulację pierwiastków oraz trwałych związków organicznych w tkankach skeratynizowanych, które stanowią główny materiał biologiczny wykorzystany w badaniach.

7.1. Metale, nie-metale oraz metalloidy

Akumulacja pierwiastków w tkance skeratynizowanej zależy od wielu czynników takich jak dieta, zajmowany poziom troficzny, płeć, gatunek oraz metabolizm [66, 72-75]. Sposób wiązania pierwiastków w piórze może się różnić, przykładowo rtęć wiązana jest bardzo silnie przez grupy funkcyjne keratyny.

Czas pierzenia w przypadku ptaków i linienia w przypadku ssaków oraz związane z tym przebywanie na danym obszarze żerowania w okresie wzrostu tkanki w dużym stopniu definiuje ilość pierwiastków zakumulowanych w piórach/sierści. Dodatkowym czynnikiem wpływającym na ilość pierwiastków akumulowanych w tkance jest rodzaj pigmentu. Główną grupą pigmentów w upierzeniu ptaków są pigmenty melaninowe oraz karotenoidy. Zdolność ptaków do magazynowania metali śladowych w na-pigmentowanych partiach piór może zostać wykorzystana do śledzenia ich dostępności w diecie poszczególnych osobników oraz zdolności do radzenia sobie ze środowiskowymi wyzwaniami w ich pozyskaniu.

W publikacji przedstawionej w Załączniku 1. przeprowadziliśmy kompleksowy przegląd badań mających na celu zbadanie zależności między zawartością wybranych metali w częściach piór zawierających pigmenty: melaninę oraz karotenoidy oraz piór bez pigmentacji. Manuskrypt został opracowany w celu zidentyfikowania obszaru, w którym nadal występują luki w wiedzy dotyczące wpływu podwyższonej ekspozycji na wybrane pierwiastki śladowe, a ich akumulacją w piórach zawierających pigmenty eumelaninowe oraz karotenoidy. Wysoka zawartość metali (zwłaszcza wapnia) występuje w częściach piór z wyższą zawartością eumelaniny – jednej z dwóch podstawowych form melaniny. Miedź oraz cynk również

wykazują pozytywną korelację, jednak ilość przeprowadzonych badań jest wciąż zbyt mała, aby jednoznacznie potwierdzić zależność depozycji od zawartości eumelaniny. Z badań wynika, iż należy osobno traktować akumulację metali istotnych dla zdrowia oraz toksycznych ze względu na różnice w mechanizmie depozycji. Osobno należy również traktować depozycję względem rodzaju pigmentu.

Załącznik 1

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REVIEW ARTICLE

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Biological responses to environmental contamination. How can metal pollution impact signal honesty in avian species?

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Abstract

Environmental pollution, for example with metals, can significantly affect the ecosystem balance leading to severe changes. Biologically active pigments are relevant for the appearance and condition of birds. Melanin and carotenoid particles are the most frequently deposited pigments in avian integument. They are responsible for the majority of colors of bird plumage. The phenotypic expression can be affected by metal contamination. It can be manifested as color bleaching or differences in the size of plumage badges. In this study, we performed a comprehensive review of related studies in order to estimate the underlying population effect of this potential dependency. The study is based on the review of the literature regarding several avian species. It was designed to identify an area where the effect of the exposure is still poorly known. The analysis was specifically conducted to investigate the correlation between trace element concentration and eumelanin deposition. Moreover, we searched for factors that could affect spectral properties of feathers with carotenoid-based pigmentation. As a result, we found carotenoid-based pigmentation to be of a good use in terms of visual condition assessment. Changes in melanin-based pattern should be analyzed separately for eu- and pheomelanin as well as for a range of essential and toxic elements. Comprehensive studies on the subject are still scarce. Therefore, the issue requires further investigation.

KEYWORDS

carotenoids, feather, melanin, pigments, trace elements

1 | INTRODUCTION

Plumage coloration is used by birds to communicate signal honesty, informing on the individual's quality status (Bortolotti, Blas, Negro, & Tella, 2006; Gunderson, Frame, Swaddle, & Forsyth, 2008; McGraw, Hill, & Parker, 2005; Zahavi, 1975). Signals must be costly to be reliable, otherwise "low-quality" individuals would easily benefit from faking (Zahavi, 1975; Olson and Owens, 1998). The ability to sequester certain minerals within melanin- or carotenoid-based parts can be used to trace dietary access to such elements. Pigment-based feather coloration can be also used to track the ability of individuals to cope with environmental and physiological challenges in gaining pigments (McGraw, 2005).

Melanin- and carotenoid-based plumage pigmentation is widespread across avian species. Numerous studies have been dedicated to enhancing knowledge about its origin, synthesis, and factors affecting its expression (e.g., McGraw, 2005; Niecke, Heid, & Krüger, 1999; Niecke, Rothlaender, & Roulin, 2003). However, information on many aspects of the effect of environmental factors such as metal pollution on trait display is still scarce. The knowledge gap is especially evident in respect to studies focusing on the correlation between trace element concentration and pigment deposition.

Enrichment with several elements was determined in melanized parts of feathers. Some studies clearly showed calcium and zinc enrichment within pigmented spots (e.g., Niecke et al., 1999, 2003). According to the study by Dauwe and Eens (2008), metal pollution, in particular lead, cadmium, copper, and zinc, enhanced the expression of the melanin-based breast stripe in great tits (*Parus major*) from the most polluted sites. At the same time, yellow carotenoid coloration was negatively correlated with metal contamination. Black sections of the feather shaft were significantly enriched with zinc, calcium, copper, and magnesium according to a study by Hanć, Zduniak, Erciyas-Yavuz, Sajnóg, and Barałkiewicz (2017). In Giraudeau, Mateos-Gonzalez, et al. (2015), the determined total content of metals was positively correlated with the size of the melanin-based black tie. Melanin pigmentation was also positively correlated with copper level and negatively with chromium concentration (Giraudeau, Mateos-Gonzalez, et al., 2015).

Melanin is synthesized in the body either from tyrosine or from phenylalanine as the end product of the biochemical pathway (Hearing, 1993). Metal ions can modulate the activity of the enzyme tyrosinase that catalyzes melanin production (McGraw, Beebee, Hill, & Parker, 2003; Zduniak, Surmacki, Erciyas-Yavuz, Chudzińska, & Barałkiewicz, 2014). Melanin carboxyl groups can also bind metal ions and serve as cation chelators facilitating body detoxification (Chatelain, Gasparini, Jacquin, & Frantz, 2014; Dauwe, Bervoets, Pinxten, Blust, & Eens, 2003; Zduniak et al., 2014). Because melanin production is associated with significant cost, it is assumed that only the healthiest individuals would be capable of producing the darkest version of a melanin-based color trait (Meunier, Figueiredo Pinto, Burri, & Roulin, 2011). For some species, however, for example, females of the common eider (*Somateria mollissima*), pale coloration could be more costly to produce than dark coloration (Hanssen, Folstad, & Erikstad, 2006). In such a situation, the trait displaying cost could be misleadingly interpreted.

In contrast to melanin, vertebrates and insects cannot synthesize carotenoids de novo, and pigment particles need to be acquired from food sources (Goodwin, 1984; Sillanpää, Salminen, Lehikoinen, Toivonen, & Eeva, 2008). Carotenoid uptake from food is limited by the species physiology and genotype (Olson and Owens, 1998). The presence of carotenoid is relevant for the condition of birds due to its role in both health maintenance and ornamental signaling (Tummeleht, Mägi, Kilgas, Mänd, & Hõrak, 2006). More colorful plumage can indicate better body condition and better ability to assimilate carotenoids from food during molt (Giraudeau, Chavez, Toomey, & McGraw, 2015). Metals can affect the abundance and quality of carotenoid-rich food sources (Eeva, Lehikoinen, & Rönkä, 1998; Eeva et al., 2008; Geens, Dauwe, & Eens, 2009). Also, birds from more polluted places are commonly believed to use more carotenoids for antioxidant defense to reduce oxidative stress levels, which leaves less to be used for feather coloration (Geens et al., 2009).

This study focuses primarily on potential effects of trace elements on melanin- and carotenoid-based coloration and therefore signal quality. Melanin-based coloration has been believed to be controlled exclusively genetically for many years (Niecke et al., 2003). Recent findings, however, confirm the hypothesis that both carotenoid (Eeva et al., 2008) and melanin deposition is condition-dependent (Guindre-Parker & Love, 2014). This study presents a brief review of the limited existing evidence of a link between environmental contamination and pigment deposition in bird feathers.

2 | METHODS

2.1 | Data sources

The study is based on the extensive search of literature concerning the associations between chemical element concentration and pigment deposition measured in feathers. Keyword searches were performed, particularly by means of Web of Science, Google Scholar, and Wiley database. The data search was last updated in October 2017. The following keyword combinations were used: "metal, element analysis, melanin ornaments," "feather, metal, melanin," "bird, melanin coloration," "feather, carotenoids," "bird, carotenoid coloration," and "feather, carotenoids, metal". A forward search was also performed for articles cited in papers such as McGraw (2003), Eeva et al. (2008) and for abstracts, book chapters, and conference papers covering the topic. We also searched for previous reviews focusing on melanin- and carotenoid-based coloration and articles cited in them (McGraw, 2008; Santos et al. 2011, Meunier et al., 2011). In total, about 70 papers were found, screened by abstract or full text. Due to their limited availability, we do not use unpublished studies in the paper to avoid charges of a publication bias (known as the "file-drawer" problem, Rosenthal, 1979). Only studies published in English were included.

Because carotenoid pigmentation is widely represented among multiple species, we searched for papers concerning metals as a factor affecting carotenoid-based coloration. Due to differences in pigment acquisition as compared to melanin, we focused on food chain disturbances resulting in potential color bleaching.

2.2 | Average effect size

We calculated an overall effect size for the correlation between concentrations of several trace elements in feathers and eumelanin deposition based on original studies performed on eight species. Comprehensive studies with simultaneous quantity determination of color and trace element concentration are still scarce; thus, we restricted the meta-analysis to eumelanin. Because the data were incomplete, all trace elements (zinc, lead, cadmium, calcium, copper) were examined separately (Table 1; Figure 1). In analysis, following studies were used: Chatelain et al., 2014; Chatelain, Gasparini, & Frantz, 2016; Giraudeau, Chavez, et al., 2015; Giraudeau, Mateos-Gonzalez, et al., 2015; Gochfeld et al. 1991; McGraw, 2007; Niecke et al., 2003; Zduniak et al., 2014 . Small sample sizes in the available research result in a high confidence interval. In the majority of cases, the relationships between variables were presented as correlation coefficient *r*. If the

TABLE 1 Results of the meta-analysis of the association between trace element concentration and eumelanin deposition in feathers

Metal	Mean effect size *r* (*Zr*)	The standard error *r* (*Zr*)	The lower limit of the confidence interval for *r* (*Zr*)	The upper limit of the confidence interval for *r* (*Zr*)	*p*	Sample size (number of birds)
Zinc	0.156 (0.163)	0.058 (0.058)	0.270 (0.282)	0.041 (0.043)	0.0038 (0.0069)	283
Calcium	0.408 (0.428)	0.109 (0.132)	0.623 (0.693)	0.195 (0.163)	0.00009 (0.0019)	60
Copper	0.294 (0.308)	0.109 (0.108)	0.508 (0.549)	0.080 (0.068)	0.0035 (0.012)	72
Lead	0.280 (0.292)	0.075 (0.074)	0.426 (0.475)	0.133 (0.108)	0.00009 (0.0019)	122
Cadmium	0.022 (−0.009)	0.132 (0.132)	0.281 (0.255)	−0.238 (−0.275)	0.4343 (0.942)	60

Note. Presented average effect sizes were calculated using correlation coefficient *r* and its normalizing transformation Fisher's Z: *Z*(*r*) (details in Method section).

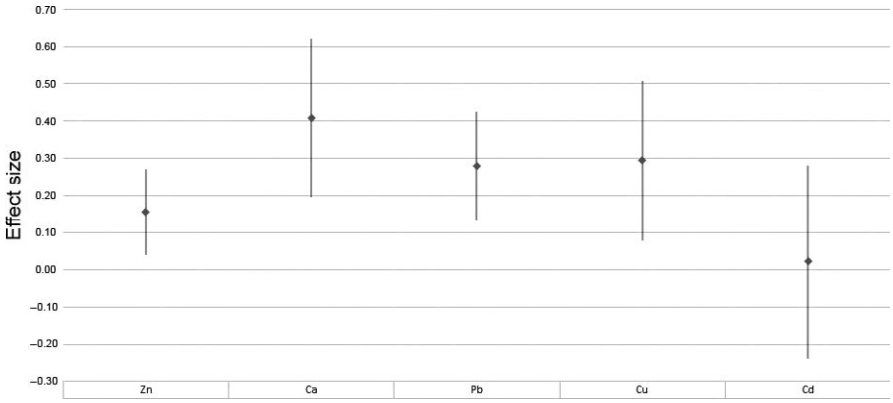

FIGURE 1 Forest plot of the effect size of correlation between the element concentration and the eumelanin deposition (based on *r*)

relationship was presented in the form of significance level *p*, and assuming that the correlation significance was tested using a *t*-test, the value was recalculated for *r*, according to the following formula (Rosenthal & Rubin, 2003):

$$r = \sqrt{\frac{t^2(p)}{t^2(p) + (N-2)}}$$

where: *t*(*p*)—Student's *t* statistic for significant level *p* and degrees of freedom equal to *N*-2.

We performed a fixed effects meta-analysis using correlation coefficient *r* and its normalizing transformation Fisher's Z: *Z*(*r*) to estimate the mean effect size. Effect sizes based on correlation coefficient *r* were calculated based on Olkin-Pratt (DSL) fixed-effect meta-analytical approach, as described by Schulze (2004) in R environment (Lumley, 2012 ("rmeta"package); R Core Team, 2017) and Fisher's Z: *Z*(*r*) were calculated in Excel (Excel, 2010).

When correlation coefficients were recalculated for its normalizing transformation Fisher's Z: *Z*(*r*), the following formula was used:

$$Z(r) = \frac{1}{2}\ln\frac{1+r}{1-r} = \operatorname{arctanh}(r)$$

If variable *X* and *Y* had a combined two-dimensional normal distribution, and if the individual pairs (X_i, Y_i) were independent, then *Z*(*r*) had approximately normal distribution with a mean:

$$m_Z = \frac{1}{2}\ln\frac{1+\rho}{1-\rho}$$

and a standard deviation:

$$s_Z = \frac{1}{\sqrt{N-3}}$$

3 | RESULTS AND DISCUSSION

3.1 | Melanin coloration

The dependency between metal abundance and pigment expression has been rarely subject to experimental or field studies. This study compiles available research from a collection of empirical studies to estimate the variance of the underlying population effect of this potential dependency. Melanin is synthesized from amino acid precursors within melanocytes (Michalik et al. 2010, McGraw et al., 2003). Its production can be costly for the bird in terms of energy and time involved (Gunderson et al., 2008). In vertebrates, melanin takes two main forms: eumelanin which gives darker black, brown, or gray color, and pheomelanin which gives reddish or buff color (McGraw, Safran, & Wakamatsu, 2005). Elements such as Ca, Cu, Fe, and Zn are necessary in the process of intermediate products formation in syntheses of both melanin

types (McGraw et al., 2003). They also play an indispensable role in multiple physiological body functions (Bogden & Klevay, 2000). Therefore, their level could be a consequence of their baseline distribution in the entire body (Zduniak et al., 2014). Due to a vast diversity of integument coloration patterns and multiple factors affecting deposition, it is not fully clear how metal distribution is shaped within melanized parts. Here, we calculated the average effect size of concentrations of five chemical elements and eumelanin deposition in spotted parts of feather. Due to the limited number of studies, however, the analysis can only be treated as support for further studies. The most significant effect size was recorded for calcium (Table 1). Calcium is relevant in many physiological processes, including skeletal mineralization (Zduniak et al., 2014). It is essential for the survival of birds. It induces aggregation of melanin particles, making feather more durable and resistant to mechanical stress (Niecke et al., 1999). Synchrotron X-ray analyses showed that the distribution of calcium is controlled by melanin pigment and is nonuniformly distributed throughout feather parts (Edwards et al., 2016; Howell et al., 2017). In a study performed on male Barn Owls, Roulin, Dauwe, Blust, Eens, and Beaud (2006) demonstrated a positive relationship between enhanced calcium deposition in humerus bone and plumage spottiness. This study provided no evidence of the production of eumelanin pigment generating additional cost for the organism in terms of extra calcium use (Roulin et al., 2006).

In an experimental study by McGraw (2007), the black breast eumelanin-based patch in male zebra finches (*Taeniopygia guttata*) increased its size after calcium supplementation. Therefore, especially in the case of species with a hierarchy linked to the size of melanin-based patches, calcium availability could be expected to be a factor affecting signal honesty.

Results regarding zinc were not homogenous for all studies ($Q = 8.940$, $\chi^2 = 7.815$ for $\alpha = 0.05$, 3 degrees of freedom; $p = 0.03$), and the overall size effect was not significant ($p = 0.081$). Zinc is an essential trace element for the physiological condition of birds, and its distribution might be focused in a repetitious banding pattern (Howell et al., 2017). Niecke et al. (2003) found zinc enhancement within spotted feathers of the barn owl (*Tyto alba*). The relationship was noticeably different for eumelanized parts ($r = 0.18$, $p = 0.26$) and reddish brown parts with pheomelanin ($r = -0.72$, $p < 0.001$). Zinc concentration was 2.7 times higher within black spots, compared to unspotted parts. It was also found in nonpigmented feather parts. Zinc appeared to have higher affinity for pheomelanin than eumelanin, and relative, not absolute concentrations correlating with pigment patterns (Edwards et al., 2016).

Similarly to zinc and calcium, copper is controlled by melanin pigment patterns in feathers (Edwards et al., 2016). Both analyses in this study employed a similar sample size in the calculation of average effect size. The values of correlation coefficients, however, significantly differed from each other. The data were homogenous as $Q = 2.671$, $\chi^2 = 3.841$ for $\alpha = 0.05$ and one degree of freedom, $p = 0.102$. The overall effect size was statistically significant (Table 1). Macro- and microminerals are scarce in the diet of many bird species (Zduniak et al., 2014). Deposition of valuable elements such as calcium, zinc, or copper in inert dead tissue enables their further usage. However, as shown in the study by Roulin et al. (2006), eumelanism may be compromised against other physiological processes such as calcium storage in bones that requires the same essential elements. Eumelanin pigmentation may therefore reflect the physiological ability to absorb and store elements in body parts (Roulin et al., 2006).

Elevated levels of toxic compounds were also found in melanized feather parts. Regarding analysis of average effect size for lead, only one of the studies (Chatelain et al., 2016) showed a statistically significant relationship. The remaining studies provided no unambiguous results, probably due to a small sample size. Data homogeneity was confirmed as $Q = 4.412$, $\chi^2 = 7.815$ for $\alpha = 0.05$; 3 degrees of freedom, $p = 0.220$. The overall effect size for all studies was statistically significant. In the case of cadmium, results of only one study including three different species were found. Data homogeneity was confirmed ($Q = 1.012$, $\chi^2 = 5.991$ for $\alpha = 0.05$ and 2-degrees of freedom, $p = 0.603$). The overall effect size was not statistically significant.

Dauwe and Eens (2008) found that lead, cadmium, copper, and zinc enhanced the expression of the melanin-based breast stripe in great tits (*Parus major*). In contrast to zinc, Chatelain et al. (2014), found no correlation between lead concentration and melanin-based coloration score in urban feral pigeons after 1 year of captivity. Because environmental factors may have hidden the correlation pattern during field studies, experimental research on domesticated species would help to understand the adaptive function of element deposition in melanized parts. The ability to cope with metals after they exceed toxic levels can be used to signal condition of an individual bird (McGraw, 2003). Knowing the pattern and effect of toxic metal deposition in melanized parts of feathers on signal honesty may shed some light on birds adaptation abilities in high polluted places.

3.2 | Carotenoids

Carotenoid deposition is most likely species-dependent. It is influenced by the abundance and quality of carotenoid-rich food, as well as the bird's ability to assimilate pigments. Giraudeau, Mateos-Gonzalez, et al. (2015) show a negative correlation of carotenoid-based coloration with mercury concentration ($p = 0.02$), as well as with the sum of metals (Hg, Cu, Pb, Cr, As, Cd, Sb, and Zn) ($F_{1,30} = 2.07$, $p = 0.16$). Chicks of great tits living nearer a pollution source (a factory complex producing Cu, Ni, and fertilizers) had paler yellow plumage than chicks living further away from the factory (Eeva et al., 1998). A study by Geens et al. (2009) shows similar results. Giraudeau, Chavez, et al. (2015) confirmed the finding in reference to house finches (*Haemorhous mexicanus*), evidencing that urban birds had paler plumage than desert birds at capture. Carotenoid composition and distribution in feathers are affected by genetic, metabolic, physiological, and dietary factors (Brush & Power, 1976). In more polluted areas, a different type of prey would be eaten, with a predominance of less carotenoid-rich food sources, for example,

spiders instead of caterpillars (Geens et al., 2009; Koivula, Kanerva, Salminen, Nikinmaa, & Eeva, 2011). Many insect species can selectively accumulate more carotenoids such as lutein than others (Ahmad, 1992; Sillanpää et al., 2008). Some birds can also have this ability (McGraw et al., 2003).

In more polluted areas, plants could contain less carotenoid pigments due to elevated oxidative stress levels. Therefore, caterpillars feeding on them would also be of lower quality (Isaksson & Andersson, 2007). This explanation, however, is not always reflected in studies. In the study by Sillanpää et al. (2008), insects had higher body carotenoid concentration when derived from more polluted places, or there was no association between polluted and unpolluted areas.

Isaksson and Andersson (2007) found that caterpillars living in the urban environment were more abundant and heavier in comparison with the rural population, although they also contained less carotenoids in their bodies. Chicks from urban areas were fed approximately twice more often by their parents, providing carotenoid availability similar to that of rural nestlings.

Coloration often increases in individuals with age (Delhey & Kempenaers, 2006). Less colorful individuals have less chance to survive until adulthood (Pagani-Nuñez & Senar, 2012). The estimation of the carotenoid content and search for carotenoid-rich food sources requires experience, not available to young birds at the beginning. Younger birds can be also more affected by parasitism and higher levels of oxidative stress; therefore, more carotenoids might need to be used as antioxidants (Geens et al., 2009; Giraudeau, Barcelo, & Senar, 2014). The ability of younger and older birds to extract and assimilate carotenoids from food may also differ (McGraw & Parker, 2006). Terms of molting and seasonal differences in food abundance and quality, as well as other dietary antioxidants protecting carotenoids from oxidation and bleaching (Del Val, Quesada, & Senar, 2010; Giraudeau et al., 2014), can also be responsible for major differences between age classes. Carotenoid-based coloration could possibly reflect only the level of other antioxidants, such as vitamin A, acting against oxidative bleaching of carotenoids (Constantini & Møller, 2008; Hartley & Kennedy, 2004) .

3.3 | Knowledge gaps and research needs

Feathers are complex biological structures that display a diversity of pigmentation patterns. Melanin bounds many compounds and retains them for long periods of time. Therefore, melanin-based pigmentation could be used to assess long-term physical condition of the individual (Hung & Li, 2015). Melanin coloration results from the co-occurrence of both eumelanin and pheomelanin pigments at different concentrations (McGraw & Parker, 2006; Zduniak et al., 2014). Differences in their bounding capacities may explain the distinct spatial distribution pattern across species for some of the elements (Howell et al., 2017). The ability of pheomelanin to store elements is still largely understudied. Only a few examples can be found in the literature (Niecke et al., 2003; Zduniak et al., 2014).

A huge diversity of within-feather pigmentation patterns exist. The mechanisms determining pigment distribution into keratinocytes are of interest to scientists (Prum & Williamson, 2002). Little is known, however, about the fundamental biological processes leading to orderly patterns and integumental tissue development. It seems that highly melanic birds should be favored in an environment with a high level of certain metals (Chatelain et al., 2014). Many birds which adapt to live in European urban areas have melanin-based plumage coloration (Chatelain et al., 2016). Yet, the mechanism and effect of toxic metals deposition are significantly understudied.

Both carotenoid- and melanin-based pigmentation is considered a way of signaling which is costly in production. Production of eumelanin is linked to a major physiological process, namely skeletal mineralization (Roulin et al., 2006). Carotenoid deposition is most likely species-dependent and is influenced by the abundance and quality of carotenoid-rich food, as well as the bird's ability to assimilate pigments.

Metal ions can affect the pigment synthesis pathway. The degree of melanin and carotenoid deposition, however, is not necessarily clearly linked to honest signaling, because additional environmental and physiological factors might correspond to it. The profound understanding of the issue requires further research concerning free-living birds, domesticated, and captivated species. They would show different pigment acquisition.

4 | CONCLUSION

New selection pressure caused by anthropogenic activity challenges the ability of birds to adapt and survive in moderately and strongly polluted habitats (Giraudeau, Chavez, et al., 2015). In the environment subject to transformations, animals have to adapt to new conditions and xenobiotic influence. The impact of trace elements on biota is intensively investigated. Several empirical studies showed that melanin bounding capacities may allow some body detoxification. Essential and nonessential elements, however, behaved differently and should be treated separately. Carotenoid-based color bleaching is a first visual signal for mates and rivals providing information on the individual's ability to cope with environmental and physiological challenges. More data are still required, however, on many aspects facilitating the understanding of the mechanisms responsible for plumage coloration and the actual cost of pigment deposition.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

ADP was responsible for concept development, literature search, work design, and manuscript writing. MR and ZP provided text corrections, and JM provided the meta-analysis. All of the authors took part in the data interpretation and critical draft revision and contributed to editing the manuscript. All authors read and approved the final manuscript.

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7.2. Trwałe zanieczyszczenia organiczne

TZO to związki organiczne na bazie węgla o właściwościach fizycznych i chemicznych, które pozwalają im pozostawać w środowisku przez bardzo długi czas, mają właściwości lipofilowe oraz są toksyczne dla organizmów żywych [34]. Klasyczne TZO obejmują związki o udowodnionej toksyczności np. z grupy polichlorowanych bifenyli oraz pestycydów chloroorganicznych [34].

Różnice w sposobie akumulacji oraz depozycji TZO mogą być spowodowane różnicą we właściwościach związków. Przykładowo, związki perfluorowane wiążą się głównie z białkami, stąd, ich duże nagromadzenie może wystąpić we frakcji białkowej krwi oraz w wątrobie. Z kolei większość klasycznych TZO wykazuje większe powinowactwo do tłuszczu i ich duże ilości można wykryć w tkankach mających duży procentowy udział tłuszczu [50].

Naturalne procesy takie jak bioakumulacja, oraz metabolizm wpływają na profil TZO w organizmie [51]. Ze względu na szybki metabolizm w ciele kręgowców, WWA nie spełniają wszystkich kryteriów, aby były klasyfikowane jako klasyczne TZO. Jednak biorąc pod uwagę ich wszechobecność w środowisku, toksyczność oraz duże powinowactwo do tłuszczu, są zwykle dodawane do tej kategorii [52; 53].

8. Gatunki wykorzystane w badaniach

Obecność zanieczyszczeń pochodzenia antropogenicznego w organizmach morskich jest miarodajnym wskaźnikiem zmian występujących w środowisku oraz odpowiedzi fauny morskiej na podwyższoną ekspozycję. Procesy zachodzące w ekosystemie lądowym są odmienne od tych w środowisku morskim, co skutkuje odmiennym profilem narażenia na ksenobiotyki [54].

8.1. Ekosystem morski

Wszystkie gatunki, zarówno przebywające na obszarach polarnych podczas sezonu letniego, jak i gatunki niemigrujące, muszą zmierzyć się z szeregiem czynników stresowych. Przykładowo ptaki morskie często zmagają się z wysoką konkurencją o pożywienie oraz

miejsce do gniazdowania, ciężkimi warunkami pogodowymi oraz zanieczyszczeniem środowiska. Ptaki morskie odgrywają szczególną rolę w ekosystemie będąc organizmami „dwu-środowiskowymi". Żerując w morzu i wracając na tereny lęgowe mogą łatwo przenosić materię, w tym zanieczyszczenia ze środowiska wodnego na ląd [23,41].

Oceanniki to małe ptaki (waga 35-65 g), których naturalny zasięg występowania obejmuje Ocean Południowy oraz Spokojny. Jako ptaki morskie, oceanniki przebywają głównie na otwartych wodach, na lądzie przebywając wyłącznie podczas sezonu lęgowego. Odżywiają się głównie małymi skorupiakami, rybami oraz kałamarnicami wyłapywanymi blisko powierzchni wody [55,56,57,58]. Próbki piór osobników dorosłych oraz piskląt (pióra właściwe oraz puch) zostały pobrane podczas sezonu lęgowego na Antarktydzie od dwóch gatunków należących do rodziny *Oceanitidae*- oceannik żółtopłetwy (*Oceanites oceanicus*) oraz czarnobrzuchy (*Fregetta tropica)*. Oceanniki zostały wybrane jako obiekt badań ze względu na ich znaczenie w antarktycznej sieci troficznej, oraz braku badań dotyczących narażenia tych ptaków morskich na zanieczyszczenia pochodzenia naturalnego oraz antropogenicznego.

Alczyk (*Alle alle*) to ważący 150–180 g najmniejszy przedstawiciel rodziny alkowatych (Alcidae) przebywający na terenach lęgowych wyłącznie na obszarze Arktyki [59, 60]. Jest kluczowym gatunkiem arktycznej sieci troficznej - uznawany jest za najważniejszego ptasiego zooplanktonożercę w atlantyckiej części Arktyki, a lęgowe kolonie alczyka stanowią istotne źródło związków biogennych [59, 60]. Alczyk został wybrany jako główny obiekt badań ze względu na swoją kluczową rolę w arktycznym łańcuchu troficznym, wysoką liczebność szacowaną na 37 mln par [60] i możliwość bioakumulacji zanieczyszczeń z lokalnego pokarmu, który w sezonie lęgowym stanowią głównie skorupiaki planktonowe. Pomimo swojej liczebności, relatywnie mało uwagi było skupione do tej pory na jego przydatności w badaniach ekotoksykologicznych, zwłaszcza prowadzonych przy zastosowaniu próbek pobranych w sposób niedestrukcyjny dla osobnika. Alczyki są organizmami migrującymi - sezon lęgowy odbywa się na Arktyce, na zimowanie ptaki przenoszą się na niższe szerokości geograficzne [61]. Podwójny system pierzenia i znajomość czasu oraz miejsca pierzenia umożliwia pozyskanie informacji o narażeniu osobnika na związki toksyczne w okresie wzrostu piór.

8.2. Ekosystem lądowy

Polarny ekosystem lądowy składa się z ograniczonej liczby gatunków wykorzystujących wyłącznie lądowe zasoby pokarmowe, ze względu na mniejszą dostępność bazy pokarmowej. Mniejsza różnorodność równoważona jest w przypadku wielu gatunków dużą liczebnością, sprawiając iż gatunki te stały się ważną częścią ekosystemu polarnego.

Renifer svalbardzki (*Rangifer tarandus platyrhynchus*) jest najmniejszym podgatunkiem renifera tundrowego i zamieszkuje wyłącznie archipelag Svalbard. Jego dieta składa się w całości z dostępnej roślinności porastającej tundrę, w tym mchów oraz porostów, co sprawia iż łańcuch troficzny jest krótki i składający się z organizmów na stałe bytujących w środowisku arktycznym. Szacowany stan populacji wynosi około 10 000 osobników [63]. Jest to jedyny duży roślinożerca żyjący na wyspie. Badania wykazały, iż renifery Svalbardzkie nastawione są głównie na ilość spożywanej biomasy, a nie jakość pożywienia [63, 64]. Gatunek ten został objęty ochroną w 1925 roku, po wytępieniu większości populacji w wyniku polowań [65]. Aktualnie, pomimo iż presja związana z polowaniem została praktycznie zlikwidowana, zmiany klimatyczne oraz zanieczyszczenie środowiska stanowią najpoważniejsze czynniki mogące wpływać na dynamikę populacji. Jako jedyny wyłącznie lądowy gatunek ssaka roślinożernego, renifer pełni w środowisku Spitzbergenu kluczową rolę, jednak badania ekotoksykologiczne oceniające wpływ zanieczyszczeń na jego funkcjonowanie są wciąż niezwykle rzadkie. Próbki sierści oraz odchodów posłużyły do oceny stopnia narażenia reniferów na zanieczyszczenia emitowane do środowiska z naturalnych źródeł oraz przenoszone z niższych szerokości geograficznych.

9. Rodzaje materiałów biologicznych wykorzystanych w badaniach

9.1. Pióra

Z biologicznego punktu widzenia pióro stanowi złożoną strukturę będącą wytworem skóry ptaków, zbudowaną głównie z keratyny, która pełni funkcje podczas lotu, przy komunikacji oraz termoregulacji [66]. Jeden ptak ma zwykle na ciele ponad 20 000 piór, często

o zróżnicowanej kolorystyce, ułożone w powtarzające się wzory charakterystyczne dla gatunku. W przypadku dorosłych osobników pióra są odnawiane w cyklicznych fazach, zwanych pierzeniem, w czasie charakterystycznym dla danego gatunku [67].

Zaletą stosowania piór w badaniach ekotoksykologicznych jest ich duża trwałość, możliwość zbierania próbek od osobników będących na różnych fazach rozwoju, oraz braku przemian metabolicznych związków zdeponowanych w tkance. Dodatkowo możliwe jest wykorzystanie okazów dostępnych w muzeach oraz innych narodowych instytucjach i analiza zmian ekspozycji na dany związek w czasie [68]. Dodatkowo, jeżeli bezpośredni kontakt z osobnikiem nie jest wskazany lub możliwy (np. w przypadku skrytych lub agresywnych gatunków), pióra mogą być zbierane z ziemi z obszaru bytowania danego gatunku oraz z gniazd. Jednak w przypadku próbek zbieranych w ten sposób wiele czynników wpływających na zawartość zanieczyszczeń w piórze jest nieznana (wiek osobnika, płeć, czas pierzenia) [69].

Związki są wbudowywane w wewnętrzną strukturę pióra wyłącznie w okresie jego wzrostu, kiedy połączenie z naczyniami krwionośnymi jest aktywne. Gdy krew jest dostarczana do pióra, zawarte w niej związki o wysokim powinowactwie do grup sulfhydrylowych wiążą się z nimi i są włączane w strukturę pióra [66]. Kiedy proces rośnięcia się zakończy i pióro jest w pełni uformowane, wówczas połączenie to zanika i zatrzymany zostaje dalszy transport substancji do pióra [70,71].

Pomimo iż w przypadku w pełni ukształtowanego pióra nie następuje dalsza depozycja wewnętrzna, poziom zanieczyszczeń może być podwyższony ze względu na depozycję zanieczyszczeń na powierzchni pióra. Pióra mogą zostać zanieczyszczone głównie wydzielinami produkowanymi przez ptaki, takimi jak wydzielina z gruczołu kuprowego lub solnego. Wydzielina z gruczołu kuprowego działa jak film natłuszczający, pokrywając pióro wodoodporną warstwą zwiększającą jego wytrzymałość, ułatwiając cząsteczkom przyleganie do powierzchni pióra. Wydzielina ta może stanowić nawet do 85% lipidów w piórze [76]. Dodatkowym źródłem zanieczyszczeń powierzchniowych na piórze jest bezpośredni kontakt z wodą, powietrzem oraz błotem i glebą [77-79].

Aby uniknąć zawyżonych wyników oraz błędnej interpretacji narażenia na badane związki, zewnętrzna powierzchnia pióra powinna być dokładnie oczyszczona z zanieczyszczeń zewnętrznych. Nie zatwierdzono jednej metodyki mycia piór, wybór środka zależy przede wszystkim od rodzaju oznaczanych związków, gdyż różnice w powinowactwie do grup funkcyjnych keratyny skutkują różnicami w trwałości i sile wiązania. W literaturze dominuje

użycie acetonu jako środka stosowanego do usunięcia zanieczyszczeń zewnętrznych głównie w połączeniu z wodą destylowaną lub dejonizowaną [80-82], czasami dodatkowo używając łaźni ultradźwiękowej [83]. Inne metody obejmują przykładowo: użycie wyłącznie wody destylowanej [84], wykorzystanie 0.25 M zasady sodowej [85, 86] oraz wykorzystanie detergentów [87, 88]. Na rysunku 3 pokazano zdjęcia piór dwóch gatunków, wykorzystane w analizie pierwiastkowej.

Rysunek 3. Próbki piór alczyka (*Alle alle*) oraz oceannika (*Oceanites oceanicus*), po przetransportowaniu ich do Polski

9.2. Sierść

Podobnie jak pióra, sierść również jest wytworem keratynowym skóry, ale występującym u ssaków. Związki są wbudowywane w wewnętrzną strukturę sierści również wyłącznie w okresie wzrostu tkanki. W przeciwieństwie do ptaków, gdzie głównym źródłem zanieczyszczenia zewnętrznego jest wydzielina z gruczołu kuprowego, w przypadku sierści reniferów będą to cząsteczki gleby i roślinności oraz zanieczyszczenia z powietrza. Ze względu na długość sierści oraz jej gęstość, cząsteczki ziemi z łatwością wplatają się w jej strukturę. Ze

względu na możliwość utraty analitów przy zastosowaniu silniejszego rozpuszczalnika, relatywnie gładką strukturę włosa oraz charakterystykę zanieczyszczeń deponowanych na sierści, przy oznaczaniu związków z grupy TZO zaleca się wykorzystanie wyłącznie wody dejonizowanej [10]. W przypadku oznaczeń metali nie ma jednoznacznie ustalonej metodyki, jednak biorąc pod uwagę powyższe powody, jeżeli sierść nie jest zabrudzona krwią lub tłuszczem zwierzęcia, wykorzystanie wody dejonizowanej jest uzasadnione.

Sierść renifera wyróżnia sztywna wypełniona powietrzem, ściśle upakowana struktura, podlegająca zmianom sezonowym. Letnia i zimowa okrywa dorosłych i młodych reniferów różni się pod względem właściwości, takich jak długość, gęstość oraz kolor [89]. Podstawową funkcją sierści jest ochrona ciała przed zimnem oraz wiatrem [89]. Wymiana sierści u jeleniowatych następuje każdego roku [90]. Sierść wykorzystana w badaniach pokazana jest na rysunku 4.

Rysunek.4 Próbka sierści renifera Svalbardzkiego wykorzystana do analizy

9.3. Skorupki powylęgowe

Skorupki ptasie są relatywnie rzadko wykorzystywane w badaniach ekotoksykologicznych, w porównaniu do innych materiałów biologicznych. Skorupka powylęgowa to bogata w wapń struktura pierwotnie otaczająca rosnący w jaju zarodek.

Zawiera informację o relatywnie krótkim okresie ekspozycji na zanieczyszczenia, między uformowaniem się jaja, a jego złożeniem, mogącą pochodzić z rozległego obszaru żerowania samicy [91,92]. Skorupka od wewnątrz otoczona jest membraną, która również może posłużyć jako narzędzie do oceny narażenia na substancje toksyczne, przykładowo Peakall [93] wykorzystał membrany pochodzące od sokoła wędrownego do oceny ekspozycji ptaków drapieżnych na związki DDT. Jednak ze względu na jej duże zanieczyszczenie, membrana nie zawsze może zostać wykorzystana i w badaniach można wykorzystać wyłącznie utwardzoną skorupkę.

Skorupka jaja chroni rozwijający się zarodek przed środowiskiem zewnętrznym oraz jest głównym źródłem wapnia oraz magnezu podczas embriogenezy. Rozwijający się ptasi embrion absorbuje wapń i inne pierwiastki początkowo z żółtka, a następnie ze skorupki [31, 102, 103]. Skorupki powylęgowe wyróżniają się kształtem, strukturą oraz kolorem zależnym od gatunku ptaka oraz środowisku życia. Skorupki mogą być zanieczyszczone zarówno na zewnątrz (ziemia, odchody, resztki roślin w gnieździe), jak i wewnątrz (resztki po wykluciu), stąd pierwszym krokiem w procedurze analitycznej jest wyczyszczenie skorupki odpowiednim roztworem. Rozpuszczalnik powinien zostać dobrany w zależności od stopnia wybrudzenia skorupki, można wykorzystać na przykład wodę dejonizowaną i aceton. Na rysunku 5 przedstawiono próbki skorupek alczyka wykorzystane w późniejszych badaniach.

Rysunek. 5 Skorupki alczyka *Alle alle* w pojemniczkach polietylenowych po przewiezieniu do laboratorium

9.4. Odchody

Jedną z głównych dróg eliminacji związków z organizmu są odchody [94,95]. Stanowią one źródło informacji o biodostępności pierwiastków w danym środowisku, przyjmowanych głównie z diety [94, 96]. Ich wykorzystanie w polarnych badaniach środowiskowych jest aktualnie niewielkie. Zaletą wykorzystania odchodów jest możliwość pobrania próbki bez bezpośredniego kontaktu ze zwierzęciem, od osobników o różnym wieku oraz płci. Informacja o biodostępności związków z diety pochodzi z krótkiego okresu czasu i dotyczy związków efektywnie eliminowanych z organizmu. W przypadku miejsc ubogich w naturalną ściółkę, odchody stanowią źródło wielu pierwiastków, umożliwiając wzrost nowych roślin. Odchody zwierząt żyjących na Arktyce ulegają powolnemu rozkładowi, ze względu na niską temperaturę i mniejszą aktywność bakteriologiczną. Jednak jeżeli próbki nie są pobrane od razu po depozycji, mogą być na nich deponowane zanieczyszczenia atmosferyczne.

10. Cel rozprawy

Celem podjęcia kompleksowych badań było poznanie stopnia akumulacji wybranych pierwiastków i trwałych związków organicznych w materiałach biologicznych pobranych niedestrukcyjnie od kluczowych gatunków żyjących w rejonach polarnych. Motywacją do prowadzenia badań w środowisku polarnym były wnioski ze studium literatury, na podstawie których stwierdzono znaczne braki w wiedzy dotyczących akumulacji oraz potencjalnego wpływu związków chemicznych na polarną faunę morską oraz lądową. Obecnie badania polarne skupiające się na wykorzystaniu tkanek pobranych nie destrukcyjnie obejmują głównie oznaczenia zawartości rtęci. W badaniach będących podstawą rozprawy doktorskiej zaplanowano szereg oznaczeń wybranych pierwiastków oraz trwałych zanieczyszczeń organicznych będących źródłem informacji na temat zanieczyszczenia środowiska polarnego, zarówno Arktyki jak i Antarktyki. Wszystkie gatunki będące obiektem badań w niniejszej pracy są według aktualnej wiedzy mało zbadane pod katem ekotoksykologicznym, są istotnym ogniwem łańcucha pokarmowego w ekosystemie w którym żyją, cechuje je duża liczebność, oraz tworzenie kolonii/stada, co umożliwia pobranie próbek od reprezentatywnej grupy osobników.

Biorąc pod uwagę postępujące ocieplanie klimatu i prognozowane zwiększenie się ilości substancji toksycznych dostających się do ekosystemów polarnych [1,2,3], istnieje potrzeba prowadzenia badań dostarczających informacji o sposobach odpowiedzi fauny morskiej oraz lądowej na zwiększoną ekspozycję. Gatunki, od których pobierane zostaną próbki w celu realizacji badań do rozprawy doktorskiej reprezentują dwa ekosystemy: morski oraz lądowy i są to 3 gatunki ptaków morskich (alczyk, oceannik czarnobrzuchy, oceannik żółtopłetwy) oraz renifer Svalbardzki, odpowiednio.

Głównym celem badań przeprowadzonych do rozprawy doktorskiej było potwierdzenie :

Hipotezy 1: Akumulacja pierwiastków w roślinności Spitzbergenu, zwłaszcza w mchach oraz porostach, wpływa na zawartość metali oraz innych pierwiastków w sierści renifera. Istnieje bezpośrednia zależność między rodzajem spożywanej roślinności, a poziomem pierwiastków w sierści, oraz ze względu na mały zasięg przemieszczania się poszczególnych populacji mogą wystąpić różnice w akumulacji pierwiastków między nimi **[A. D. Pacyna, K. Koziorowska, S. Chmiel, J. Mazerski, Ż. Polkowska, Svalbard reindeer as an indicator of ecosystem changes in the Arctic terrestrial ecosystem, Chemosphere, 2018, DOI: 10.1016/j.chemosphere.2018.03.158]**

Hipotezy 2: Odchody stanowią efektywny sposób pozbycia się zarówno dużej ilości pierwiastków toksycznych, jak i innych np. fosforu, i mogą stanowić użyźnienie tundry ubogiej w pierwiastki biogenne. Ze względu na różnice w diecie zimowej i letniej wystąpią różnice w zawartości metali ciężkich w odchodach z dwóch sezonów. Ze względu na depozycję atmosferyczną wystąpią różnice między zawartością pierwiastków w świeżo pozbieranych odchodach, oraz starszych. Ze względu na brak istotnych różnic w diecie nie wystąpi różnica w zawartości pierwiastków w odchodach pozbieranych od samca, samicy oraz osobnika młodego **[A.D. Pacyna, M. Frankowski, K. Kozioł, M. H. Węgrzyn, P. Wietrzyk-Pełka, S. Lehmann-Konera, Ż. Polkowska, Evaluation of the use of reindeer droppings for monitoring essential and non-essential elements in the polar terrestrial environment, Science of the Total Environment, 2019, DOI: 10.1016/j.scitotenv.2018.12.232]**

Hipotezy 3: Sierść stanowi drogę eliminacji szeregu pierwiastków oraz może być wykorzystana do oceny ekspozycji reniferów na wybrane trwałe związki organiczne. Związki które trafiły na Arktykę w wyniku transportu transgranicznego, w tym pestycydy chloroorganiczne, wciąż są obecne w środowisku lądowym Arktyki i mogą mieć wpływ na zwierzęta zamieszkujące obszary polarne **[A. D. Pacyna-Kuchta, P. Wietrzyk-Pełka, M. H. Węgrzyn, M. Frankowski, Ż. Polkowska, A screening of select toxic and essential elements and persistent organic pollutants in the fur of Svalbard reindeer, Chemosphere (przyjęte do druku)]**

Hipotezy 4: Pióra dorosłych i młodych oceanników będą charakteryzowały różnice w profilu pierwiastkowym, ze względu na różne obszary żerowania, podczas okresu pierzenia i karmienia młodych piskląt. Dwa gatunki synantropijne oceanników odżywiają się podobnym pokarmem, jednak w przypadku oceannika czarnobrzuchego procentowy udział ryb w diecie jest wyższy, co może skutkować wyższą zawartością rtęci w organizmie
[A. D. Pacyna, D. Jakubas, A.N.M.A. Ausems, M. Frankowski, Ż. Polkowska, K. Wojczulanis-Jakubas, Storm petrels as indicators of pelagic seabird exposure to chemical elements in the Antarctic marine ecosystem, 2019, Science of The Total Environment, DOI: 10.1016/j.scitotenv.2019.07.137]

Hipotezy 5: Profil pierwiastkowy obecny w piórach najliczniejszego ptaka Arktyki europejskiej będą cechowały znaczące różnice między sezonem przed- i po-lęgowym, ze względu na aktywność migracyjną. Pióra osobników dorosłych, piskląt oraz skorupki mogą zostać wykorzystane w celu określenia ekspozycji alczyka w przeciągu roku na różnych fazach rozwoju i na różnych obszarach żerowania.
[A. D. Pacyna-Kuchta, D. Jakubas, M. Frankowski, Ż. Polkowska, K. Wojczulanis-Jakubas, Exposure of a small Arctic seabird, the little auk (*Alle alle*), to selected elements throughout the course of a year (w recenzji)]

Ponadto uzyskane wyniki badań posłużą do:
-zdobycia informacji na temat przenoszenia zanieczyszczeń w warunkach polarnych wykorzystując próbki pozyskane od żywych ptaków morskich oraz renifera Svalbardzkiego;
- oceny narażenia na substancje toksyczne ptaków morskich podczas dwóch stadiów rozwoju (pisklęta oraz osobniki dorosłe);

- oceny źródeł zanieczyszczeń (naturalne, antropogeniczne) wykrywanych w poszczególnych gatunkach.

11. Metodyka badań i przebieg pracy badawczej

Przeprowadzone w ramach pracy doktorskiej badania mają charakter interdyscyplinarny. Do współpracy oprócz ekspertów z zakresu chemii analitycznej włączono także specjalistów z zakresu biologii, w szczególności ornitologii oraz botaniki. Dzięki interdyscyplinarnemu zespołowi badawczemu możliwe było uzyskanie bogatej interpretacji otrzymanych danych chemicznych. Tkanki skeratynizowane, będące głównym materiałem do badań, stanowią źródło informacji o związkach dostarczonych wraz z krwią w okresie wzrostu tkanki. Jednym z czynników mającym wpływ na ilość zdeponowanych pierwiastków w tkance skeratynizowanej jest obecność lub brak pigmentów w grupy melanin. Nie wzięcie pod uwagę tego czynnika może wpłynąć na znaczące różnice w poziomie oznaczanych pierwiastków, nawet w przypadku próbek pochodzących od jednego osobnika. Różnice w upierzeniu i ubarwieniu ptaków oraz ssaków utrudniają standaryzację tego typu badań. Biorąc pod uwagę powyższe procesy przyjęto, iż w przypadku renifera Svalbardzkiego do analizy wykorzystana zostanie wyłącznie biała sierść, która jest łatwiejsza do pozyskania i porównania w badaniach prowadzonych w przyszłości. Z kolei u ptaków, zarówno u alczyka jak i u dwóch gatunków oceanników, wykorzystane zostały wyłącznie czarne zawierające głównie eumelaninę pióra, ponieważ występują one zarówno u piskląt jak i u osobników dorosłych.

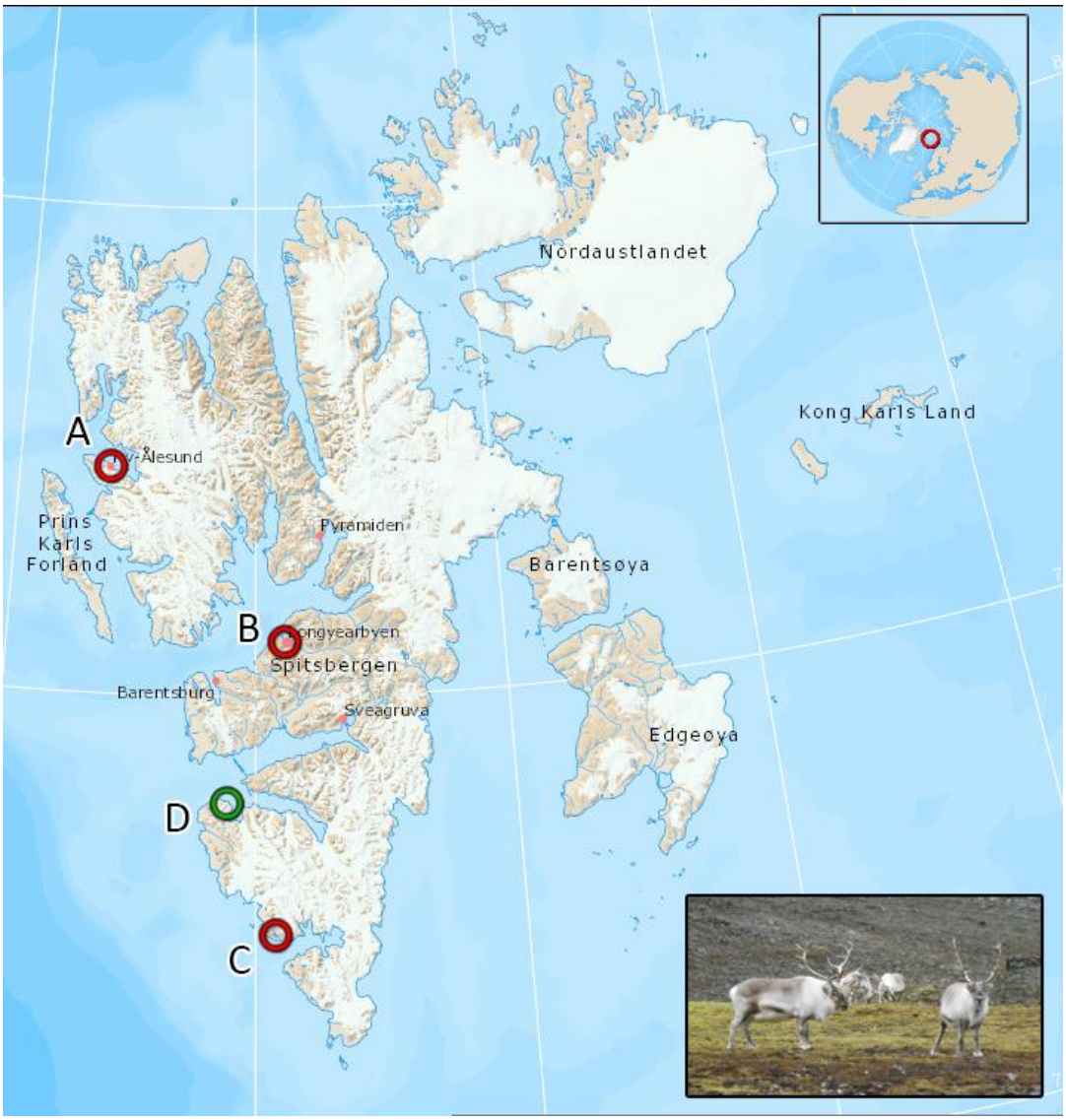
11.1. Miejsca prowadzenia badań i pobrania próbek

Próby materiałów biologicznych do badań zostały pobrane od reprezentacyjnych gatunków dwóch polarnych ekosystemów: lądowego (Arktyka Europejska) oraz morskiego (Arktyka Europejska oraz Antarktyda). W przypadku ekosystemu morskiego ocenie ekotoksykologicznej poddane zostały gatunki z dwóch osobnych środowisk polarnych, zajmujące podobny poziom troficzny. Wszystkie próbki były docelowo przetransportowane do laboratorium Katedry Chemii Analitycznej, Politechniki Gdańskiej, gdzie zostały odpowiednio przygotowane do analizy.

11.1.1. Arktyka Europejska

Próbki sierści renifera Svalbardzkiego pobrane zostały na obszarze trzech miejsc położonych na Spitzbergenie (Rysunek 6). Wszystkie próbki zostały pobrane w sezonie letnim; w sierpniu 2015 roku w okolicy stolicy wyspy, Longyearbyen (78°N 015°E, n=11, punkt B na Rysunku 6), we wrześniu 2016 roku od populacji żerującej w pobliżu Polskiej Stacji Polarnej, Hornsund (77°N 015°E, n=16; punkt C na Rysunku 6), oraz w lipcu 2017 roku ponownie w okolicy Longyearbyen (n=5) oraz w okolicy stacji badawczej Ny-Ålesund (11°48'/12°04'E, 78° 54'/78°55'N; n=3, punkt A na Rysunku 6). Próbki pobrane zostały przez autorkę rozprawy doktorskiej (próbki z Hornsundu), w ramach współpracy wewnątrz-katedralnej (przez dr inż Katarzynę Kozak oraz dr inż. Sarę Lehmann-Konera) oraz międzyuczelnianej (współpraca z botanikami z Uniwersytetu Jagiellońskiego w Krakowie). Wszystkie próbki sierści po zebraniu przechowywane były w osobnych woreczkach strunowych i przetransportowane statkiem do Polski.

W ramach rozprawy przeprowadzone zostały również badania mające na celu określić zmienność biodostępnych pierwiastków w diecie w zależności od rodzaju tundry, na której żerują renifery. W tym celu między 6-24 sierpnia 2016 roku pobrano próbki odchodów renifera w okolicy fjordu Bellsund (próbki pobrane przez dr inż. Sarę Lehmann-Konera; Calypsostranda i dolina Chamberlin) (n=54; punkt D na Rysunku 6). Próbki pobierane były manualnie w polietylenowych rękawiczkach, do czystych woreczków strunowych i wstępnie suszone. Następnie próbki ponownie spakowano do woreczków strunowych, zabezpieczonych przed zanieczyszczeniem poprzez umieszczenie ich w dwóch dodatkowych woreczkach i przetransportowane statkiem do Polski .



Rysunek 6. Miejsca pobierania próbek pochodzących od renifera Svalbardzkiego; punkty A-C wskazują na miejsca bytowania populacji od których pobrano sierść, punkt D-oznacza miejsce pobrania odchodów; źródło mapy: toposvalbard.npolar.no; zdjęcie Aneta Pacyna-Kuchta

Kolonia alczyka znajduje się na zboczach Ariekammen, w południowo-zachodniej części Spitzbergenu (Rysunek 7). Próbki pobrane zostały w sezonie letnim 2017 od osobników dorosłych oraz piskląt w ramach współpracy międzyuczelnianej z Uniwersytetem Gdańskim przez dr hab. Katarzynę Wojczulanis-Jakubas oraz Dariusza Jakubas. Rodzaj pobranych próbek to pióra dorosłych alczyków wyrośnięte przed okresem lęgowym (pióra z podgardla, n=74) oraz po sezonie lęgowym (pióra okrywowe pobrane z tyłu korpusu ciała, n=74), pióra puchowe (n=16), oraz właściwe (n=18) pochodzące od piskląt oraz skorupki powylęgowe (n=18). Pióra

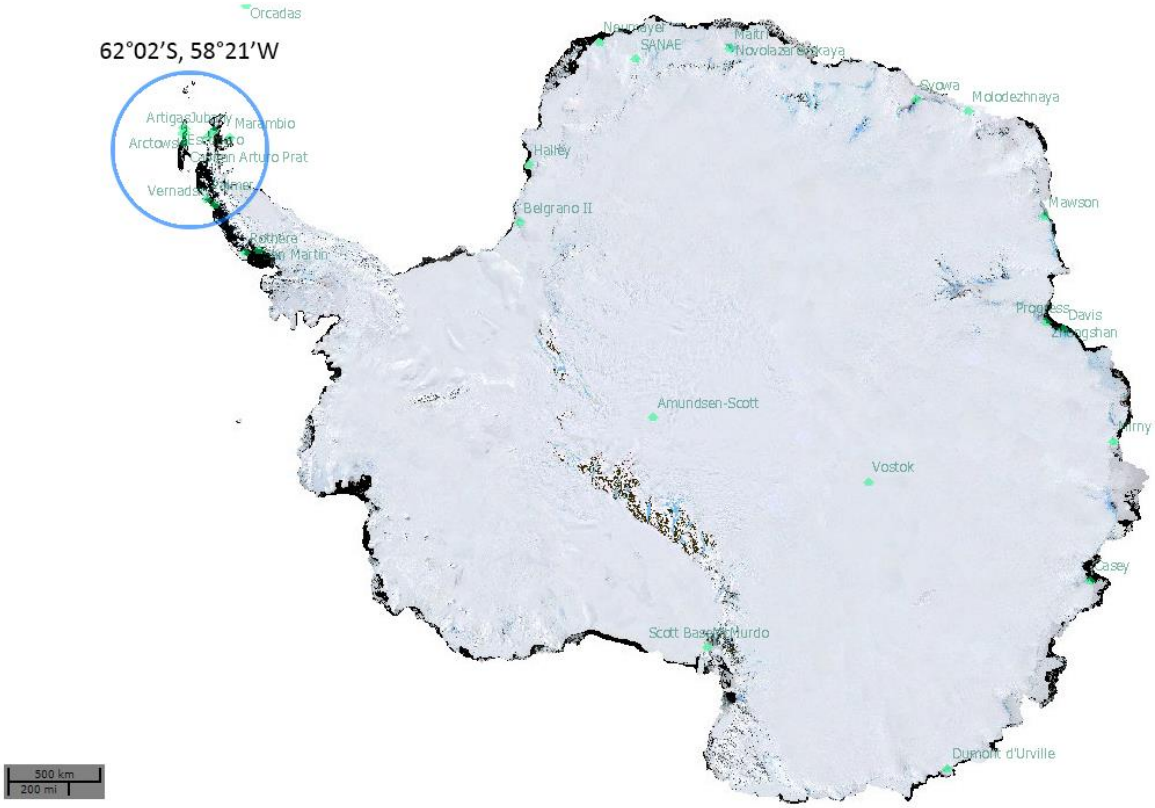
pochodzące od piskląt oraz skorupki pobrane zostały z tych samych gniazd. Po pobraniu pióra przechowywane były w woreczkach strunowych w temperaturze pokojowej, a skorupki zamrożone w polietylenowych pojemnikach.

Rysunek 7. Miejsca pobierania próbek pochodzących od alczyka (Alle alle); źródło mapy: toposvalbard.npolar.no; zdjęcie Katarzyna Wojczulanis-Jakubas

11.1.2. Antarktyda

Oceanniki to ptaki morskie gniazdujące na trudno dostępnych klifach, spędzające sezon lęgowy na Antarktydzie. Próbki piór dwóch synantropijnych gatunków oceannika żółtopłetwego *Oceanites oceanicus* oraz oceannika czarnobrzuchego *Fregetta tropica* pochodzące od osobników dorosłych oraz piskląt (pióra właściwe oraz puch) pobrane zostały od ptaków gniazdujących na Wyspie Króla Jerzego w okolicy Polskiej Stacji Polarnej im.

Henryka Arctowskiego (62°02’S 58°21’W, Rysunek 8). Badane gatunki mają podobną obszar żerowania i dietę, różniącą się udziałem procentowym ryb. Oba gatunki gniazdują w podobnym środowisku, a po okresie lęgowym oba migrują na północ, spędzając czas na otwartym morzu i przechodząc wymianę upierzenia [104]. Ilość pobranych próbek zależała od gatunku, wieku oraz rodzaju przeprowadzonej analizy, dokładne dane przedstawione są w załączniku 5 do rozprawy doktorskiej. Po pobraniu pióra przechowywane były w oddzielnych woreczkach strunowych.

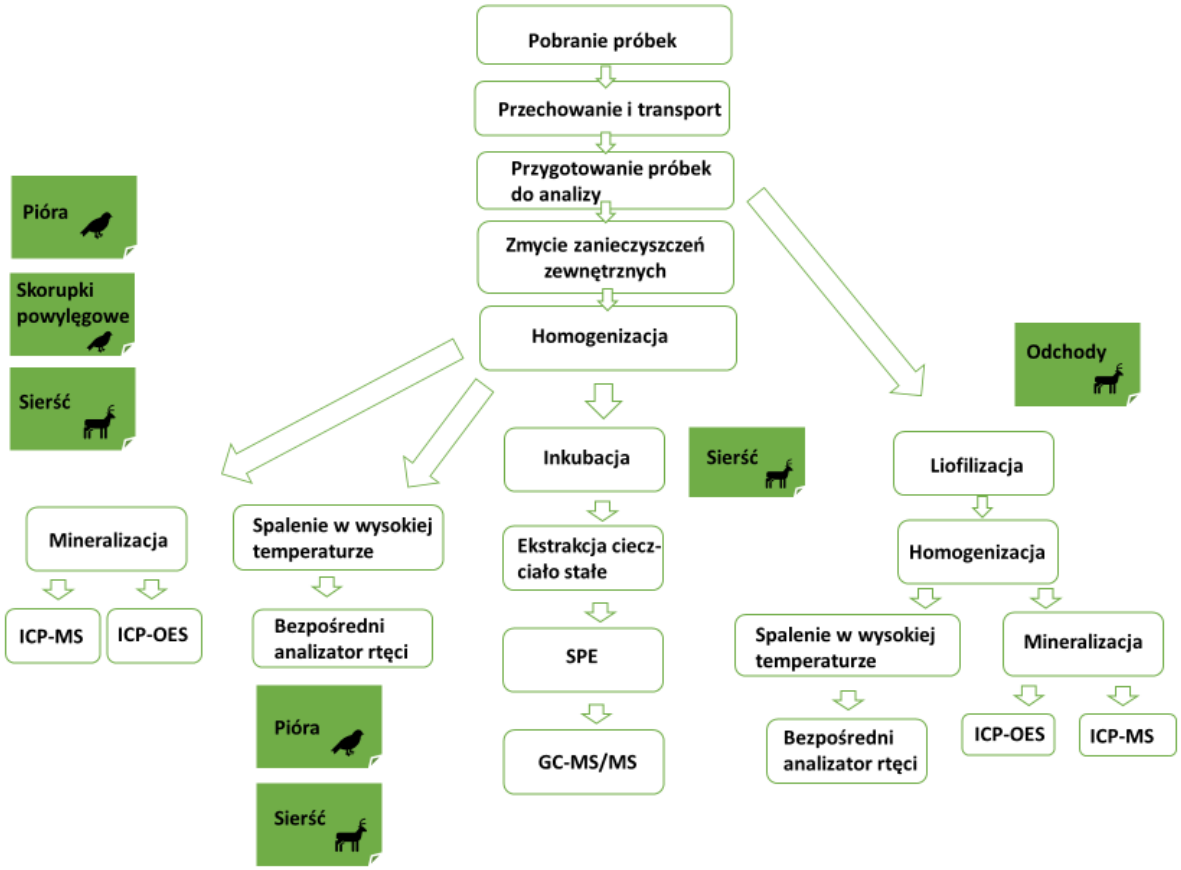


Rysunek 8. Miejsce kolonii lęgowych oceanników, mapa z zaznaczonymi stacjami polarnymi; źródło mapy: https://lima.usgs.gov/ (Landsat Image Mosaic of Antarctica)

11.2. Procedura analityczna

Dzięki wykorzystaniu nowoczesnych technik analitycznych umożliwiających wykrycie i oznaczenie wielu analitów podczas jednego cyklu pomiarowego możliwe było uzyskanie

rozbudowanej bazy danych chemicznych. Na rysunku 9 przedstawiono schemat procedury analitycznej. W tabeli 1 przedstawiono jakie pierwiastki były oznaczane w poszczególnych tkankach. Trwałe związki organiczne były oznaczane wyłącznie w sierści renifera, dokładny spis przedstawiony jest w tabeli 2. Podczas stosowania każdej metody analitycznej konieczna jest jej walidacja. Walidacja metod analitycznych obejmuje testowanie podczas pomiaru istotnych cech charakteryzujących metodę takich jak: LOD, LOQ, odtwarzalność, poprawność, czułość, niepewność, dokładność, zakres liniowości, odzysk, selektywność, specyficzność, zanieczyszczenie tła. Parametry te były wyznaczane dla każdej metody na podstawie roztworów wzorcowych, analizy prób ślepych oraz certyfikowanych materiałów odniesienia. Otrzymane wyniki badań chemicznych zostały ostatecznie poddane analizie statystycznej i zinterpretowane, z uwzględnieniem schematu pierzenia/linienia oraz dostępnych informacji o diecie, wieku, płci oraz obszarze żerowania.



Rysunek.9 Ogólny schemat procedury analitycznej

Tabela 1. Pierwiastki oznaczane w badaniach będących podstawą rozprawy doktorskiej

Grupa pierwiastków	***Tkanka***	***Sprzęt używany do oznaczeń***	***Laboratorium w którym przeprowadzono analizy***
Fe, Zn, Ba, Ca, Cu, K, Mg, Mn, Se, Sr, Pb, Cr, Ni, V, Ga, La, Rb, As, Li, Co, Hg, Cd, Cs, Be	Sierść renifera	ICP-MS Xseries2 by Thermo; ICP-MS 2030 Shimadzu, Japan	Uniwersytet Marii Curie Skłodowskiej w Lublinie, Wydział Nauk o Ziemi i Gospodarki Przestrzennej Uniwersytet im. Adama Mickiewicza w Poznaniu, Wydział Chemiczny, Pracownia Analizy Wody i Gruntów
Hg	Sierść renifera	Direct Mercury Analyzer MA-3000 Nippon Instruments Corporation	Politechnika Gdańska, Wydział Chemiczny, Katedra Chemii Analitycznej
Ca, Fe, K, Mg, P, S, Si	Odchody renifera	ICP-OES 9820 Shimadzu, Japan	Uniwersytet im. Adama Mickiewicza w Poznaniu, Wydział Chemiczny, Pracownia Analizy Wody i Gruntów
Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, La, Li, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, V, Zn	Odchody renifera	ICP-MS 2030 Shimadzu, Japan	Uniwersytet im. Adama Mickiewicza w Poznaniu, Wydział Chemiczny, Pracownia Analizy Wody i Gruntów

Hg	Odchody renifera	Direct Mercury Analyzer MA-2000 Nippon Instruments Corporation	Politechnika Gdańska, Wydział Chemiczny, Katedra Chemii Analitycznej
As, Bi, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mo, Ni, Pb, Sb, Se, Sr, Zn	Pióra oceanników	ICP-MS 2030 Shimadzu, Japan	Uniwersytet im. Adama Mickiewicza w Poznaniu, Wydział Chemiczny, Pracownia Analizy Wody i Gruntów
Hg	Pióra oceanników	Direct Mercury Analyzer MA-3000 Nippon Instruments Corporation	Politechnika Gdańska, Wydział Chemiczny, Katedra Chemii Analitycznej
As, Ba, Bi, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr, V, Zn, K	Pióra alczyka, skorupki powylęgowe	ICP-MS 2030 Shimadzu, Japan	Uniwersytet im. Adama Mickiewicza w Poznaniu, Wydział Chemiczny, Pracownia Analizy Wody i Gruntów
Ca, Mg	Skorupki powylęgowe	ICP-OES 9820 Shimadzu, Japan	Uniwersytet im. Adama Mickiewicza w Poznaniu, Wydział Chemiczny, Pracownia Analizy Wody i Gruntów

Tabela 2. TZO oznaczane w badaniach sierści renifera Svalbardzkiego będących podstawą rozprawy doktorskiej

Grupa związków	*Homologi*	*Sprzęt wykorzystany przy analizie*	*Laboratorium*
WWA	Naftalen; acenaftylen; 2 bromo-naftalen; acenaften; fluoren; fenantren; antracen; fluoranten; pyren; benz[a]antracen; chrysen; benzo[b]fluoranten; benzo[a]pyren; indeno[1,2,3-cd]pyren; dibenz[a,h]antracen; benzo[ghi]perylen	Shimadzu GC-MS-TQ8050 wyposażony w automatyczny podajnik próbek AOC-20i	Politechnika Gdańska, Wydział Chemiczny, Katedra Chemii Analitycznej
PCB	28, 52, 77, 101, 118, 126, 138, 153, 169, 180	GC-MS Agilent 7000D GC/TQ (7890B GC System)	Politechnika Gdańska, Wydział Chemiczny, Katedra Chemii Analitycznej
OCs	alfa-, beta-, delta-hch, hcb, heptachlor, mirex, aldrin, dieldrin, endrin, op-DDT, pp-DDT, op-DDE, pp-DDE, op-DDD, pp-DDD	GC-MS Agilent 7000D GC/TQ (7890B GC System)	Politechnika Gdańska, Wydział Chemiczny, Katedra Chemii Analitycznej

11.2.1. Analiza pierwiastkowa - Rtęć

Do oznaczeń zawartości rtęci całkowitej wykorzystana została technika zimnych par (CV-AAS). Metoda polega na amalgamacji par rtęci po termicznym rozkładzie próbki w rurze ceramicznej. Próbki zostały umieszczone w ceramicznych łódeczkach i zasypane neutralną zasypką. Podgrzanie do wysokiej temperatury (850°C) powoduje rozkład próbki i przekształcenie rtęci do postaci atomowej, która absorbowana jest na złożu złota w formie

amalgamatu. Następnie w wyniku ponownego podgrzania (do około 600/650°C), rtęć jest uwalniana ze złoża i w komorze absorpcyjnej mierzona jest absorbancja fali przy długości fali równej 253.7 nm. Kolektor złoty zostaje ochłodzony przed rozpoczęciem kolejnych pomiarów. Zawartość rtęci była obliczana na podstawie pięciostopniowej krzywej kalibracyjnej.

Zarówno NIC MA 2000 (wykorzystany przy analizie odchodów renifera), jak i NIC MA 3000 (wykorzystany przy analizie sierści i piór) wyposażony jest w automatyczny podajnik próbek. Każda próbka analizowane było w dwóch lub trzech powtórzeniach. Akceptowalne CV% wyznaczone pomiędzy powtórzeniami dla danej próbki wyznaczone zostało na 15%, wyniki powyżej tej wartości były odrzucane.

11.2.2. Analiza pierwiastkowa - Inne pierwiastki

W celu analizy pierwiastkowej umyte odpowiednim rozpuszczalnikiem i wysuszone próbki piór, sierści, skorupek powylęgowych oraz liofilizowane próbki odchodów zważono na wadze analitycznej (dokładność 0.1 mg) i umieszczano osobno w czystych teflonowych pojemnikach, umytych wcześniej wodą dejonizowaną oraz roztworem kwasu azotowego (V). Po dodaniu kwasu azotowego(V) o stężeniu 65%, próbki poddawano mineralizacji wspomaganej promieniowaniem mikrofalowym wykorzystując odpowiedni program temperaturowy (Anton Paar). Następnie zmineralizowany roztwór przenoszono do czystych plastikowych fiolek i rozcieńczano wodą dejonizowaną (czystość Mili-Q) do objętości 25 ml.

Dodatkowo do każdej partii sporządzono próbki ślepe, aby kontrolować zanieczyszczenie tła (dla każdej tury mineralizacji wstawiano minimum jedną próbkę ślepą). Zawartość pierwiastków analizowana była przy zastosowaniu ICP-MS oraz ICP-OES w laboratorium w Lublinie oraz w laboratorium w Poznaniu (szczegóły w tabeli 1. oraz w publikacjach załączniki 2-6).

11.2.3. Wielopierścieniowe węglowodory aromatyczne

Próbki sierści renifera Svalbardzkiego przeanalizowano pod kątem zawartości związków z grupy WWA. Po trzykrotnym umyciu wodą dejonizowaną sierść była suszona, cięta na mniejsze kawałki oraz ważona. Następnie próbki inkubowano w 45°C przez 12 godzin z dodatkiem mieszaniny aceton:heksan (1:2), kwasu solnego o stężeniu 15% oraz wzorca

wewnętrznego (znakowany izotopowo naftalen-d8, benzo(a)antracen-d12; c=0.1 µg/ml). Następnie próbki wytrząsano i górną warstwę organiczną zebrano do szklanych fiolek przemytych wcześniej 3-krotnie n-heksanem. Do próbek ponownie dodano mieszaninę aceton:heksan i wytrząsano około 30 s. Po dokładnym rozdzieleniu faz, drugi raz zebrano warstwę organiczną. Ekstrakty odparowano do około 3 ml., a następnie oczyszczono na wcześniej przygotowanych kolumienkach SPE zawierających filtr, aktywowaną krzemionkę, oraz bezwodny siarczan sodu. Kolumienki były kondycjonowane heksanem, do elucji wykorzystano mieszaninę heksan:DCM (7:3 v:v). Objętość roztworu została zredukowana do sucha w strumieniu azotu i natychmiast odtworzona w 300 µl heksanu. Tak przygotowane próbki poddawane były analizie ilościowej przy zastosowaniu GC-MS/MS (Shimadzu GC-MS-TQ 8050) w trybie MRM. Parametry metody oraz specyfikacja są przedstawione w tabeli 3.

Tabela 3. Parametry metody oraz specyfikacja techniczna użytej w procedurze analitycznej aparatury pomiarowej dla Shimadzu GC-MS-TQ 8050

Aparat pomiarowy	***Specyfikacja***
Shimadzu GC-MS-TQ8050 z automatycznym podajnikiem próbek AOC-20i	Kolumna Zebron ZB-Semivolatiles 20 m L x 0.18 mm I.D. x 0.18 µm df, coated with 5% polysilarylene – 95% Polydimethylsiloxane dostarczona przez Phenomenex Tryb splitless, temperatura nastrzyku 280 °C, objętość nastrzyku 2 µL Temperatura interfejsu I źródła jonów: 320 °C i 230 °C. Początkowa temperatura kolumny to 50 °C utrzymywana przez 1 min, następnie podniesiona do 260 °C z przyrostem temperatury 20 °C/min, następnie do 300 °C z przyrostem 5 °C/min, ostatecznie do 320 °C z przyrostem 20 °C/min, utrzymane przez 15 min. Hel (99.999%) został użyty jako gaz nośny, ze stałym przepływem 1 mL/min. Jako gaz kolizyjny wykorzystano argon. Napięcie detektora 1.5 kV.

11.2.4. Polichlorowane bifenyle i pestycydy chloroorganiczne

Sierść była czyszczona trzykrotnie wodą dejonizowaną, suszona, cięta na mniejsze kawałki i ważona. Następnie próbki inkubowano w 45°C przez 12 godzin z dodatkiem mieszaniny heksan:DCM (4:1), 4M HCl oraz mieszaniną wzorców wewnętrznych (DDT-D8, PCB-28 C13, PCB-180 C13; c=0.1 µg/ml). Następnego dnia próbki były wstawione na ultradźwięki przez 1 minutę, po czym próbki wytrząsano i górną warstwę organiczną zebrano do szklanych fiolek przemytych wcześniej 3-krotnie n-heksanem. Do próbek ponownie dodano mieszaninę heksan:DCM (4:1) i wytrząsano około 30 s. Po dokładnym rozdzieleniu faz, drugi raz zebrano warstwę organiczną. Próbki odparowano do około 3 mililitrów. Ekstrakty oczyszczono na wcześniej przygotowanych kolumienkach SPE zawierających filtr, zakwaszoną krzemionkę, oraz bezwodny siarczan sodu. Kolumienki były kondycjonowane, do elucji wykorzystano mieszaninę heksan/DCM (4:1, v/v). Objętość roztworu została zredukowana do sucha w strumieniu azotu i natychmiast odtworzona w 200 µl izooktanu. Tak przygotowane próbki poddawane były analizie jakościowej i ilościowej przy wykorzystaniu chromatogramu gazowego (Agilent 7890B) sprzężonego z tandemowym spektrometrem mas Agilent (7000D) działającego w trybie MRM w Katedrze Chemii Analitycznej Wydziału Chemicznego Politechniki Gdańskiej. Parametry metody oraz specyfikacja są przedstawione w tabeli 4.

Tabela 4. Parametry metody i specyfikacja techniczna użytej w procedurze analitycznej aparatury pomiarowej dla Shimadzu GC-MS-TQ 8050

Aparat pomiarowy	***Specyfikacja***
GC-MS Agilent 7000D GC/TQ (7890B GC System)	Kolumna HP-5MS 5% Phenyl Methyl Silox 30 m x 250 µm x 0.25 µm, Agilent Technologies, Inc. Tryb splitless, temperatura nastrzyku 250 °C, objętość nastrzyku 5 µL N_2 użyty jako gaz kolizyjny (1.5 ml/min), He jako gaz nośny (ze stałym przepływem 1 ml/min) oraz jako Quench Gas (2.5 ml/min)

Temperatura interfejsu I źródła jonów: 300 °C i 230 °C. Początkowa temperatura kolumny to 70 °C utrzymywana przez 2 min, następnie podniesiona do 150 °C z przyrostem temperatury 25 °C/min, następnie do 200 °C z przyrostem 3 °C/min, następnie do 280 z przyrostem 8°C/min ostatecznie do 300 °C z przyrostem 100 °C/min, utrzymane przez 5 min.

Informacje o podstawowych parametrach walidacyjnych metod badawczych, wykorzystanych w trakcie realizacji rozprawy doktorskiej przedstawiono w tabeli 5, szczegółowy opis znajduje się w publikacjach [załączniki 2-6].

Tabela 5. Podstawowe parametry walidacyjne metod użytej w procedurze analitycznej aparatury pomiarowej (*dla roztworu zmineralizowanej próbki)

Oznaczane anality	*LOD*	*LOQ*	*Jednostka*	*CV*	*Tkanka*	*Aparatura pomiarowa*
Hg	0.54	1.62	ng/g dw	>15%	Pióra, sierść	Direct Mercury Analyzer MA-3000 NIC
Hg	0.54	1.62	ng/g dw	3.83%	Odchody renifera	Direct Mercury Analyzer MA-2000 NIC
Li, Fe, V, Cr, Ni, As, Rb, Ba, Pb, Be, Co, Ga, Cs, Cd, La, Cu, Zn	0.01-0.5	0.03-1.5	ng/L*	>15%	Sierść	ICP-MS Xseries2 by Thermo; ICP-MS 2030 Shimadzu, Japan

Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, La, Li, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, V, Zn	0.004-0.013	0.92-3.07	ng/g dw	>15%	Odchody, pióra, skorupki powylęgowe, sierść	ICP-MS 2030 Shimadzu, Japan
Ca, Fe, K, Mg, P, S, Si	0.0004-0.031	0.001-0.104	mg/L*	3.82%	Skorupki powylęgowe, odchody	ICP-OES 9820 Shimadzu, Japan
ΣWWA	0.013–0.38	0.039-1.14	ng/g dw	>5%	Sierść renifera	Shimadzu GC-MS-TQ8050
ΣPCB, ΣOC	0.011–1.38	0.033-4.14	ng/g dw	>5%	Sierść renifera	GC-MS Agilent 7000D GC/TQ (7890B GC System)

11.3. Narzędzia wykorzystane do analizy i interpretacji wyników

W celu analizy oraz interpretacji wyników wykonano szereg testów statystycznych, między innymi test t-Studenta, metody statystyczne dwuparametrowe (matryca korelacji Pearson‘a), jak i wieloparametrowe PCA, PERMANOVA i SIMPER. Użycie konkretnych typów testów opisane jest w załącznikach 2-6.

Test t-Studenta posłużył w analizie pierwiastkowej do sprawdzenia różnic statystycznych między wybranymi grupami (np. wiekowymi). Matryca korelacji Pearson‘a została użyta głównie do wykrycia zależności pomiędzy wynikami badań chemicznych. Do analizy głównych składowych wykorzystano te zmienne, które wykazały istotne związki korelacyjne. Aby znaleźć grupy pierwiastków o wysokim stopniu powiązania wykorzystano analizę klastrową (HCA). PERMANOVA, SIMPER zostały wykonane w celu porównania składu pierwiastkowego jakościowego oraz ilościowego, względem wybranych faz życiowych i/lub gatunku. Większość testów statystycznych wykonana została na danych przekształconych logarytmicznie [log(x+1)].

12. Otrzymane wyniki i dyskusja

Weryfikacja słuszności sformułowanych w rozdziale 6 „Cel rozprawy doktorskiej" hipotez badawczych nastąpiła na podstawie badań przedstawionych szczegółowo w załącznikach 2-6. Poniżej przedstawiono główne wyniki.

12.1. Weryfikacja hipotezy 1

Hipoteza 1: Akumulacja pierwiastków w roślinności Spitzbergenu, zwłaszcza w mchach oraz porostach, wpłynie na zawartość metali toksycznych oraz innych pierwiastków w sierści renifera. Istnieje bezpośrednia zależność między rodzajem spożywanej roślinności, a poziomem pierwiastków w sierści, oraz ze względu na mały zasięg przemieszczania się poszczególnych populacji mogą wystąpić różnice w akumulacji pierwiastków między nimi

Weryfikację **pierwszej hipotezy** wykonano w oparciu o badania próbek sierści pobranej od dwóch populacji renifera Svalbardzkiego. Wyniki badań zostały opublikowane w czasopiśmie *Chemosphere* (załącznik 2) **[A. D. Pacyna, K. Koziorowska, S. Chmiel, J. Mazerski, Ż. Polkowska, Svalbard reindeer as an indicator of ecosystem changes in the Arctic terrestrial ecosystem, Chemosphere, 2018, DOI: 10.1016/j.chemosphere.2018.03.158]**

Badania będące podstawą publikacji stanowią pierwszy udokumentowany opis zawartości 18 pierwiastków (Fe, Zn, Ba, Cu, Pb, Cr, Ni, V, Ga, La, Rb, As, Li, Co, Hg, Cd, Cs, Be) w próbkach sierści renifera Svalbardzkiego z dwóch populacji żyjących w pobliżu Polskiej Stacji Polarnej oraz w pobliżu stolicy Svalbardu (Longyearbyen). Dodatkowo w próbkach wykonano analizy stałych izotopów (SI) azotu (δ15N) oraz węgla (δ13C), w celu określenia wpływu diety na rodzaj oraz ilość akumulowanych pierwiastków.

W przypadku kilku próbek z okolic Longyearbyen stwierdzono bardzo wysokie zawartości żelaza skorelowane z profilem izotopowym azotu wskazującym na duży udział gorszej jakościowo roślinności w diecie. Wysoki poziom żelaza był skorelowany również z podwyższonym poziomem ołowiu, miedzi oraz innych pierwiastków mogących mieć działanie toksyczne dla organizmu. Stałe izotopy azotu (δ15N) oraz węgla (δ13C) są coraz częściej stosowane jako niezbędne narzędzie w badaniach ekologicznych. Różne typy roślin cechuje stały stosunek izotopów (13C/12C i 15N/14N), które zależą od ich fizjologii (na przykład ścieżki fotosyntetycznej), a także środowiska (np. temperatury, natężenia światła, wilgotności

powietrza, opadów). Stałe izotopy są włączane do tkanki skeratynizowanej z diety i mogą być wykorzystane do oceny przestrzennej i czasowej zmiany składników odżywczych, oraz charakteryzują niszę troficzną.

Ze względu na trudne warunki atmosferyczne oraz ukształtowanie powierzchni i naturalne przeszkody w postaci lodowców, śledzenie reniferów w terenie jest zadaniem bardzo trudnym i często logistycznie niemożliwym. Wykorzystując analizę stałych izotopów węgla oraz azotu w sierści, można pozyskać informację o preferowanym typie roślinności reniferów w danym czasie. Z kolei analizując próbki sierści pod kątem pierwiastkowym, można zdobyć cenne informacje o ekspozycji ssaków lądowych na zanieczyszczenia. Z badań prowadzonych przeze mnie na dwóch populacjach renifera Svalbardzkiego wynika, iż różnice w profilu izotopowym między populacjami są znaczące i dieta wyraźnie wpływa na rodzaj oraz stopień zanieczyszczenia metalami śladowymi.

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Svalbard reindeer as an indicator of ecosystem changes in the Arctic terrestrial ecosystem

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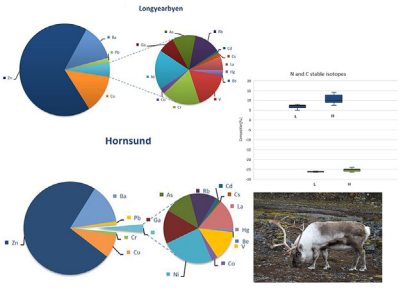
HIGHLIGHTS

- The first communication concerning trace element concentration in hairs of two separate subpopulations.
- Iron overload correlated with high levels of other elements.
- Similarity in trends in the studied subpopulations observed for many metals.
- A high variation in nitrogen isotopes signatures.

GRAPHICAL ABSTRACT



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ABSTRACT

Over the years, noticeable effort has been directed towards contaminant determination in multiple biotic samples collected from the inhabitants of the Arctic. Little consideration has been given to polar herbivores, however, especially those from the European parts of the Arctic. To provide a broader perspective, we aimed to decipher trace element concentration in hairs of the key species in the Arctic, namely the Svalbard reindeer (*Rangifer tarandus platyrhynchus*), and to recognise whether diet variations could correspond with forward exposure. The effect of habitat and diet was investigated using the ratios of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), and previous literature studies on vegetation from the areas of interest. Analysis was performed for eighteen elements in total, both toxic and essential. Metals were present in a decreasing order Fe > Zn > Ba > Cu > Pb > Cr > Ni > V > Ga = La > Rb > As > Li > Co > Hg > Cd > Cs > Be. Similarity in trends in the studied subpopulations was observed for many metals. A significant log-linear correlation was observed for most of the elements, excluding nitrogen and carbon isotopes signature. Extremely high iron levels were determined in some of the samples, suggesting past iron overload. Zinc, in contrast to the remaining metals, did not correlate well with any other element. Mercury was determined at very low levels, in accordance with previous literature regarding its concentrations in moss and lichen species in Svalbard. The analysis of stable

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isotopes showed a high variation in nitrogen isotopes signatures. Further research is required to properly evaluate the potential health risks and ecological implications of elevated exposure.

1. Introduction

Constant pollutant emission is undeniably a serious problem and it is considered a huge threat to ecosystem stability. Anthropogenic activities undoubtedly have significant ecological consequences worldwide. The Arctic is an invaluable source of information on the global-scale impact due to long-range contaminant transport (Davis, 1996; Halbach et al., 2017). The accumulation of trace elements, particularly heavy metals, and the resulting enrichment in higher trophic levels, raise questions about its impact on native fauna. Due to its unique geographical location, the Svalbard Archipelago has become a significant recipient of pollutants emitted in the Northern Hemisphere. Natural sources of heavy metal emissions include volcanic activities, biogenic sources, soil-derived dusts, and sea salt aerosols. It is anthropogenic emissions, however, that are assumed to account for the observed heavy metal levels in the Arctic to the greatest degree (AMAP, 2005; Halbach et al., 2017). With only several local sources of pollution (such as mining activities, airport, ship traffic), most contaminants including heavy metals are atmospherically transported long-range from mid- and low-latitudes (Bard, 1999).

A growing amount of evidence arose in the recent years concerning the deposition of pollutants in polar, particularly marine biota (e.g. Burger et al., 2007). Physiological and ecological factors affecting the bioaccumulation process vary between terrestrial and aquatic ecosystems (van den Brink et al., 2015). Terrestrial species are often weakly investigated and yet crucial parts of any polar ecosystem. Reindeers are a key component of the Arctic terrestrial ecosystem (Duffy et al., 2005). Because they are a part of a simple food chain, the species is ideal for monitoring changes in the terrestrial trophic network (Elkin and Bethke, 1995).

In this paper, we investigate the usefulness of molten fur collected from a broadly distributed resident of the European part of the Arctic, namely - the smallest reindeer subspecies (*Rangifer tarandus platyrhynchus*). This large herbivore, endemic to Svalbard, can be found in the majority of non-glaciated areas of the island. The Svalbard reindeer has certain adaptations to the polar environment, including relatively short legs and thick fur with colouring and thickness varying between the seasons (Cuyler and Øritsland, 2002; npolar.no; mosj.no). Its total population size is estimated for 10,000 animals (npolar.no). Monitoring studies conducted in Brøggerhalvøya, Reindalen, Adventdalen, and Edgeøya suggest high annual fluctuations (mosj.no; Reimer, 2012) primarily caused by variations in climate condition (such as snow depth and rain-on-snow events), and partially by competition for food resources.

The primary function of the fur of the Svalbard reindeer is body insulation from cold and wind (Cuyler and Øritsland, 2002). In cervids, the coat is replaced annually. New fur developes from late spring/early summer to late fall. The trace element composition of fully grown hairs largely reflects summer and fall deposition (Drucker et al., 2010). Reindeer hairs develop a hollow, air-filled, stiff, close-packed structure with a primary heat transfer function. It also undergoes seasonal changes. Summer and winter fur of adults and calves is characterised by different properties such as hair length, density, and colour (Cuyler and Øritsland, 2002).

The Svalbard reindeer is the only large grazing mammal in the European High Arctic (Hayashi et al., 2014). It is exposed to contaminants particularly through its diet, composed of different types of vegetation, including lichen and moss (Robillard et al., 2002). Terrestrial plants receive metals sprayed from seawater (if they grow within the distance of sea spray influence), by dry and wet deposition, and from melting glaciers as trapped particles are released from ice (Xie et al., 2006; Samecka-Cymerman et al., 2011). Birds can also be an additional vector for contaminant transport (Savinov et al., 2003), as well as reindeer guano (Van der Wal et al., 2004). The Svalbard subpopulation eats almost all types of vegetation available. During the growing season, selection for plant quantity rather than quality is observed (Van der Wal et al., 2000).

Plants show variable stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) depending on their physiology and environmental conditions, e.g. temperature, light intensity, air humidity, or precipitation (Drucker et al., 2010). Stable isotopes are incorporated into growing hair from diet, and can be used to assess spatial and temporal variation in diet components, to characterise the trophic niche (Boecklen et al., 2011), unravel the migration path (Hobson and Wassenaar, 2008), or determine habitat selection (Newsome et al., 2009). The ecology of the animal can be therefore investigated based on stable isotope analysis, as their abundance in tissues reflects that in the diet (Drucker et al., 2010).

The available data on exposure assessment in polar herbivores is still limited, particularly to the Alaskan and Canadian populations. Also studies concerning stable isotope analysis in reindeer tissues are scarce. To fill this gap in knowledge, the present study focused on the investigation on 18 trace elements (Fe, Zn, Ba, Cu, Pb, Cr, Ni, V, Ga, La, Rb, As, Li, Co, Hg, Cd, Cs, Be), and nitrogen and carbon stable isotopic composition in hairs collected in the summer season from reindeer herds. The Svalbard reindeer is a sedentary species, migrating only in the case of significantly reduced food resources (Hansen et al., 2010b). It is therefore vulnerable to any changes in local foraging conditions. Hairs can be used as a long-range record of contaminants deposition as they accumulate elements continuously by bounding them to sulphur-rich hair proteins during the hair growth period (Duffy et al., 2005).

The primary objective of this paper is to provide new background data on the levels of metals in reindeer fur, and a comparison between two subpopulations living in distant areas in order to establish the pollution level and determine variations in nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotope composition.

2. Materials and methods

2.1. Study area and sampling

Fur samples were collected in two consecutive summer seasons: in August 2015 from Longyearbyen region (N78° E015°, n = 11) and in September 2016 from the Fuglebekken catchment in the vicinity of the Polish Polar Station in Hornsund (N77° E015°, n = 16) (Fig. 1).

Samples were collected from the ground, after a herd moved to a new place. To avoid pseudoreplication, only freshly molten fur was collected (one sample per at least 4 m^2 distance). We assumed that samples were from separate individuals. All samples were individually packed in clean zip bags, and stored at a temperature of 4 °C prior to analysis. Long, straight, white on entire length (except

Fig. 1. Study area with main coordinates, A-Longyearbyen area, B- Hornsund area [map source: toposvalbard.npolar.no]; Svalbard reindeer (*Rangifer tarandus platyrhynchus*).

darker tip) guard hairs were collected. Mean temperature during the period of sample collection amounted to 2.9 °C in August 2015 (Longyearbyen) and 3.9 °C in September 2016 (Hornsund) (yr.no). Sample weight varied from 16 to 80 mg for samples collected from Longyearbyen, and from 9 to 100 mg for samples collected from the Hornsund area.

The Svalbard reindeer, unlike other reindeer subspecies, is highly stationary. It is reluctant to migrate beyond its territory range mostly established by natural barriers (thin sea ice, glaciers, steep mountains) (Hansen et al., 2010b). Genetic differences between populations might occur even at distances <50 km^2 (Côté et al., 2002). Therefore, the studied herds are most likely from completely separate populations. Predation is almost non-existing, with the exception of local hunting and occasional evidence of polar bear hunting attempts (Hansen et al., 2011).

2.2. Analytical methods

18 trace elements and nitrogen and carbon stable isotopes composition were analysed. The basic course of the analytical procedure involves removal of external contamination and then elemental analysis preceded by acid mineralization in microwave emitter (trace elements except for mercury), thermal vaporization (mercury) and high temperature oxidation ($\delta^{13}C$ and $\delta^{15}N$).

2.2.1. Trace elements (except for mercury)

First, each hair strand was separated manually from the collected sample with clean tweezers to separate from any parts of moss collected with the fur ball. To remove the adherent external contamination such as dust and loosely bound particulate matter, each pooled sample from one individual was cleaned by vigorous shaking at least 2 times in double deionised water for 15 min in an automatic shaker, and then air-dried for 24 h. Only white hairs were used, and all visible dust particles were washed out. Next, dry hairs were homogenised by cutting into small parts, weighed to the nearest 0.1 mg, and placed in a clean teflon vessel with 65% HNO_3 (Merck, 99% purity). Digestion was carried out using a high-pressure microwave emitter (Microwave Digestion System, Anton Paar). The temperature was increased from room temperature to 90 °C (app. 6–8 °C/min). Such conditions were maintained for 25 min. After that, temperature was gradually cooled down. Subsequently mineralised samples were diluted with deionised water into 25 ml in clean plastic flasks. To ensure quality control, blank samples were run with every batch. The metals were determined by means of a quadrupole spectrometer ICP-MS Xseries2 by Thermo with inductively-coupled plasma. For the purpose of reduction of isobaric and polyatomic interferences, a collision/reaction cell was used with the application of a mix of helium and hydrogen gases, and the kinetic energy discrimination function (KED). Detail information about analytical instrumentation can be found in Table 4, Supplementary Material.

The accuracy of the analyses was verified by means of certified material Standard Reference Material NIST 1643e Trace Elements in Water and Analytical Reference Material EnviroMAT ES-H-2 CRM

SCP SCIENCE. The retrieval of the elements water ranged from 87% to 109%.

The determination was performed at the Department of Hydrology, Faculty of Earth Sciences and Spatial Management, Marie Curie-Skłodowska University in Lublin.

2.2.2. Mercury analysis

External contamination was washed out using the same procedure as for other trace elements. The pooled dry sample was cut into smaller pieces using sterilised stainless scissors, weighed (to the nearest 0.01 mg), and analysed by the thermal vaporization atomic absorption method (MA-3000 Nippon Instruments Corporation). The samples were heat decomposed in a ceramic boat, first heated to 180 °C for 120 s, and then to 850 °C also for 120 s. The mercury collector collects the atomised mercury gas in a form of gold amalgam, condensing and purifying the mercury. After heat decomposition, the mercury collection tube was heated to 650 °C to liberate the mercury gas. Absorbance at a wavelength of 253.7 nm was then measured. Oxygen flow amounted to 0.4 L/min. Total mercury concentration was determined in triplicates, and based on them the variation coefficient was calculated. Quality control included blank samples every 5–6 subsamples run. The median of the coefficient of variation between replicates was equal to 10.0 (7.91–13.95) in samples collected from Longyearbyen, and 3.65 (1.64–8.98) in samples from Hornsund. Reference materials MODAS-4 Cormorant Tissue (M-3 CornTis), MODAS-3 Herring Tissue (M-3 HerTis), MODAS-5 Cod Tissue (M-5 CodTis) were used to determine analytical accuracy, and to perform method and quality control. Recovery of reference materials measured on three replicates of each RM varied from 94 to 100%.

2.2.3. Stable isotopes

The analyses of carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were done in an Elemental Analyzer Flash EA 1112 Series combined with an Isotopic Ratio Mass Spectrometer IRMS Delta V Advantage (Thermo Electron Corp., Germany). Details of these measurements are described by Kuliński et al. (2014). In short, the samples were dried, homogenised, and weighed into silver capsules (about 1 mg). This sample weight guarantees C and N loads significantly higher than those given by the limit of quantification (C = 20 μg, N = 20 μg). Next, samples were oxidised in 1020 °C in presence of Cr_2O_3 and Co_3O_4. After catalytic oxidation, gases including CO_2, NO_x and H_2O, were transported to the second reactor, where NO_x was reduced to N_2 on the metallic Cu (650 °C). Subsequently, the analysis products were dried with $Mg(ClO_4)_2$ and separated on GC (45 °C). The separated gases (CO_2 and N_2) were transported to the IRMS. The isotopic composition of carbon and nitrogen was calculated using laboratory working pure reference gases (CO_2 and N_2) calibrated against IAEA standards: CO-8 and USGS40 for $\delta^{13}C$ and N-1 and USGS40 for $\delta^{15}N$. Results of $\delta^{13}C$ and $\delta^{15}N$ were given in the conventional delta notation, i.e., versus PDB for $\delta^{13}C$ and versus air for $\delta^{15}N$ as parts per thousand (‰) according to the following equation:

$$\delta X\ (‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000$$

where: X is the stable isotope ratio of $\delta^{13}C$ or $\delta^{15}N$; R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The measurement precision was better than 0.20‰ for $\delta^{13}C$ and 0.18‰ for $\delta^{15}N$ (n = 5).

2.3. Quality assurance/quality control (QA/QC)

To ensure high quality of results, the obtained data were subject to strict quality control procedures. All the analytical equipment was carefully washed before analysis. Based on duplicate and triplicate samples, the variance coefficient of metal concentration was calculated. If the coefficient >15%, samples were excluded from the analyses, assuming unreliable estimation of metal concentration. Background contamination was present in metal method blanks prepared after mineralization, therefore blank correction was performed for all elements. Blank correction involved subtracting the total amount of analyte detected in the method blank from the total amount of analyte detected in the hair samples. Negative numbers and numbers below the limit of detection were reported as half of the limit of detection for statistical analysis. The obtained results were also corrected for sample weights and method dilution factor, and are reported as μg/g dw. All reagents were of the highest purity. Ultrapure water was produced from a Mili-Q Gradient A10 (Milipore, France).

ICP-MS equipment calibration employed the multi-element standard by Inorganic Ventures ANALITYK - CCS-1, CCS-4, CCS-6. The optimised and validated methods showed good linearity (R2>0.999) over a wide range with low limits of detection. Both the method limit of detection (LOD) and the limit of quantitation (LOQ) were calculated based on the standard deviation of the response (s), and the slope of the calibration curve (b) according to the following formulas: LOD = 3.3(s/b), LOQ = 10(s/b) (LOD/LOQ - Li, Fe, V, Cr, Ni, As, Rb, Ba, Pb 0.1/0.3 ppb; Be, Co, Ga, Cs, Cd, La 0.01/0.03 ppb; Cu, Zn 0.5/1.5 ppb). For mercury the method limit of detection and quantification was equal to 0.54 and 1.62 ppb, respectively.

Due to the fact that metals are bound to the keratin structure with variable affinity, removal efficiencies differ significantly among compounds when stronger solvents such as acetone are used. Therefore, only double deionised water was used as a washing agent. Some part of surface contamination might not have been removed. Because it is difficult to distinguish between internal and external exposure, it can be assumed that hairs provide information of integral exposure.

2.4. Statistical methods

Data were log-transformed to meet the assumptions of normality, and consequently parametric tests were performed. A T–difference test of means was performed for trace metals and stable isotopes. A Pearson's correlation test was performed to investigate the relationships between metals and continuous explanatory variables (hair $\delta^{13}C$ and $\delta^{15}N$ values). High correlation values between the primary values of the metals in the analysed samples justify the principal component analysis. Two main components have been designated for interpretation, accounting for 81.79% of the cases. However, the analysis provides no meaningful information for the interpretation of data analysis. Therefore, data clustering was performed to provide an insight into the data structure. Clustering was done by the nearest neighbour's method, adopting tangent distance as a measure of distance.

3. Results

Median, mean, and standard error, log transformed mean, and t-difference test of means are presented in Table 1. For compiled samples, correlation coefficients are mostly high, many are close to one (Table 3). The correlation of variables with regard to the sampling site was also tested. In the majority of cases, stronger correlations between metals were observed in samples from the Longyearbyen area, compared to the Hornsund samples. For zinc, correlations with other metals were notably lower (the highest occurs with gallium content: $R^2_{tot} = 0{,}54$). Those coefficients were used to measure similarity of variables by data clustering (Fig. 2). As a result, two groups were obtained: zinc as an isolated element, and

Table 1
Trace element concentration in reindeer fur samples collected from two separate populations (μg/g dw).

Element	Longyearbyen (n = 11)			Hornsund (n = 16)			t–difference test of means ($p < 0.05$)
	Median	Mean ± standard error (Cl95%)	Log transformed mean	Median	Mean ± standard error (Cl95%)	Log transformed mean	
Li	0.43	4.36 ± 2.18	−0.04	0.51	0.49 ± 0.08	−0.49	2.15
Be	0.01	0.09 ± 0.05	−1.11	0.02	0.025 ± 0.004	−1.80	1.79
V	0.83	3.05 ± 1.20	0.14	0.73	0.94 ± 0.21	−0.24	2.08
Cr	0.89	2.82 ± 1.17	0.08	2.24	3.28 ± 0.81	0.06	−0.34
Co	0.13	1.31 ± 0.65	−0.48	0.15	0.34 ± 0.11	−0.97	1.76
Ni	0.89	3.81 ± 1.72	0.13	1.26	1.90 ± 0.54	−0.05	1.23
Ga	0.37	0.97 ± 0.38	−0.32	0.81	1.00 ± 0.20	−0.12	−0.07
As	0.54	1.06 ± 0.39	−0.21	0.65	0.74 ± 0.13	−0.24	0.91
Rb	0.62	3.12 ± 1.42	−0.01	0.66	0.76 ± 0.10	−0.19	2.02
Cd	0.05	0.30 ± 0.23	−1.08	0.11	0.17 ± 0.04	−1.01	0.68
Cs	0.09	0.73 ± 0.40	−0.83	0.03	0.04 ± 0.01	−1.61	2.08
La	0.32	2.22 ± 1.08	−0.18	0.72	0.79 ± 0.14	−0.34	1.59
Pb	1.68	5.14 ± 2.19	0.37	1.96	3.20 ± 0.82	0.29	0.95
Hg	0.13[a]	0.34 ± 0.23[a]	0.29[a]	0.06[a]	0.06 ± 0.01[a]	−1.17[a]	-[b]
Fe	602	3300 ± 1550	3.03	494	530 ± 97	2.54	2.17
Zn	65.9	90.6 ± 24.8	1.82	141	154 ± 16	2.15	−2.23
Cu	13.2	19.95 ± 4.63	1.19	15.2	18.45 ± 3.04	1.18	0.28
Ba	12.5	27.50 ± 8.85	1.24	26.3	26.50 ± 3.73	1.33	0.11

[a] Longyearbyen (n = 4), Hornsund (n = 5).
[b] -low sample size.

Table 2
Nitrogen and carbon stable isotopes concentration in Svalbard reindeer hairs.

	Longyearbyen (n = 10)		Hornsund (n = 22)	
	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]
Arythmetic Mean	6.73	−26.19	10.96	−25.47
SD	1.40	0.24	2.01	0.76
Median	7.41	−26.22	10.66	−25.17
Min	3.73	−26.48	7.49	−26.67
Max	8.00	−25.82	14.04	−24.02

other elements forming a single cluster. After further division, we obtained a five-elemental cluster (containing V, Fe, Li, Cs, and La), a three-elemental cluster (As, Ga and Ba), and the remaining elements as isolated items. High variation was observed for nitrogen isotope composition. T– difference test of means ($p < 0.05$) for nitrogen isotopes ($\delta^{15}N$) was equal to −5.16, and for carbon ($\delta^{13}C$) to −3.12 (Table 2). Three individuals from the Longyearbyen area showed elevated contents of all the measured elements, with extremely high levels of iron, chromium, nickel, and lead. The average value of nitrogen isotope $\delta^{15}N$ for those outliers was equal to 6.95 [‰]. Outliers were not excluded from statistical analysis.

4. Discussion

This study reports the levels of essential and toxic elements and stable isotope composition in Svalbard reindeer hair samples collected from herds living in distant parts of the island. Keratinised tissues such as hairs, fur, or feathers can be collected non-lethally, and have been successfully used for stable isotopes and heavy metal analysis for many years (Duffy et al., 2005; Burger et al., 2007; Sergiel et al., 2017). Hair tissue has several advantages in practical use. Owing to its stability, samples can be stored for a long time, they are relatively metabolically inactive (Duffy et al., 2005), and elements are accumulated over extended periods of time. Therefore, the exposure assessment covers several weeks or months. Molten hairs can be collected without direct contact, avoiding difficulties related to capturing a free-living individual. However,

Table 3
Pearson correlation values indicating correlation between the various trace elements measured (n = 26).

Variable	15 N	13C	Li	Be	V	Cr	Fe	Co	Ni	Cu	Zn	Ga	As	Rb	Cd	Cs	Ba	La	Pb
15 N	1.00	−0.06	−0.16	−0.25	−0.21	−0.02	−0.26	−0.16	−0.03	−0.02	0.64	0.24	0.04	−0.10	0.20	−0.36	0.17	−0.04	0.01
13C		1.00	0.08	0.25	0.08	−0.10	0.09	0.07	−0.09	−0.10	−0.32	−0.19	−0.06	−0.07	−0.09	0.12	−0.10	−0.01	−0.07
Li			1.00	0.95	0.97	0.77	0.97	0.77	0.89	0.79	0.44	0.79	0.87	0.92	0.75	0.94	0.81	0.94	0.94
Be				1.00	0.87	0.74	0.86	0.52	0.68	0.59	0.49	0.71	0.79	0.83	0.57	0.90	0.78	0.86	0.56
V					1.00	0.82	0.98	0.76	0.88	0.80	0.39	0.78	0.88	0.87	0.74	0.92	0.82	0.95	0.81
Cr						1.00	0.80	0.48	0.80	0.71	0.52	0.76	0.74	0.65	0.76	0.70	0.82	0.88	0.64
Fe							1.00	0.73	0.88	0.78	0.38	0.75	0.86	0.91	0.71	0.97	0.81	0.95	0.78
Co								1.00	0.73	0.71	0.33	0.65	0.72	0.72	0.66	0.67	0.61	0.64	0.89
Ni									1.00	0.85	0.55	0.80	0.80	0.80	0.85	0.79	0.81	0.89	0.88
Cu										1.00	0.50	0.79	0.80	0.75	0.82	0.67	0.78	0.82	0.86
Zn											1.00	0.74	0.60	0.53	0.57	0.27	0.67	0.55	0.52
Ga												1.00	0.94	0.84	0.81	0.64	0.97	0.84	0.83
As													1.00	0.91	0.74	0.78	0.94	0.87	0.82
Rb														1.00	0.66	0.89	0.85	0.87	0.80
Cd															1.00	0.63	0.83	0.79	0.82
Cs																1.00	0.71	0.87	0.69
Ba																	1.00	0.88	0.79
La																		1.00	0.78
Pb																			1.00

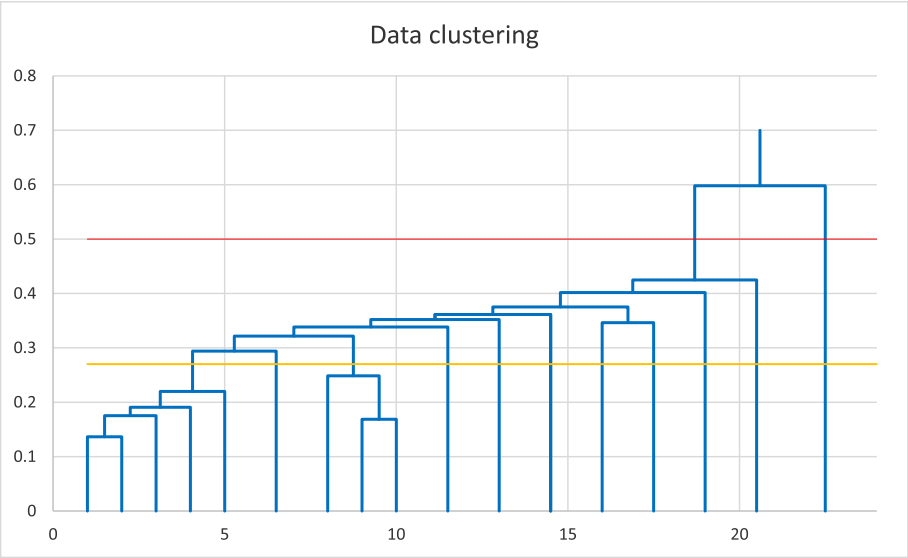

Fig. 2. Hierarchical dendrogram for clustering the chemical elements. Lines indicate distance 0.27 and 0.5, respectively. From the left: 1-V, 2-Fe, 3-Li, 4-Cs, 5-La, 6,5-Rb, 8-As, 9-Ga, 10-Ba, 11,5-Be, 13-Ni, 14,5-Cr, 16-Co, 17,5-Pb, 19-Cu, 20,5-Cd, 22,5-Zn.

because factors such as specimen age and gender are often unknown, this mode of sampling also limits the possibility of result interpretation.

Svalbard reindeers consume various plants, including vascular plants, bryophytes, and lichens, all determined to accumulate high levels of essential and heavy elements (Jóźwik, 1990; Samecka-Cymerman et al., 2011; Garty, 2001). Their levels found in polar plant species can be elevated due to natural processes (such as volcano eruptions, rock weathering) or atmospheric deposition, mainly from long distance transboundary transport from lower latitudes (Grodzińska and Godzik, 1991). Sea aerosol can be an additional source of elements such as lead, mercury, and cesium (Kłos et al., 2017).

Spatial and temporal heterogeneity in diet components might be responsible for significant seasonal differences in contaminant distribution across studies (Robillard et al., 2002). In our study, the majority of elements showed a strong positive correlation with multi-element totals, excluding zinc. High variability in trace element composition was observed even above an order of magnitude within samples of reindeer from one location. This is probably related to differences in age (herds were composed from both young and older individuals), gender, and food preference. Due to lack of previous studies regarding trace elements in reindeer hairs, our data can be used as a reference for future investigations in the Svalbard Archipelago concerning reindeer and closely related species.

4.1. Accumulation route

Vegetation covers only 6–7% of the area of Svalbard. The growing season lasts approximately 90 days (Kłos et al., 2015). Because of the short grazing season, the Svalbard reindeer must restore its body reserves after winter, and accumulate fat at this time (Staaland, 1984). The plant species-specific physiology, age, and sampling location will correspond with forward exposure. Lower trace element levels are observed in vascular plants as compared to mosses and lichens (Wojtuń et al., 2013). This may be related to their higher morphological similarity, and more selective accumulation process (Chiarenzelli et al., 2001). Due to the lack of root system, slow growth rate, longevity, vast surface area, and lack of well-developed cuticle, plants such as lichens and bryophytes are prone to accumulating a varied cocktail of toxic compounds from the atmosphere (Robillard et al., 2002; Gamberg et al., 2005; Samecka-Cymerman et al., 2011). Essential elements such as copper and zinc, necessary for plant growth, can also be accumulated beyond physiological demands (Samecka-Cymerman et al., 2011; Jóźwik, 1990). For instance, for zinc, enhanced exposure in lichens is above 500 μg/g, cadmium can be tolerated between 1 and 30 μg/g, and copper between 1 and 50 μg/g (Nieboer et al., 1978).

The accumulation route can be passive by water transpiration passage (e.g. Cu in lichens), active (e.g. zinc), and metabolic (e.g. manganese), or a mix of those factors (Jóźwik, 1990). Mosses are evidenced to accumulate notably high levels of Cd, Co, Cr, Cu, Fe, Mn, and Zn, even higher than lichens (Wojtuń et al., 2013). Particularly moss species such as *Aulacomium palustre, A. turgidum, Hylocomium splendens, Sanionia uncinata*, and *Tortula ruralis* are suspected to be very good heavy metal accumulators in Svalbard (Grodzińska and Godzik, 1991).

4.2. Toxic elements

Mercury is a global pollutant that enters the Arctic terrestrial ecosystem mainly through rock weathering and long-range atmospheric deposition (Gamberg et al., 2015). During spring, atmospheric Hg(0) is oxidised into Hg(II), and deposited in the snow, ice, or ocean surfaces from where can be partly reemitted or further retained, transformed, and transported (Schroeder et al., 1998; Halbach et al., 2017). In addition to snow and ice, soil is believed to be a major land mercury reservoir in the Arctic (Gamberg et al., 2015). Our study shows low mercury contents in both studied subpopulations. Elevated mercury level is indeed usually found in marine biota, in contrast to terrestrial mammals, especially herbivores with a short food chain.

To the best of our knowledge, no studies are available regarding contaminant deposition in the hair of the Svalbard reindeer subspecies. Duffy et al. (2005) conducted a study on mercury levels in the hair of the Alaskan reindeer population, indicating low exposure (mean total mercury for free ranging individuals was equal to 0.055 μg/g). Mercury was also a major research interest in Lokken et al. (2009) pilot study performed on lichen and the Alaskan

caribou population (mean hair levels varied from 0.0146 to 0.0834 μg/g). In the present study, the highest level was found in the Longyearbyen population. It does not exceed 0.160 μg/g (median equal to 0.112 and 0.060 μg/g).

Mercury and cadmium previously showed a clear pattern of accumulation towards higher trophic levels in the terrestrial ecosystem (Dietz et al., 2000). Cadmium binds to the low molecular weight sulphur-rich proteins, and accumulates mostly in kidneys (Chan et al., 2001). It also may significantly increase with age (Danielsson and Frank, 2009). In our study, however, age differences were not analysed, and hair bounding capacities are different than in internal tissues. Literature studies on both areas showed low cadmium exposure in vegetation (Wojtuń et al., 2013; Samecka-Cymerman et al., 2011; Węgrzyn et al., 2013; Kłos et al., 2015), and as expected we found low levels in reindeer hair. To our best knowledge, no study has been published concerning cadmium accumulation in Svalbard mammal herbivores, therefore no comparison is possible.

On the other hand, high lead levels were found in the majority of samples, suggesting an accumulation path by vegetation. High levels of lead were also previously found in Greenland soils (Fig. 3). However, it does not tend to accumulate towards higher trophic levels, as reindeers had lower lead levels than lichens (summarized in Dietz et al., 2000 based on Greenlandic studies of the AMAP programme). Notice that only reindeer internal tissues were used. In Svalbard area, levels of lead in vegetation is highly variable. Threshold values for lead in lichens are from 5 to 100 μg/g and 15 μg/g is a boundary for enhanced exposure (Nieboer and Richardson, 1981). In hairs, lead is accumulated both externally and internally over a long period of time, until molting. It is possible that apart from internal contamination accumulated by foraging on high-lead level food sources, part of external contamination was not washed out during the cleaning procedure.

4.3. Other elements

The studied samples showed particular patterns such as high intra-individual variations in the level of several compounds (iron, chromium, zinc etc.). All the analysed elements occur in broad concentration ranges. Relatively high levels of mean nickel in the Longyearbyen subpopulation, before also observed in the population of moss *Hylocomium splendens*, could be associated with past mining activities in the area (Kłos et al., 2015). The main source of nickel in Longyearbyen is most likely rock waste derived from mining activities and aviation emissions, although discharges transported long-range from the Kola Peninsula are also suspected (Kłos et al., 2017). Iron was significantly elevated in some of the samples from the Longyearbyen area, with the highest level at 14640 μg/g dw. Other two samples were also above 5000 μg/g dw of iron. The effect of spontaneous iron overload was previously described in liver tissues of Svalbard reindeer (Borch-Iohnsen and Nilssen, 1987; Borch-Iohnsen and Thorstensen, 2009). It was caused by high uptake of dietary iron consumed with iron-rich forage plants (Borch-Iohnsen and Thorstensen, 2009). In Svalbard reindeers, spontaneous seasonal iron overload with massive siderosis is considered natural, and occurs mostly in winter when available vegetation is of poorer quality (Borch-Iohnsen and Thorstensen, 2009). It is possible that when reindeers' nutritional conditions improved after winter (Borch-Iohnsen and Thorstensen, 2009), accessory iron was redistributed from the liver to hairs. If that is the case, hairs can be used to reveal past iron overload. All other elements were also significantly elevated in those individuals, suggesting some health implications (with examples presented in Table 5, Supplementary material). Mercury was not analysed in those samples. Levels of iron in samples from the Hornsund area were lower, not exceeding 5000 μg/g. In two cases, more than 1100 μg/g of iron was detected.

Because reindeer subspecies *Rangifer tarandus platyrhynchus* lives exclusively in the Svalbard Archipelago, the nominative species was expected to receive more attention. Studies on Canada and Greenland caribou and reindeer populations mostly concerned internal tissues (Elkin and Bethke, 1995; Robillard et al., 2002; Larter and Nagy, 2000; Aastrup et al., 2000). Medvedev (1995) reported cadmium and lead levels in the bone, teeth, and antlers of forest reindeer (*Ragnifer tarandus fennica*) from north-west Russia. The highest mean levels of cadmium and lead were found in the bone tissue (2.1 ± 1.1 and 41.6 ± 23.7 μg/g dw, respectively). The levels did not depend on sex or age of individuals. Heavy metal levels were also reported for North Norway population in samples collected from semi-domesticated reindeer. Cadmium, lead, arsenic, nickel, and vanadium were determined in the muscle, liver, tallow, and bone marrow tissues, with the highest level of all the elements in the liver (except nickel) (Ali Hassan et al., 2012). A reliable comparison between those studies is not possible, however, because the relationship between deposition of compounds in hairs and internal tissues is not always clear. Svalbard is an Arctic semi-desert compared to other places inhabited by reindeers, with low precipitation and humidity, cold winter temperatures, and high wind speed, resulting in different feeding behaviour and patch choice (Lindner, 2002). The Svalbard reindeer also differs from other reindeer subspecies in its anatomy and physiology (Lindner, 2002).

4.4. Stable isotopes of carbon and nitrogen

Stable isotopes (SI) of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are increasingly employed as an indispensable tool in ecological studies (Sergiel et al., 2017). The main sources of nitrogen in the Arctic include atmospheric discharge of NO_x, NH_x, primary N2-fixation from the atmosphere, and bird guano (Skrzypek et al., 2015). In nitrogen-limited terrestrial ecosystems such as Arctic tundra, soil microbes are recognised to function as main nitrogen pools, competing for nitrogen with plants (Bardgett et al., 2007). Plant growth is limited by nitrogen availability. Consequently, the capacity for carbon sequestration is also restricted (Skrzypek et al., 2015). Arctic tundra contains a significant percent of the global soil carbon reserve. Its storage is controlled by factors such as e.g. temperature, vegetation type, soil hydrology, or shifts in vegetation state. The latter can be induced by herbivores (Van der Wal, 2006; Speed et al., 2010).

Forage patch choice by reindeers and nitrogen content in plants are largely influenced by the timing of snowmelt (Van der Wal et al., 2000). In Svalbard, seasonal variability of plant and soil nitrogen pools are mostly controlled by changes in temperature and soil moisture over the growing season. Such changes, however, are markedly lower than in the other seasonally cold ecosystems (Bardgett et al., 2007). Also Arctic tundra has a high capacity to retain nitrogen transported after extreme events, with non-vascular plants acting as a short-term sink, and vascular plants as a long-term reservoir (Choudhary et al., 2016). Our results indicate high variability in the $^{15}N:^{14}N$ ratio, suggesting that reindeers consume vegetation with different ^{15}N values. In the Fuglebekken catchment (Hornsund), high loads of nutrients are deposited by large bird colonies such as little auk (*Alle alle*). This influx impacts soil fertility and subsequently plant productivity and structure (Skrzypek et al., 2015). As a result, the available vegetation differs in protein, sugar composition, and digestibility (Staaland, 1984). Bird guano and additional N-sources from colonies, such as carcasses, dead chicks, and eggshells, constitute a huge N-load compared to

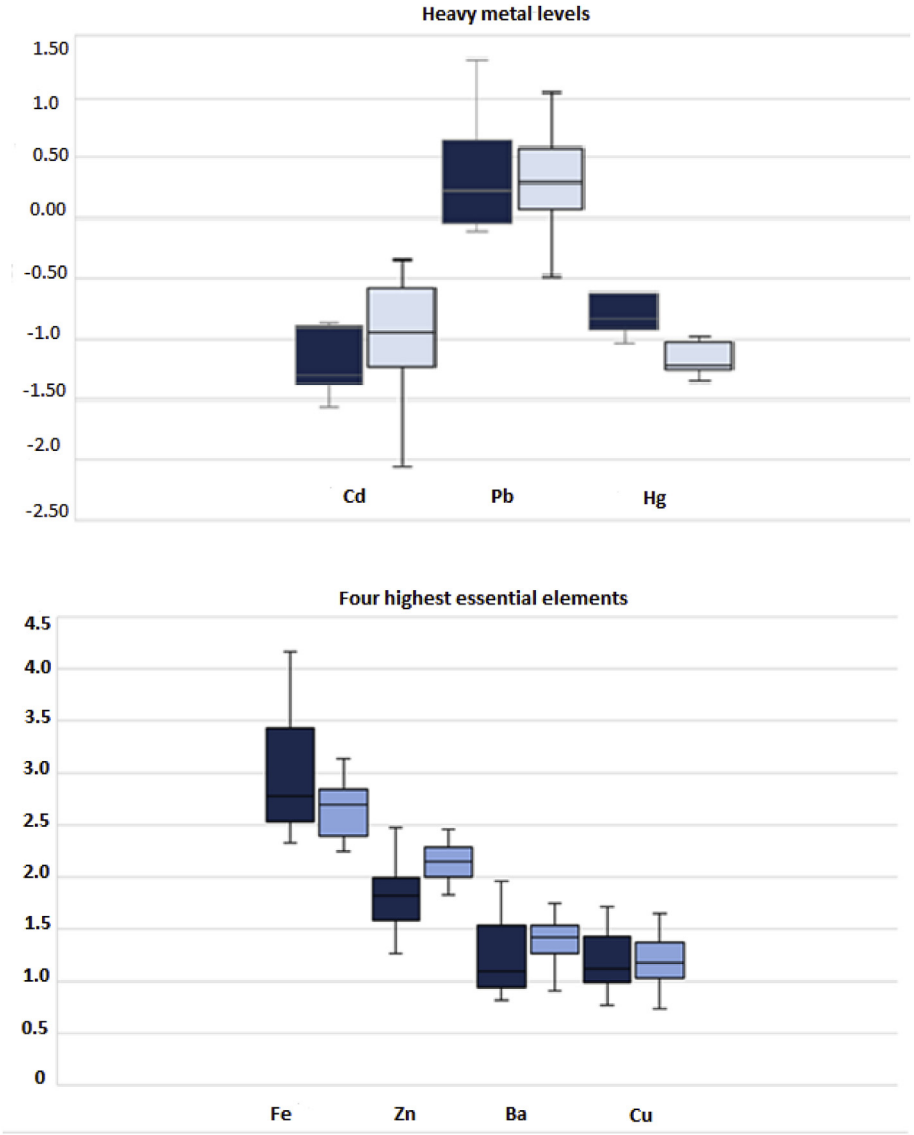

Fig. 3. ab. Plot of average Cd, Pb and Hg and Fe, Zn, Ba and Cu concentration in Longyearbyen (dark colors) and Hornsund (light colors) reindeer hair samples. Values are log transformed. The horizontal lines represent medians, the boxes – upper and lower (25–75% quartiles) and whiskers – minimum and maximum values. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

other sources (Skrzypek et al., 2015). It could account for significant differences between the two subpopulations (Fig. 4).

Moss tundra serves as an important sink for carbon sequestration (Nakatsubo et al., 2015). Here, relatively low variability was observed for stable carbon isotopes. No significant correlation was observed between C and N values and metal concentration, apart from zinc.

No previous studies are available concerning stable isotope analysis in the keratinised tissues of the Svalbard reindeer. Mosbacher (et al., 2016) showed high inter- and intra-annual seasonality in the diet of the Greenland muskox (*Ovibos moschatus*) by the application of sequential data on nitrogen stable isotopes derived from guard hairs. Drucker (et al., 2010) studied the dietary references and habitat use of moose (*Alces alces*) and caribou (*Rangifer tarandus*) in plucked hair samples from Canada populations. The dietary strategies of those species differ in spite of the same habitat range. Differences in stable isotope abundance were significantly linked to the species' dietary specialisation (Drucker et al., 2010).

The long-term variation in weather conditions may impact vegetation quality, consequently affecting the ungulates' nutritional profile and foraging conditions. Lower snow layer hardening in winter leads to changes in snow-pack properties, including ground icing, resulting in snowpack with impenetrable vegetation underneath (Hansen et al., 2011; Loe et al., 2016). Food availability can also be restricted by overgrazing (Węgrzyn et al., 2016). Therefore, some populations are more likely to expand their foraging area, or alternatively use less preferred food sources such as goose droppings (Van der Wal and Loonen, 1998) or marine algae (Hansen and Aanes, 2012). Because many factors are responsible for seasonal availability of various food sources, and Svalbard reindeers tend to forage for plant quantity rather than quality (Van der Wal et al., 2000), a complex study program concerning trace element levels in vegetation may help assess their future potential exposure.

5. Conclusion

The Svalbard reindeer is one of the least studied subspecies

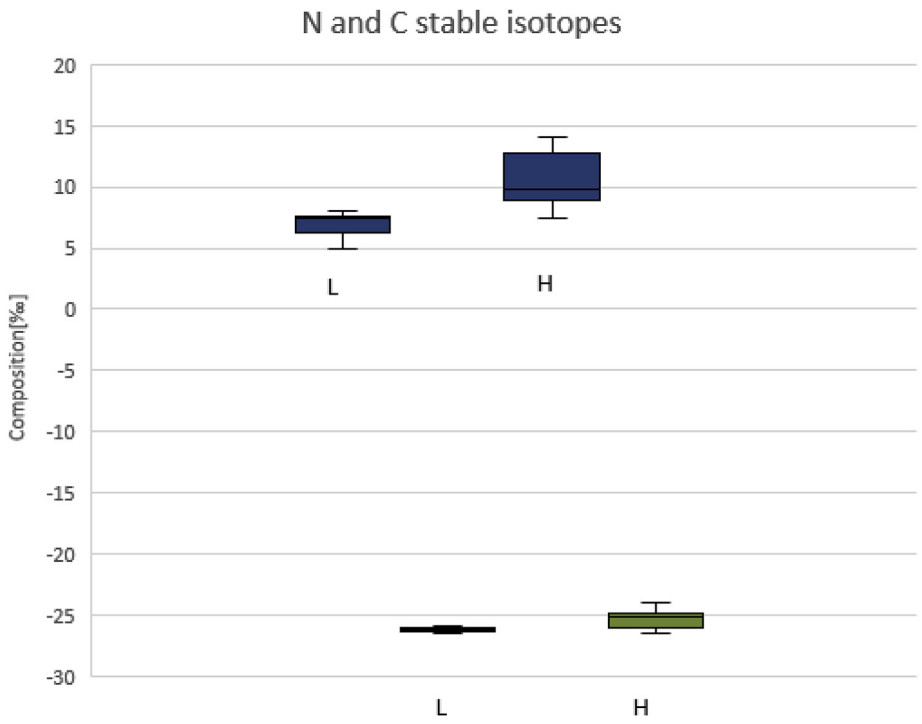

Fig. 4. Plot of average nitrogen (blue) and carbon (green) stable isotope composition in Longyearbyen (L) and Hornsund (H) reindeer hair samples. The horizontal lines represent medians, the boxes – upper and lower (25–75% quartiles) and whiskers – minimum and maximum values excluding outliers. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

amongst family *Rangifer*. In this paper, we present to the best of our knowledge the first communication concerning trace element concentration in hairs of two separate subpopulations. Better knowledge of the potential impacts of metal on the terrestrial ecosystem is needed in polar mammal populations, especially to identify levels related to health dysfunction. In the present study, mercury is indicated as an insignificant thread in terrestrial ecosystem, although levels of lead, chromium, and nickel were noticeably elevated in some of the samples. Because hairs are a dead tissue accumulating elements over long period of time, reindeer may use it in a detoxification process for instance for depositing past iron overload.

Future climate changes will induce higher pressure on all terrestrial species. Rising temperatures, more frequent extreme weather events, heavy rain-on-snow events, and variations in seasonal precipitation patterns may cause negative implications for herbivores (Hansen and Aanes, 2012). In spite of their remarkable abilities to locate food beneath the snow-pack, severe icy conditions may induce changes in reindeer behaviour, including range expansion to mountainous terrain (Hansen et al., 2010a, 2010b), and eating marine algae (Hansen and Aanes, 2012) resulting in potential changes in the foraging profile and contaminant accumulation. The research presented so far provides evidence that keratinised tissues can be a valuable source of information in ecotoxicological studies. Monitoring studies should involve not only marine species, but concurrently more terrestrial key species as an important part of the trophic network.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.03.158

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12.2. Weryfikacja hipotezy 2

Hipoteza 2: Odchody stanowią efektywny sposób pozbycia się zarówno dużej ilości pierwiastków toksycznych, jak i innych np. fosforu, i mogą stanowić użyźnienie tundry ubogiej w pierwiastki biogenne. Ze względu na różnice w diecie zimowej i letniej wystąpią różnice w zawartości metali w odchodach pochodzących z dwóch sezonów. Ze względu na depozycję atmosferyczną wystąpią różnice między zawartością pierwiastków w świeżo pozbieranych odchodach, oraz starszych. Ze względu na brak istotnych różnic w diecie nie wystąpi różnica w zawartości pierwiastków w odchodach pozbieranych od samca, samicy oraz osobnika młodego.

Weryfikację **drugiej hipotezy** wykonano w oparciu o badania próbek odchodów pobranych renifera Svalbardzkiego. Wyniki badań zostały opublikowane w czasopiśmie *Science of The Total Environment* (załącznik 3) **[A.D. Pacyna, M. Frankowski, K. Kozioł, M. H. Węgrzyn, P. Wietrzyk-Pełka, S. Lehmann-Konera, Ż. Polkowska, Evaluation of the use of reindeer droppings for monitoring essential and non-essential elements in the polar terrestrial environment, Science of the Total Environment, 2019, DOI: 10.1016/j.scitotenv.2018.12.232]**

W badaniach będących podstawą publikacji wykorzystano odchody renifera w celu zbadania szklaku eliminacji 30 pierwiastków. Próbki pobrane zostały na dwóch obszarach tundry o odmiennych gatunkach dominujących. Pierwszym rodzajem była tundra zdominowana przez gatunki o umiarkowanym zapotrzebowaniu na wodę, takie jak karłowate krzewy, rośliny kwitnące, graminoidy, mszaki oraz porosty [105]. W pobliżu rzeki przepływającej przez dolinę, w rejonie o wyższej wilgotności dominował drugi rodzaj tundry, składająca się głównie z mszaków [105]. Próbki pobrane z tych dwóch obszarów, zostały dodatkowo podzielone względem sezonu na letnie, zimowe oraz zimowe-przejściowe. Dla ponad połowy analizowanych pierwiastków wystąpiły znaczące różnice w poziomie dla próbek pobranych z dwóch typów tundry. Ocenie poddany został wpływ depozycji atmosferycznej oraz czynników środowiskowych na poziom pierwiastków w próbkach, porównując poziomy w świeżo zdeponowanych odchodach letnich z tundry pierwszego typu z odchodami starszymi. Różnice statystyczne wykryte zostały wyłącznie dla As, K, Mn, Na, Ni oraz Sb. Za wyjątkiem cynku oraz potasu, nie wykryto znaczących różnic w poziomie pierwiastków w próbkach

pobranych od dorosłych samic, samców oraz osobnika młodego. Wyniki badań pokazują iż renifery biorą udział w przenoszeniu wielu metali, niemetali i metaloidów w środowisku w tym wapnia, fosforu, cynku, aluminium i ołowiu.

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Evaluation of the use of reindeer droppings for monitoring essential and non-essential elements in the polar terrestrial environment

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HIGHLIGHTS

- Reindeer droppings were used to assess bioavailability of 30 elements.
- Significant differences in element concentration between foraging sites
- Seasonal differences occurred for part of the elements.
- Usefulness of older as opposed to freshly collected droppings was assessed.

GRAPHICAL ABSTRACT

Method	Elements analysed	Concentration levels (means)
ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry)	Ca, Fe, K, Mg, P, S, Si	0.44-47.9 mg g^{-1} dw
ICP-MS (Inductively Coupled Plasma Mass Spectrometry)	Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, La, Li, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, V, Zn	0.027-2518 μg g^{-1} dw
Thermal vaporization atomic absorption	Hg	0.082 μg g^{-1} dw

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ABSTRACT

Excess or toxic metals, non-metals and metalloids can be eliminated from the organism by deposition in inert tissue (e.g. fur) or excretion with body secretions, urine and faeces. Droppings are one of the main routes for the elimination of multiple elements and they can be collected without direct contact with the animal. Contaminant concentration has been examined in non-lethally collected tissues of several species (especially reptilian, avian and mammalian). However, studies on species residing in polar areas are still limited, especially of mammals from the European Arctic. Reindeers are the only large herbivores living in Svalbard, being an essential part of the Arctic terrestrial ecosystem. Although reindeer presence has a high impact on their surroundings, those huge mammals are rarely part of ecotoxicological studies regarding metal pollution. In this paper, the droppings of Svalbard reindeer were used as a non-invasively collected tissue to examine the excretion pathway of 30 elements. Samples were collected in mesic and moss tundra, representing summer, winter and winter-transitional excretion. For more than a half of the studied elements, significant differences occurred between the samples collected in the two tundra types. The feasibility of older and fresh samples was assessed based on summer droppings, and significant differences were found for K, As, Mn, Na, Ni, and Sb concentrations. No relevant differences in element levels were observed for samples collected from adult females, adult males and calves, except for zinc and potassium. Results show that reindeer droppings are an important vector for the transfer of many metals, non-metals and metalloids including calcium, phosphorus, zinc, aluminium and lead. As a sedentary species, feeding on local food sources, Svalbard reindeer is a valuable indicator of trace element presence in the polar terrestrial ecosystem.

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1. Introduction

Tundra is a characteristic biome of the Arctic, covering about one-tenth of the total Earth surface (Poissant et al., 2008). Although local fauna is composed of a limited number of species, low biodiversity is often counterbalanced by high local abundance. The arctic terrestrial ecosystem may be exposed to an enhanced level of metals and metalloids of natural and anthropogenic origin (AMAP, 2005; Ruman et al., 2012; Kozak et al., 2013; Halbach et al., 2017). Thus, studies employing terrestrial fauna and flora are crucial for a comprehensive understanding of ecological consequences to the polar ecosystem. Svalbard Archipelago is inhabited by a number of birds, by polar bears and polar foxes, but Svalbard reindeer (*Rangifer tarandus platyrhynchus)* is the only large mammal herbivore there (Hayashi et al., 2014). In contrast to other *Rangifer* subspecies, Svalbard reindeer is highly stationary and does not migrate long distances under normal circumstances (Tyler and Øritsland, 1989). It eats almost all types of available vegetation with the selection based on plant quantity rather than quality (Van der Wal et al., 2000). Their territory is established in a great part by natural barriers thus genetic differences might occur even at distances <50 km^2 (Hansen et al., 2010; Côté et al., 2002). The restricted foraging area, longevity and easiness to spot make the Svalbard reindeer a suitable species to monitor the local pollution level.

A non-lethal sampling includes samples taken with direct contact with an animal (e.g. blood and fur collection) and without direct contact (molten fur, droppings). The non-lethal monitoring of biota is recently gaining a lot of attention worldwide, e.g. in birds (Jerez et al., 2011; Burger et al., 2008; Becker et al., 2016), reptiles (Ikonomopoulou et al., 2011; Schneider et al., 2011; Villa et al., 2015), and mammals (Duffy et al., 2005; Pacyna et al., 2018). Some studies have based their conclusions on completely non-destructive sampling procedures, such as collecting shed skins of cobras (Kaur, 1988), faeces (Xu et al., 2006; Yin et al., 2008), feather (Burger et al., 2008; Jerez et al., 2011), urine (Hart et al., 2018), or fur (Duffy et al., 2005). However, the Arctic has been so far represented to a smaller extent, mostly by bird species (e.g. Burger et al., 2008; Fort et al., 2016).

Faeces generally contain a high concentration of metals and are one of the main routes for the elimination of the majority or excess consumed elements (Yin et al., 2008; Orłowski et al., 2015). Thus, droppings can be used as an indicator of the environmental availability of metals in food items consumed by higher-trophic-level animals (Costa et al., 2012; Yin et al., 2008). This approach was used in previous research, including birds and marine mammals (e.g. Costa et al., 2012; Orłowski et al., 2015; Yin et al., 2008). However, data reporting concentrations of essential and non-essential elements in reindeer living in the Arctic tundra is scarcely available. Previously, reindeer excrements were used to assess exposure close to the station located in Ny Ålesund (Yin et al., 2008). A low level of mercury was found, however, lead levels were unexpectedly high, as compared to the levels found in seabirds and marine mammals. Here, we examined the concentration of 30 elements, including toxic metals of environmental concern (Al, As, Ni, Cd, Pb, Hg) in samples collected from a previously not studied population in the Bellsund region of Svalbard. The key aim of this study is to evaluate the elemental composition of reindeer faeces and to contrast the concentration levels found in: 1) mesic and moss tundra; 2) seasonal excretion (winter, transitional and summer faeces), 3) the droppings of calves, females and males. We also provide information about the usefulness of older samples as opposed to fresh ones.

2. Material and methods

2.1. Study area

Field sampling was conducted between 6th and 24th of August 2016 on the marine terrace Calypsostranda (south margin of the Bellsund Fjord) and in the valley Chamberlin (south margin of the Recherche Fjord) (Fig. 1). The presence of selected metals in various components of the environment has already been identified in this area (Lehmann et al., 2016; Lehmann-Konera et al., 2018; Szumińska et al., 2018). The meteorological conditions of the study area (the south margin of the Bellsund Fjord, vicinity of the polar station in Calypsobyen) for the years 1986–2011 were as follows: mean air temperature equalled 5.0 °C, mean total precipitation = 32.4 mm and mean wind velocity = 4.3 m s^{-1} (Mędrek et al., 2014).

According to Elvebakk (2005), the vegetation of the study area belongs to mesic tundra and is dominated by dwarf shrubs, flowering plants and graminoids, mainly *Luzula nivalis*. However, also bryophytes and lichens are common components of vegetation. The following species were dominant in Chamberlindalen in the dryer part of the valley: *Saxifraga oppositifolia* L., *S.axifraga cespitosa* L., *Salix polaris* Wahlenb., *Cetrariella delisei* (Bory ex Schaer.) Kärnefelt & A. Thell, *Collema ceraniscum* Nyl., *Lecidea ramulosa* Th. Fr., *Ochrolechia frigida* (Sw.) Lynge, *Stereocaulon alpinum* Laurer., *Thamnolia vermicularis* (Sw.) Schaer. Near the river flowing through the valley, in places with higher humidity, moss tundra was predominant, consisting mainly of bryophyte species, such as *Aulacomnium turgidum* (Wahlenb.) Schwägr., *Campylium polygamum* (Schimp.) C.E.O. Jensen, *Dicranum fuscescens* Turner, *Dicranum groenlandicum* Brid., *Distichium capillaceum* (Hedw.) Bruch & Schimp., *Drepanocladus fluitans* (Hedw.) Warnst., *Hylocomium splendens* (Hedw.) Schimp., *Pohlia cruda* (Hedw.) Lindb., *Polytrichum alpinum* (Hedw.) G.L.S, *Ptilidium ciliare* (L.) Hampe, *Racomitrium canescens* (Hedw.) Brid., *Racomitrium lanuginosum* (Hedw.) Brid., *Sanionia uncinata* (Hedw.) Loeske, and *Sarmentypnum sarmentosum* (Wahlenb.) Tuom. & T.J. Kop.

2.2. Sample collection

54 samples were collected manually to polyethylene zip bags, previously washed with deionized water. To avoid contamination, personnel taking the samples wore polyethylene gloves. After collection, samples were initially dried in the vicinity of the polar station in Calypsobyen. Subsequently, the dried samples were packed into their original zip bags, secured from potential contamination by two more zip bags and transported to the laboratory in Poland. Although all samples were collected within one month, the differences in the sample structure allow for the approximate evaluation of their origin (for further references, see Węgrzyn et al., 2018b). Samples were assessed by their appearance and divided into three groups, representing summer, winter and winter-transitional excretion. Winter faeces were characteristic pellet-shaped droppings and their age was determined (to be within the 2015/2016 season) on the basis of their compact structures, namely no clear cavities as a result of ageing, no cover of cyanobacteria layer on their surface, and their black or black-grey surfaces being shiny with no clear matt coating, which would occur on faeces older than last winter. Summer samples were compact, homogeneous pieces (Fig. 2 presents a schematic drawing with major differences included). The winter-transitional samples had features of both winter and summer faeces (pellet-shaped droppings, but irregular and with the pieces connected to one another).

Opportunistically, fresh samples were collected from individuals with the separation of male adult, female adult and calf. Herein, fresh means that samples were collected immediately after the excretion happened.

2.3. Laboratory analysis and QA/QC

Samples (in total 54) were freeze-dried to remove all water residues, then ground with mortar and pestle. Approximately 0.5 g was weighted to the nearest 0.1 mg, and placed in a clean Teflon vessel with 65% HNO_3 (Merck, Suprapur, Germany). Digestion was carried out using a high-pressure microwave emitter (Microwave Digestion System, Anton Paar). The temperature was increased from room

Fig. 1. Study site A - mesic tundra, B - moss tundra (map source: toposvalbard.npolar.no); fot. (Sara Lehmann-Konera): moss tundra.

temperature to 180 °C (at the rate of approximately 8 °C/min). Such conditions were maintained for 10 min. After that, the temperature was gradually reduced. Subsequently, the mineralized samples were diluted with deionized water (Millipore Milli-Q, France) to 25 mL in clean plastic flasks. To ensure quality control, blank samples were run with every batch.

A subset of the samples was analyzed with a mercury analyser by thermal vaporization atomic absorption method (NIC MA-2000). Freeze-dried samples were weighted to the nearest 0.1 mg and heat decomposed in a ceramic boat with the addition of activated Al_2O_3, Na_2CO_3 and $Ca(OH)_2$, used to eliminate substances possibly interfering with the measurement. Purified air was used as a carrier gas. The mercury concentration was expressed in μg per 1 g dry weight of droppings. Quality control included blank samples inserted between every batch of 8–9 samples, and sample runs performed in duplicate (and in triplicate when necessary). Measurement precision, presented as the coefficient of variation of the concentrations found in the duplicates or triplicates of a single sample was 3.83 (min-max 0.00–9.61). All samples tested for Hg concentrations were well above the detection limit of 0.54 ppb.

Ca, Fe, K, Mg, P, S, and Si were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES 9820 Shimadzu, Japan) and the following 22 elements: Ag, Al, As, B, Ba, Be, Cd, Co, Cr,

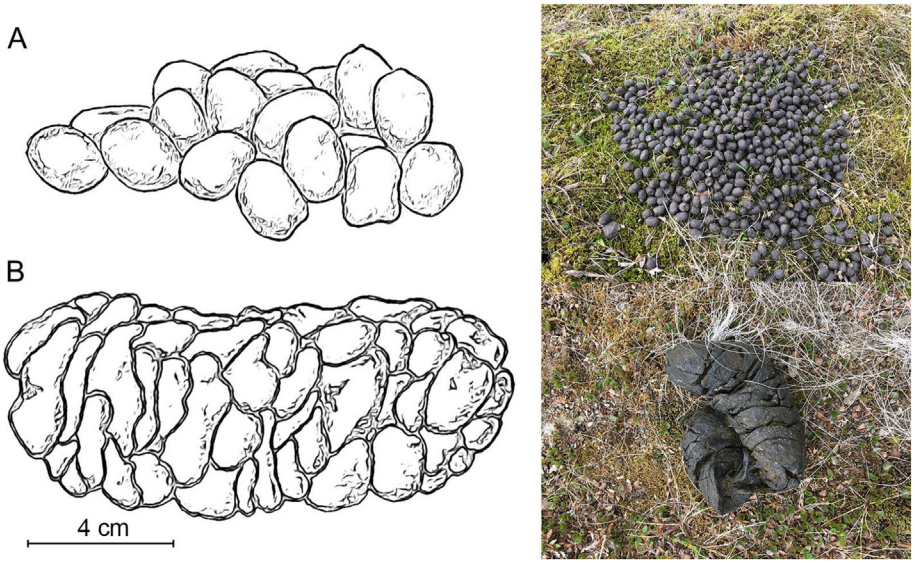

Fig. 2. Winter (A) and summer (B) droppings of the Svalbard reindeer.

Cu, La, Li, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, V, and Zn, were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS 2030 Shimadzu, Japan). Analyses were performed in three replicates (mean RSD 3.82% and 6.43% for ICP-OES and ICP-MS measurements, respectively). All measurement conditions and parameters are listed in Tables 1a and 1b. The results were corrected for mean value from 6 procedural blanks in the case of K, Be, Cr, La, Pb and Sb. In the rest of the samples, blank correction was not required, as background contamination was negligibly low. Both the method limit of detection (LOD) and its limit of quantification (LOQ) are listed in Tables 2a and 2b. For statistical analyses, results below the LOD were assigned a half of the LOD value. Results of the ICP-OES analysis of six samples from mesic tundra were excluded from interpretation due to detected technical problems with the instrument.

Calibration of the ICP-MS was performed with the ICP IV multi element standard (Merck, USA) and with single-element standards for As, Sb, Se, Mo and V (Sigma-Aldrich, USA). Additionally, for ICP-MS Sc, Rh, Tb and Ge in supra pure 1% HNO3 (Merck, USA) were used as internal standards. Deionized water was obtained from the Milli-Q Direct 8 Water Purification System (Merck Millipore) and applied for sample (pre)treatment and dilution.

The ICP-OES 9820 (Shimadzu, Japan) calibration was completed with single-element standards (Sigma Aldrich, USA) containing 1000 mg L^{-1} of Ca, Mg, K, Fe, P, S and Si, of which calibration standard solutions were made. The accuracy of the analyses was verified by means of two certified reference materials (CRMs): Trace metals ICP - sample 1 and Trace metals ICP - sample 2. The recovery of the selected elements in both CRMs ranged from 96% to 104%.

2.4. Statistical methods

Samples were separated according to their location and time of origin. Ag was below LOD in 70% of all samples, thus it was rejected from statistical analysis. The analysis was performed separately for samples collected in mesic and moss tundra with the season differentiation (MeS – mesic tundra summer, MeW – mesic tundra winter, MoS – moss tundra summer, MoW – moss tundra winter). Statistical differences between groups were tested using the Analysis of Variance (ANOVA) and then by the non-parametric U Mann-Whitney test, as the normal distribution and homogeneity of variance were not achieved for all groups of samples. Statistical differences were also tested for fresh and older samples from the summer season, collected in the mesic tundra.

We employed principal component analysis (PCA) for the whole dataset as an exploratory technique to detect the closely correlated variables and to search for seasonal characteristics of the studied samples. The variables were log-transformed to bring their distribution closer to normality, although in the case of Fe, Al, Ba, Co, Cr, La, Li, Ni, Sb, V and Zn, the log-transformed dataset was still showing deviations from normality in a Q-Q plot. Due to the missing values in the dataset, the variables were divided into two groups with a different number of cases (corresponding to different laboratory analysis methods: ICP-OES and ICP-MS). The PCA was conducted separately for each of these variable sets, with the exception of aluminium being grouped together with the ICP-OES results, since its concentrations were much higher than for all other variables analyzed by ICP-MS.

The statistical analysis was performed using two software: Statistica 13.1 for ANOVA and Mann-Whitney test, and R version 3.4.4 for the PCA (*prcomp* function).

Table 1a
ICP-MS 2030 (Shimadzu, Japan) measurement conditions and parameters.

Parameter and accessories	Value
Radio frequency power generator	1.2 kW
Gas type	argon
Plasma gas flow rate	8.0 L min^{-1}
Auxiliary gas flow rate	1.1 L min^{-1}
Nebulization gas flow rate	0.6 L min^{-1}
Torch	Mini-torch (quartz)
Nebulizer	Coaxial
Spray chamber temperature	3 °C
Drain	Gravity fed
Internal standard	Automatic addition
Sampling depth	5 mm
Collision cell gas flow (He)	6 mL min^{-1}
Cell Voltage	−21 V
Energy Filter	7.0 V
Number of replicates	3

Table 1b
ICP-OES 9820 measurement parameters (Shimadzu, Japan).

Parameter and accessories	Value
Radio frequency power generator	1.2 kW
Gas type	Argon
Plasma gas flow rate	10.0 L min^{-1}
Auxiliary gas flow rate	0.6 L min^{-1}
Nebulization gas flow rate	0.7 L min^{-1}
Plasma view	Vertical torch; axial/radial view
Torch	Mini-torch (quartz)
Nebulizer	Coaxial
Chamber	Cyclone (glass)
Drain	Gravity fed
Injector tube	Quartz (1.2 mm i.d.)
Background correction	2-points
Number of replicates	3
Exposure time	15 s

3. Results

Significant differences in element concentration occurred between samples derived from mesic and moss tundra (without season differentiation). Statistically significant differences (non-parametric U Mann-Whitney test $p < 0.05$) occurred for: Ca ($p < 0.0002$), Fe($p < 0.0001$), S ($p < 0.0380$), Al ($p < 0.0001$), Ba ($p < 0.0000$), Be ($p < 0.0001$), Co ($p < 0.0000$), Cr ($p < 0.0000$), Cu ($p < 0.0004$), La ($p < 0.0005$), Li ($p < 0.0001$), Mn ($p < 0.0063$), Mo ($p < 0.0036$), Ni ($p < 0.0000$), Pb ($p < 0.0239$), Sb ($p < 0.0133$), Sr ($p < 0.0000$), and V ($p < 0.0001$). Thus it suggests that differences in vegetation type growing in mesic and moss tundra and the meteorological conditions affecting the soil surface have a prevalent role in reindeer exposure assessment.

With the data divided only by season, statistically significant differences (non-parametric U Mann-Whitney test; $p < 0.05$) occurred for K ($p < 0.0024$), P ($p < 0.0001$), S ($p < 0.0016$), Cd ($p < 0.0017$), Cu ($p < 0.0044$), Mn ($p < 0.0167$), Se ($p < 0.0341$), and Zn ($p < 0.0010$).

Analyzing simultaneously by tundra type and season enables a clearer view of the whole data set. Several significant differences occurred in samples representing summer deposition from mesic and moss tundra and, in parallel, winter deposition from mesic and moss tundra (Tables 3a and 3b), with more dissimilarities occurring during the summer season. Between older and freshly collected summer faeces, statistically significant differences (Tables 4a and 4b) were found for K ($Z = -2.3372$, $p < 0.0195$), As ($Z = -2.3176$, $p < 0.0205$), Mn

Table 2a
Limit of detection and limit of quantification from ICP-OES [mg L^{-1}].

Element	WL (nm)	LOD (3 s)	LOQ (10 s)
Ca	396.8	0.005	0.016
Fe	259.9	0.001	0.003
K	766.4	0.008	0.026
Mg	285.2	0.0004	0.001
P	177.4	0.010	0.035
S	180.7	0.031	0.104
Si	251.6	0.001	0.004

Table 2b
Limit of detection and limit of quantification from ICP-MS [μg L^{-1}].

Element	Quantified isotope	LOD (3 s)	LOQ (10s)
Ag	107	0.0006	0.0019
Al	27	0.1554	0.5183
As	75	0.0128	0.0427
B	11[a]	0.6734	2.244
Ba	138	0.0089	0.0296
Be	9[a]	0.0003	0.0009
Cd	111	0.0016	0.0055
Co	59	0.0024	0.0082
Cr	52	0.0113	0.0377
Cu	63	0.0836	0.2787
La	139	0.0004	0.0014
Li	7[a]	0.0023	0.0078
Mn	55	0.0137	0.0457
Mo	98	0.0007	0.0023
Na	23[a]	0.6569	2.189
Ni	60	0.0043	0.0144
Pb	208	0.0031	0.0102
Sb	121	0.0008	0.0025
Se	78	0.0399	0.1330
Sr	88	0.0033	0.0111
V	51	0.0015	0.0050
Zn	66	0.0389	0.1299

[a] Analysis was performed without gas in the collision cell.

($Z = 2.1559$, $p < 0.0311$), Na ($Z = -3.2877$, $p < 0.0011$), Ni ($Z = 2.4793$, $p < 0.0132$), and Sb ($Z = 2.6948$, $p < 0.0071$). Finally, concentrations in calf, female and male reindeer excrements were at a similar level, except zinc and potassium (Fig. 3), although due to small sample sizes, no further analysis was performed.

To reveal a synthetic picture of the connections between variables, principal component analysis (PCA) was performed. In the variable set with the higher concentrations and with a few missing records, the two main PCs explained 67.5% of the total variability in the dataset. In the space defined by those two components, clusters related to seasons could be found (Fig. 4a), as well as clusters of similar tundra types (Fig. 4b). The further PCs (3 and 4, explaining 20.6% of the total variability), despite their potential importance suggested by the scree plot, did not exhibit any clear division with respect to season or tundra type.

Based on a scree plot, we analyzed 3 PCs for the second variable set (with lower concentrations and no missing data records; the total variability explained by the three factors was 74.9%) and marked the points on the graph according to the collection season and tundra type. The first PC, explaining 39.2% of the dataset variability, was connected to the type of environment in which the sample was collected (mesic or moss tundra, Fig. 5b). None of the principal components, however, has divided the values clearly by season (Fig. 5a).

4. Discussion

Svalbard Archipelago is affected by long-range-transported pollution, including multiple heavy metals (Pacyna, 1995). Contaminants may be delivered into land by wet and dry deposition, redeposited from melting glaciers, sea aerosol and biota (Wojtuń et al., 2013). Through droppings, metals excreted by fauna reach the Arctic soil. Animal droppings can serve as a biomonitoring tool, as they exhibit significant spatial differences in trace element accumulation (Yin et al., 2008). Thus, it is necessary to understand the place of reindeer droppings in the elemental cycles of this environment.

A direct source of trace metal intake for reindeer is their diet which consists of mosses, lichens and vascular plants, all of which may accumulate significant amounts of metals and metalloids (Wojtuń et al., 2013; Węgrzyn et al., 2016, 2018a). Moss is a dominant form of vegetation in the Arctic tundra ecosystems, and due to its physiology, it may easily intercept, retain, and accumulate metals from dry and wet deposition (Wojtuń et al., 2013, 2018). Some elements in the faeces, including Cu, may also correspond to the geochemical background levels in the area of interest (Yin et al., 2008). It was suggested that through their long-term interaction with lichens and mosses, reindeer have adapted evolutionarily to tolerate compounds occurring at high levels in lichens and moss, e.g. phenolic compounds or iron (Borch-Iohnsen and Thorstensen, 2009; Sundset et al., 2010). However, the interactions and effects of excess exposure to many compounds in not well known, thus it cannot be concluded yet that the high levels of elements are not harmful to their health.

4.1. Differences by location: mesic and moss tundra

Differences in the plant types growing in mesic and moss tundra may imply differences in metal exposure. High levels of Co, Cr, Cu, Fe, Hg, Mn, Ni, and Pb were found in moss *Racomitrium lanuginosum, Sanionia uncinata, and Straminergon stramineum* from variable wet tundra environments (Wojtuń et al., 2013). The moss tundra receives water from spring runoff and melting ice or snow, which increases the load of available metals and metalloids (Wojtuń et al., 2013). Sea aerosol is an additional source of elements including sodium, lead, mercury and cesium (Kłos et al., 2017; Wojtuń et al., 2018). Thus we suspected higher element levels in droppings collected from the moss tundra type. Since some of the elements accumulate very efficiently in mosses (e.g., Cd, Co, Cr, Cu, Fe, Mn, and Zn) (Wojtuń et al., 2013), the Zn-Cd-Cu-Mn and Mo element correlation in our studies (Fig. 5) may be explained by their dietary intake from moss tundra. Al-Fe could be correlated (Fig. 4) due to a common geological source, and be introduced to reindeer diet through rock weathering and subsequent absorption by vegetation.

Mercury contamination in polar regions has raised substantial concerns, as it may easily enter the food chain (Poissant et al., 2008). Primary sources of mercury in the Arctic are transport via air, ocean

Table 3a
Levels of the analyzed macro-elements [mg g^{-1} dw] in reindeer droppings. Values reported are: mean ± SD (median) for groups of samples referring to their location and season. The significance level of the statistical difference between seasons (based on the U Mann-Whitney test) with p-values <0.05 is highlighted. Abbreviations: MeS – mesic tundra summer, MeW – mesic tundra winter, MoS – moss tundra summer, MoW – moss tundra winter.

Element	Mean ± SD (median) [mg g^{-1} dw] [droppings grouped by tundra type and season]					Statistical difference between mesic and moss tundra [U Mann-Whitney test; p-values]	
	Mesic tundra summer (n = 22)	Mesic tundra winter (n = 9)	Mesic tundra winter transitional (n = 3)	Moss tundra summer (n = 7)	Moss tundra winter (n = 7)	p (MeS-MoS)	p (MeW-MoW)
Ca	54.9 ± 12.9 (57.3)	58.3 ± 16.0 (61.9)	36.1 ± 6.05 (33.3)	23.4 ± 5.32 (22.8)	41.8 ± 17.9 (37.8)	**0.0001**	0.112
Fe	2.85 ± 0.76 (2.71)	3.36 ± 0.72 (3.36)	2.37 ± 0.61 (2.01)	9.23 ± 3.48 (10.7)	8.41 ± 6.50 (4.93)	**0.0002**	0.112
K	2.55 ± 1.58 (2.18)	0.80 ± 0.85 (0.32)	1.57 ± 0.24 (1.45)	1.41 ± 0.97 (0.88)	1.18 ± 0.73 (1.37)	0.120	0.397
Mg	6.37 ± 2.91 (5.33)	3.37 ± 1.25 (2.81)	6.15 ± 3.23 (4.88)	6.48 ± 2.07 (6.53)	6.33 ± 2.47 (5.76)	0.899	**0.015**
P	3.32 ± 1.03 (3.05)	1.88 ± 0.52 (1.55)	2.84 ± 0.53 (2.59)	3.10 ± 1.12 (2.62)	2.21 ± 0.87 (1.91)	0.558	0.341
S	4.63 ± 0.44 (4.65)	4.04 ± 0.55 (3.88)	4.29 ± 0.36 (4.14)	4.26 ± 0.72 (4.06)	3.67 ± 0.63 (3.58)	0.252	0.397
Si	0.46 ± 0.04 (0.45)	0.44 ± 0.06 (0.44)	0.48 ± 0.02 (0.47)	0.39 ± 0.08 (0.44)	0.44 ± 0.08 (0.46)	0.161	0.916

Table 3b
Levels of analyzed elements (μg g^{-1} dw) in reindeer droppings: mean ± SD (median). Columns represent division by sampling location or season. The level of statistical difference between seasons (based on the U Mann-Whitney test) with p-values < 0.05 was highlighted. Abbreviations: MeS – mesic tundra summer, MeW – mesic tundra winter, MoS – moss tundra summer, MoW – moss tundra winter. Sample size listed in the table heading, except for Hg (see footnote).

Element	Mean ± SD (median) [μg g^{-1} dw] [droppings grouped by tundra type and season]					Statistical difference between mesic and moss tundra [U Mann-Whitney test; p-values]	
	Mesic tundra summer (n = 26)	Mesic tundra winter (n = 11)	Mesic tundra winter transitional (n = 3)	Moss tundra summer (n = 7)	Moss tundra winter (n = 7)	p (MeS-MoS)	p (MeW-MoW)
Al	1857 ± 481 (1807)	2080 ± 470 (2091)	1526 ± 266 (1457)	4188 ± 1436 (3993)	4413 ± 3088 (2610)	**0.0002**	0.174
As	0.737 ± 0.328 (0.660)	0.942 ± 0.384 (0.854)	0.60 ± 0.08 (0.57)	1.04 ± 0.27 (1.20)	1.042 ± 0.533 (0.913)	**0.045**	0.856
B	14.82 ± 3.16 (14.40)	13.9 ± 6.26 (12.2)	14.8 ± 2.28 (13.8)	13.1 ± 4.32 (11.9)	12.3 ± 3.76 (10.8)	0.523	0.717
Ba	38.5 ± 7.55 (38.2)	35.4 ± 5.04 (36.9)	33.4 ± 7.2 (28.5)	104.8 ± 42.5 (80.0)	121.6 ± 35.1 (124.8)	**0.0001**	**0.0006**
Be	0.051 ± 0.038 (0.047)	0.054 ± 0.016 (0.056)	0.03 ± 0.01 (0.04)	0.127 ± 0.051 (0.134)	0.137 ± 0.080 (0.091)	**0.003**	**0.005**
Cd	1.023 ± 0.417 (0.918)	0.755 ± 0.340 (0.665)	1.04 ± 0.38 (0.98)	1.88 ± 1.20 (1.64)	0.885 ± 0.518 (0.715)	**0.018**	0.587
Co	0.898 ± 0.169 (0.898)	0.699 ± 0.110 (0.717)	0.94 ± 0.155 (0.84)	4.79 ± 2.02 (5.03)	3.54 ± 1.91 (3.67)	**0.00007**	**0.0006**
Cr	1.849 ± 0.469 (1.82)	2.24 ± 0.544 (2.12)	1.45 ± 0.22 (1.41)	17.1 ± 8.67 (23.6)	14.1 ± 11.8 (10.8)	**0.00007**	**0.0086**
Cu	8.57 ± 3.13 (9.13)	4.62 ± 2.18 (3.42)	9.81 ± 2.79 (9.96)	17.2 ± 3.99 (16.0)	10.8 ± 5.70 (10.9)	**0.0002**	**0.003**
La	2.82 ± 0.650 (2.72)	3.24 ± 0.847 (3.33)	2.24 ± 0.34 (2.15)	5.43 ± 1.86 (5.68)	5.70 ± 3.39 (4.47)	**0.0001**	0.174
Li	1.22 ± 0.434 (1.18)	1.36 ± 0.363 (1.37)	1.03 ± 0.26 (1.00)	2.97 ± 1.06 (2.56)	3.50 ± 2.74 (1.76)	**0.0028**	0.147
Mn	241 ± 108 (227)	141 ± 65.6 (123)	208 ± 120 (135)	334 ± 104 (278)	278 ± 109 (227)	0.050	**0.002**
Mo	0.517 ± 0.181 (0.503)	0.548 ± 0.419 (0.354)	0.65 ± 0.20 (0.53)	0.896 ± 0.245 (0.834)	0.671 ± 0.226 (0.662)	**0.0006**	0.239
Na	164 ± 89.9 (142)	105 ± 52.5 (79.4)	113 ± 49.6 (115)	112 ± 27.5 (97.7)	133 ± 39.6 (128)	0.708	0.174
Ni	2.015 ± 0.339 (1.93)	1.72 ± 0.243 (1.76)	2.51 ± 0.55 (2.77)	19.04 ± 5.94 (20.6)	13.4 ± 7.10 (12.3)	**0.033**	**0.033**
Pb	4.58 ± 2.43 (3.63)	7.58 ± 3.75 (7.92)	2.88 ± 0.54 (2.99)	2.47 ± 1.26 (1.85)	3.88 ± 1.84 (4.33)	**0.0007**	0.070
Sb	0.028 ± 0.026 (0.022)	0.036 ± 0.043 (0.022)	0.05 ± 0.01 (0.05)	0.009 ± 0.011 (0.00)	0.022 ± 0.026 (0.013)	**0.0112**	0.205
Se	0.988 ± 0.247 (0.972)	1.25 ± 0.325 (1.25)	0.73 ± 0.03 (0.72)	1.30 ± 0.457 (1.24)	1.45 ± 0.705 (1.31)	0.226	0.717
Sr	84.9 ± 53.9 (72.2)	110 ± 80.8 (74.3)	63.6 ± 12.4 (66.5)	142.8 ± 36.6 (145)	211 ± 114 (154)	**0.00007**	**0.0112**
V	2.78 ± 0.709 (2.77)	3.79 ± 1.36 (3.64)	2.35 ± 0.28 (2.50)	10.9 ± 5.36 (13.2)	9.97 ± 8.37 (6.91)	**0.00007**	0.147
Zn	153 ± 72.8 (155)	81.8 ± 44.6 (61.5)	165 ± 62.3 (165)	236 ± 93.3 (246)	115 ± 66.6 (93.7)	0.055	0.239
Hg[a]	0.069 ± 0.016 (0.067)	0.129	–	0.100 ± 0.028 (0.100)	0.082 ± 0.022 (0.085)	–	–

[a] MeS n = 11, MeW n = 1, MoS n = 6, MoW n = 5.

currents, and rivers, and subsequently dry and wet deposition (Poissant et al., 2008). Here, the highest mercury level was 0.129 μg g^{-1} dw in the winter samples collected in the mesic tundra. Moss tundra dropping levels did not exceed 0.100 μg g^{-1} dw. This suggests low exposure, since also the level in the vegetation described in previous studies did not exceed 0.190 μg g^{-1} dw (Wojtuń et al., 2013). However, most of the mercury in the body is assimilated, and may easily biomagnify (Yin et al., 2008), thus it would not be excreted effectively with droppings.

Previous studies by Yin et al. (2008) showed a high level of lead in Arctic reindeer excrements (>11 μg g^{-1}), probably as a consequence of past industrial activities and leaded gasoline use (Sturges and Barrie, 1989). Although since the 1980s the usage of leaded gasoline was significantly reduced, large amounts of Pb emitted into the atmosphere were still deposited in the Arctic (Sturges and Barrie, 1989). The current atmospheric deposition of lead in the Norwegian Arctic originates mostly from Eastern Eurasia (spring input) and Northern America (summer input), with local crustal inputs playing a minor role (Bazzano et al., 2015). Here, Pb levels were significantly higher ($p < 0.0239$) in mesic tundra, with the mean concentrations of 4.58/7.58 μg g^{-1} dw (summer/winter), than in wet moss tundra (2.47/3.88 μg g^{-1} dw).

Multiple elements of toxicological concern were found at higher concentrations in the moss tundra in this study. The aluminium concentration in samples derived from moss tundra was more than two times higher compared to mesic tundra ($Z = -3.9181$, $p < 0.0001$). Chromium concentration was the highest during summer in the moss tundra setting, with the mean of 17.1 ± 8.67 μg g^{-1} dw (mesic tundra mean ± SD 1.85 ± 0.47 μg g^{-1} dw; $Z = -5.0037$, $p < 0.0000$). Also nickel

Table 4a
Mesic tundra summer samples: comparison of the old and freshly collected droppings (units: μg g^{-1} dw; values reported: mean concentration ± 1 SD and median in brackets). Sample size listed in the table heading, except for Hg (see footnote).

Element	Mean ± SD (median) [μg g^{-1} dw]/samples grouped by the length of period between excretion and collection/	
	Older (n = 17)	Fresh (n = 9)
Al	1828 ± 529 (1720)	1913 ± 368 (1861)
As	0.694 ± 0.371 (0.650)	0.819 ± 0.204 (0.782)
B	15.6 ± 3.16 (15.1)	13.3 ± 2.57 (13.3)
Ba	39.2 ± 8.61 (39.1)	37.1 ± 4.65 (37.5)
Be	0.059 ± 0.042 (0.056)	0.036 ± 0.022 (0.028)
Cd	1.11 ± 0.395 (1.15)	0.866 ± 0.411 (0.677)
Co	0.931 ± 0.184 (0.944)	0.836 ± 0.213 (0.869)
Cr	1.84 ± 0.49 (1.89)	1.86 ± 0.427 (1.79)
Cu	9.40 ± 2.92 (9.69)	7.01 ± 2.91 (5.21)
La	2.69 ± 0.569 (2.56)	3.05 ± 0.725 (3.40)
Li	1.17 ± 0.484 (1.13)	1.303 ± 0.301 (1.26)
Mn	274 ± 119 (275)	179 ± 34.7 (176)
Mo	0.513 ± 0.213 (0.439)	0.525 ± 0.095 (0.569)
Na	121 ± 67.4 (85.9)	245 ± 69.9 (247)
Ni	2.13 ± 0.354 (2.06)	1.79 ± 0.141 (1.81)
Pb	4.24 ± 1.94 (3.43)	5.23 ± 3.05 (4.56)
Sb	0.037 ± 0.028 (0.027)	0.012 ± 0.008 (0.014)
Se	0.946 ± 0.206 (0.879)	1.07 ± 0.296 (1.01)
Sr	89.8 ± 65.1 (68.5)	75.5 ± 15.6 (77.1)
V	2.69 ± 0.743 (2.74)	2.93 ± 0.609 (2.80)
Zn	171 ± 69.5 (187)	120 ± 66.9 (82.1)
Hg[a]	0.068 ± 0.015 (0.075)	0.069 ± 0.016 (0.067)

[a] Old n = 3, fresh n = 8.

Table 4b
Concentration levels of elements in mg g^{-1} dw. Values reported are means ± 1 SD (median in brackets).

Element	Mean ± SD (median) [mg g^{-1} dw]/samples grouped by the length of period between excretion and collection/	
	Older (n = 13)	Fresh (n = 9)
Ca	50.85 ± 12.7 (51.6)	60.65 ± 10.8 (63.5)
Fe	2.67 ± 0.76 (2.65)	3.11 ± 0.68 (2.82)
K	1.85 ± 1.16 (1.67)	3.56 ± 1.55 (3.82)
Mg	6.05 ± 3.20 (5.10)	6.83 ± 2.36 (5.57)
P	3.52 ± 1.16 (3.34)	3.04 ± 0.72 (2.94)
S	4.66 ± 0.53 (4.79)	4.58 ± 0.26 (4.57)
Si	0.46 ± 0.05 (0.47)	0.44 ± 0.03 (0.45)

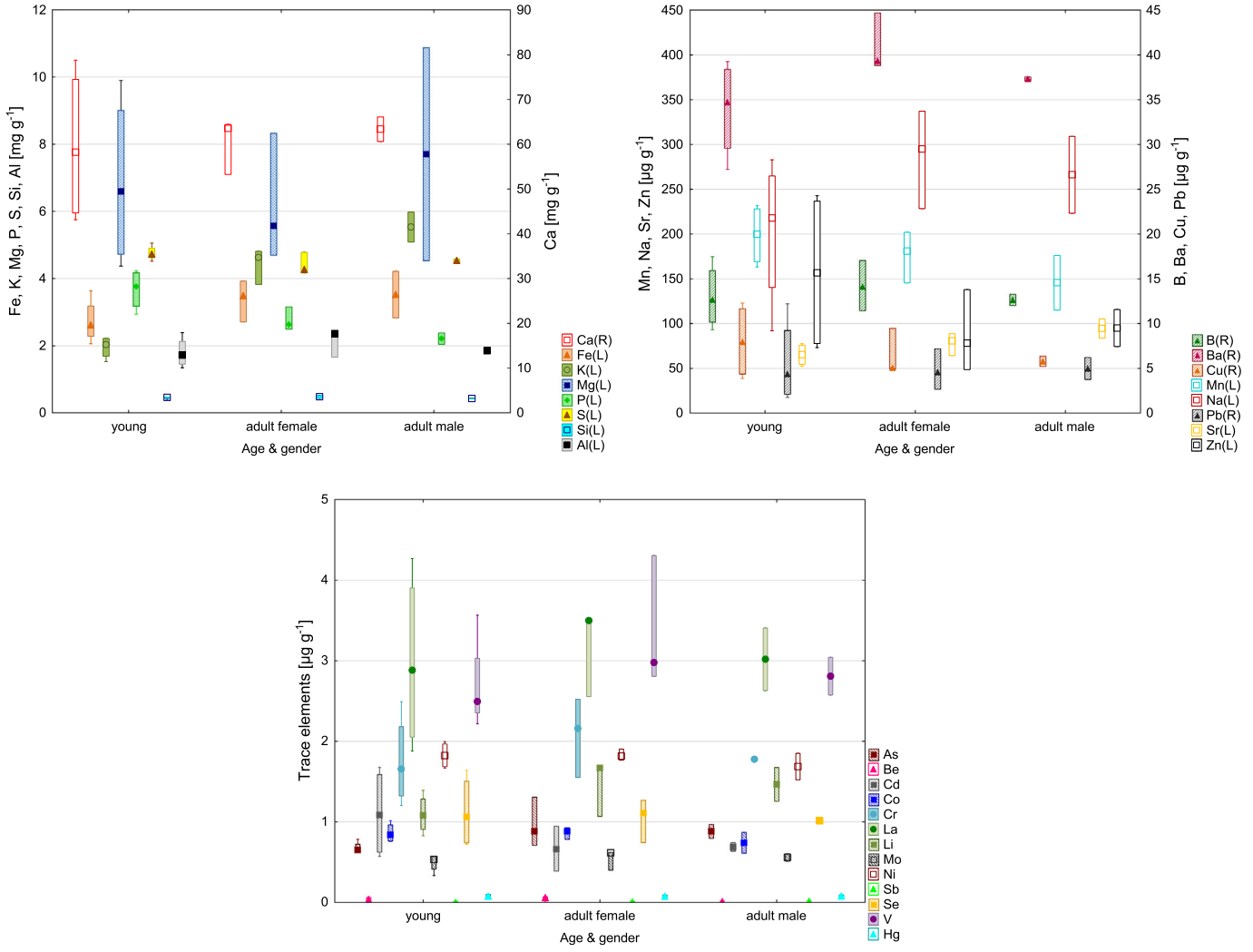

Fig. 3. a,b,c. Concentration levels of selected elements in reindeer calves (n = 4), adult females (n = 3) and males (n = 2). The box signifies quartiles (the point marker in the middle is median), whiskers show the full range of values. In the legend, R and L in brackets mean right or left axis of the graph. The variables for each graph were chosen depending on their concentration range, to improve the clarity of presentation.

concentration was significantly lower in the mesic tundra ($Z = -5.5169$, $p < 0.0000$). Cadmium and arsenic levels were also higher in the moss tundra type, although the difference was not statistically significant.

Among all elements, the highest concentration was found for calcium (Table 3a) in the mesic tundra, where it was much higher than in the moss type ($Z = 3.7313$, $p < 0.0002$). Magnesium, potassium and phosphorus concentrations did not differ significantly, in contrast to iron and sulphur (Fe: $Z = -4.0942$, $p < 0.0001$, S: $Z = 2.0755$, $p < 0.0380$).

4.2. Feasibility of comparison between the older and fresh samples

Samples collected immediately after excretion and older but excreted during summer (Tables 4a and 4b) were analyzed to compare the concentration levels between them and to assess the utility of the older sample usage. Due to several elemental concentrations departing from the assumptions of ANOVA, such as the normal distribution (in the case of As, B, Be, Na and Sr) and the homogeneity of variance (Mn, Mo, Ni, S, Sb and Sr), we explored the results with a non-parametric technique (the Mann-Whitney test). The statistically significant differences ($p < 0.05$) were found then for K ($Z = -2.3372$, $p < 0.0195$), As ($Z = -2.3176$, $p < 0.0205$), Mn ($Z = 2.1559$, $p < 0.0311$), Na ($Z = -3.2877$, $p < 0.0011$), Ni ($Z = 2.4793$, $p < 0.0132$), and Sb ($Z = 2.6948$, $p < 0.0071$). ANOVA confirmed such statement for potassium, which met its assumptions ($p < 0.0105$, $F = 7.98$), as well as possibly for sodium, manganese, nickel and antimony. Due to the small sample sizes, mercury was not analyzed. For all the other elements, the differences were not statistically significant.

Potassium (and sodium) were more abundant in fresher samples, even up to two times higher than in older droppings. The difference between samples could be caused by atmospheric precipitation, as both elements are readily washed out with water. Apart from arsenic, sodium and potassium, the differences in element concentrations favoured older samples, perhaps due to extra atmospheric deposition over time. The lack of significant differences in lead and cadmium concentrations suggests that older samples are suitable to evaluate exposure to these metals if fresh samples are unavailable.

4.3. Season

Within each season, distinct features of element accumulation can be seen, especially in the summer (Tables 3a and 3b). Although Svalbard reindeer are foraging for biomass mostly (Van der Wal et al., 2000), they have certain preferences in choosing foraging areas, dependent on the season (Węgrzyn et al., 2018b). Reindeer prefer open plains and wetlands in winter, whereas after April they mostly choose slopes and

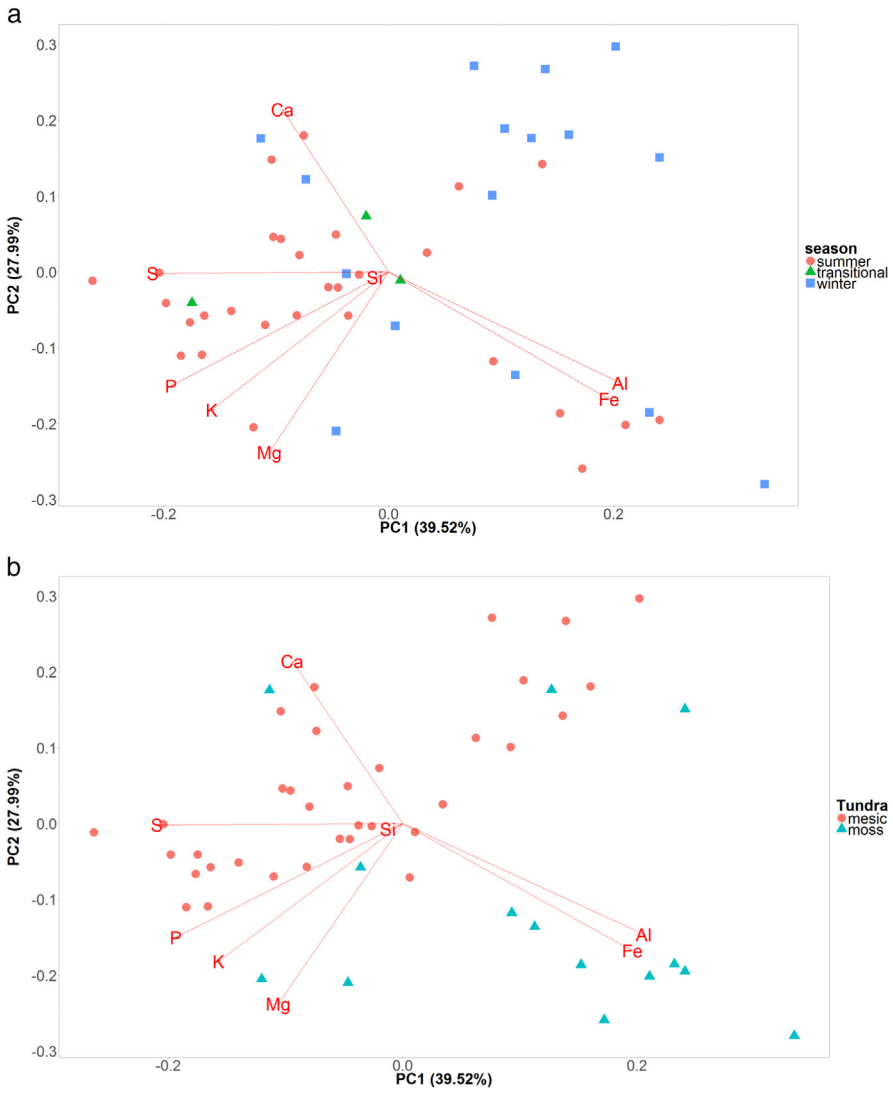

Fig. 4. a. PCA of the variable set with higher concentrations. The space defined for PCs 1 and 2 with points marked by season. b. PCA of the variable set with higher concentrations. The space defined for PCs 1 and 2 with points marked by tundra type.

areas with dry vegetation types (Lindner, 2002). Furthermore, food density may be temporarily reduced due to competition or snow accumulation (Lindner, 2002). Thus, the inter-seasonal differences between element levels may result from the restricted food availability and different element concentrations of various vegetation types.

Meteorological conditions shape the seasonal differences in element supply through wet and dry deposition, e.g., Bazzano et al. (2015) show the concentrations of atmospheric lead and anthropogenic sulphates were higher in the spring than in the summer. However, very few studies model seasonal deposition sums in the Arctic, so the full atmospheric influence is difficult to discern.

No inter-seasonal difference (summer vs winter) was found for zinc, sodium, boron and selenium. It may be because they are affected by metabolic regulation more than the long-range transport. In the case of sodium and selenium, sea-related emissions may also play a role (Kabata-Pendias and Szteke, 2015; Kłos et al., 2017). Further factors, such as water-soil exchange, precipitation concentration and volume may affect levels in vegetation, and thus the load of metals consumed by reindeer.

4.4. Comparison with previous studies

Previous research on fur samples of Svalbard reindeer showed high concentrations of lead, and relatively low mercury and cadmium levels (Pacyna et al., 2018). Yin et al. (2008) studies showed a high level of lead in Arctic reindeer excrements (>11 μg g^{-1}). For comparison with other reindeer species, we report data on Alaskan caribou after O'hara et al. (2003). The mean lead level in their faeces (6.18 μg g^{-1} ww), although elevated, was within the normal range for cattle (2.0 to 35.0 μg g^{-1} ww). Cadmium and arsenic were at the level of 0.06 and 0.14 μg g^{-1} ww, respectively. Copper, zinc and iron levels were 11.06, 39.1 and 299 μg g^{-1} ww, respectively.

Samples of liver, teeth and antlers of forest reindeer (*Rangifer tarandus* fennica) (Medvedev, 1995), analyzed for heavy metal and sulphur content, exhibited high lead and cadmium levels in bone tissue (41.6 ± 23.7 and 2.1 ± 1.1 μg g^{-1} dw, respectively). Internal tissues were subject to studies on caribou and reindeer populations from Canada, northern Norway and Greenland (Elkin and Bethke, 1995; Robillard et al., 2002; Larter and Nagy, 2000; Aastrup et al., 2000; Ali Hassan et al., 2012). However, as element excretion is tissue-specific, it is difficult to make a concise comparison between previous research and the current study.

Due to the specific vegetation and climate of Svalbard, which results in a particular feeding behaviour and patch choice by reindeer, and the isolation of the archipelago, the Svalbard reindeer data obtained here should not be directly compared to other reindeer subspecies, such as caribou (Lindner, 2002). However, it can be noted that several elements

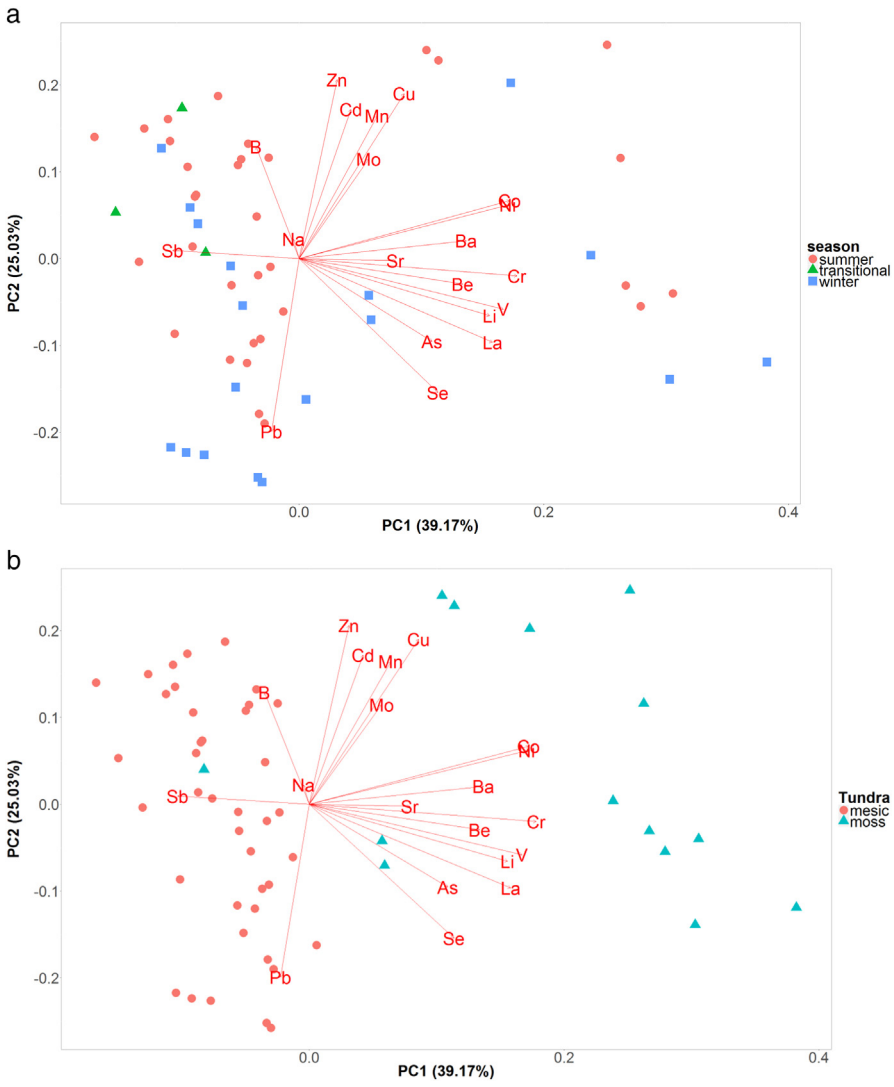

Fig. 5. a. PCA of the variable set with lower concentrations, analyzed by ICP-MS. The space defined for PCs 1 and 2, with points marked by season. b. PCA of the variable set with lower concentrations, analyzed by ICP-MS. The space defined for PCs 1 and 2 with points marked by tundra type.

of ecotoxicological interest, including lead and mercury, are found at analogous levels and probably come from similar sources.

4.5. Age and gender as an interfering factor

With few samples assigned to calf, female and male adults, statistical analysis was not feasible, hence we present the data only in a visual form (Fig. 3). The young reindeer were still fed with mother's milk, simultaneously introducing fresh vegetation into their diet. Only for potassium, the level in calves was lower, compared to adult individuals, and in the case of zinc, it was higher. Thus, the age of the excreting reindeer can be a source of uncertainty in the present study, and further studies on more individuals should take place.

5. Conclusion

Various biological materials are used as environmental indicators, with special attention given recently to low-cost, noninvasive and easy-to-perform sampling procedures. Droppings are a route of elimination for toxic and excess elements, giving information on the dietary availability of compounds, the level of metal contamination and organism excretion possibilities. In the case of terrestrial species, especially those shy and endangered, it gives a possibility to monitor their population over years, without direct contact with an animal during sample collection. The distribution pattern of metals and metalloids can be affected by the season and the tundra type where the reindeer forage, thus such factors should be taken into account in the study design. Several metals, including cadmium and lead, occurred without significant differences in older and freshly collected samples. Nonetheless, the sample collection should be carried out carefully, with the description of factors affecting reindeer exposure. The presence of metals and metalloids in the terrestrial biota has potential consequences for the local biogeochemical cycle and the quality of the local environment. Since climate change may induce increased exposure from released contaminants, e.g. from melting glaciers and permafrost, we need to understand the ecosystem response to such higher exposure.

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12.3. Weryfikacja hipotezy 3

Hipoteza 3: Sierść stanowi drogę depozycji szeregu pierwiastków oraz może być wykorzystana do oceny ekspozycji reniferów na wybrane trwałe związki organiczne. Związki, które trafiły na Arktykę w wyniku transportu transgranicznego, w tym na pestycydy chloroorganiczne, wciąż są obecne w środowisku lądowym Arktyki i mogą wpływać na zwierzęta zamieszkujące obszary polarne

Weryfikację **trzeciej hipotezy** wykonano w oparciu o badania próbek sierści pobranych od renifera Svalbardzkiego. Wyniki badań zostały zaakceptowane do druku w czasopiśmie *Chemosphere* (załącznik 4) **[A. D. Pacyna-Kuchta, P. Wietrzyk-Pełka, M. H. Węgrzyn, M. Frankowski, Ż. Polkowska, A screening of select toxic and essential elements and persistent organic pollutants in the fur of Svalbard reindeer, Chemosphere]**

Zwierzęta lądowe Arktyki Europejskiej rzadko są obiektem badań ekotoksykologicznych, a mogą być cennym źródłem informacji o depozycji i biodostępności różnych związków chemicznych. Renifer Svalbardzki jest długowiecznym ssakiem roślinożernym, będącym częścią krótkiego łańcucha pokarmowego. W badaniach wykorzystano próbki sierści zebrane w 2017 roku od populacji żyjących w okolicy stolicy Spitzbergenu (Longyearbyen) oraz okolic stacji badawczej w Ny-Ålesund. Spośród 18 analizowanych pierwiastków tylko rtęć była poniżej limitu detekcji. Najwyższe poziomy pierwiastków odnotowano w kolejności dla metali takich jak Fe, Mg, Zn, K, Ca, Cu. Spośród trwałych związków organicznych w próbkach sierści tylko nieliczne były powyżej granicy oznaczalności, włączając PCB28, p,p-DDD oraz wybrane WWA, głównie dwu- i trzypierścieniowe. Wyniki badań sugerują, iż w okolicy stolicy Longyearbyen lokalne źródła emisji mogą znacząco wpływać na poziom zanieczyszczeń, tworząc lokalne miejsca o zwiększonej zawartości pierwiastków toksycznych. Jednak ze względu na wstępny charakter badań, wykorzystana została mała liczba próbek. Wykorzystanie nowoczesnych technik analitycznych oraz sprzętów takich jak GC-MS/MS pracujący w trybie MRM, pozwala na zwiększenie selektywności oraz dokładności oznaczania związków. Jest to szczególnie ważne w przypadku próbek wobec których spodziewamy się niskich poziomów oznaczanych związków. Wykorzystanie sierści ma wiele zalet, zarówno praktycznych jak i etycznych, redukując koszty i umożliwiając przechowanie i transport próbek w temperaturze pokojowej.

Załącznik 4.

Dear Miss Pacyna,

I am pleased to inform you that the manuscript "A screening of select toxic and essential elements and persistent organic pollutants in the fur of Svalbard reindeer" (Miss Aneta Pacyna) has now been accepted by the editor for publication.

Your accepted manuscript will now be transferred to our production department and work will begin on creation of the proof. If we need any additional information to create the proof, we will let you know. If not, you will be contacted again in the next few days with a request to approve the proof and to complete a number of online forms that are required for publication.

Thank you for considering our journal for the publication of your research.

Kind regards,
Professor Willie J. G. M. Peijnenburg
Editor
Chemosphere

A screening of select toxic and essential elements and persistent organic pollutants in the fur of Svalbard reindeer

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Abstract

Reindeers play an important role in the polar ecosystem, being long-lived sole vegetarians feeding on local vegetation. They can be used as a valuable bioindicator, helping us to understand contaminants' impact on the polar terrestrial ecosystem. Still, scarce data exist from research in which polar herbivores (especially those from the European parts of the Arctic) were a major study subject for trace elements and persistent organic pollutant determination. Here, Svalbard reindeer fur has been

used to determine metals, non-metals and metalloids using ICP-MS, and several persistent organic pollutants including polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) using gas chromatography coupled to a tandem mass spectrometer (GC-MS/MS). Samples were collected from reindeer populations living in the area near Ny-Ålesund and Longyearbyen. Essential elements like Fe, Mg, Zn, K, Ca, Cu predominated in the trace elements profile. Median values of As, Cd, Co, Li, Ni, Se and V were all below 0.5 µg/g dw. Mercury was below detection limit in all samples, while the Pb median varied from 0.35 to 0.74 µg/g dw. Except acenaphthylene and fluorene, PAHs were detectable only in samples collected in the vicinity of Longyearbyen. Of 15 studied pesticides, only DDT and its metabolites were above the detection limit, and, of PCBs, only PCB28.

Keywords: *Rangifer tarandus platyrhynchus, tundra, metal, DDT, PAHs, PCB28*

1. Introduction

Arctic herbivores, though they have a crucial impact on polar ecosystems, are rarely included in ecotoxicological studies, especially those from the European part of the Arctic. Due to the prevalent climate conditions, with cold, dense air and darkness in winter time, the possibility of compound deposition and accumulation in Arctic is increased, which may lead to the accumulation of those compounds in higher trophic level species (Halbach et al., 2017). Svalbard (74–81°N), is part of the European Arctic, and subject to the long-range transport of contaminants. Due to its unique geographical location, it has become a significant recipient of contaminants emitted at mid and low latitudes and transported by air and sea currents into Arctic areas (Braune et al., 2005; de Wit et al., 2004; AMAP, 2005; Halbach et al., 2017). While contaminants are to some extent introduced into the Arctic environment from local natural sources, it is assumed that emission transported long-range from Europe and Asia is majorly responsible for the non-essential metals and persistent organic pollutants levels observed in the polar region (AMAP, 2005; 2011; Reimann and de Caritat, 2005; Eckhardt et al., 2007; Halbach et al., 2017; Zaborska et al., 2017). Contaminants may also be

transported from the marine environment to land by biovectors such as seabirds (Choy et al., 2010) and by volcanic activity, as well as being redeposited from glaciers and soil (Samecka-Cymerman et al., 2011; Zaborska et al., 2017; Kozioł et al., 2017). Additionally, increasing research and tourist activities, and runoffs from coal mines, may add to the local contamination (Granberg et al., 2017; Aslam et al., 2019; Wojtuń et al., 2019).

Feeding ecology and preferences have a major effect on the intake of contaminants (Bocharova et al., 2013). In terrestrial ecosystems, the bioaccumulation process would be affected by different physiological and ecological factors than in marine ecosystems (van den Brink et al., 2015). Trace elements can be divided into groups, as some of them are essential for proper functioning (e.g. Zn, Se, Cu), while others are toxic without any known beneficial role in the organism (e.g. Hg or Pb) (Eisler, 1987; Burger and Gochfeld, 2000a,b; Peakall and Burger, 2003). Essential elements may also be toxic in excessive quantities (Burger and Gochfeld, 2000b; Peakall and Burger, 2003). Persistent organic pollutants are a group of contaminants with properties that provide them a high bioaccumulation potential (lipophilicity, persistence in the environment, volatility). They are usually produced in processes associated with anthropogenic activities, and transported long distances to the Arctic. Several studies confirmed the presence of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) in the polar biota (see references in Letcher et al., 2010 and Marquès et al., 2017). PCBs and OCPs have potential negative biological effects and thus are frequently studied compounds in polar research (Jones and Voogt, 1999; Jaspers et al., 2010; Letcher et al., 2010). PAHs may have natural or anthropogenic origin, since, for example, pyrogenic PAHs can be emitted during incomplete combustion of fossil fuels and organic matter (Balmer et al., 2019). The PAHs found in terrestrial compartments (including soil and sediments) mostly originate from atmospheric and combustion-derived sources (Balmer et al., 2019).

Contaminant upload and redistribution is an essential factor that may affect a species' ability to survive changes connected with climate warming. Non-lethally and non-invasively collected samples

have been gaining much attention recently, including keratinised tissues such as mammal fur or the feathers of bird species (D'Havé et al., 2005; Duffy et al., 2005; Jaspers et al., 2006; Pacyna et al., 2018; Pacyna et al., 2019a). Fur collection does not imply any harm to the animal, storage is relatively easy, compounds do not metabolise and tissue levels correspond to long-term exposure (Duffy et al., 2005; Jaspers et al., 2010). Hair samples have been proven to be a useful matrix for biomonitoring of trace elements and several POPs (Duffy et al., 2005; Jaspers et al., 2010; Pacyna et al., 2018). However, due to the low lipid content of hair, POP levels in them can be low, comparing to internal tissue (D'Havé et al., 2005; Jaspers et al., 2010), and thus, depending on contaminant concentration, hair mass should be appropriately high for reliable quantification (Covaci et al., 2002; Jaspers et al., 2006).

Here, we intend to add new data related to the only large grazing mammal in the European High Arctic – the Svalbard reindeer (*Rangifer tarandus platyrhynchus*). This endemic, widespread resident of Svalbard is exposed to contaminants mostly through its diet, which is composed of different types of locally grown vegetation, lichen and moss (Robillard et al., 2002; Węgrzyn et al., 2018; Węgrzyn et al. 2019). The present study describes the concentrations of 18 elements, 16 polycyclic aromatic hydrocarbons (PAHs), 10 polychlorinated biphenyls (PCBs), and 15 organochlorine pesticides (OCPs) in the moulted fur of Svalbard reindeer. Moulted fur (shed by the reindeer during the natural process of renewing their coats) was used, as it does not require direct contact with an individual, eliminating the stress factor for an animal. The main goal of this study was to investigate the distribution pattern of contaminants, and to depict their possible co-exposure and/or similar bioaccumulation and excretion patterns. It also serves to provide background data on the levels of POPs and six elements not studied before in reindeer fur. Moreover, comparison analysis between elemental contents in reindeer fur collected in 2015 in a previous study (Pacyna et al. 2018) and here was performed to investigate differences in element concentration in time. To the best of our knowledge, the present study is the first to examine organic compounds and Ca, K, Mg, Mn, Se and Sr in Svalbard reindeer fur and the second for the other elements analysed.

2. Materials and methods

2.1 Study site and sampling

Sampling was conducted in the 2017 summer season in Spitsbergen in two locations, in the vicinity of Longyearbyen (15°35'/15°39'E, 78°02'/78°13'N) and in the vicinity of Ny-Ålesund (11°48'/12°04'E, 78° 54'/78°55'N) (Fig. 1). Spitsbergen is the largest island in the Svalbard archipelago, and Longyearbyen, being the administrative centre of Svalbard, represents the largest settlement on the island. The Longyearbyen area can be affected by local contamination sources, including exhausts from power plants, industrial waste dumps, and coal mines (Wojtuń et al., 2019). The northernmost settlement of Ny-Ålesund has visitors only for research purposes.

Fig. 1 Study site showing area where Svalbard reindeer fur was collected: A – Svalbard Archipelago, 1 – Ny-Ålesund, 2 – Longyearbyen, map source: Norwegian Polar Institute, www.npolar.no

Reindeer fur grows at a specific time and the coat is replaced seasonally. Contaminants deposited in hair tissue reflect blood concentrations at the time of hair growth (Jaspers et al., 2010). Freshly moulted fur was collected manually from the ground into separate string bags. All reindeer fur samples (n=8 in total) were collected at a given location within one day. Samples were collected in places where reindeers wiped their winter fur on the ground. The sampling localities were about 1 km apart. Individual reindeers were identified by observation from a distance so as to avoid having the same animal's fur collected on repeat occasions. Samples were stored at ambient temperature.

2.2 Chemical analysis

To prevent possible interferences caused by different melanin contents, only white hairs were analysed. Hair strands were separated manually from the collected sample with clean tweezers to separate them from any moss debris or soil collected with the fur ball. To remove adherent external contamination such as dust particles and loosely bound particulate matter, each pooled sample from one individual was cleaned at least 3 times in double-deionised water (Milipore Mili-Q, France) and then air-dried for 24 h. Organic solvents were not used for cleaning purposes, as they may wash out elements of interest from the hair's internal structure (Jaspers et al., 2010). Then, an appropriate sample amount from each individual (sample mass is given below for each group) was taken for separate analysis. Analysis was performed for: 18 elements (As, Ba, Ca, Cd, Co, Cu, Fe, Hg, K, Li, Mg, Mn, Ni, Pb, Se, Sr, Zn, V); 16 PAHs (naphthalene; acenaphthylene; 2-bromo-naphthalene; acenaphthene; fluorene; phenanthrene; anthracene; fluoranthene; pyrene; benz[a]anthracene; chrysene; benzo[b]fluoranthene; benzo[a]pyrene; indeno[1,2,3-cd]pyrene; dibenz[a,h]anthracene; benzo[ghi]perylene); 10 PCBs (28, 52, 77, 101, 118, 126, 138, 153, 169, 180); and 15 OCPs (alfa-, beta-, delta-hch, hexachlorobenzene, heptachlor, mirex, aldrin, dieldrin, endrin, op-DDT, pp-DDT, op-DDE, pp-DDE, op-DDD, pp-DDD).

2.2.1 Metals, non-metals and metalloids

Dried samples were cut with clean stainless-steel scissors. About 150–170 mg of sample was then weighed, and 10 ml of 65% HNO_3 was added. Samples were digested using a high-pressure microwave emitter (Microwave Digestion System, Anton Paar). The temperature was increased from room temperature to 90°C and such conditions were maintained for 25 min. After that, the temperature was gradually lowered. Subsequently, mineralised samples were diluted with double-deionised water to 25 ml in clean plastic flasks. To check accuracy, 3 randomly chosen samples were prepared in duplicates. Every batch contained blank samples, to ensure quality control and check for background contamination. Certificate reference material (CRM, Human hair ERM-DB001, Sigma-Aldrich) was used in triplicate to check the accuracy of obtained results. Analysis was performed

using Inductively Coupled Plasma Mass Spectrometry (ICP-MS 2030, Shimadzu, Japan). Measurement conditions and parameters are reported in a previous study (Pacyna et al., 2019a)

2.2.2 Polycyclic aromatic hydrocarbons

Dried samples were cut with clean, acetone-rinsed stainless-steel scissors. Samples (mean 273 mg) were incubated overnight with 20 ml hexane:acetone (2:1), 7 ml 15% HCl and 10 µl internal standards (naphthalene-d8, benzo(a)anthracene-d12; c=0.1 µg/ml). Next day, samples were vortexed and the organic layer was collected. A fresh solvent layer (5 ml of hexane:acetone 2:1 v:v) was added, the sample was vortexed again, and the organic layer was collected and combined with the previously collected layer into the same tube. Samples were evaporated until approx. 3 ml.

Clean-up was performed on SPE columns filled from the bottom with activated silica gel, and anhydrous Na_2SO_4. Columns were conditioned with 2 volumes of hexane (2*2 ml), sample extract was added, and eluted using 2 volumes of DCM: hexane (3:7 v:v) (2*3 ml). The volume was evaporated in a nitrogen stream until dry, and reconstituted in 300 µl of hexane. Determination was performed on a gas chromatography instrument coupled to a tandem mass spectrometer (Shimadzu GC-MS-TQ 8050) operating in Multiple Reaction Monitoring (MRM) mode. Measurement conditions and parameters are given in Supplementary Table 1.

2.2.3 Pesticides and polychlorinated biphenyls

Dried samples were cut with clean, acetone-rinsed stainless-steel scissors. A modified procedure used by Jasper et al. (2010) was applied. About 300 mg of the samples was incubated overnight with 25 ml hexane:DCM (4:1), 7 ml 4M HCl and 10 µl internal standards (DDT-D8, PCB-28 C13 and PCB-180 C13, c=0.1 µg/ml). Next day, samples were put in ultrasounds for 1 min, vortexed, and the organic layer was collected into a tube. A fresh solvent layer (2*10 ml of hexane:DCM (4:1v:v) was added, and the organic layer was collected again and combined into the same tube. Samples were

evaporated until approx. 3ml. Clean-up was performed on SPE columns filled from the bottom with acidified silica gel and anhydrous Na_2SO_4. Columns were conditioned with 4 ml of hexane/DCM mixture (4:1, v/v), then sample extract was added and eluted using 2 volumes of hexane/DCM (4:1 v:v) (2*4 ml). The volume was evaporated in a nitrogen stream until almost dry, and reconstituted in 200 µl of isooctane. Determination was performed by a gas chromatograph (Agilent 7890B) coupled to a tandem mass spectrometer (Agilent 7000D) (GC-MS/MS) operating in MRM mode. Measurement conditions and parameters are given in Supplementary Table 1.

2.3 Quality QA/QC

The limit of detection (LOD) for trace elements, OCs and PCBs was calculated as the concentrations corresponding to a signal equal to three times the standard deviation of blank solution signal. For PAHs and compounds not detected in blank sample, LODs were calculated based on the standard deviation of the response (s), and the slope of the calibration curve (b) according to the formula: LOD 3.3(s/b). LOD was 0.004–0.92 ng/g dw for all elements, 0.013–0.38 ng/g dw for PAH, and 0.011–1.38 ng/g dw for OC and PCBs. Details for trace element quality control are reported in a previous study (Pacyna et al., 2019a). Mean recovery for internal standards was 133% for benzo[a]anthracene, 83% for DDT, 89% for PCB28 and 92% for PCB180. All studied compounds were blank corrected by a mean procedural blank value. Parameters used in MRM analysis are given in Supplementary Tables 2a,b.

2.4 Data analyses

The U Mann–Whitney test was used to assess differences in elemental contents in reindeer fur collected in Longyearbyen in 2015 (previous study by Pacyna et al., 2018; excluding three outliers with Fe level higher than 5000 µg/g dw) and 2017. Levene's test was performed to assess the equality of variances, while the Shapiro–Wilk test helped determine the normality of the data set. The statistical analyses were carried out using STATISTICA 13 (Statsoft, Tulsa, OK, USA).

3. Results

Mean±SD and median for all elements are listed in Table 1 and in Table 2 for organic pollutants. The highest concentration based on median value was found for the essential elements Fe, Mg, Zn, K, Ca, Cu. The median values of As, Cd, Co, Li, Ni, Se and V were all below 0.5 µg/g dw. A Pb level above 0.5 µg/g dw was found only in Ny-Ålesund samples (median 0.743 µg/g dw). This is the first report on Se in Svalbard reindeer fur, which was at a similar level in all samples (median 0.163–0.187 µg/g dw). Sr, Mn and Ba were all below 2 µg/g, with the exception of Mn from Ny-Ålesund. Mercury was <LOD in all samples.

Comparison of metal contents in reindeer fur was performed for samples collected in the vicinity of Longyearbyen in 2015 (study by Pacyna et al., 2018) and 2017. The majority of studied elements significantly differed between 2015 and 2017 (Fig. 2; Supplementary Table 3). Among eleven analysed elements (As, Ba, Cd, Co, Cu, Fe, Li, Ni, Pb, V, Zn) only Co, Li and Zn did not reveal significant differences in their contents between the compared years (Fig. 2; Supplementary Table 3).

Of the 15 studied pesticides, only DDT and its metabolites were above detection limit, with the highest values found for p,p-DDD, in a single sample from Longyearbyen (57.5 ng/g dw). However, with the exception of this one outlier, p,p-DDD level was much lower in the rest of the samples (median value 1.44 ng/g dw). PAHs and PCBs were mostly below LOD in samples collected in Ny-Ålesund, and the only exceptions wwere fluorene (0.152±0.166 ng/g dw) and PCB28 (1.39±0.40 ng/g dw). Fifty percent of studied PAHs were detected in at least one fur sample from Longyearbyen, but for acenaphthylene, naphthalene, 2-bromo- and fluorene only, more than 65% of the samples were above LOD (Table 2). Of 10 PCB congeners, only PCB28 was found (1.38±1.17 ng/g dw).

Table 1. Results for 18 analysed elements, mean± SD (median) µg/g dw

Table 2. Results for the found persistent organic pollutants, mean±SD, and minimum/maximum value, results in ng/g dw. Mean is calculated only when 65% of the samples were above LOD

Fig 2. Comparison between elemental concentration in fur samples collected from Longyearbyen (n=5) with samples from Longyearbyen collected in 2015 in previous study by Pacyna et al. (2018) (excluding three individuals with Fe level higher than 5000 µg/g dw; n=8), µg/g dw

Table 3. Comparison between element profiles in samples collected from studied populations and previous study from Pacyna et al. (2018), based on median value (µg/g dw), L1 – samples from Longyearbyen (n=5), Ny-A – samples from Ny-Ålesund (n=3), L2 – samples from Longyearbyen collected in 2015 (n=11), H – samples from Hornsund, collected in 2016 (n=16)

4. Discussion

The Svalbard reindeer is highly stationary, and reluctant to migrate beyond its territory, which is mostly established by natural barriers such as glaciers and steep mountains (Hansen et al., 2010). It eats locally growing vegetation including vascular plants, bryophytes and lichens, all determined to accumulate high levels of essential and non-essential elements (e.g. Samecka-Cymerman et al., 2011; Wojtuń et al., 2019). Thus it can be a valuable indicator of local contamination.

Svalbard plants have high levels of Zn, Fe and Mn, resulting in high availability of those elements for herbivores (Hanaka et al., 2019; Aslam et al., 2019). Previously significant differences between the concentrations of several elements were found between foraging sites and seasons, based on reindeer droppings (Pacyna et al., 2019b). The elemental profile in soil and vegetation from Svalbard was shown to be similar, but with differences in elemental concentrations occurring between the sample types (Aslam et al., 2019). Here, seven elements had concentrations above 5 µg/g dw, with more than 40 µg/g dw found for Fe, Mg, Ca, K and Zn (Table 1, 3). The level of Mn in fur was also relatively high, with mean values of 2.85–4.50 µg/g dw. The elemental profile in plants growing in the

244 High Arctic (Fe>Zn≥Mn>Ni≥Pb>Cu≥Cd; Hanaka et al., 2019) is mostly in agreement with our findings
245 on element profiles in reindeer fur (Table 4). This suggests that reindeers can deposit excess amounts
246 of elements accumulated from their diet, and that fur can be used to track their exposure.

247 In a previous study by Pacyna et al. (2018) the concentrations of 18 elements were analysed in
248 Svalbard reindeer fur samples collected from two separate subpopulations (living in proximity to
249 Longyearbyen and the Polish Polar Station in Hornsund). Of those, 12 (As, Ba, Co, Cd, Cu, Fe, Hg, Li,
250 Ni, Pb, V and Zn) were also studied here, thus comparison between them is possible. Cu, Fe and Zn
251 were found in all samples in high concentrations, with Fe having been at a much higher level in a
252 previous study (median value 494–602 µg/g dw, Pacyna et al., 2018). In samples collected in 2015
253 (those reported in Pacyna et al., 2018), Fe levels in samples from around Longyearbyen were almost
254 6.5 times higher than those reported here, and 3 times higher than the present results in samples
255 from Ny-Ålesund. This huge difference may be partially caused by the difference in sample size, as
256 here only 8 samples in total were analysed. In a previous study 3 outliers with extraordinary levels of
257 Fe were found, indicating that some individuals were feeding on more contaminated food. This
258 difference may also be caused by differences in local geological conditions in Svalbard (Halbach et al.,
259 2017), and the ability of moss to sequester high amounts of trace elements (Wojtuń et al., 2019). In
260 Longyearbyen, mining activities and a power plant may be sources of local contamination, creating
261 hotspots of increased element abundance (Marquès et al., 2017, Wojtuń et al., 2019). The runoffs
262 from mines are characterised by high levels of elements such as Fe, Mn, Ni, Cu and Zn (Sřndergaard
263 et al., 2007; Granberg et al., 2017; Aslam et al., 2019). Thus, depending on sample site, high
264 differences in element concentrations can be found in soil and vegetation, e.g. in moss *Sanionia*
265 *uncinata* levels varied from 4,240 to 13,300 µg/g dw for Fe, from 30 to 261 µg/g dw for Zn, and from
266 3.4 to 55 µg/g dw for Cu (Marquès et al., 2017, Wojtuń et al., 2019).

267 Mercury accumulates in the surface soil layer, and is available for vegetation. It was found in
268 concentrations of 0.111 ±0.036 µg/g in Svalbard surface soils, and mostly originated from

atmospheric deposition (Halbach et al., 2017). Hg levels in moss *Sanionia uncinata* and the dwarf-shrub *Salix polaris*, a widely distributed species, were found to be low, at <0.013 to 0.12 µg/g (Samecka-Cymerman et al., 2011; Wojtuń et al., 2013; 2019; Krajcarová et al., 2016). Here, mercury was undetectable, and in the previous study was also at a very low level (median 0.06–0.13 µg/g dw) (Pacyna et al., 2018). As keratinised tissue is usually a target tissue for mercury (Monteiro and Furness, 1995), it seems that it is not currently a concern for Svalbard reindeer.

Several factors affect atmosphere transport of Cd, with the most significant being aerosol particle size, temperature, height of release, wind speed and precipitation conditions (AMAP, 2005; Aslam et al., 2019). Both here and in the previous study (Pacyna et al., 2018) Cd were at low levels, below 0.5 µg/g dw. Cobalt, lithium and zinc levels were comparable between studies (Fig. 2, Pacyna et al., 2018). Lead levels were higher in a previous study, with seven individuals having more than 4 µg/g dw Pb in fur. Here, Pb level was more equally distributed, with a concentration range of 0.12–1.26 µg/g dw.

PAHs are a class of lipophilic semi-volatile organic compounds, originating mainly from incomplete combustion processes such as burning of fossil fuels and biomass (Wang et al., 2009; Balmer et al., 2019). Soil is one of the major sinks for PAH deposition (Wang et al., 2009, Marquès et al., 2017), but atmospheric PAHs may also be deposited in sediments and surface water by wet and dry deposition, and as a secondary emission re-volatised from ground surfaces (Balmer et al., 2019). PAHs can also be accumulated in the snow and be transferred into soil during ice-snow melting process (Kozioł et al. 2017).

Moss tissue can also accumulate PAHs. A study from Ny-Ålesund found the concentration of ΣPAH_{16} in moss to vary from 158 to 244 ng/g dw, while levels in reindeer dung were from 49 to 340 ng/g dw (Wang et al., 2009). Soil was characterised by a higher percentage of median and higher molecular weight PAHs, whereas both moss and reindeer dung had a higher percentage of low molecular weight PAHs, such as naphthalene, acenaphthylene, acenaphthene and fluorene (Wang et al., 2009).

This difference in distribution between those three components was probably caused by the physicochemical properties of individual PAHs, meteorological conditions, different uptakes, as well as differences in accumulation routes, with soil accumulating PAHs mostly through dry/wet deposition, and moss sequestering PAHs from the vapour phase. The composition profiles of PAHs in reindeer dung and moss varied only insignificantly (Wang et al., 2009).

Although they have lipophilic properties, PAHs are subject to biotransformation processes, and can be readily metabolised in vertebrates and seem not to biomagnify through food chains (Hylland et al., 2006; Wan et al., 2007). Here, mostly lighter PAHs with 2 and 3 rings were detected, predominantly in samples collected from the area close to Longyearbyen where beside long-range transport, coal mining activities and human settlement may also be sources of parent PAHs. Coal mining has gone on in Svalbard for almost 100 years, but most of the mines are now closed, with the exception of Mine 7 in Longyearbyen (Aslam et al., 2019; Wojtuń et al., 2019). Previous reports from this region revealed high PAH levels in soils collected close to the power plants (coal- and diesel-fired) (Marquès et al., 2017).

As primary emissions of several POPs were significantly reduced, the concern about secondary re-emissions on the atmospheric levels in the High Arctic have arisen (Hung et al., 2005; Eckhardt et al., 2007; Balmer et al., 2019). Biomass-burning emissions are an important source of long-range transported PCBs and other POPs into the High Arctic (Eckhardt et al., 2007). PCB congeners – both lower and higher chlorinated (PCB 52, 66/95, 101, 118, 138, 153, 180) – can be bioaccumulated in reindeers (Kelly and Gobas, 2001), but some, such as PCB 52, can also be eliminated efficiently from the reindeer body (Kelly and Gobas, 2001).

PCB congeners were found in environmental samples from Ny-Ålesund and in the vicinity of Longyearbyen. The mean concentration of ΣPCBs was 0.57–10.8 ng/g dw in soils, 0.30–56.3 ng/g dw in vegetation (Zhang et al., 2014; Zhu et al., 2015; Aslam et al., 2019) and 0.56–39.6 ng/g dw in

318 reindeer dung (Zhang et al., 2014; Zhu et al., 2015). The PCB concentrations in vegetation from the
319 Canadian high Arctic were lower – ΣPCB_{70} varied from 0.19 to 4.82 ng/g dw (Cabrerizo et al., 2018).

320 Here, levels of most PCB congeners were below quantification level, apart from PCB28. PCB28
321 presence in Svalbard is affected by emission from Western Russia and partially from Scandinavia and
322 Eastern Europe (Ubl et al., 2012). Its concentration in the Arctic air is generally higher during
323 summer, and correlated with temperature, which could suggest re-volatilisation of PCB28 from the
324 surface deposits into the air when temperatures are high (Wania et al., 1998; Eckhardt et al., 2007).
325 In the future there is a high possibility of increasing concentration of lighter PCB congeners in the
326 Arctic components due to re-emission from the ocean and ice, as well as the microbial degradation
327 of heavier PCBs (Hung et al., 2016; Fagervold et al., 2007; Aslam et al., 2019).

328 Several organochlorine pesticides have been detected in High Arctic biota (Letcher et al. 2010), but to
329 the best of our knowledge, no OCPs have been analysed before in the fur of Svalbard reindeer.
330 Organochlorine compounds were previously examined in the hairs of polar bears living in East
331 Greenland (Jaspers et al., 2010). As the diet of those carnivores is composed in most part of fat-rich
332 seals, the determined OCP levels in their internal tissue can be high (Gebbink et al., 2008; Letcher et
333 al., 2010), but a much smaller number of compounds was found in clean hair samples (Jaspers et al.,
334 2010). As a possible reason for that, the authors suggest that the sample amount was too low for
335 reliable determination of analysed pesticides above the limit of quantification (13–140 mg),
336 inefficient uptake into the hair of polar bears and the unique capacity of polar bears to metabolise
337 p,p´-DDT and to a lesser extent p,p'-DDE (Letcher et al., 1998; Polischuk et al., 2002; Jaspers et al.,
338 2010). Here, out of 15 studied OCs, only DDT and its metabolites were detected, mostly at low levels.
339 Some studies suggest that reindeers also have the ability to metabolise or eliminate p,p-DDT, but
340 cannot effectively metabolise the persistent metabolite p,p-DDE (Kelly and Gobas, 2001). More
341 research is needed to understand mechanisms enabling this herbivore to eliminate OCs from its
342 body.

5. Conclusion

The Svalbard reindeer is a long-lived herbivore that is part of a relatively simple food chain and can be used for monitoring changes in the terrestrial trophic network. Beside mercury, all studied elements could be quantified in the hair of Svalbard reindeer. In future studies, samples collected from the area close to Longyearbyen should be analysed in higher numbers, as the elemental concentration can be affected by locally created hotspots with elevated levels of contaminants. Here, essential elements like Fe, Mg, Zn, K, Ca, Cu were found in the highest concentrations. Further studies should be performed to examine whether high levels of those elements can be related to adverse health effects. Levels of most elements of ecotoxicological interest, such as As, Cd, Co, Hg and Ni were low, and currently not a threat to studied individuals. Only a few POPs could be determined, including PCB28, p,p-DDD and some of the parent PAHs. Reindeers living close to Longyearbyen had higher levels of POPs than those living close to Ny-Ålesund, where mostly research activities are performed. Thus it seems that local activities close to Svalbard's largest settlement may affect the contaminant levels in the local ecosystem. Further research with a larger sample size is recommended to confirm our findings and to draw more definitive conclusions on using Svalbard reindeer fur for biomonitoring of POPs.

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Table

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Tab.1 Results for 18 analysed elements, mean± SD (median) µg/g dw

Element	***Longyearbyen (n=5)***	***Ny-Alesund (n=3)***
As	0.102±0.067 **(0.072)**	0.196±0.167 **(0.118)**
Ba	1.42±0.68 **(1.01)**	1.46±0.38 **(1.40)**
Ca	42.00±10.03 **(42.0)**	109.4±128.4 **(44.8)**
Cd	0.026±0.008 **(0.023)**	0.309±0.469 **(0.044)**
Co	0.424±0.558 **(0.178)**	0.768±0.823 **(0.402)**
Cu	5.23±0.52 **(5.37)**	6.57±0.47 **(6.43)**
Fe	180.3±238.2 **(92.9)**	379.9±406.4 **(190.3)**
Hg	<LOD	<LOD
K	55.0±22.0 **(59.9)**	31.1±32.2 **(18.2)**
Li	0.191±0.250 **(0.089)**	0.251±0.181 **(0.214)**
Mg	86.0±90.8 **(45.6)**	1275.5±2088.4 **(85.3)**
Mn	2.85±3.00 **(1.48)**	4.50±4.30 **(3.05)**
Ni	0.134±0.173 **(0.047)**	0.519±0.496 **(0.244)**
Pb	0.471±0.327 **(0.354)**	0.796±0.444 **(0.743)**
Se	0.167±0.022 **(0.163)**	0.182±0.029 **(0.187)**
Sr	2.04±1.26 **(1.79)**	8.24±13.3 **(0.642)**
V	0.198±0.302 **(0.098)**	0.440±0.581 **(0.147)**
Zn	52.7±7.25 **(49.6)**	73.1±22.2 **(73.7)**

Table

Table 2. Results for the found persistent organic pollutants, mean±SD, and minimum/maximum value, results in ng/g dw. Mean is calculated only when 65% of the samples were above LOD

Compound	*Longyearbyen (n=5)*	*min-max*	*Ny-Alesund (n=3)*	*min-max*
Naphthalene	<LOD	<LOD-0.068	<LOD	<LOD
Acenaphthylene	0.034±0.022	<LOD-0.058	<LOD	<LOD-0.040
Naphthalene, 2-bromo-	0.035±0.019	<LOD-0.053	<LOD	<LOD
Fluorene	0.385±0.236	0.020-0.595	0.152±0.166	<LOD-0.334
Phenanthrene	<LOD	<LOD-0.034	<LOD	<LOD
Anthracene	<LOD	<LOD-0.093	<LOD	<LOD
Fluoranthene	<LOD	<LOD-0.257	<LOD	<LOD
Indeno[1,2,3-cd]pyrene	<LOD	<LOD-0.025	<LOD	<LOD
Benzo[ghi]perylene	<LOD	<LOD-0.022	<LOD	<LOD
PCB28	1.38±1.17	<LOD-2.65	1.39±0.40	0.84-1.79
p,p-DDE	<LOD	<LOD-1.54	<LOD	<LOD-1.29
p,p-DDT	<LOD	<LOD-4.24	<LOD	<LOD
o,p-DDT	<LOD	<LOD-4.21	<LOD	<LOD-0.51
p,p-DDD	2.66±2.85*	0.84-57.5	<LOD	<LOD-3.61

***one outlier removed from the mean, but is shown as the maximum value**

Table

Table 3. Comparison between element profiles in samples collected from studied populations and previous study from Pacyna et al. (2018), based on median value (µg/g dw), L1 – samples from Longyearbyen (n=5), Ny-A – samples from Ny-Ålesund (n=3), L2 – samples from Longyearbyen collected in 2015 (n=11), H – samples from Hornsund, collected in 2016 (n=16)

	>5 µg/g	***5.0 - 0.5 µg/g***	***<0.5 µg/g***
L1	Cu, Ca, K, Mg, Fe, Zn	Ba, Mn, Sr	As, Cd, Co, Hg, Li, Ni, Pb, Se, V
Ny-A	Cu, Ca, K, Mg, Fe, Zn	Ba, Mn, Pb, Sr	As, Cd, Co, Hg, Li, Ni, Se, V
L2	Ba, Cu, Fe, Zn	As, Pb, Ni, V, Cr, Rb	Co, Li, Hg, Cd, Be, Ga, Cs, La
H	Ba, Cu, Fe, Zn	As, Pb, Ni, V, Li, Cr, Ga, Rb, La	Co, Hg, Cd, Be, Cs

Figure

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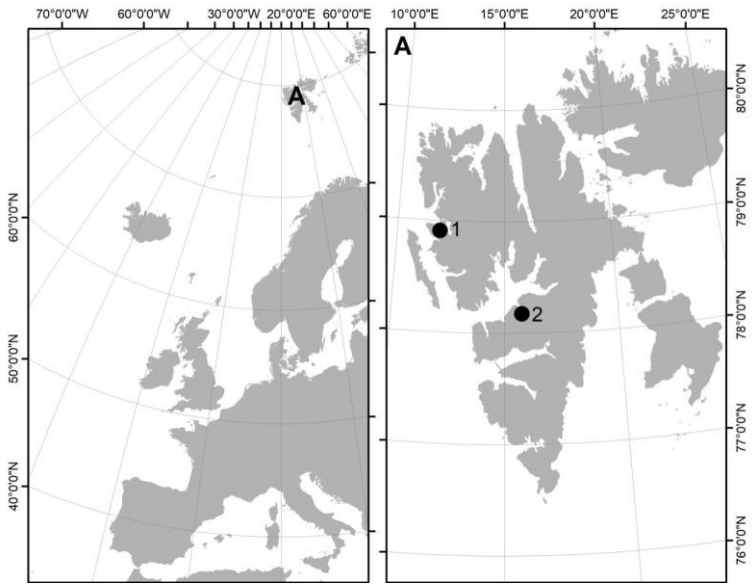
Fig. 1 Study site showing area where Svalbard reindeer fur was collected : A – Svalbard Archipelago, 1 – Ny-Ålesund, 2 – Longyearbyen, map source: Norwegian Polar Institute, www.npolar.no



Figure

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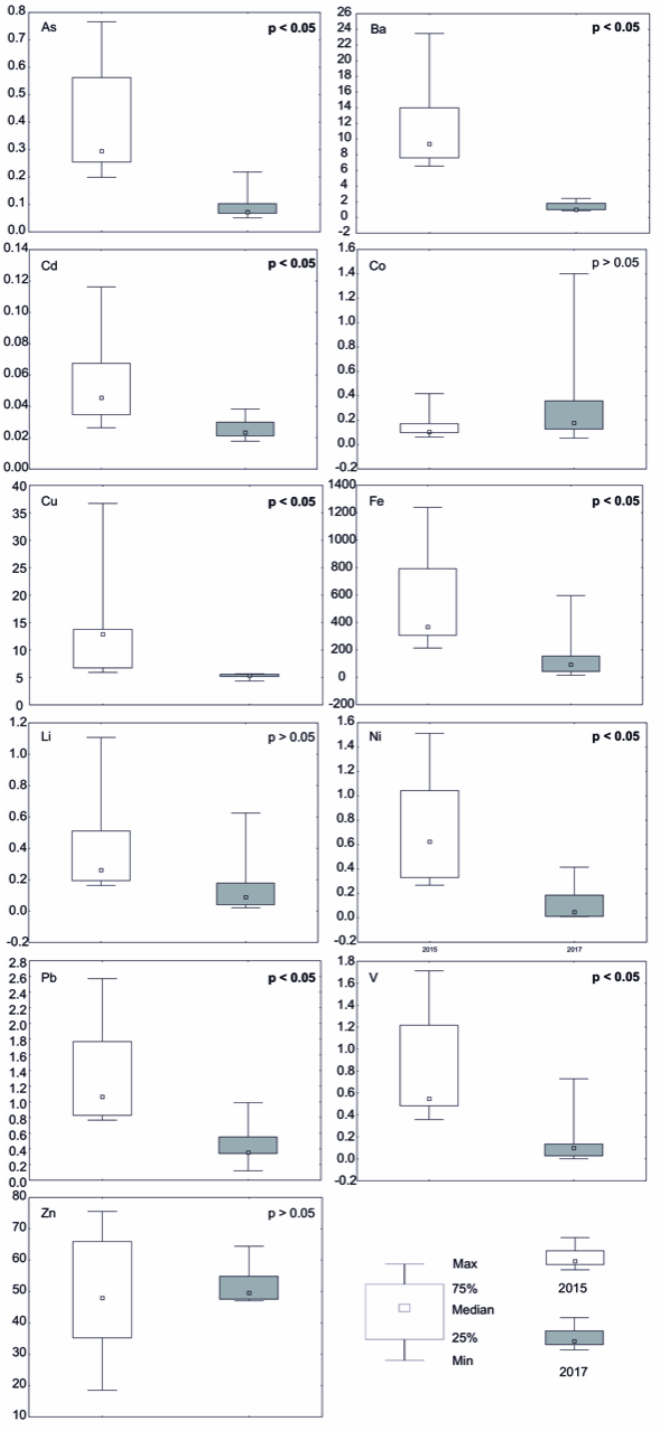
Fig 2. Comparison between elemental concentration in fur samples collected from Longyearbyen (n=5) with samples from Longyearbyen collected in 2015 in previous study by Pacyna et al. (2018) (excluding three individuals with Fe level higher than 5000 µg/g dw; n=8), µg/g dw



Supplementary Material

Click here to download Supplementary Material: Supplementary Table3.docx

12.4. Weryfikacja hipotezy 4

Hipoteza 4: Pióra dorosłych i młodych oceanników (Oceannik czarnobrzuchy *Fregetta tropica*, oceannik żółtopłetwy *Oceanites oceanicus*) będzie cechował inny profil pierwiastkowy, ze względu na różne obszary żerowania podczas wzrostu piór u dorosłych i piskląt. Oba gatunki odżywiają się podobnym pokarmem, jednak w przypadku oceannika czarnobrzuchego procentowy udział ryb w diecie jest wyższy, co może skutkować wyższą zawartością rtęci w organizmie

Weryfikację **czwartej hipotezy** wykonano w oparciu o badania próbek piór osobników dorosłych oraz piskląt dwóch gatunków oceanników. Wyniki badań zostały opublikowane w czasopiśmie *Science of the Total Environment (*załącznik 5) **[A. D. Pacyna, D. Jakubas, A.N.M.A. Ausems, M. Frankowski, Ż. Polkowska, K. Wojczulanis-Jakubas, Storm petrels as indicators of pelagic seabird exposure to chemical elements in the Antarctic marine ecosystem, 2019, Science of The Total Environment, DOI: 10.1016/j.scitotenv.2019.07.137]**

Badania obejmowały analizę zawartości 18 pierwiastków w piórach piskląt oraz osobników dorosłych morskich 2 gatunki oceanników *Oceanites oceanicus* oraz *Fregetta tropica* reprezentujące Antarktykę. W efekcie wykryto różnice międzygatunkowe między poziomem pierwiastków w piórach osobników dorosłych. Wystąpiły również znaczące różnice między pisklętami, a osobnikami dorosłymi.

W przypadku oceannika czarnobrzuchego, znajdującego się wyżej w łańcuchu pokarmowym, odnotowano znacząco wyższy poziom rtęci, miedzi oraz selenu. Puch piskląt zawierający informację o związkach przekazanych przez samicę podczas wzrostu w jaju oraz pierwszych tygodni życia wskazał relatywnie wysoki poziom wielu pierwiastków włączając w to rtęć. Znaczące różnice między poziomami oznaczonymi w próbkach w piór piskląt oraz puchu wskazują na użyteczność wykorzystania piór puchowych w biomonitoringu oraz różnicach w ekspozycji w ciągu pierwszych tygodni życia. Analiza klastrowa badanych pierwiastków wskazała na powiązania sugerujące głównie naturalne źródła pochodzenia pierwiastków. Dalsza szczegółowa analiza wyników badań z podziałem na płeć osobników dorosłych pozwoli na lepsze zrozumienie czynników wpływających na bioakumulację metali przez ten mało zbadany gatunek antarktycznego ptaka.

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Storm petrels as indicators of pelagic seabird exposure to chemical elements in the Antarctic marine ecosystem

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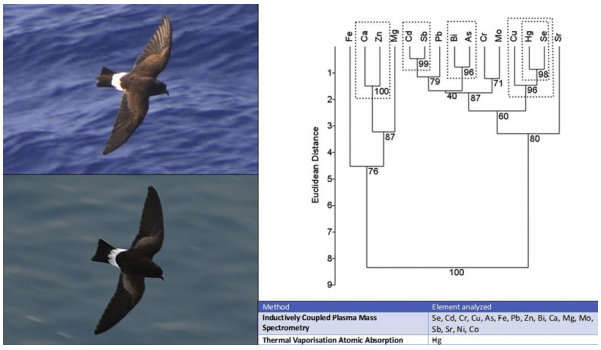
HIGHLIGHTS

- We examined 17 element levels in adults and chicks feathers of two storm petrel species.
- We found interspecies differences, between black-bellied and Wilson's storm petrel.
- Hg accumulation in black-bellied storm petrel could be classified as intermediate.
- The contaminant profile was different for adults and young.
- There was also evidence on maternal transfer of Hg in both species.

GRAPHICAL ABSTRACT



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ABSTRACT

Data on trace element bioavailability in the south-polar marine ecosystem is still scarce, compared to that relating to temperate zones. Seabirds can be used as indicators of ecosystem health and sentinels of environmental pollution, constituting a link between marine and terrestrial environments. Here, we analysed the concentration of 17 elements (with special emphasis on mercury, Hg) in feathers of adults and chicks of two pelagic seabirds – the Wilson's storm petrel *Oceanites oceanicus* and the black-bellied storm petrel *Fregetta tropica* – breeding sympatrically in the maritime Antarctic. Since adult feathers are formed during the non-breeding period away from the breeding grounds, but down and body feathers of chicks grow at the breeding sites, we were able to evaluate the birds' exposure to contaminants at various stages of their annual life cycle and in various marine zones. We found that of the two studied species, adult black-bellied storm petrels had significantly higher mercury, selenium and copper levels (5.47 ± 1.61; 5.19 ± 1.18; 8.20 ± 0.56 μg g^{-1} dw, respectively) than Wilson's storm petrels (2.38 ± 1.47; 1.81 ± 0.98; 2.52 ± 2.35 μg g^{-1} dw, respectively). We found that Wilson's storm petrel chicks had a significantly different contaminant profile than adults. Arsenic, bismuth and antimony were detected exclusively in the chick feathers, and the Se:Hg molar ratio was higher in chicks than in adults. Our study also suggests considerable maternal transfer of Hg (to down feathers) in both species. As global contaminant emissions are expected to increase, birds inhabiting remote areas with sparse anthropogenic pollution can indicate the temporal trends in global contamination.

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1. Introduction

Organisms living in Antarctica are exposed to a number of environmental factors that may affect their health and survival. Of those, the most influential are harsh climate conditions, competition for food, and predation, but pollutants may also play an important role (Santos et al., 2006; Metcheva et al., 2006). Contaminants in the polar zone may originate from natural processes [i.e. volcanic activity, the input of sea-spray, mechanical and chemical rock weathering (Malandrino et al., 2009)] and biota [e.g. mammals or bird colonies can be a source of nutrients/organic matter (N, F) and several elements such as Cd, Hg, As, Se and Zn to the terrestrial and coastal ecosystem (Cipro et al., 2018)]. Anthropogenic sources of Antarctic ecosystem contamination are often located outside the region, e.g. lead from industrial emissions is transported from South America (Sañudo-Wilhelmy et al., 2002; Gaiero et al., 2003; Bargagli, 2008). However, local sources, too, may contribute due to increasing research and tourism activities resulting in fuel combustion, accidental oil spills, waste disposal sites, sewage and paint residues (Bargagli, 2008; Jerez et al., 2011; Mão de Ferro et al., 2013).

Pelagic seabirds living in the southern polar zone can be used in ecotoxicological studies to assess trace element pollution and marine ecosystem health (Carravieri et al., 2014). They constitute a valuable link between terrestrial and marine zones of the Antarctic (Santos et al., 2006). As they often cover vast distances in search of suitable foraging areas, they are exposed to pollutants in various geographical locations. They may also carry these contaminants between wintering and/or stop-over staging and breeding, due to migratory connectivity (Webster et al., 2002).

Seabirds' feathers are often used to evaluate their exposure to contaminants (e.g. Jerez et al., 2011; Bustamante et al., 2016; Philpot et al., 2019), providing a record of contaminant uptake at the time of feather growth and development (Bearhop et al., 2002; Jaspers et al., 2004). High metals affinity to sulfhydryl groups of the feather structural proteins are making them a suitable biomonitoring tool (Thompson et al., 1998). Elemental deposition in feather tissue is species-specific and depends on multiple factors, including diet, age, detoxification abilities and moulting pattern (Burger and Gochfeld, 1997; Evers et al., 2008; Cipro et al., 2014; Pacyna et al., 2017). Knowledge of avian moulting sequences is essential to the reconstruction of the contamination period (Bustamante et al., 2016; Cherel et al., 2018). Adult feathers may provide a wider perspective on metal exposure over the annual cycle, but as seabirds may cover a vast area during the moulting period it is challenging to properly interpret their exposure over time. Also seasonal shifts in element concentrations can occur (Øverjordet et al., 2015). By contrast, chick feathers may provide information over a shorter period of exposure for a more defined area (Evers et al., 2005). Chick down is formed in the egg from maternal nutrients and as such represents female contamination during the pre-laying period (Ackerman et al., 2016). Thus, analysis of feathers collected at various life stages allows the elemental concentrations of various areas to be reconstructed, indicating temporal and spatial trends in pollution in the ecosystems being occupied at the time (Becker, 2003).

Despite the growing number of studies on Antarctic and sub-Antarctic food web contamination, still little is known about elemental concentrations in seabirds feeding at low trophic levels (i.e. preying on zooplankton and krill), likely due to their relatively lower exposure to contaminants compared to top predators. For instance, petrels (i.e. species from three families of Procellariiformes: Procellariidae, Oceanitidae, and Hydrobatidae) are still one of the most poorly examined seabird groups, mostly due to their small body size, their nesting predominantly on isolated and inaccessible islands, and their high mobility at sea (Rodríguez et al., 2019). However, even this group is exposed to a multitude of contaminants (e.g., Anderson et al. 2010; Bocher et al., 2003; Cipro et al., 2014; Fromant et al., 2016; Philpot et al., 2019).

In this study we determined levels of elements in feathers of two storm-petrel species breeding in the maritime Antarctica, the Wilson's storm petrel (*Oceanites oceanicus*, hereafter WSP) and the black-bellied storm petrel (*Fregetta tropica*, hereafter BBSP). We focused both on elements of wider ecotoxicological interest (i.e. arsenic [As], cadmium [Cd], chromium [Cr], copper [Cu], lead [Pb], mercury [Hg], selenium [Se], and zinc [Zn]) and on those rarely studied in avian tissues (i.e. antimony [Sb], bismuth [Bi], calcium [Ca], cobalt [Co], iron [Fe], nickel [Ni], magnesium [Mg], molybdenum [Mo], and strontium [Sr]). Gathering data about the concentration of various elements in tissues of living animals is crucial in order to properly assess ecosystem health and to comprehend pollutants' abilities for potential bioaccumulation and biomagnification. By studying rarely analysed elements, the results should provide background data for research detecting future inputs of elements in remote polar regions (Santos et al., 2006).

We aimed to:

1) present reference values for the concentrations of 17 elements that can be used in the future for monitoring contamination level in Antarctic marine predators;
2) compare elemental concentrations between feathers collected from different age groups representing various life-history stages (i.e. chick feathers representing the chick-growth period, chick down representing maternal input, and adult feathers representing part of the non-breeding period, when the feathers grew); by considering the spatial and temporal differences in feather growth between these groups, we expected to detect differences in elemental concentrations between the various types of feathers;
3) compare elemental concentrations in feathers grown during the non-breeding season between adults of the two species, with special emphasis on Hg and Se:Hg molar ratio (linked to protective action against Hg bioaccumulation and toxicity by creation of Hg—Se compounds [Nigro and Leonzio 1996; Khan and Wang, 2009]). Considering inter-specific differences in trophic level (see Materials and Methods) and in the location of non-breeding areas (Fig. 1), we expected to detect differences in elemental concentrations between the species;
4) identify patterns in concentrations of elements, and thus identify possible common sources of contamination.

2. Materials and methods

2.1. Studied species

The two study species – the Wilson's storm petrel and the black-bellied storm petrel – are small pelagic seabirds, with circumpolar breeding distributions including sub-Antarctic islands and the maritime Antarctic. Both species breed sympatrically in the study area (see below) during the austral summer (from December to March), with similar breeding biology: single-egg clutch, incubation lasting 38–44 days, and chick rearing up to 71 days. Although both species are among the smallest endotherms living in the Antarctic, they play an important role as predators preying on Antarctic krill, myctophid fish and amphipods (Hahn, 1998; Quillfeldt, 2002; Quillfeldt et al., 2005; Wasilewski, 1986). Preying on fish and crustaceans in equal proportions (Hahn, 1998), BBSP feeds at a higher trophic level than WSP, which eats mainly crustaceans (80–90% of meals) (Quillfeldt, 2002; Quillfeldt et al., 2017). After breeding, both species migrate northwards, where they spend the non-breeding period at open sea and complete their moult (Beck and Brown, 1972). They moult in the Atlantic Ocean in a wide range of habitats: WSP from sub-Antarctic to subtropical waters and BBSP primarily either in sub-Antarctic–subtropical waters or at the continental shelf (Phillips et al., 2009) (Fig. 1).

2.2. Sample collection

We studied the two storm-petrel species in the breeding colonies located in the vicinity of Henryk Arctowski Station in Admiralty Bay, King

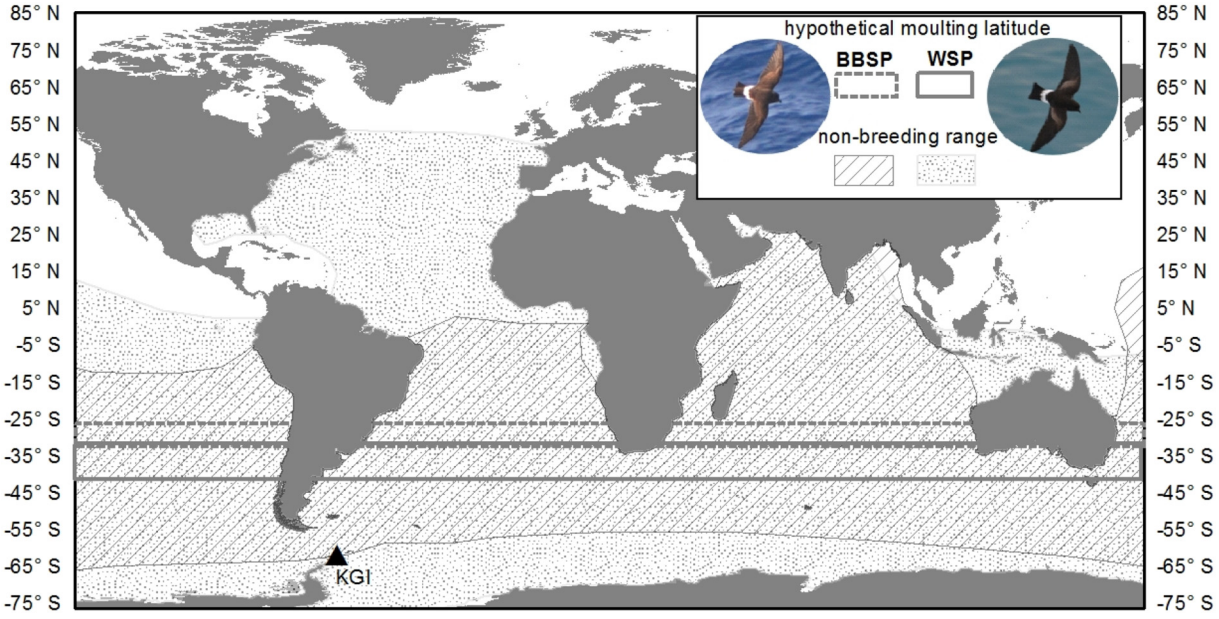

Fig. 1. Range of the studied species and possible areas of elemental input: triangle – study area, King George Island (KGI), grey rectangles – moulting latitudes for adult storm-petrels (dotted for black-bellied storm-petrel (BBSP) and solid for Wilson's storm-petrel (WSP); according to isotopic data from Quillfeldt et al., 2005 and Phillips et al., 2009 calculated based on equation proposed by Quillfeldt et al., 2005). Storm-petrels non-breeding range map source: BirdLife International and Handbook of the Birds of the World, 2018. Photos by DJ.

Gorge Island, South Shetlands, Antarctica (62°02′S 58°21′W, Fig. 1) in 2017. King George Island is the largest island in the South Shetlands Archipelago, 90% ice-covered, with rocks mainly formed by andesitic and basaltic magma (Santos et al., 2006). We captured adult birds in the breeding colony (in their nests, using mist-nets spread in the colony area) during the incubation period and collected 4–5 body feathers from the back. Back body feathers represent mostly trace element input from food and water intake during part of the non-breeding period when the feathers grew, which takes place outside the colony in the Atlantic Ocean (Fig. 1). To sample chicks we caught them by hand in the nest and collected down (at the time they were starting to lose it, i.e. when their body feathers were well grown, thus minimising the risk of affecting thermoregulation), and 4–5 body feathers from the back (when the nestlings were about to fledge). Down feathers represent trace elements passed on by the female to the embryo, reflecting their input during the pre-laying period, probably from areas around the breeding colony (and likely predominantly reflecting the food intake). Chick body feathers represent the nesting period and input from marine environments (as food and water intake). We stored all the samples in individual plastic zip-lock bags until chemical analysis.

2.3. Analytical procedure

Prior to chemical analysis, we cleaned all feather samples to remove external contamination, firstly with acetone (Sigma-Aldrich, USA) and then two times with deionised water (Mili-Q Gradient A10, Milipore, France) (procedure of Jaspers et al., 2004, modified). We air-dried the washed feathers for 24 h. If the total mass of the sample permitted, we used an aliquot of the collected material for the analysis of all trace elements, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

We determined concentrations of 17 trace elements by ICP-MS analytical technique in the following feather samples types: adult WSP (n = 12), chick body WSP (n = 4), chick down WSP (n = 4) and adult BBSP (n = 4). Mean feather mass was: for adults 10 mg (4–18 mg), for chick body feathers 6 mg (5–8 mg) and for chick down feathers 16 mg (6–39 mg). Due to insufficient amount of chick body BBSP and down BBSP feathers, we measured only Hg content in these samples using the cold vapour technique. In total, we determined Hg concentration in the following types of feather samples: adult WSP (n = 35), chick body WSP (n = 10), down WSP (n = 16), adult BBSP (n = 11), chick body BBSP (n = 6) and down BBSP (n = 6).

2.3.1. Trace element concentration

We homogenised dry feathers by cutting them up, then weighed them to the nearest 0.01 mg, and placed them in a clean Teflon vessel with 7 ml 65% HNO_3 (Merck, Suprapur). We carried out digestion using a high-pressure microwave emitter (Microwave Digestion System, Anton Paar). We increased the temperature from the ambient value to 90 °C (approximately 6–8 °C/min). We maintained these conditions for 25 min, after which we gradually lowered the temperature. Subsequently, we diluted the fully mineralised samples with deionised water to 25 ml in clean plastic flasks. To ensure quality control and check background contamination, we ran blank samples with every batch. To ensure accuracy of obtained results we ran certified reference material (CRM, Human hair ERM-DB001) in triplicate. We analysed samples using an ICP-MS 2030 (Shimadzu, Japan) (for measurement conditions and parameters see Table 1, Supplementary material).

2.3.2. Mercury concentration (cold vapour technique)

We weighed the dry samples to the nearest 0.01 mg in a ceramic boat, then we covered them with activated Al_2O_3 and analysed them using the thermal vaporisation atomic absorption method (MA-3000 Nippon Instruments Corporation). We analysed at least two feather aliquots (1–10 mg dry weight) for each individual. The details of the program used and the equipment specification are described in Pacyna et al. (2018). We determined total Hg concentration in duplicates or triplicates when possible, taking sub-samples of the homogenised feathers. We calculated the coefficient of variation (CV) based on these. If the CV was above 15%, we excluded samples from the analyses, deeming the estimation of Hg concentration unreliable. Thus, for statistical analysis we used: adult WSP (n = 25), chick body WSP (n = 5), down WSP (n = 16), adult BBSP (n = 8), chick body BBSP (n = 5) and down BBSP (n = 6). Mean CV was 8.64 ± 4.65% for adults, 6.32 ± 5.40% and 2.92 ± 2.64% for chick body feathers and down, respectively. To check background contamination, we performed a quality control including blank samples every 5–6 subsamples. We analysed CRM every 10th subsample run.

2.4. Quality control

We found that results for CRM analysis were in agreement with the certified values (mass used for analysis: for trace elements 200 mg, for mercury 14–28 mg). Recoveries were high: As 98%, Cd 106%, Cu 104%, Hg 92%, Pb 96%, Se 92%, Zn 97%. To check accuracy and recoveries of other elements absent in this CRM we applied a treatment used before by Pacyna et al. (2019). We blank-corrected samples analysed on the ICP-MS (by a mean value of all blank samples). For Hg analysis, we found that background contamination was negligible and we did not perform blank correction. The limit of detection (LOD) and quantification (LOQ) values were calculated as the concentrations corresponding to signals equal to three and ten times the standard deviation of blank solution signal, respectively. For Hg LOD/LOQ were calculated based on the standard deviation of the response (s), and the slope of the calibration curve (b) according to the following formulas: LOD 3.3(s/b), LOQ 10(s/b). Method LOD/LOQ were in range of 0.004–0.92 and 0.013–3.07 ng/g respectively. We reported our results as $\mu g \cdot g^{-1}$ dry weight (dw). For statistical analysis results below the LOD we assigned half of the LOD value.

For calibration of the ICP-MS we used the ICP IV multi-element standard (Merck, USA) and As, Sb, Se, Mo and V (Sigma-Aldrich, USA), Hg (Merck, USA) as single standards. As internal standards we used: Sc, Rh, Tb and Ge in supra pure 1% HNO_3 (Merck, USA). For sample pretreatment and sample dilution we used deionised water obtained from the Milli-Q Direct 8 Water Purification System.

2.5. Statistical analyses

To investigate variation in the qualitative and quantitative composition of trace elements in feathers, we firstly performed multivariate analyses for all elements to find general patterns and then we did univariate analyses for particular elements.

To compare the qualitative and quantitative compositions of all trace elements in feathers among the life-history stages and species, we applied the following multivariate methods:

1) a multivariate (for all trace elements together) PERMANOVA (non-parametric MANOVA based on the Bray–Curtis measure; Anderson, 2001) with concentrations of all elements as a response variable and birds' age (adult WSP, adult BBSP, chick down WSP, chick body WSP) as the explanatory variable;
2) A similarity percentage breakdown procedure (SIMPER) to assess the average percentage contribution of individual elements to the dissimilarity in all elements concentrations between age groups in a Bray–Curtis dissimilarity matrix (Clarke, 1993).

To compare the qualitative and quantitative compositions of particular trace elements in feathers between the life-history stages and species, we used an unimodal Kruskal–Wallis test with a *U* Mann–Whitney test as a *post-hoc* test for all group pairs, excluding adult BBSP vs chick down WSP and adult BBSP vs chick body WSP. In a separate analysis, we compared Hg concentration among all categories for a larger sample size.

Then, to find the groups of elements with high degrees of association in feather elemental concentrations, we performed a Hierarchical Cluster Analysis (HCA). A high degree of association between element concentrations, expressed by clustering in one group, can be used to identify common sources of elements (e.g. Hashmi et al., 2013), but it does not require the formulation of any a priori hypothesis considering the nature of the relationships (Bianchi et al., 2008). We performed HCA with Euclidean distance as a distance measure, and the paired group method as the linkage method. For each cluster obtained, we calculated Bootstrap Probability (BP) using multiscale bootstrap resampling. BP of a cluster may take a value between 0 and 100, indicating how well the data supported the cluster, with a higher value indicating a better fit (Hammer et al., 2001). We only considered clusters with BP ≥ 95. To determine how well the generated clusters represented dissimilarities between objects, we calculated the cophenetic correlation coefficient. Values close to 0 indicate poor clustering, and values close to 1 show strong clustering.

We performed PERMANOVA, SIMPER and HCA analyses on log(x + 1) transformed data. We classified the strength of the correlation according to Hinkle et al. (2003): strong correlation with r = |0.90–1.00|, high correlation with r = |0.70–0.90|, moderate correlation with r = |0.50–0.70|, and low correlation with r = |0.30–0.50|.

We performed separate SIMPER and PERMANOVA analyses for three groups of elements:

1. all elements
2. essential elements, i.e. As, Ca, Cr, Cu, Fe, Mg, Mo, Se and Zn; and
3. non-essential elements, i.e. Bi, Cd, Hg, Pb, Sb, and Sr.

We calculated Se:Hg molar ratios based on the mean Hg values and the mean Se values from our study. The Se:Hg molar ratio was obtained using the molecular weight (200.59 and 78.9 for Hg and Se, respectively) (Burger et al., 2013). We compared Se:Hg molar ratios between the studied age categories in both species using a chi-squared test.

We performed PERMANOVA, SIMPER, and HCA analyses in PAST software (Hammer et al., 2001) and the Kruskal–Wallis and Mann U Whitney test in R software (R Core Team, 2018), using the ggpubr package (Kassambara Alboukadel, 2018).

Table 1
Elemental concentrations of the studied elements in feathers of storm-petrels, mean ± SD (min–max) $\mu g\ g^{-1}$ dw, N = the number of individuals sampled, LOD = detection limit, LOQ = quantification limit, Se:Hg – Se:Hg molar ratio.

Element	Adult WSP (N = 12)	Adult BBSP (N = 4)	Chick down WSP (N = 4)	Chick body WSP (N = 4)
As	75% < LOD	100% < LOQ	1.76 ± 1.10 (0.45–3.16)	6.11 ± 1.43 (4.77–8.38)
Bi	92% < LOD	100% < LOD	1.57 ± 1.78 (<LOD–3.92)	14.16 ± 5.92 (6.50–20.50)
Ca	96.0 ± 21.0 (77.7–156.0)	80.0 ± 11.2 (72.8–99.3)	242.8 ± 60.4 (165.0–326.0)	136.50 ± 11.79 (124.9–156.0)
Cd	<LOD (<LOD–0.45)	<LOQ	0.45 ± 0.22 (<LOQ–0.68)	0.51 ± 0.24 (<LOD–0.70)
Cr	0.67 ± 0.45 (0.11–4.36)*	0.71 ± 0.45 (0.09–1.22)	1.54 ± 0.48 (0.88–2.08)	3.46 ± 0.78 (2.73–4.78)
Cu	2.52 ± 2.35* (<LOD–13.9)	8.12 ± 0.56 (7.52–9.06)	1.52 ± 0.60 (0.67–2.26)	6.68 ± 3.15 (2.49–10.96)
Fe	20.40 ± 18.00 (<LOD–263.0)*	10.73 ± 5.04 (<LOD–16.16)	74.30 ± 19.30 (50.80–102.4)	131.7 ± 81.8 (63.1–270.0)
Mg	478 ± 130 (315–773)	429 ± 101 (306–529)	538 ± 354 (316–1152)	378 ± 91 (285–513)
Mo	2.41 ± 4.09 (<LOD–14.6)	0.56 ± 0.27 (0.16–0.84)	1.92 ± 1.77 (0.36–4.90)	7.13 ± 7.42 (1.52–19.79)
Pb	0.33 ± 0.37* (<LOD–5.06)	0.36 ± 0.24 (0.11–0.74)	1.77 ± 0.91 (0.75–2.76)	1.43 ± 1.32 (0.36–3.67)
Sb	100% < LOD	75% < LOD	0.22 ± 0.15 (<LOD–0.43)	1.17 ± 0.53 (0.58–1.92)
Se	1.81 ± 0.98 (<LOD–4.65)	5.19 ± 1.18 (3.74–6.62)	4.06 ± 0.50 (3.29–4.69)	3.63 ± 1.01 (2.39–5.13)
Sr	5.77 ± 3.62 (2.47–13.53)	2.79 ± 1.21 (1.35–4.58)	23.19 ± 8.75 (13.4–37.1)	9.57 ± 1.020 (8.63–11.27)
Zn	109.20 ± 18.50 (70.3–141)	99.95 ± 13.01 (85.5–120)	48.30 ± 7.08 (40.30–59.60)	93.00 ± 11.30 (79.4–110.8)
Se:Hg	1.92	2.41	6.00	13.77

* For Cr, Cu, Fe and Pb one outlier was excluded from the mean calculation, but it is shown as the maximal value.

Table 2
Intergroup differences (one-way PERMANOVA, Bonferroni-corrected *p* values) of elemental concentration, log(x + 1) transformed, in the feathers of the four studied groups of storm-petrels: body feathers of adult Wilson's (WSP_Ad) and back-bellied (BBSP_Ad) storm-petrels, down from Wilson's storm-petrel chicks (WSP_down) and body feathers from Wilson's storm petrel fledglings (WSP_CHF).

PERMANOVA, F = 11.48, $p = 0.0001$				
All elements	WSP_CHF	WSP_down	BBSP_Ad	WSP_Ad
WSP_CHF	–	0.182	0.176	0.007
WSP_down		–	0.157	0.004
BBSP_Ad			–	0.037
WSP_Ad				–
PERMANOVA, F = 9.26, p = 0.0001				
Essential	WSP_CHF	WSP_down	BBSP_Ad	WSP_Ad
WSP_CHF	–	0.160	0.181	0.003
WSP_down		–	0.166	0.005
BBSP_Ad			–	0.136
WSP_Ad				–
PERMANOVA, F = 12.93, p = 0.0001				
Non-essential	WSP_CHF	WSP_down	BBSP_Ad	WSP_Ad
WSP_CHF	–	0.160	0.181	0.003
WSP_down		–	0.166	0.012
BBSP_Ad			–	0.043
WSP_Ad				–

3. Results

3.1. Element concentrations

Of all the metals examined, Ni and Co were below the limit of detection in all samples, and were thus excluded from further analysis. As, Bi and Sb were detected exclusively in chick feathers, both body and down. Concentrations of all elements (mean ± SD) are presented in Tables 1 and 4 (for Hg). Concentration chain based on mean values for particular groups are:

a) For adult WSP Mg > Zn > Ca > Fe > Sr > Cu > Mo > Hg > Se > Cr > Pb
b) For adult BBSP Mg > Zn > Ca > Fe > Cu > Hg > Se > Sr > Cr > Mo > Pb
c) For chicks body feathers Mg > Ca > Fe > Zn > Bi>Sr > Mo > Cu > As>Se > Cr > Pb > Sb > Hg > Cd
d) For chicks down feathers Mg > Ca > Fe > Zn > Sr > Se > Mo > Pb = Hg = As>Bi = Cr = Cu > Cd > Sb

3.2. Inter-group differences in all elements concentration

3.2.1. All elements

The concentrations of all combined studied elements differed significantly between adult WSP and all other categories (PERMANOVA, Bonferroni-corrected $p < 0.04$) (Table 2). SIMPER analysis showed that the overall average dissimilarity was 18.5%. Fe and Bi contributed most (14% and 11%, respectively) to the pattern of overall dissimilarity (Table 3). Bi, As and Fe contributed most (19%, 14% and 14%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and chick WSP body feathers. Fe and Se contributed most (14% and 12%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and chick WSP down. Cu, Fe, Hg and Se contributed most (16%, 15%, 14% and 12%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and BBSP.

3.2.2. Essential elements

The concentrations of all combined studied elements differed significantly between adult WSP and all WSP chick categories (PERMANOVA, Bonferroni-corrected $p < 0.006$). We found no differences between adult WSP and BBSP ($p = 0.136$) (Table 2). The SIMPER analysis showed that the overall average dissimilarity was 14.2%. More than half of the pattern of overall dissimilarity observed in elemental concentrations was explained by Fe, As, Cu and Mo (22%, 15%, 13% and 13%, respectively) (Table 3). As, Fe and Mo together contributed over 50% (23%, 22% and 15%, respectively) to the pattern of dissimilarity observed in elemental concentrations between adult WSP and chick WSP body feathers. Fe, Ca, As, Zn and Mo together contributed over 50% (21%, 13%, 12%, 11% and 11%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and chick WSP down (Table 3). Cu, Fe, Se and Mo together contributed over 50% (23%, 21%, 18% and 13%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and BBSP (Table 3).

Table 3
Sources of variability (average percentage dissimilarity) in the elemental concentrations (log(x + 1) transformed) in: body feathers of adult Wilson's (WSP_Ad) and back-bellied (BBSP_Ad) storm-petrels, down from Wilson's storm-petrel chicks (WSP_down) and body feathers from Wilson's storm petrel fledglings (WSP_CHF), according to the SIMPER analysis. Only elements with a contribution >10% are shown. ADis - Average Dissimilarity, Contr. (%) – percentage contribution, Overall - overall average similarity.

	Overall dissimilarity		WSP_Ad vs WSP_CHF			WSP_Ad vs WSP_down			WSP_Ad vs BBSP_Ad		
	ADis	Contr.		ADis	Contr.		ADiss	Contr.		ADis	Contr.
All elements											
Fe	2.57	13.9	Bi	4.18	19.1	Fe	2.67	14.0	Cu	2.12	16.1
Bi	2.07	11.2	As	3.06	14.0	Sr	2.36	12.4	Fe	1.94	14.7
			Fe	3.02	13.8				Hg	1.89	14.3
									Se	1.63	12.3
Overall	18.46			21.87			19.30			13.23	
Essential											
Fe	3.10	21.9	As	3.72	22.6	Fe	3.24	21.5	Cu	2.43	22.8
As	2.06	14.5	Fe	3.68	22.4	Ca	1.94	12.9	Fe	2.22	20.8
Cu	1.83	12.9	Mo	2.40	14.6	As	1.85	12.3	Se	1.87	17.6
Mo	1.83	12.9	Cr	1.87	11.3	Zn	1.71	11.3	Mo	1.41	13.3
			Cu	1.82	11.0	Mo	1.66	11.0			
Overall	14.16			16.46			15.07			10.65	
Non-essential											
Bi	11.47	29.2	Bi	23.67	50.3	Sr	13.96	36.4	Hg	14.42	49.1
Sr	10.01	25.5	Sb	6.70	14.2	Pb	7.43	19.4	Sr	8.68	29.5
Hg	7.02	17.9	Sr	6.26	13.3	Bi	7.27	19.0	Pb	4.74	16.1
Pb	5.59	14.2	Pb	5.38	11.4	Hg	5.25	13.7			
Overall	39.31			47.09			38.33			29.41	

3.2.3. Non-essential elements

The concentrations of all combined studied elements differed significantly between adult WSP and all other categories (PERMANOVA, Bonferroni-corrected $p < 0.05$; Table 2). The SIMPER analysis showed that the overall average dissimilarity was 39.3%. Bi, Sr, Hg and Pb together contributed over 50% (29%, 25%, 18% and 14%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations (Table 3). Bi, Sb, Sr and Pb were responsible for 50%, 14%, 13% and 11%, respectively, of the dissimilarity pattern in elemental concentrations observed between adult WSP and chick WSP body feathers. Sr, Pb, Bi and Hg together produced the majority of dissimilarity in elemental concentrations observed between adult WSP and chick WSP down (36%, 19%, 19% and 14%, respectively). Hg, Sr and Pb together contributed over 50% (49%, 30% and 16%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and BBSP (Table 3).

3.3. Intergroup differences for particular elements

Kruskal–Wallis inter-group tests comparing the concentration of particular elements revealed significant differences for all elements ($p < 0.05$) (Supplementary Materials 2, Fig. ES1–ES7) except Mg ($p = 0.44$) and Mo ($p = 0.12$). *Post-hoc* tests revealed the following pattern of significant inter-group differences (Supplementary Materials 2, Fig. ES1– ES7):

1. lower concentration of Cu, Hg and Se in adult WSP compared to adult BBSP;
2. lower concentration of As, Bi, Ca, Cr, Fe, Sb and Se in adult WSP compared to chick WSP body feathers
3. higher concentration of Zn in adult WSP compared to chick WSP down
4. higher concentration of As, Bi, Ca, Cd, Fe, Pb, Sb, Se and Sr in chick WSP down compared to adult WSP
5. higher concentration of As, Bi, Cr, Cu, Sb and Zn in chick WSP body feathers compared to chick WSP down
6. lower concentration of Ca and Sr in chick WSP body feathers compared to WSP down

Other studied inter-group differences were not significant ($p > 0.05$).

3.4. Inter-group differences for Hg concentration determined by cold vapour technique

The Kruskal–Wallis test revealed significant inter-group differences ($p < 0.05$) in the concentration of Hg determined by cold vapour technique. *Post-hoc* tests results and pattern of significant inter-group differences are presented in Fig. 2.

3.5. Grouping of elements

The Hierarchical Cluster Analysis for all studied groups combined (cophenetic correlation 0.902) recognised four main significant clusters grouping the trace elements (Fig. 3). The first cluster included Ca and Zn (BP = 100), while the second cluster contained Cd—Sb (BP = 99). Then, the third was Bi—As (BP = 96) and the fourth was Cu-Hg-Se (BP = 96), with a subcluster of Hg—Se (BP = 98).

4. Discussion

Our study provides reference values for concentration of 17 elements in feathers of two pelagic seabird species from the maritime Antarctic. We revealed several differences in elemental concentrations between the two species, as well as differences in exposure between life-cycle stages. We also identified some patterns in concentrations of particular elements.

4.1. Contaminant patterns of selected elements and comparisons with other seabirds from south polar areas

Although reference values of 17 elements are provided in our study, below, we discuss only the those considered most relevant in terms of possible effects on birds' health and survival.

4.1.1. Mercury

Hg is an endocrine disruptor associated with several adverse effects, including decreased body condition, immune responses and hormonal secretion (Wolfe et al., 1998; Scheuhammer et al., 2007; Tartu et al., 2014, 2015). As such, it affects birds reproduction and survival, and so may impact birds' population dynamics (Tartu et al., 2013; Goutte et al., 2014). Bird feathers are perceived as the main route for Hg

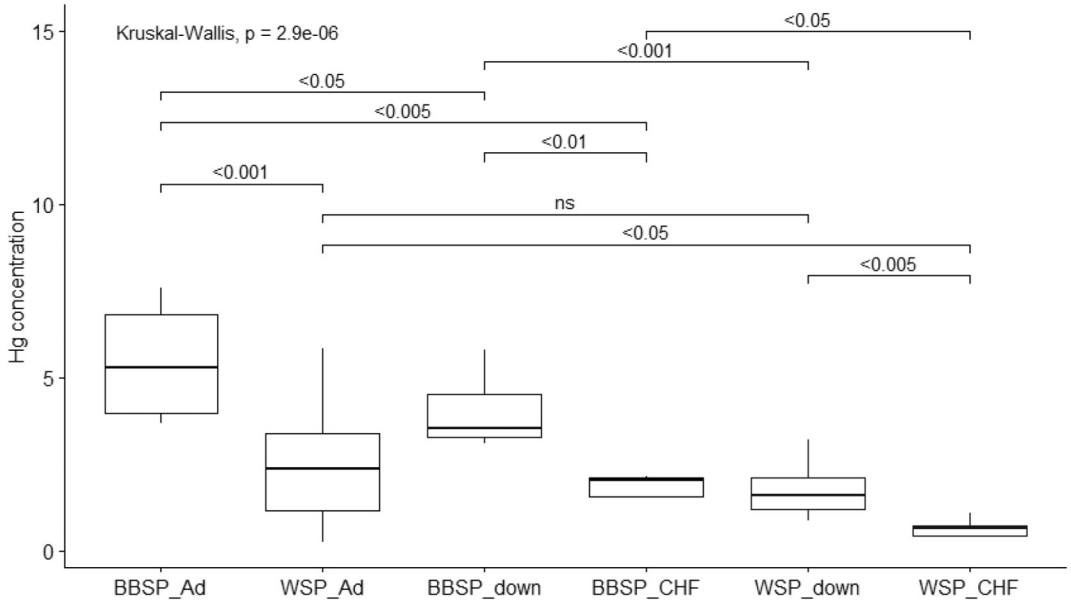

Fig. 2. Concentration of Hg ($\mu g \cdot g^{-1}$ dw) in feathers of the six studied groups of storm-petrels: body feathers of adult Wilson's (WSP_Ad) and back-bellied (BBSP_Ad) storm-petrels, down from Wilson's (WSP_down) and black-bellied (BBSP_down) storm-petrel chicks and body feathers from Wilson's (WSP_CHF) and black-bellied (BBSP_CHF) storm-petrel chicks. Boxplots show the median (band inside the box), the first (25%) and third (75%) quartile (box), and the lowest and the highest values within 1.5 interquartile range (whiskers).

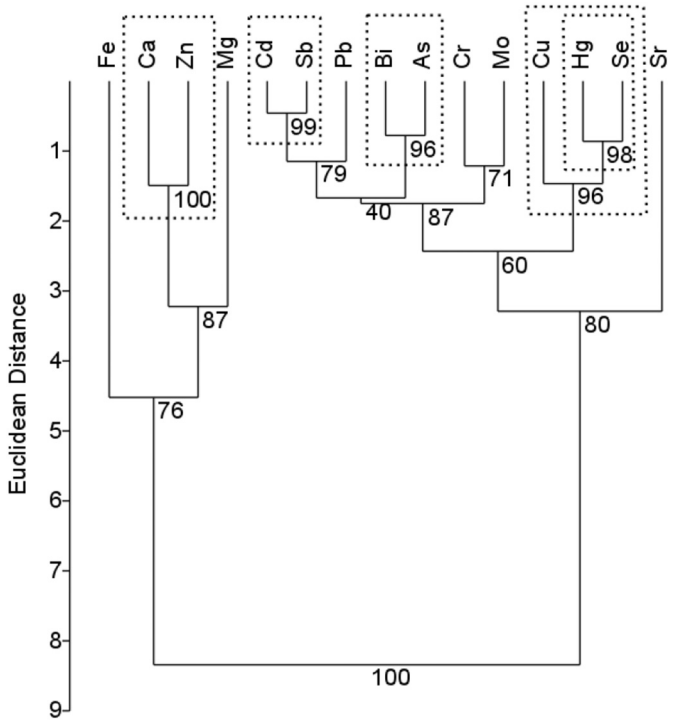

Fig. 3. Hierarchical dendrogram of the studied elements in the feathers of the studied storm-petrels (all age and feather type groups combined), obtained using a paired group method and Euclidean distance matrix (the distance reflects degree of association between different elements). Numbers below branches indicate bootstrap probability values (bootstrap $n = 1000$). Clusters with bootstrap support ≥95 denoted with a dotted rectangle.

excretion (Monteiro and Furness, 1995; Santos et al., 2006), but its level would depend on multiple factors, including diet, excretion capacities in the feathers and moulting pattern (Becker et al., 2016; Bustamante et al., 2016). The Hg concentration reported in our results for BBSP adults (5.47 ± 1.61 μg g^{-1} dw) are in a range of values reported previously by Carravieri et al. (2014; 4.22±2.53 μg g^{-1} dw). However, for adult WSP, our values (2.38 ± 1.47 μg g^{-1} dw) were much higher compared to other studies (0.42 ± 0.13 μg g^{-1} dw; Carravieri et al., 2014). Nevertheless, Hg levels in adult WSP from our study were comparable to mean levels reported for another low-trophic-level seabird, the Antarctic prion *Pachyptila desolata* (1.73–2.80 μg g^{-1} dw). In general, there is a high variability between petrel species (0.42–12.43 μg g^{-1} dw; Table 4). Here, the inter-species difference in Hg concentration is most likely associated with diet (Thompson and Furness, 1989; Bustamante et al., 2016; Blévin et al., 2013) as BBSP feeds at a higher trophic level than WSP (Quillfeldt et al., 2017). Such a dietary explanation was suggested in the study of Blévin et al. (2013), where chicks of 21 various species breeding in the Southern Ocean were been found to vary greatly in terms of Hg concentration (from 0.05 ± 0.01 μg g^{-1} in the South Georgian diving petrel *Pelecanoides georgicus* to 5.31 ± 1.12 μg g^{-1} in the northern giant petrel *Macronectes halli*).

Examining Hg concentrations in age groups, in both species we found that it was significantly higher in adults than in chicks of the same species (excluding WSP down; Fig. 2) probably due to the longer exposure time of adults. This is similar to white-chinned petrels *Procellaria aequinoctialis* (Carvalho et al., 2013), for which the same explanation has been suggested. In contrast, in the wandering albatross *Diomedea exulans*, Hg contamination was higher in immatures than adults, which may be associated with moulting intensity and detoxification capacities varying between adults and immatures (Bustamante et al., 2016).

4.1.2. Selenium and its interaction with mercury

Se is an essential trace element for proper organism functioning, including thyroid function (Burger et al., 2013), and it is known for its protective action against Hg bioaccumulation and toxicity through the creation of Hg—Se compounds (Nigro and Leonzio 1996; Khan and Wang, 2009). However, excess Se may as well have toxic effects on vertebrates (Burger et al., 2013).

We found that Se levels significantly differ between the two studied species, with almost three times higher values found in adult BBSP compared to adult WSP (5.19 ± 1.18 μg g^{-1} dw vs 1.81 ± 0.98 μg g^{-1} dw). These values add to a wide range reported so far from other seabirds of the Southern Ocean (3.40–19.40 μg g^{-1} dw; Anderson et al., 2010, Fromant et al., 2016, Philpot et al., 2019). Interestingly, Se levels in *Pygoscelis* sp. penguins living in King George Island were similar to values found in our study (2.46–6.37 μg g^{-1} dw; Jerez et al., 2011),

Table 4
Variability of mercury (Hg) levels in feathers of *Procellariiformes*. N – number of individuals.

Species	Study area	Tissue	N	Age	Concentrat. mean ± SD μg g^{-1} dw	Reference
Antarctic prion (*Pachyptila desolata*)	Kerguelen archipelago	Body feathers	10	Unknown	2.8 ± 1.2	Fromant et al., 2016
			10	Adult	1.73 ± 0.50	Carravieri et al., 2014
White-headed petrel (*Pterodroma lessonii*)	Kerguelen archipelago	Body feathers	10	Adult	12.43 ± 2.01	Carravieri et al., 2014
			10	Chicks	1.54 ± 0.34	Blévin et al., 2013
Spectacled petrel (*Procellaria conspicillata*)	Southwestern Atlantic Ocean off the Brazilian coast	Contour feathers	38	Unknown	11.17 ± 3.78	Carvalho et al., 2013
White-chinned petrel (*Procellaria aequinoctialis*)	Kerguelen archipelago	Body feathers	22	Unknown	7.63 ± 3.87	Cipro et al., 2014
	Southwestern Atlantic Ocean off the Brazilian coast	Contour feathers	9	Adults	3.45 ± 2.84	Carvalho et al., 2013
			21	Juveniles	1.14 ± 2	
	Kerguelen archipelago	Body feathers	14	Chicks	1.82 ± 0.51	Blévin et al., 2013
Leach's storm-petrel (*Oceanodroma leucorhoa*)	Machias Seal Island, New Brunswick, Canada	Breast feathers	15	Adult	7.01*	Bond and Diamond, 2009
			20	Chicks	1.42*	
Wilson's storm-petrel (*Oceanites oceanicus*)	Kerguelen Islands	Body feathers	12	Adult	0.42 ± 0.13	Carravieri et al., 2014
	King Gorge Island, South Shetlands, Antarctica	Body feathers	25	Adult	2.38 ± 1.47	Present study
		Body feathers	5	Chick	0.67 ± 0.27	
		Down	16	Chick	1.72 ± 0.65	
Black-bellied storm-petrel (*Fregetta tropica*)	Kerguelen Islands	Body feathers	10	Adult	4.22 ± 2.53	Carravieri et al., 2014
	King Gorge Island, South Shetlands, Antarctica	Body feathers	8	Adult	5.47 ± 1.61	Present Study
		Body feathers	5	Chick	1.87 ± 0.29	
		Down	6	Chick	3.99 ± 1.07	

* Estimated marginal mean.

while for penguin *Pygoscelis* from other area Se levels were lower, from <0.80 to 2.0 μg g^{-1} (Metcheva et al., 2006).

The studied WSP chicks had higher Se levels compared to adults (Table 1). This trend was not observed in gadfly petrels *Pterodroma spp*, where Se levels were significantly lower in chicks than in adults (Philpot et al., 2019). These differences are difficult to explain given the currently limited knowledge about Se distribution and metabolism.

Worldwide studies quantifying Hg—Se co-exposure and interaction in seabirds are still rare, but have increased in recent years, and show that seabirds' ability to deal with high mercury and selenium levels is still not fully understood, and may depend on age and species (e.g., Carvalho et al., 2013; Cipro et al., 2014; González-Solís et al., 2002; Carravieri et al., 2017; Philpot et al., 2019). Se—Hg molar ratios in our study differed between chicks and adults, being highest in WSP chick body feathers (Table 1). However, all ratios reported here were >1, suggesting activation of a defence mechanism against high Hg concentrations and a health impact associated with potential Se toxicity (Lucia et al., 2016).

4.1.3. Cadmium

Cd is another toxic element that readily bioaccumulates in food webs (Cipro et al., 2014), and Cd has been reported at even higher levels in Antarctic species including plankton, marine benthic invertebrates, fishes, seabirds and marine mammals (see references in Jerez et al., 2011), than in their counterparts sampled in polluted coastal areas (Petri and Zauke, 1993). In our study, Cd levels in adults were mostly below the quantification limit (Table 1), but it is not exceptional (e.g. penguin feathers were also generally low (<LOD–0.10 μg g^{-1} dw, Jerez et al. [2011]; <0.15–0.21 μg g^{-1} dw [Metcheva et al., 2006]) and may be related to relatively low deposition of Cd in feathers (Lucia et al., 2010; Cipro et al., 2014). Indeed, in Antarctic prions, Cd levels in internal tissues (kidney 105 ± 37 μg g^{-1} dw) were considerably higher than in feathers (mean 0.06 ± 0.03 μg g^{-1} dw) (Fromant et al., 2016). Thus, feathers would give only partial information of bird exposure; i.e. only when it reaches high levels.

4.1.4. Lead

After Hg, Pb is another major contaminant of toxicological concern (Burger and Gochfeld, 2009), affecting breeding success, migratory behaviour and survival of animals at various trophic levels (Burger, 1995). It may affect food web dynamics e.g. by decreasing the abundance and availability of food prey, or by interfering with its natural hiding or escape behaviour (Burger, 1995). Pb is accumulated in feathers at higher rate compared to Cd (Jerez et al., 2011), but can be elevated due to exogenous contamination (Jaspers et al., 2004). Adverse effects from lead toxicity might occur at levels of 4 μg g^{-1} in feathers (Burger and Gochfeld, 2000) but levels in adult seabirds are usually lower (0.51–1.68 μg g^{-1} dw, Mendes et al., 2008, Burger and Gochfeld, 2009). In our study the Pb concentration was generally low in adults (<1.17 μg g^{-1} dw, with one outlier reaching 5.06 μg g^{-1} dw), and higher in chicks (0.36–3.67 μg g^{-1} dw). Similarly low values of Pb concentration have been reported for other seabirds from the Southern Ocean (Metcheva et al., 2006; Anderson et al., 2010; Jerez et al., 2011; Fromant et al., 2016). However, for penguins breeding on King George Island, high Pb values have been also reported, which has been explained by local human activities (many scientific bases and a small airport in the study area; Jerez et al., 2011).

4.1.5. Zinc

Zn can be bioaccumulated in polar organisms, but most likely does not biomagnify (Santos et al., 2006). Variation of this element concentration in adult storm petrels was relatively low (WSP 109.20 ± 18.50 μg g^{-1} dw, BBSP 99.95 ± 13.01 μg g^{-1} dw), and in chicks was even lower, and with small inter-individual variability (WSP down and body feathers 48.30 ± 7.08 and 93.00 ± 11.3 μg g^{-1} dw, respectively). The reported Zn concentration falls well within the range reported from other seabirds (6.95–301 μg g^{-1} dw) (Anderson et al., 2010; Cipro et al., 2014; Fromant et al., 2016; Philpot et al., 2019; Metcheva et al., 2006; Jerez et al., 2011; Santos et al., 2006).

4.1.6. Copper

Cu, like Zn, is also an essential element, with concentrations in seabird tissues controlled mostly in homeostasis processes (Bocher et al., 2003). Variation in the concentration variation of Cu in the two species was much larger than for Zn (adults, WSP: 2.52 ± 2.35 μg g^{-1} dw, BBSP: 8.12 ± 0.56; chick body, WSP: 6.68 ± 3.15, chick down WSP: 1.52 ± 0.60 μg g^{-1} dw). These values also seem to fall well within the range reported so far for other seabirds (6.0–12.7 μg g^{-1} dw; Metcheva et al., 2006, Jerez et al., 2011).

4.2. Potential sources of elements

The contamination of Antarctic biota may have both natural and anthropogenic sources (Jerez et al., 2011; Lu et al., 2012; Deheyn et al., 2005). Our cluster analysis revealed some interesting groupings of elements that suggested common source of contamination.

The Bi—As cluster suggests a volcanic origin of the two elements. Worldwide emissions from volcanoes are deemed a considerable source of atmospheric Bi and As (Candelone et al., 1995; Kabata-Pendias and Szteke, 2015), and the soils on King George Island are mostly composed of mineral and rock fragments with some volcanic ashes (Lee et al., 2004). The ashes were blown from Deception Island, a volcanic island located ~130 km south-west of King George Island (Jeong and Yoon, 2001), where the most recent eruption occurred in the late 1960s (Orheim, 1972). Storm petrels may additionally gain As from food sources, as low-trophic organisms (as petrels diet items) easily assimilate this element (Rahman et al., 2012). Mão de Ferro et al. (2013) found As enrichment in several Antarctic abiotic and biotic samples to probably be a result of past volcanic activity and sediment petrologic characteristic, as well as As leaching processes. They also indicated that during a high tide, leaching processes of As can occur to shore and semi-submerged areas, thus being available to aquatic organisms (Mão de Ferro et al., 2013). All feathers were cleaned by the exact same procedure, but we cannot exclude the possibility of external contamination by soil particles, as both As and Bi were only detected in chicks feathers.

Both elements of the Ca—Zn cluster are necessary components in the synthesis of the feather pigment melanin (McGraw et al., 2003). They also play an essential role in multiple physiological body functions (Bogden and Klevay, 2000). Thus, this cluster may reflect both co-exposure from diet and the similar co-regulation mechanisms responsible for element deposition. Both Ca and Zn accumulation in feathers may depend on melanin type and content, as shown by element enrichment in pigmented feather parts (Niecke et al., 1999, 2003).

The Cd—Sb cluster may represent common food and/or water input. Cd may originate from anthropogenic pollution but also from rock weathering and/or natural sources, as it is more mobile in seawater than in other water bodies and is easily absorbed by aquatic biota (Kabata-Pendias and Szteke, 2015). Natural sources (diffusive fluxes, upwelling and continental weathering) can be responsible for higher abundance of Cd in Antarctic water samples (Sañudo-Wilhelmy et al., 2002). High Cd concentrations were found in Antarctic krill *Euphasia superba*, which is the main dietary component for adults and chicks of both storm-petrel species (Wasilewski, 1986; Petri and Zauke, 1993; Hahn, 1998; Nygård et al., 2001; Quillfeldt, 2002). The natural sources of Sb and its compounds are volcanic eruptions, sea spray, forest fires and wind-blown dust, suggesting a non-anthropogenic source (Kabata-Pendias and Szteke, 2015). Considering its clustering with Cd, we would suggest a natural source of both elements in the feathers of the studied birds.

The common clustering of Cu-Hg-Se may be explained by the properties of Se and the high concentration of all these elements in aquatic

organisms, including fish. Marine aerosols are enriched in Se resulting from the formation of volatile Se-organic compounds. Volcanic emissions were suggested as a prevalent source of Hg in Deception Island (Mão de Ferro et al., 2014). Also, summer input from the Southern Ocean may be a net source for the gaseous element Hg in the marine boundary layer (Wang et al., 2017).

4.3. Species and age differences in elemental concentrations

Significant differences in concentration of various elements (i.e. Cu, Hg, and Se) between the WSP and BBSP found in our study are most likely to be associated with inter-species differences in foraging (different trophic levels with a different contribution of fish in their diet [Quillfeldt et al., 2017]).

Significant differences in concentrations of various elements (Supplementary Materials 2, Fig. ES1–ES7) between WSP age groups are also likely to be associated with diet, although here not with the difference in diet composition but more with the location of food resources exploited during the period of growth of relevant feathers. Chick feathers are more suitable for local exposure assessment, as levels are not affected by moulting patterns, because chicks receive food collected by parents in the vicinity of the breeding colony, and have a shorter exposure time. Thus, in cases when adult and offspring diet do not differ significantly, chick feathers may also be used to reconstruct adults' foraging ecology and adults' exposure to several pollutants during the chick-rearing period (Blévin et al., 2013).

Down feathers have been successfully used to estimate Hg concentrations in eggs (Santos et al., 2017), suggesting its potential as a suitable proxy for contaminant determination. A strong correlation between the levels of both Hg and Se in eggs and the liver of incubating females has been found in *Charadriiformes* (Ackerman et al., 2016). In our study, all examined elements except Ni and Co were detected in down feathers, enabling exposure assessment at the earliest phase of life. The highest Ca level was found in WSP down, probably because the developing embryo absorbs Ca and other elements, initially from the yolk and subsequently from the eggshell (Castilla et al., 2010). We found the lowest Zn level in down (48.30 ± 7.08 μg g^{-1} dw), at almost two times lower than the level in chick body feathers (93.00 ± 11.30 μg g^{-1} dw). Other elements, such as Pb, Hg, Se, Mg and Sr, were higher in down than in chick body feathers, suggesting that exposure changes over time. Maternal transfer of contaminants may be a reason for the increased levels of several metals in chick down feathers, as the maternal transfer is species- and element-specific (Ackerman et al., 2016).

5. Conclusions

Our study provides a reference values for concentration of 17 elements in feathers of two pelagic seabird species from the maritime Antarctic. Such data may serve to monitor contaminant levels in marine systems and to evaluate variability in contaminant levels in tissues throughout birds' annual cycle (Rodríguez et al., 2019). We also revealed several differences in elemental concentrations between the two species, as well as differences in exposure between life-cycle stages. These inter-species and inter-age differences are attributed to the various diet compositions and geographic areas of feather growth. Finally, we identified some patterns in concentration of particular elements that suggest a primarily natural origin of most elements. We believe that our study contributes to understanding spatial and temporal patterns of contaminant accumulation in the Maritime Antarctic ecosystem. As emissions and global transport of elements such as Hg, Pb and Cd are expected to increase in the future, monitoring studies on seabirds breeding in the Southern Hemisphere may be a warning system for global changes and the consequences of elevated emissions into the marine food web. Despite the limitations of our study, such as: the relatively small sample size; sample collection being restricted to one site and one season; and the lack of data on elemental concentrations in prey items, our study delivered important reference values for elemental concentrations in various age groups of the two study species. The Antarctic Treaty members urge long-term monitoring and sustained observations of the Antarctic environment and the associated data management, to detect, understand and forecast the impacts of climate-change-driven environmental variability (ATCM, 2007).

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12.5. Weryfikacja hipotezy 5

Hipoteza 5: Profil pierwiastkowy obecny w piórach najliczniejszego ptaka Arktyki europejskiej będą cechowały znaczące różnice między sezonem przed- i po-lęgowym, ze względu na aktywność migracyjną. Pióra osobników dorosłych, piskląt oraz skorupki mogą zostać wykorzystane w celu określenia ekspozycji alczyka w przeciągu roku na różnych fazach rozwoju i na różnych obszarach żerowania.

Weryfikację **piątej hipotezy** wykonano w oparciu o badania próbek piór osobników dorosłych oraz piskląt najliczniejszego ptaka Arktyki Europejskiej- alczyka *Alle alle*. Wyniki badań zostały wysłane do czasopisma *Science of the Total Environment* (załącznik 6) **[A. D. Pacyna-Kuchta, D. Jakubas, M. Frankowski, Ż. Polkowska, K. Wojczulanis-Jakubas, Exposure of a small Arctic seabird, the little auk (Alle alle), to selected elements throughout the course of a year (w recenzji)]**

W 2017 roku pobrane zostały pióra od 74 osobników dorosłych (obie płcie), oraz od 18 piskląt. Alczyki znoszą tylko jedno jajo, i wracają do gniazda na zmiany z pokarmem zdobytym w morzu. Umożliwiło to pobranie próbek piór od samca, samicy i pisklęcia z tego samego gniazda. Dodatkowo dzięki próbkom piór pobranych w roku 2016 wytypowano 20 osobników które powtórzyły się w roku 2017. Umożliwiło to ocenę ekspozycji tego samego osobnika w ciągu dwóch sezonów lęgowych. Statystyczne różnice widoczne między poziomami w piórach okrywowych wyrośniętych na zimowisku (Grenlandia, Morze Północne), a piórami z podgardla wyrośniętymi tuż przed sezonem lęgowym (Svalbard), wystąpiły wyłącznie dla Ca, Hg, Mn oraz Sr. Pióra piskląt cechował inny profil pierwiastkowy niż osobniki dorosłe.

Exposure of a small Arctic seabird, the little auk (*Alle alle*), to selected elements throughout the course of a year

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Abstract

The arctic marine ecosystem can be altered by processes of natural and anthropogenic origin. Recently, there has been special interest in contaminant determination in polar biota. However, many aspects of the spatio-temporal contamination dynamics of exposure and parental transfer are still poorly understood. Here, we used non-lethally collected samples from the most abundant seabird in the European part of the Arctic to enhance our understanding of this issue. The little auk (*Alle alle*) is a long-lived seabird species that is expected to bioaccumulate trace elements including toxic metals across its life in the high-Arctic zone during the breeding season, and in sub-Arctic areas during the wintering period. We used only non-destructively collected samples, including feathers from adult little auks,

representing exposure during part of a breeding and non-breeding time, as well as feathers from nestlings, representing local contamination, plus down feathers and eggshells, representing transfer of elements from females to their offspring. The aim of the study was to investigate differences in elemental concentration in little auk tissues representing various life-history stages, with various exposition to contamination. Significant differences between elements accumulated in adult feathers representing pre- and post-breeding exposure were found only for Ca, Hg, Mn and Sr. One third of adult birds exceeded the toxicity threshold value for mercury during the pre-breeding time, as represented by head feathers. Chick down feathers were characterised by high levels of several essential elements, an intermediate level of Hg (1.11±0.41 µg/g dw) and a high level of Se (2.53±0.59 µg/g dw), potentially reducing the toxicity of Hg. Chick body feathers had the highest level of Cu (28.5±6.44 µg/g dw), and K (819±458 µg/g dw) among all studied groups.

Key words: Arctic; environmental exposure; Svalbard; metal; feather; eggshell

1. Introduction

The Arctic is undergoing constant climate changes, which have an impact on biological systems and contaminant pathways (MacDonald et al. 2005). Although remote Arctic areas are mostly non-industrialised and sparsely occupied, they can be still exposed to multiple pollutants (MacDonald et al. 2005; AMAP 2005). Long-range transport of contaminants is considered to be a significant factor affecting heavy metal levels in the High Arctic zone (AMAP 2005; 2011; Halbach et al. 2017; Zaborska et al. 2017). Additionally, elements entering the ecosystem can originate from natural sources (volcanic eruptions on

Iceland, rock weathering) and can also be produced by local past and present human activities including runoffs from active and shut-down coal mines (Rose et al. 2004; Granberg et al. 2017), ship transportation and increased tourism. When contaminants enter the marine ecosystem, they become a subject of biological processes such as bioaccumulation, biomagnification (the process whereby contaminant concentration increases with increasing trophic level/position), as well as biotransformation (a change of concentration and chemical form of a compound) (Braune et al. 2002). For instance, mercury is methylated by bacterial activity, becoming more bioavailable for other organisms, and thus may easily enter a trophic network and potentially affect Arctic ecosystems (Dietz et al. 2013; Bustamante et al. 2016).

Being high (often on the top) of a food chain, seabirds are good model species for studying element bioaccumulation and biomagnification (Burger et al. 2008). Moreover, they play an important role in the Arctic ecosystems by being two-environmental organisms – foraging at sea and breeding on land, they may easily transport various types of matter, including contaminants, from marine areas to terrestrial (Stempniewicz et al. 2007; Burger et al. 2007).

Important aspect of examining level of contaminants is that it varies across tissue types and a tissue's propensity to accumulate xenobiotics (Finger et al. 2015). Feathers, being a tissue that can be collected almost non-invasively are often used for biomonitoring of birds exposure to contamination (e.g. Jaspers et al. 2004; Burger et al. 2008; Fort et al. 2016). Feathers are linked to the way and timetables by which they are produced and replaced by birds. When blood is delivered to the feather, compounds with a high affinity to sulfhydryl groups are bound to disulphide bonds and thereby included in the structure (Burger and Gochfeld, 1997). When the feather is fully grown the blood vessel disappears,

and the feather becomes isolated from any further internal element uptake (Jaspers et al. 2004). Feather formation and growth, and consequently metal deposition into feathers, last usually approximately 2–3 weeks (Burger et al. 2008). Thus, a feather represents an archive of the presence of elements in the blood during feather growth, which may have happened weeks or months earlier (Jaspers et al. 2004; Burger et al. 2008). Screening feathers for contaminants, while knowing where and when they were moulted, gives an insight into exposure pathway of the species. Several factors may have an impact on contaminant uptake, bioaccumulation and elemental deposition in feathers, and these include foraging behaviour, age, gender, exposure pathway, metal bioavailability, detoxification abilities and moulting pattern, with this last being crucial to the reconstruction of the contamination period (Burger et al. 2008; Bustamante et al. 2016; Pacyna et al. 2017).

In this study we analyzed concentration of various chemical elements in the High Arctic seabird little auks (*Alle alle*)- eggshells and feathers of individuals in regard to the birds age (adults and chicks), feather type (body and head feathers of adults, body feathers and down of chicks) and sex. We sampled little auk population breeding in Hornsund (Svalbard). Examining eggshells and various type feathers in adults and chicks, as well as males and females allowed us to reconstruct so far the most complete picture of spatio-temporal exposure of the local population to various elements including contaminants. By sampling the same individuals in the two consecutive breeding season we were also able to examine inter-individual level of contaminants concentration, thus to have an insight into possible birds resistance to local contaminations.

2. Methods

2.1 Studied species

The little auk (*Alle alle*) is a small seabird (body mass 150–180 g), breeding colonially, exclusively in the High Arctic (Stempniewicz, 2001). It forages mainly on zooplankton associated with Arctic water masses; the diet is supplemented by fish, especially during the non-breeding season (Stempniewicz 2001; Rosing-Asvid et al. 2013). It is a seabird with a single-egg clutch, with eggs laid in early to mid-June and hatched around 30 days later after being incubated by both parents (Stempniewicz, 2001).

Little auks perform a partial pre-breeding moult, which involves the replacement of head and throat feathers during the spring when the birds are still outside the breeding grounds (Fort et al. 2014; SEATRACK). Thus elemental concentrations in those feathers reflect part of the non-breeding season when the feathers grow (around April). A complete moult that involves replacement of the entire body plumage is exhibited shortly after the breeding season (September), probably in the vicinity of the breeding areas (Fort et al. 2014; 2016; SEATRACK). Thus, body feathers represent trace elements that have been accumulated during the previous year post-breeding period, but only during the time of new feather growth (Mosbech et al. 2012; Fort et al. 2016). Body feathers collected from chicks reflect birds' exposure from breeding grounds (Evers et al. 2005). Chick down is formed in the egg from maternal nutrients and, alongside eggshells, represent female contamination during the pre-laying time (Ackerman et al. 2016).

The little auk has been proven to be a valuable bio-indicator of temporal trends of environmental contamination within the Arctic pelagic zone (Borgå et al. 2006; Jæger et al. 2009; Fife et al. 2015; Fort et al. 2016). Since it has been recognized as a keystone species for the functioning of the Arctic terrestrial ecosystem (Stempniewicz et al. 2007), knowledge of

how it copes with the contaminants accumulated in the aquatic environment is a central issue in its conservation, and important information for predicting future environmental scenarios.

2.2 Study site and sample collection

The study was performed in a breeding colony at the Ariekammen slope in Hornsund (SW Spitsbergen, 77◦00′ N, 15◦33′ E) considered to be one of the largest little auk breeding aggregations in Svalbard (Keslinka et al. 2019; Fig. 1). During the incubation and chick-rearing period (in 2017) adults (usually breeding pairs, thus both male and female) were captured in nest chambers by hand. From each bird, two types of feathers were collected: from the back (hereafter called “body feathers”) and from the throat (hereafter called “head feathers”). Two types were also collected from nestlings of the sampled adults: down from chicks at age 7–14 d and then body feathers from the same but older chicks (14–23 d). Residual eggshells were collected from focal nests (little auks remove them from nests a few days after hatching). After collection, feathers were kept at ambient temperature in sealed string bags. Eggshells were kept in plastic containers in a refrigerator (-20°C).

For 20 individuals sampled in 2017, we had their body feathers collected in the previous season (2016), and so we used them to investigate an inter-annual difference in elemental concentration. We analysed the feathers of the same individuals identified by ring number.

Fig. 1. Study site; map source: toposvalbard.npolar.no; photo by KWJ

147 **2.3 Sample preparation**

148 All feather samples were cleaned with acetone and two times with deionised water (Mili-Q
149 water, France) to remove external contamination, and air-dried for about 24 h. Next, the dry
150 feathers were homogenised by cutting into small parts and weighed to the nearest 0.01 mg
151 (mean sample mass adult body 24.33±5.81 mg, throat 7.65±2.07 mg, chick feathers
152 16.09±4.32 mg, chick down feathers 27.21±12.16 mg). In the case of eggshells, the internal
153 membrane was discarded, as it was too contaminated, and samples were washed with
154 acetone and deionised water. Samples were left to dry for 24 h, then crushed into small
155 pieces and weighed (mean 203.3±4.1 mg). All samples were placed individually into clean a
156 Teflon vessel with 65% HNO_3 (Merck, Suprapure). Digestion was carried out using a high-
157 pressure microwave emitter (Microwave Digestion System, Anton Paar) (for a detailed
158 description, see Pacyna et al. 2019a). For quality control, blank samples were run with every
159 batch. To ensure accuracy of obtained results, a certified reference material (CRM, Human
160 hair ERM-DB001, Sigma Aldrich) was run in triplicate. The elemental concentration was
161 determined by means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS 2030
162 Shimadzu, Japan), with the exception of calcium and magnesium in eggshells which were
163 analyzed on ICP-OES 9820 (Shimadzu, Japan). Details regarding measurement conditions and
164 parameters are described in previous studies (for ICP-MS, see Pacyna et al. 2019a; for ICP-
165 OES, see Pacyna et al. 2019b).

166 Due to the lack of considerable morphological dimorphism in the little auk (Jakubas and
167 Wojczulanis, 2007) we determined sex molecularly. We extracted DNA using the Blood Mini
168 kit (A&A Biotechnology, Gdynia, Poland), after ethanol evaporation. We performed PCR
169 using the primer pair F2550 and R2718, with 50^{o}C annealing temperature (Griffiths et al.
170 1998). These primers amplify a 430-bp fragment on the W chromosome (in females only),

and a 600-bp fragment on the Z chromosome (in both sexes) (Griffiths et al. 1998). This size difference was clearly visible in UV-light when dyeing the fragments with Midori green and separating on a 2% agarose gel.

2.4 Quality control (QA/QC)

Results for CRM analysis were in agreement with the certified values, with recoveries reaching 92–106% (values were certified for As, Cd, Cu, Hg, Pb, Se and Zn). To check accuracy and recoveries of other elements absent in this CRM, a treatment used before by Pacyna et al. (2019b) was applied. The limit of detection (LOD) and quantification (LOQ) values were calculated as the concentrations corresponding to signals equal to, respectively, three and ten times the standard deviation of the blank solution signal. LOD and LOQ were in the ranges 0.004–0.92 and 0.013–3.07 ng/g, respectively. Values were blank corrected by a mean value of all blank samples. Values below LOD were replaced with half the LOD value. All results are reported as $\mu g \cdot g^{-1}$ dry weight (dw). For statistical analysis arithmetic mean was calculated if at least 65% of the samples had concentrations of the compound >LOD. In total feathers from 74 adults birds were analysed, but reliable sex determination was possible only for 54 individuals (26 males and 28 females).

2.5 Statistical analyses

To examine level of little auk contamination with chemical elements, we applied data mining approach, using various multivariate methods. Although feather samples were collected from family members potentially imposing dependency in the data set, we treated them as independent data points in all the analyses. This is because feathers of both types (pre- and post-breeding) collected from adult males and females, as a matter of fact, have grown

195 independently (both in geographical and temporal sense). Then, all the individuals were
196 represented in each sample groups in a similar number, thus the issue of possible
197 pseudoreplication could be considered as negligible.
198 To compare the qualitative and quantitative compositions of trace elements in samples
199 among the life-history stages, we applied the following methods:
200 1) A multivariate (for all trace elements together) PERMANOVA (non-parametric MANOVA
201 based on the Bray–Curtis measure; Anderson 2001) with concentrations of all elements as a
202 response variable and little auk life-history stage [body feathers from pre-breeding adults
203 (hereafter PREBR), body feathers from post-breeding adults (hereafter POSTBR), chick down
204 (hereafter CHDOW), chick body feathers (hereafter CHBFR), and egg-shell (hereafter
205 EGGSCH) as the explanatory variable;
206 2) A similarity percentage breakdown procedure (SIMPER) to assess the average percentage
207 contribution of individual elements to the dissimilarity in all elements concentrations
208 between groups in a Bray–Curtis dissimilarity matrix (Clarke 1993).
209 3) Kruskal–Wallis test to compare concentrations of particular elements in the studied
210 groups
211 Then, to find the groups of elements with high degrees of association in elemental
212 concentrations, we performed a Hierarchical Cluster Analysis (HCA). A high degree of
213 association between element concentrations, expressed by clustering in one group, can be
214 used to identify common sources of elements (e.g. Hashmi et al. 2013), but it does not
215 require the formulation of any *a priori* hypothesis considering the nature of the relationships
216 (Bianchi et al. 2008). We performed HCA with Euclidean distance as a distance measure, and
217 the paired group method as the linkage method. For each cluster obtained, we calculated
218 Bootstrap Probability (BP) using multiscale bootstrap resampling. The BP of a cluster may

take a value between 0 and 100, indicating how well the data supported the cluster, with a higher value indicating a better fit (Hammer et al. 2001). We only considered clusters with BP≥95. To determine how well the generated clusters represented dissimilarities between objects, we calculated the cophenetic correlation coefficient. Values close to 0 indicate poor clustering, and values close to 1 show strong clustering.

To compare elemental concentrations between various types of feathers or feathers collected from the same individuals in various periods we used the t-test for dependent variables. Sex differences were compared by a t-test between adult individuals from feathers grown during a post-breeding period. We performed PERMANOVA, SIMPER and HCA analyses on log(x + 1) transformed data in PAST software (Hammer et al. 2001) and all other analyses in R software.

3. Results

Elemental concentration in all samples is reported in Table 1. In the case of Bi in chick feathers, the results suggested possible external contamination, as extraordinary variations were found (65% of samples were <LOQ, range was from <LOD–92.4 µg/g dw). No significant sex differences were found in POSTBR body feathers, which representing part of the post-breeding time, except for Ca and Zn, both of which were significantly higher in females (Table 2). Concentrations of Hg also tended to be higher in females, but results were not statistically significant (p=0.057).

The following patterns of elemental profile based on median values were found:

a) Adult body feathers:

Mg > Zn > Ca > Fe > Cu > Sr > Ba > Hg > Se > Pb > Mn ≥ Cd > As > Cr > V

b) Adult head feathers:

Mg > Zn > Ca > Fe > Cu > Sr > Hg > Se > Ba > Mn > Pb > Cr > V

c) Chick down:

Mg > Ca > Zn > Sr ≥ Fe > Cu > Mn > Se > As > Hg > Bi > Cd > Pb ≥ V

d) Chick body feathers:

K > Mg > Zn > Cu > Ca > Fe > Sr > Hg > As > V

e) Eggshells:

Ca > Mg > Sr > K > Ba ≥ Fe > Zn > Mn > Cu > Se ≥ Co > As > V

Comparison of elemental concentrations in body feathers (POSTBR) and head feathers (PREBR) of all adult individuals revealed statistical differences in four elements only (Table 3), with higher concentrations of Ca and Sr and lower concentrations of Hg and Mn during POSTBR than during PREBR. Comparison of elemental concentration of the same individuals sampled both in 2016 (side body feathers) and 2017 (back feathers) revealed no statistically significant differences, except of Pb with higher values in 2017 compared to 2016 (Table.4)

3.1 Inter-group differences in all elements

The concentrations of all studied elements differed significantly between the groups (PERMANOVA, F=133.6, p=0.001; Bonferroni–corrected *post hoc* pairwise comparisons: all p=0.001).

The SIMPER analysis showed that the overall average dissimilarity was 25.8%. K, Ca and Sr contributed the most (18%, 13%, and 9%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations.

The average dissimilarity between PREBR and POSTBR was 15.4%. Fe, Ba, Ca and Mn contributed the most (16%, 11%, 10% and 9%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations. The average dissimilarity between PREBR and CHDOW was 20.6%. K, Ca and Fe contributed the most (21%, 9%, and 9%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations. The average dissimilarity between PREBR and CHBFR was 29.5%. K contributed the most (36%) to the pattern of overall dissimilarity observed in elemental concentrations. The average dissimilarity between PREBR and EGGSCH was 48.2%. Ca, K, Sr and Zn contributed the most (19%, 18%, 16% and 12%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations. The average dissimilarity between POSTBR and CHDOW was 18.7%. K, Mn, Fe, and Ba contributed the most (24%, 12%, 10% and 9%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations. The average dissimilarity between POSTBR and CHBFR was 27.9%. K and Ca contributed the most (39%, and 10%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations. The average dissimilarity between POSTBR and EGGSCH was 27.9%. K, Ca, Sr and Zn contributed the most (20%, 19%, 16% and 13%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations. CHDOW and CHBFR was 24.7%. K, Ca, Mn, and Sr contributed the most (24%, 13%, 10% and 9%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations. CHDOW and EGGSCH was 38.1%. Ca, Sr, K and Zn contributed the most (19%, 16%, 12% and 12%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations. CHBRF and EGGSCH was 42.3%. Ca, Sr, Zn and Cu contributed the most (25%, 20%, 13% and 10%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations.

3.2 Intergroup differences for particular elements

Kruskal–Wallis inter-group tests comparing the concentrations of particular elements revealed significant differences for all elements ($p<0.05$) (Supplementary Materials 1, Fig. ES1–ES8). *Post-hoc* Mann-Whitney tests revealed the all inter-group differences were significant ($p < 0.05$) except for the following pairs and elements (Electronic Supplementary Materials 1, Fig. ES1–ES7):

1. PREBR vs. POSTBR: Ba, Cd, Pb, K, Mg, Zn
2. PREBR vs. CHDOW: Cu, Fe, Mg, Se
3. PREBR vs. CHBFR: As, Fe
4. PREBR vs. EGGSCH: Mn
5. POSTBR vs. CHDOW: Fe, Mg, V
6. POSTBR vs. CHBFR: As, Pb, Mn
7. CHDOW vs. CHBFR: Ba, Pb, Cr, Fe, V
8. CHBFR vs. EGGSCH: Fe

Individual elements were found in their highest concentrations in the following groups:

1. Ba, Sr, Ca, Mg in EGGSCH
2. Hg, Se in PREBR
3. Cd, Mn, Se in CHDOW
4. Cu, K in CHBFR

Se:Hg molar ratio was highest in chicks down feathers, with significant statistical differences between groups (Fig.2).

Fig.2 Se:Hg molar ratio in studied groups (PREBR- feathers grown during pre-breeding period; POSTBR- feathers grown during post-breeding period; CHDOW- chicks down)

3.3 Potential sources of elements

The Hierarchical Cluster Analysis for all studied groups combined (cophenetic correlation 0.901) recognised some significant clusters grouping the studied trace elements (Fig. 3): Ca-Mg (BP=99), Cu-Fe (BP=99), Hg-Se (BP=100), Cd-V-As (BP=99) with inner subcluster Cd-V (BP=100) (Fig. 3).

Table 1. Element concentration in little auk samples, median (mean±sd) µg/g dw; all sampled individuals

Table 2. Summary of element concentrations [ug/g dw] in adult body feathers. Difference between males (n=26) and females (n=27) analysed with t-test

Table 3. Results of paired t-test for back (post-breeding) and throat (pre-breeding) feathers of adult little auks

Table 4. Inter-annual consistency in element concentrations (analysis of correlation); based on back feathers

Fig. 3. Hierarchical dendrogram of studied elements in samples collected from little auks (all groups combined), obtained using paired group method and Euclidean distance matrix (distances reflect the degree of association between different elements). Numbers below branches indicate bootstrap probability values (bootstrap n=1000)

4. Discussion

In adult little auks, moulting comprises of two phases and is completed in two areas, so feathers can be used to trace changes in exposure to various elements over an annual cycle and to examine changes in food-chain contamination (Fort et al. 2016). Elemental concentrations in nestlings are affected by fewer factors, as food comes from a more restricted foraging area, but they would depend on excretion rates, degrees of tissue growth and maternal transfer (Wenzel et al. 1996). Until now, studies of contamination in little auks have mostly been performed on livers and muscles (Savinov et al. 2003; Øverjordet et al. 2015; Fife et al. 2015; Borgå et al. 2006; Jæger et al. 2009). Non-lethal techniques make it possible to work with the same individuals over seasons and to monitor survival chances in relation to contaminant exposure (Fenstad et al. 2017).

4.1 Dietary exposure

Seabirds are exposed to contaminants mainly through their diet. Little auks are specialised feeders, and their diet is composed mostly of pelagic zooplankton such as copepods, amphipods, krill, supplemented by small fish (Stempniewicz 2001; Rosing-Asvid et al. 2013; Øverjordet et al. 2015). During chicks' rearing period their diet is dominated by copepods of the Calanus genus (Steen et al. 2007; Wojczulanis et al. 2006, Jakubas et al,. 2011). A strong dietary shift occurs between summer and autumn. After the summer season the availability of their main prey (caught during the breeding period) decreases dramatically, as Calanus spp. migrate to greater depths, becoming inaccessible to little auks (Fort et al. 2010). Thus, birds must feed on alternate prey, such as larger amphipods feeding at higher trophic levels, or small fish what is reflected by higher nitrogen isotopic signatures

(Fort et al. 2010; Stempniewicz 2001; Rosing-Asvid et al. 2013). This change may affect contaminant profile over time.

Evidence for both Hg and Cd biomagnification was found in the food webs of the Arctic fjord (Øverjordet et al. 2015). In particular, high Cd levels could be found in polar zooplankton, which has been referred to as a polar Cd-anomaly (Petri and Zauke 1993; Nygård et al. 2001; Zauke and Schmalenbach 2006), caused by the process of upwelling a naturally Cd-rich deep water (Nygård et al. 2001). A high Cd level was found in some polar crustaceans sampled in the Barents Sea north of Svalbard (up to 4.7 µg/g; Zauke and Schmalenbach 2006), however still little data exist for Little auks main food prey items. Although, in our study Cd was mostly at very low levels, it was nevertheless the highest in chicks down feathers (0.62±0.46 µg/g dw), suggesting at least partial bioaccumulation during a first phase of life.

The background value for Pb in polar copepods was suggested to be low, at about 0.4 µg/g (<0.3–2.7 µg/g). Cu concentration can be high in polar krill (Nygård et al. 2001), but levels reported for copepods was relatively low (6–9 µg/g), whereas Zn level was higher in copepods than in other taxa (108–509 µg/g) (Zauke and Schmalenbach 2006, and references therein). In polar areas close to Canada high levels of several elements was found in *Calanus hyperboreus* eg. As (1.42±0.42 µg/g ww), Cd (1.62±0.79 µg/g ww), Cu (1.55±0.57 µg/g ww), Se (1.87±0.99 µg/g ww), Sr (7.05±1.65 µg/g ww), Zn (17.7±0.65 µg/g ww) (Campbell et al. 2005).

Hg level in major zooplankton prey species collected in East Greenland in the summer season showed a general increase over the years 2007 and 2013, possibly caused by changes in environmental exposure to this element (Fort et al. 2016). Concentration vary between species from 0.032 to 0.081 µg/g dw (Fort et al. 2016). On the other hand total Hg

was not detected in four zooplankton species collected in Kongsfjorden (Svalbard) and low levels were found in fish (<LOD–0.02 μg/g ww) (Jæger et al. 2009). Other studies have also shown low levels of Hg in zooplankton (0.006–0.025 μg g^{-1} ww; Campbell et al. 2005). Metal accumulation in polar zooplankton is clearly species-dependent, with significant differences found within taxa.

4.2 Element deposition in adult feathers (pre- and post-breeding period)

Heavy metals can affect breeding success and the health of an individual, having an adverse effect on endocrine systems at higher concentrations (Nam and Lee 2006; 2011; Lee et al. 2012). A significant change was observed in mercury concentration between feathers grown during the pre- and the post-breeding periods. Hg is a non-essential element of particular concern, due to its high toxicity and impact on breeding success and individuals' health (Fort et al. 2016). It can easily bioaccumulate in organisms, and biomagnifies through food webs (Bustamante et al. 2016; Lucia et al. 2016), and its concentration can be high in Arctic species. To our knowledge, Hg is the only element to have previously been studied in little auk feathers. Fort et al. (2016) measured temporal changes (between 2006 and 2014) in Hg in little auks breeding in East Greenland, finding contamination of birds to be increasing at a rate of 3.4% per year, as determined by body feather analysis. Additionally, researchers also found a decreasing trend in Hg exposure during winter time spent in the northwest Atlantic (as determined by head feather analyses), which was nonetheless still consistently high. The range of Hg concentration in little auk from Greenland were, respectively, 1.00- 2.11 μg/g dw in body feathers and 2.27- 3.73 μg/g dw in head feathers (means between years 2007-2014; Fort et al. 2016). We found even higher values, i.e 1.45-17.2 (mean±SD 5.02±3.32 μg/g dw) and 0.585-2.36 (mean±SD 1.39±0.40 μg/g dw) for head

and body feathers, respectively. Both in our study from Spitsbergen and Fort et al. (2016) study from Greenland, concentration of Hg during the pre-breeding period, which reflects contamination from areas outside breeding grounds, was significantly higher compared to the post-breeding period, which reflects High-Arctic areas. Those results may be interpreted by higher exposition of little auks for contamination on the sub-Arctic foraging grounds being more exposed to human activities (oil drilling, oil spills, etc.) compared to high-Arctic breeding areas. Here no significant difference was found between the levels in body feathers from two years' seasons (Table 4); however, it should be noted that only two consecutive seasons were compared. Thus several factors, including a variation in prey availability, may have affected the final elemental concentrations found in birds.

Isotopic niches occupied by little auks from the colonies in East Greenland and Spitsbergen change throughout their annual cycle (Fort et al. 2010). After the breeding season little auks breeding in Spitsbergen go to moult in the West Greenland Sea (Stempniewicz 2001; Fort et al. 2010; SEATRACK). Females and males exposure for contaminants may differ due to sex-specific diet, spatial and/or temporal differences in stop-over or wintering strategies (Hargreaves et al. 2010). Also females may eliminate some toxic elements by sequestration into eggs (Lam et al. 2005). However, previously no difference in isotopic niche was observed for Little auk males and females through the year (Fort et al. 2010). Here, no significant sex differences in Hg concentration were found for body feathers, representing post-breeding exposure. In fact, significant differences were found only of Ca and Zn, both essential elements, regulated mostly in homeostasis processes. This suggest that both females and males are having similar exposure/foraging areas during a post-breeding period.

Both Hg and Se can be bioaccumulated in significant amounts (Borghesi et al. 2016). Se is an essential element, which level is physiologically regulated within the body and has an important role in the organism's proper functioning (Berry and Ralston 2008). It may also protect an organism against the adverse effects of Hg, by the creation of an Hg-Se complex, as Hg binds to Se with extraordinarily high affinity (Berry and Ralston 2008; Khan and Wang 2009; Øverjordet et al. 2015). Hg toxicity is observed when Hg has a substantial molar excess of Se (Berry and Ralston 2008). A high hepatic Se:Hg molar ratio has been observed in the little auk (17.23–29.63 depending on sampling year and region), suggesting a low risk of Hg toxicity (Øverjordet et al. 2015). In the present study, levels of both Hg and Se were much higher in adult head feathers, but with a molar ratio of 1.91 in head feathers and 2.27 in body feathers. At the same time Se:Hg ratio was below 1 in 35% of head feather samples and 13 % of body feather samples, suggesting in those birds possible toxic effects of Hg.

In general, apart from Hg and Sr, concentrations of other non-essential elements in the studied adult little auks were low. Sr, being a Ca analogue, accumulates mainly in calcium-rich structures (Chowdhury and Blust, 2001), with some evidence for trophic biomagnification (Campbell et al. 2005). Here little variation was found in Sr, as in POSTBR feathers Sr concentration was 14.0±3.60 µg/g dw, whereas in PREBR 11.2±4.44 µg/g dw.

Cd is naturally abundant in the North Pacific, which may result in a net flux of Cd-rich water to the Atlantic side of the Arctic Ocean, potentially leading to high Cd levels in Arctic water (Øverjordet et al. 2015). Cd is easily absorbed by the chitinous structure of zooplankton (Dietz et al. 1996; Øverjordet et al. 2015). However, feathers are not its target tissue, as only about 30% of body load is deposited in them, while the highest concentration is usually found in the kidneys and liver (Friberg et al., 1979; Burgos-Núñez et al. 2017). Here, the Cd concentration in adult feathers was relatively low (mean 0.306±0.214 µg/g dw in

455 body feathers; <LOQ in head feathers; no significant difference). Conversely to Cd, a high
456 body Pb load can be accumulated in feathers (Burger, 1993; Burgos-Núñez et al. 2017). Here,
457 no difference in Pb concentration was found between body and head feathers, nor between
458 females and males during post-breeding period. However, comparing results from two
459 seasons for the same group of birds, statistically significant inter-annual variances were
460 found only for Pb. Since our data have been collected in just two seasons, a further long-
461 term study should be performed to investigate changes in the food chain contamination.

462 Arsenic is a ubiquitous metalloid classified as toxic, and although it does not
463 biomagnify (Hargreaves et al. 2011), it can accumulate in bird tissue (such as the liver, bone
464 and feathers) in a dose-dependent way (Sánchez-Virosta et al. 2018). Here, levels of As were
465 low – <0.2 µg/g dw in body feathers, and <LOD in head feathers. When excluding outliers,
466 levels of As, Pb, and Cd did not suggest any potential ecotoxicological risk.

467 The highest absolute elemental concentration in both back and throat feathers
468 were found for Mg, Ca and Zn. Those elements are necessary for proper feather formation,
469 and are regulated mostly by homeostasis processes (Bocher et al. 2003). Cu, Fe and Zn have
470 multiple biological functions in the body, and its internal levels are in general well-regulated
471 (Friberg et al. 1979). Of those three elements Fe was characterised by a highest variability,
472 from <LOD-1289 and <LOD-598 µg/g in head and body feathers, respectively.

473

474 **4.3 Toxicity thresholds**

475 Toxicity thresholds were examined so far only in some species. Based on laboratory
476 studies, feathers are known to have shown element levels associated with adverse effects in
477 the cases of Hg, Pb and Cd (5, 4 and 2 µg/g dw, respectively; Burger and Gochfeld 1997). In
478 our study, 35% of all analysed adult birds exceed the toxicity threshold value for Hg during

the pre-breeding period, with 11% individuals having concentrations > 10 μg/g dw with the maximal level of 17.2 μg/g dw. No individuals exceeded 5 μg/g dw during the post-breeding time.

For Cd, only one individual exceeded 2 μg/g dw during the post-breeding period, and none during pre-breeding time. For Pb, 10% of adult individuals exceeded 4 μg/g dw during the post-breeding period, with the maximum value of 11 μg/g dw. During the pre-breeding period 16% of individuals has concentrations >4 μg/g dw, with the highest value >20 μg/g dw. Although the percentage of outliers for Pb is relatively low, a special attention should be paid in future studies, as long-term conditions may alter in the Arctic, resulting in a changes in birds' exposure.

4.4 Maternal transfer (chicks down, eggshells) and regional (chick body feathers) elemental input

Seabirds often increase their feeding rates and food niche width during or shortly before egg formation, making local food the main source of metal burden in eggs (Wenzel et al. 1996). As females can deposit a substantial proportion of contaminants in eggs, chicks are exposed from the beginning to some defined input of contaminants. Feathers collected from chicks, can be used to investigate birds' exposure from breeding grounds (Evers et al. 2005). Chick down feathers are formed in the egg from maternal nutrients, and eggshells thus represent female contamination during the pre-laying time (Ackerman et al. 2016).

In the newly hatched nestlings, elemental allocation to the down feathers serve as the major elimination pathway for mercury delivered by the mother (Wenzel et al. 1996). Here, Hg level in down feathers was higher than in chicks' body feathers, but lower than in adults. Se level in down feathers was higher comparing to chick body feather with 2.53±0.59

µg/g and >66% below detection limit, respectively. Also, the highest Se:Hg molar ratio was found in down feathers (5.79). Se transferred from females plays a significant role during prenatal mercury intoxication (Berry and Ralston 2008). The proportion of maternal transfer depends on the female body's load, as an increase in female Hg concentration resulted in a decreased proportion being transferred to chicks, and this effect was even stronger for Se (Ackerman et al. 2016). Female overall elemental concentration and contaminant type may have an impact on maternal transfer (Ackerman et al. 2016). Although correlations between mother Hg levels in feathers and in eggs were not found (Lewis et al. 1993; Ackerman et al. 2016), this was not true for head feathers in some species, for which feather Hg levels also correlated with internal Hg concentration (e.g in some waders, Ackerman et al. 2016).

The newly-grown feathers of nestlings represent the body load of elements, unaffected by migration or moulting (Sánchez-Virosta et al. 2018). In chicks, of all feather samples, the concentrations of several non-essential elements such as As, Bi, and Cd were highest in down feathers, being even higher than in adults. The concentration of Sr was about twice as high as in the adult feathers.

The nutritional deficiencies in the chicks diet, especially in Ca, could result in delayed growth. Ca level tends to increase in little auk chick's body after the 10^{th} day of its life, suggesting intense ossification of the skeleton at that time (Taylor and Konarzewski 1992). This could also be a reason for the Ca concentration being very high in down feathers (238±65.6 µg/g dw), and much lower in body feathers, collected at the later phase of the nesting period (30.2±12.1 µg/g dw). Mg and K levels were suggested to be relatively stable throughout the little auk's early development (Taylor and Konarzewski 1992). However, in feathers we found high variability in the levels of those two elements, with Mg being 3.5

higher in down feathers than in the later-formed body feathers, and K being exceptionally high in body feathers, but below the quantification limit in down feathers.

Both Cu and Zn levels in organism can be regulated metabolically (Wenzel et al. 1996), and both were found in high concentrations in chick body feathers (28.5±6.44 and 105±18.1 µg/g dw, respectively). Nestling plumage still has blood connection therefore it may contain more active metals like copper and zinc compared to pure keratin (Costa et al. 2013). Nygård et al. (2001) found very high Cu levels in the Antarctic petrel *Thalassoica antarctica* nestlings during the first 28 days spend in the nest. High concentration has been found in liver, suggesting that it may by connected with physiological development processes (Nygård et al. 2001). Also it could be a result of diet exposure, as Cu and Zn can be high in herbivorous calanoids (Battuello et al. 2017).

An elevated level of As in nestlings may affect skeletal and wing growth rate, possibly caused by an interaction between As and the mineral fraction of the bone. Also, it may lower fledging success and chick survival (Sánchez-Virosta et al. 2018). Here around 20% of chicks had > 3 µg/g dw of As in body feathers, but with median for all chicks being 0.31 µg/g dw. Arsenic concentration in down feathers was higher with a median being 1.33 µg/g. More studies would be useful to conclude whether As constitute a toxicological risk for little auks.

Although eggshells serve as a potential tool for non-lethal sampling, they are less commonly used in exposure assessment studies, comparing to other biomaterials. Eggshells protect embryo from external environment and is a source of Ca for its skeletal growth and calcification (Reynolds et al., 2004; Reynolds and Perrins, 2010). The developing embryo is absorbing Ca and other elements firstly from the yolk, and then from the eggshell (Orłowski et al. 2017). The eggshell is a main source of Ca and Mg during embryogenesis (Orłowski et al. 2017).

Eggshells were proven to be suitable as bioindicators for terrestrial birds, with a discrepancy between essential and non-essential elements accumulation (Dauwe et al. 1999). The metabolic pathway of calcium in the eggshell may be interacted by toxic elements (Dauwe et al. 1999). Higher levels of Pb and As in eggshells of terrestrial birds than in the egg content have been reported (Dauwe et al. 1999). Egg contents have significantly higher Hg concentrations than the hardened part of the eggshell (Peterson et al. 2017). However, strong positive correlations have been found between total Hg concentration in eggshells and in egg content, with the relationship being species-dependent, and also dependent on the age of the embryo and cleanliness of the eggshell (Peterson et al. 2017). Here, the hardened eggshell of the little auk, beside being calcium-rich, was also characterised by high levels of Mg, Sr and K, and relatively high levels of Ba and Fe. Several elements including Bi, Cd, Cr, Hg, Ni and Pb were below reliable analytical limits of quantification.

4.6 Limitations of our study

Beside Hg, most elemental concentrations in feathers of adults may be biased because of an external contamination, which cannot be fully removed even after extensive cleaning (Dauwe et al. 2003; Borghesi et al. 2016). Thus, it should be noted that adult feathers in particular are less reliable as indicators for internal uptake in case of Cd, Co, Fe, Li, Pb, Se, Mn, Cr, V (Hargreaves et al. 2010; Borghesi et al. 2016), and instead give information on integrated exposure. This may be the reason for several outliers observed in our samples collected from adult birds, especially for As, Ba, Bi, Cd, Cr, Cu, Fe, Mn, Pb and Se. However, it can be difficult to differentiate whether an outlier is caused by external contamination, or due to high internal uptake. Here, all feathers were treated by the same

cleaning procedure that has proven useful in previous studies (Dauwe et al. 2003; Jaspers et al. 2004; Pacyna et al. 2019a). Thus, although we cannot fully reject the possibility of externally contaminated samples, this risk was reduced as much as possible.

5. Conclusion

By collecting different types of little auk feathers, one may establish contaminant exposure during various phases of the annual cycle. Adults cover vast areas during their migration, and particular types of feather growing in various periods give information about their exposure during pre- and post-breeding time. Here, statistically significant differences between feathers collected at representative times, was only for Hg, Ca, Mn and Sr. Almost one third of adult birds exceeded the toxicity threshold value for Hg during the pre-breeding time, however for other toxic elements levels in birds were mostly low. Contaminant profile between chicks body and down feathers differs, with higher level of K, Zn, Cu in chicks body feathers and 50% of analysed elements below analytical limits of detection. Chicks down were characterized by higher level of several elements including Hg and Se.

Understanding the impacts of human activities and future climate change on the exposure of polar species to contaminants is treated as a research priority by the Arctic Council (AMAP 2011, 2012). Studies on avian species are becoming an effective way to understand global processes occurring in the marine ecosystem. There is still scarce information available concerning elemental exposure throughout the course of the year in seabirds along the coastal areas of the European Arctic, thus our study aims to enhance our understanding of this issue.

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Table

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Tab.1 Elemental concentration in little auk samples median (mean±sd) µg/g dw; all sampled individuals; Se:Hg molar ratio calculated only when more than 65% of both Se and Hg were above LOD

Element	*Adult body (n=74)*	*Adult throat (n=74)*	*Chick feather (n=18)*	*Chick feather down (n=16)*	*Eggshells (n=18)*
As	0.146 (0.199±0.196)**	**68%<LOD ; 80%<LOQ**	0.31 (1.38±2.83)*	1.33 (1.43±1.08)	0.050 (0.061±0.039)
Ba	2.63 (2.88±2.99)	0.651 (3.59±5.41)*	**65%<LOD**	**65%<LOD**	9.77 (9.53±3.48)
Bi	**90%<LOD**	**90%<LOD**	**65%<LOQ (possibly contaminated)**	0.91 (2.43±3.21)	**88%<LOD**
Ca	151.2 (154.5±59.2)	83.1 (101.2±82.6)*	26.9 (30.2±12.1)	235.0 (238±65.6)	43571 (41669±8583)
Cd	0.242 (0.306±0.214)*	**92%<LOQ**	**72%<LOQ**	0.53 (0.62±0.46)	**83%<LOQ**
Co	**75%<LOD**	**75%<LOD**	**82%<LOD**	**82%<LOD**	0.211 (0.227±0.093)
Cr	0.115 (0.737±1.40)*	0.296 (1.38±2.20)*	**72%<LOQ**	**68%<LOD**	**66%<LOD**
Cu	16.7 (17.3±5.98)	14.3 (16.7±10.9)**	28.5 (28.5±6.44)	13.03 (12.5±2.99)	0.522 (0.513±0.314)
Fe	20.4 (58.3±111)*	25.4 (102.3±200.9)**	15.8 (44.6±55.9)	22.1 (23.5±12.6)*	9.42 (11.1±6.94)
Hg	1.38 (1.39±0.397)	4.27 (5.02±3.32)	0.52 (0.57±0.17)	1.08 (1.11±0.41)	**100%<LOD**
Mg	530.8 (536±112)	521.4 (542±156)	155.6 (152±29.2)	546.1 (544±110)	1557 (1563±237)
Mn	0.269 (0.515±0.664)**	0.573 (3.12±4.29)	**84%<LOD**	4.29 (5.49±4.13)	0.653 (0.821±0.429)
Ni	**85%<LOD**	**85%<LOD**	**100%<LOD**	**100%<LOD**	**88%<LOD**
Pb	0.675 (1.23±1.66)*	0.411 (1.53±2.43)**	**66%<LOD**	0.08 (0.32±0.42)	**80%<LOD**
Se	1.028 (1.03±0.44)*	2.55 (2.63±1.61)	**66%<LOD**	2.51 (2.53±0.59)	0.267 (0.277±0.112)
Sr	13.4 (14.0±3.60)	10.4 (11.2±4.44)	4.54	24.1	2052 (2093±267)

			(4.80±1.11)	(23.4±4.76)	
V	0.082 (0.104±0.096)**	0.123 (0.181±0.164)**	0.02 (0.31±0.66)*	0.06 (0.08±0.09)*	0.004 (0.007±0.007)
Zn	153.8 (154.5±50.2)	136.3 (158±99.4)	106.5 (105±18.1)	61.1 (63.9±15.1)	1.35 (1.65±0.92)
K	**98%<LOQ**	**98%<LOQ**	829.3 (819±458)	**83%<LOQ**	411.6 (428±72.7)
Se:Hg	**2.27**	**1.91**	-	**6.49**	-

*One outlier excluded; ** two outliers excluded

Table

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Tab.2 Summary of elements concentration [ug/g dw] in adults body feathers. Males (N=26) and females(N=27) difference analysed with t-test

Element	*Male_mean*	*Male_SD*	*Female_mean*	*Female_SD*	*t*	*p*
As	0.768	2.80	0.278	0.489	-0.882	0.386
Ba	2.44	3.30	3.12	2.89	0.807	0.424
Ca	135	49.6	169	57.9	2.31	**0.025**
Cd	0.405	0.424	0.278	0.238	-1.35	0.185
Cr	0.937	1.66	0.731	1.45	-0.483	0.631
Cu	17.2	3.60	18.0	8.81	0.440	0.662
Fe	61.6	130.4	62.2	122	0.017	0.986
Hg	1.27	0.441	1.49	0.355	1.95	0.057
Mg	532	116	552	108	0.657	0.514
Mn	0.526	0.637	0.521	0.791	-0.027	0.979
Pb	1.56	2.58	1.45	1.88	-0.179	0.859
Se	1.26	1.73	1.06	0.45	1.87	0.12
Sr	13.6	3.90	14.6	3.89	1.03	0.309
V	0.614	2.65	0.115	0.124	-0.960	0.346
Zn	137	46.9	168	51.0	2.39	**0.021**

Table

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Tab.3 Results of paired t-test for body (POSTBR) and throat (PREBR) feathers of adult little auk

Element	*t*	*p*
As	-0.61	0.542
Ba	-1.38	0.173
Ca	2.65	**0.01**
Cd	1.32	0.192
Cr	-1.64	0.105
Cu	-1.28	0.206
Fe	-0.86	0.394
Hg	-9.24	**0.0**
Mg	-0.32	0.753
Mn	-3.2	**0.002**
Pb	-1.3	0.197
Se	0.613	0.544
Sr	4.75	**0.0**
V	0.54	0.594
Zn	-0.44	0.658

Table

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Tab.4 Interannual consistency in elements concentration (analysis of correlation); based on body feathers

Element	*r*	*t*	*p*
Ba	0.2	0.84	0.414
Ca	0.39	1.75	0.098
Cd	-0.04	-0.18	0.861
Cr	0.14	0.58	0.573
Cu	0.31	1.35	0.196
Fe	0.42	1.91	0.074
Hg	0.04	0.18	0.862
Mg	0.14	0.58	0.571
Mn	0.26	1.1	0.289
Pb	0.55	2.68	**0.016**
Se	0.35	2.65	0.16
Sr	0.36	1.6	0.129
V	0.22	0.91	0.374
Zn	0.2	0.85	0.408

Figure

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Fig. 1 Study site; map source: toposvalbard.npolar.no; photo by KWJ

Figure

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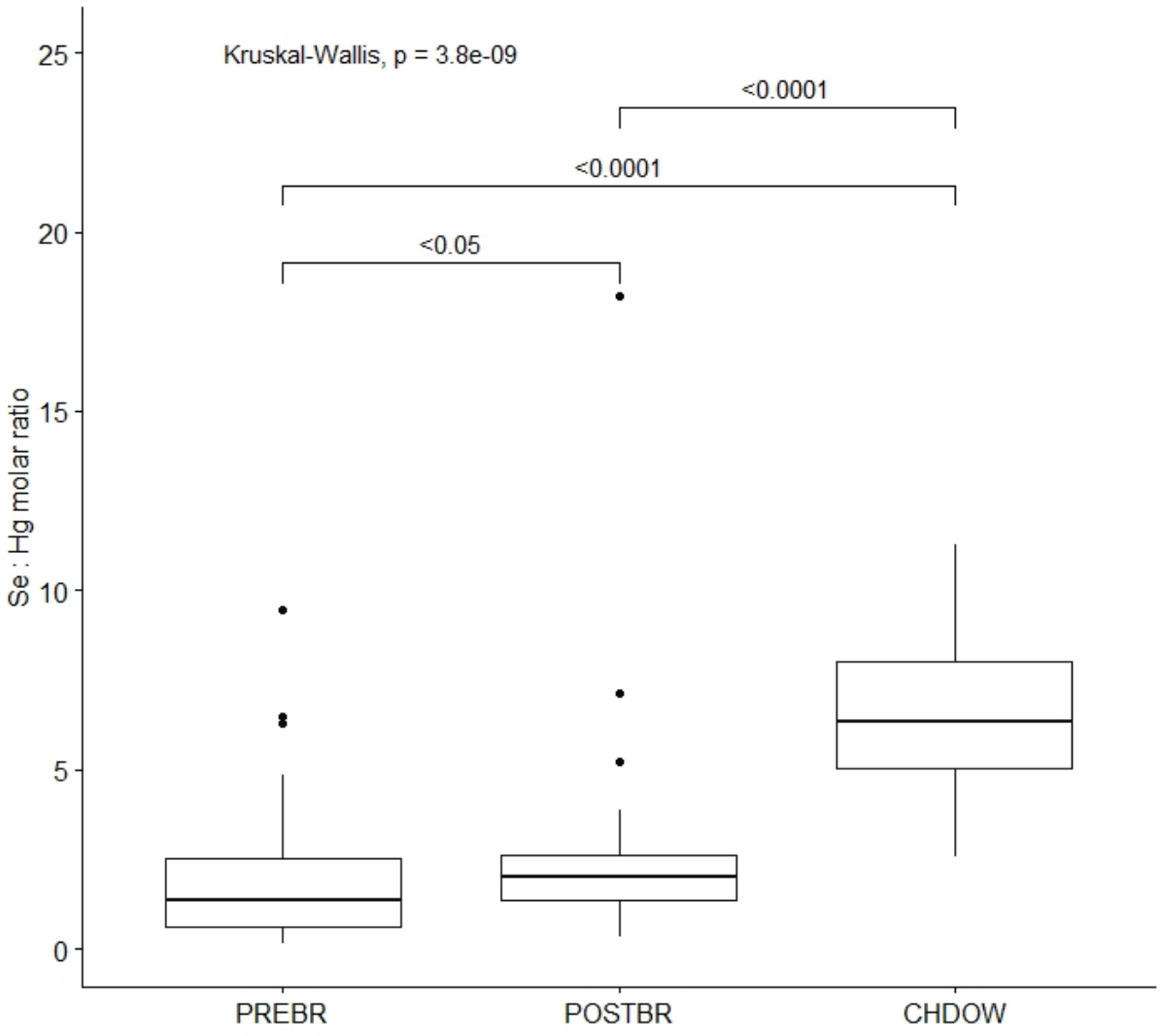
Fig.2 Se:Hg molar ratio in studied groups (PREBR- feathers grown during pre-breeding period; POSTBR- feathers grown during post-breeding period; CHDOW- chicks down)



Figure
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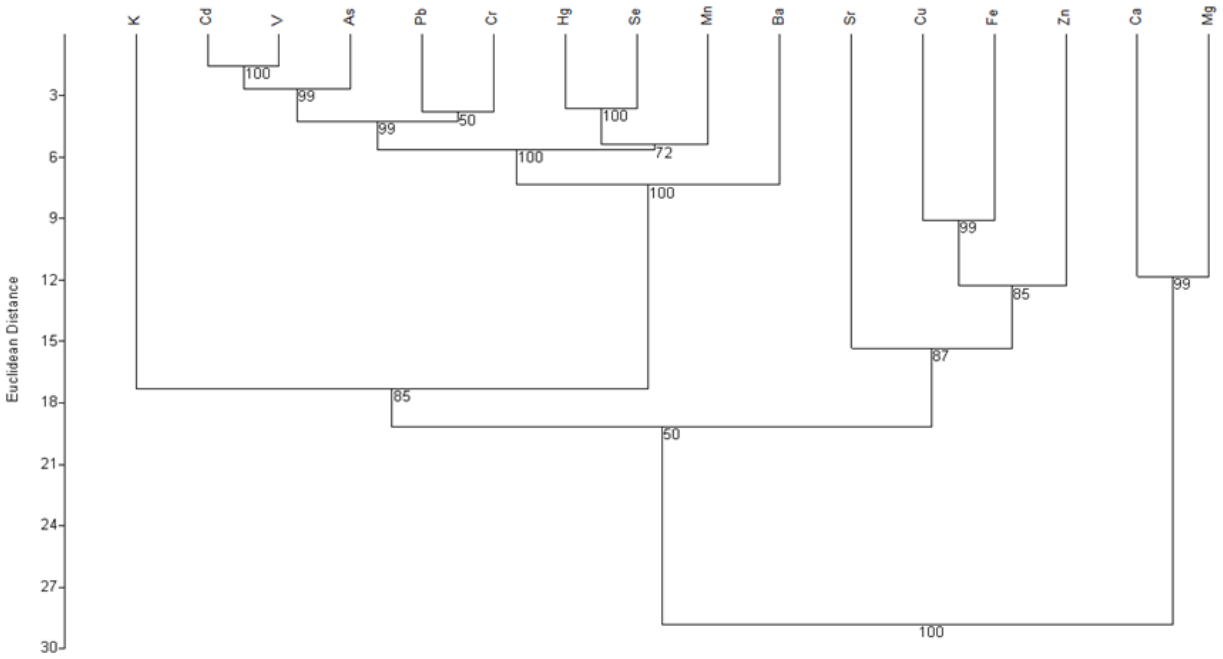
Fig. 3 Hierarchical dendrogram of the studied elements in the samples collected from the little auks (all groups combined), obtained using a paired group method and Euclidean distance matrix (the distances reflect the degree of association between different elements). Numbers below branches indicate bootstrap probability values (bootstrap n = 1000)



Supplementary material for on-line publication only

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13. Podsumowanie

Obszary polarne znajdujące się na półkuli północnej oraz południowej to rejony odizolowane geograficznie, o wyjątkowo surowym klimacie. Prowadzenie badań na obszarach polarnych jest utrudnione ze względu na bardzo zmienne warunki pogodowe, trudności z poruszaniem się w terenie, oraz logistykę związaną z transportem ludzi oraz próbek. Jednocześnie badania tam prowadzone są niezwykle cenne ze względu na globalny charakter zjawisk zachodzących na obszarach polarnych. Trwałe związki organiczne, takie jak wielopierścieniowe węglowodory aromatyczne, polichlorowane bifenyle oraz pestycydy, jak również wybrane metale i metalloidy mogą znacząco wpłynąć na równowagę ekologiczną ekosystemu polarnego. Wykorzystanie nie-destrukcyjnie pobranych tkanek takich jak sierść oraz pióra umożliwia pobieranie próbek od żywych osobników, nie naruszając struktury populacji oraz kwestii moralnych związanych z odłowem.

Geograficzne odizolowanie oraz specyficzne warunki klimatyczne sprawiają, iż rejony polarne są zamieszkiwane przez ograniczoną liczbę gatunków. Mniejsza różnorodność równoważona jest w przypadku wielu gatunków niezwykle dużą liczebnością, sprawiając iż gatunki te stały się ważną częścią ekosystemu polarnego. Dodatkowy czynnik stresowy w postaci większego zanieczyszczenia środowiska może osłabić zdolności przetrwania i bezpośrednio wpłynąć na stan populacji. Stąd badania nad poznaniem stopnia akumulacji oraz sposobów eliminacji z organizmu związków toksycznych dla reprezentatywnych gatunków polarnych, przyczynią się do lepszego zrozumienia wpływu emisji zanieczyszczeń na faunę Arktyki oraz Antarktyki oraz zrozumieniu odpowiedzi fizjologicznej organizmów polarnych na zwiększające się zanieczyszczenie środowiska.

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15. Spis dorobku naukowego

a) Publikacje

<u>Artykuły z listy JCR</u>

1) **Aneta Dorota Pacyna**, Carlos Zumalacárregui Martínez, David Miguélez , Frédéric Jiguet, Żaneta Polkowska, Katarzyna Wojczulanis-Jakubas, Mercury contamination, a new potential threat to the globally threatened Aquatic Warbler *Acrocephalus paludicola*?, Environmental Science and Research Pollution, 2017, https://doi.org/10.1007/s11356-017-0201-1 [IF: 2,914; Q2]

2) **Aneta Dorota Pacyna,** Katarzyna Koziorowska, Stanisław Chmiel, Jan Mazerski, Żaneta Polkowska, Svalbard reindeer as an indicator of ecosystem changes in the Arctic terrestrial ecosystem, Chemosphere, 2018, DOI: 10.1016/j.chemosphere.2018.03.158 [IF: 5,108; Q1]

3) **Aneta Dorota Pacyna**, Marek Ruman, Jan Mazerski, Żaneta Polkowska, Biological responses into environmental contamination. How metal pollution may impact signal honesty in avian species? Ecology and Evolution, 2018, DOI:10.1002/ece3.4192 [IF:2,415; Q1]

4) **Aneta Dorota Pacyna**, Marcin Frankowski, Krystyna Kozioł, Michał Hubert Węgrzyn, Paulina Wietrzyk-Pełka, Sara Lehmann-Konera, Żaneta Polkowska, Evaluation of the use of reindeer droppings for monitoring essential and non-essential elements in the polar terrestrial environment, Science of the Total Environment, 2019, DOI: 10.1016/j.scitotenv.2018.12.232 [IF: 5,589; Q1]

5) **Aneta Dorota Pacyna**, Dariusz Jakubas, Anne N.M.A. Ausems, Marcin Frankowski, Żaneta Polkowska, Katarzyna Wojczulanis-Jakubas, Storm petrels as indicators of pelagic seabird exposure to chemical elements in the Antarctic marine ecosystem, Science of The Total Environment, DOI: doi.org/10.1016/j.scitotenv.2019.07.137 [IF: 5,589; Q1]

6) **Aneta Dorota Pacyna-Kuchta** Paulina Wietrzyk-Pełka, Michał Hubert Węgrzyn, Marcin Frankowski, Żaneta Polkowska, A screening of select toxic and essential elements and persistent organic pollutants in the fur of Svalbard reindeer, Chemosphere [przyjęty do druku] [IF: 5,108; Q1]

7) **A. D. Pacyna-Kuchta**, D. Jakubas, M. Frankowski, Ż. Polkowska, K. Wojczulanis-Jakubas, Exposure of a small Arctic seabird, the little auk (*Alle alle*), to selected elements throughout the course of a year (w recenzji czasopisma Science of the Total Environment) [IF: 5,589; Q1]

Publikacje z listy MNiSW

Aneta Pacyna, Żaneta Polkowska, 2017. Materiały biologiczne pochodzące od ptaków jako źródło informacji o zanieczyszczeniu środowiska Cz. I. Wykorzystanie piór ptaków w celu biomonitoringu środowiska, Analityka- Nauka i Praktyka

b) Rozdziały w monografii:

Aneta Pacyna, Krystyna Kozioł, Marek Ruman, Żaneta Polkowska, The occurrence of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in the chosen area of Svalbard, New perspectives in polar research, 85-96. ISBN 978-83-62673-47-6

Katarzyna Kozak, Małgorzata Szopińska, **Aneta Pacyna,** Krystyna Kozioł, Sara Lehmann, Żaneta Polkowska, Anthropopressure's intensification with reference to Arctic ecosystems, New perspectives in polar research, 69-84. ISBN 978-83-62673-47-6

c) Udział w konferencjach (wystapienia ustne oraz postery):

Proceedings of the 11th ISC Modern Analytical Chemistry, 2015, Praga, Czechy (prezentacja, praca pokonferencyjna)

UK Arctic Science Conference 2015, Sheffield, Wielka Brytania (poster)

XXXVI Sympozjum Polarne "Progress in polar research – new experiences and challenges", 2016, Lublin, Polska (prezentacja)

Impact of climate change and pollution on vegetation distribution and condition in the temperate, boreal, alpine and polar zones, 2016, Warszawa, Polska (prezentacja)

The Arctic Science Summit Week 2017, Praga, Czechy (poster)

Interdisciplinary Polar Studies in Poland, 2017, Warszawa, Polska (poster)

XXXVII Sympozjum Polarne Polar Change- Global Change, 2018, Poznań, Polska (poster)

The 39th International Symposium on Halogenated Persistent Organic Pollutants, 2019, Kyoto, Japonia (poster)

16. Wyjazdy stażowe

01.09.2015-30.12.2015 Odbycie stażu zagranicznego na Wydziale Bionauki Uniwersytu Aarhus, w Danii w ramach projektu „Advanced PhD" , Roskilde, Dania

09. 2015 Udział w wyprawie polarnej w ramach projektu grantowego nr 2013/09/N/ST10/04191, finansowanego przez Narodowe Centrum Nauki, Polska Stacja Polarna im. Stanisława Siedleckiego, Spitsbergen, Arktyka

17. Uzyskane stypendia

2014/2015 Stypendium Rektora dla najlepszych doktorantów, Politechnika Gdańska

09.2015- 12.2015 Stypendium na odbycie stażu zagranicznego w wybranym ośrodku naukowym (Uniwersytet Aarhus, Dania) w ramach programu *Advanced PhD*

2017 Stypendium Santander Universidades dla pracowników i doktorantów Politechniki Gdańskiej

2017/2018 Stypendium Rektora dla najlepszych doktorantów, Politechnika Gdańska

2019/2020 Stypendium zadaniowe z programu POWER 3.5, Politechnika Gdańska

08.2019 Sfinansowanie udziału w konferencji The 39th International Symposium on Halogenated Persistent Organic Pollutants, 2019, Kyoto, Japonia z programu *PROM- Międzynarodowa wymiana stypendialna doktorantów i kadry akademickiej w zakresie nowoczesnych technologii*

18. Oświadczenia współautorów o współudziale w pisaniu publikacji