

Seabird transported contaminants are dispersed in island ecosystems

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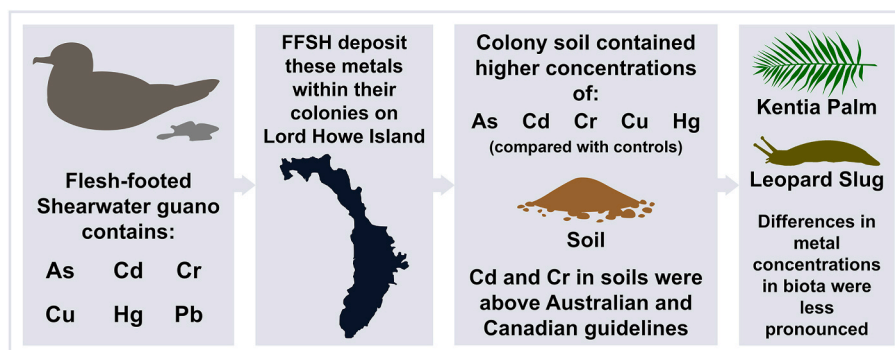
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HIGHLIGHTS

- Flesh-footed Shearwaters are vectors for metals and metalloids to Lord Howe Island.
- Guano metal concentrations were greater than Procellariiformes in the literature.
- As, Cd, Cu and Hg concentrations were higher in soils from colonies versus controls.
- The concentration of Cd and Cr in colony soils exceeds soil guidelines.

GRAPHICAL ABSTRACT



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ABSTRACT

Seabirds are long-range transporters of nutrients and contaminants, linking marine feeding areas with terrestrial breeding and roosting sites. By depositing nutrient-rich guano, which acts as a fertiliser, seabirds can substantially influence the terrestrial environment in which they reside. However, increasing pollution of the marine environment has resulted in guano becoming similarly polluted. Here, we determined metal and metalloid concentrations (As, Cd, Cr, Cu, Hg, Pb) in Flesh-footed Shearwater (*Ardenna carneipes*) guano, soil, terrestrial flora, and primary consumers and used an ecological approach to assess whether the trace elements in guano were bioaccumulating and contaminating the surrounding environment. Concentrations in guano were higher than those of other Procellariiformes documented in the literature, which may be influenced by the high amounts of plastics that this species of shearwater ingests. Soil samples from shearwater colonies had significantly higher concentrations of all metals, except for Pb, than soils from control sites and formerly occupied areas. Concentrations in terrestrial primary producers and primary consumers were not as marked, and for many contaminants there was no significant difference observed across levels of ornithogenic input. We conclude that Flesh-footed Shearwaters are transporters of marine derived contaminants to the Lord Howe Island terrestrial environment.

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1. Introduction

Long-range transport of contaminants refers to the movement of potentially toxic substances (e.g., metals, persistent organic pollutants (POPs), radioactive material) over long distances through the atmosphere and waterways (Franklin, 2006). Typically, these contaminants are released from industrial, agricultural, and municipal regions and are often deposited to areas where they were never produced or used (e.g., the Arctic; Macdonald et al., 2000). Thus, long-range transport can pose a risk to fragile environments that are otherwise relatively pristine or untouched by humans (Kallenborn, 2006). Deposited contaminants can have a large effect on ecosystems through contaminating soil, waterbodies, and biota (Santamans et al., 2017). Contaminants can bioaccumulate and biomagnify in fauna and flora which can lead to a range of negative consequences, including adverse effects on their growth, development, reproductive capabilities, and survival (Connan and Stengel, 2011; Aulsebrook et al., 2020), while prolonged exposure can have significant long-term impacts on entire ecological systems (Peter-son et al., 2003).

While physical systems are responsible for the bulk movement of contaminants throughout the world, mobile organisms also play a considerable role, and the impact can be greater when the organisms responsible congregate in large numbers at recipient sites (Blais et al., 2007; Cipro et al., 2018). Organisms such as birds, fish, and insects can transport pollutants through their consumption and excretion of contaminated food (Wing et al., 2014), and can move contaminants great distances, especially if they are migratory (Bauer and Hoye, 2014). The transfer of contaminants across geographic boundaries by organisms has been reported for fish and seals (e.g., salmon (*Oncorhynchus* spp.) transport marine derived contaminants to their spawning grounds; Ewald et al., 1998; elephant seals (*Mirounga leonine*) deposit metals obtained through their prey to their terrestrial haulouts; Celis et al., 2022), but perhaps the most well-known vector of metals from the marine environment to land are seabirds (Marmen et al., 2017).

Seabirds often have elevated concentrations of contaminants in their tissues because of their high trophic position (Shoji et al., 2019). When seabirds return to land to breed or roost, they can deposit these contaminants in their guano and other allochthonous inputs such as feathers, egg remains and carcasses (Otero et al., 2018). As seabird colonies are often dense aggregations of individuals, the deposition of guano can lead to hotspots of contamination (Evenset et al., 2007), with concentrations of some metals being orders of magnitude higher than in surrounding areas without the influence of seabirds (Headley, 1996; Brimble et al., 2009).

For islands that are often host to vast seabird colonies, the island ecosystems can benefit from the nutrients deposited by the birds through improved soil nutrient status and increased primary production (Polis and Hurd, 1996; Gonzalez-Bergonzoni et al., 2017). Much of the research on seabirds as vectors has focused on the nutrient inputs from guano (Grant et al., 2022), however, with increasing pollution of the marine environment (Riechers et al., 2021), for many seabird populations worldwide their nutrient-rich guano and other inputs are increasingly being found to contain metals and POPs which can contaminate their terrestrial breeding and roosting grounds (Perfetti-Bolaño et al., 2018; Shoji et al., 2019). Thus, many islands that are known wildlife refuges from human habitation or destruction may potentially be inadvertently negatively impacted by the presence of seabirds.

While there have been a number of studies that have examined seabird-derived contaminants in recipient environments, including measuring the concentration and impacts of contaminants in soils, flora, and fauna (Headley, 1996; Blais et al., 2005), few have specifically measured the concentration in seabird guano (Geizer et al., 2021; Grant et al., 2022), which is the main seabird input into terrestrial breeding and roosting grounds (Bukancinski et al., 1994). Thus, the aim of this paper was to take a holistic approach by measuring the concentrations of

potentially harmful metals and metalloids (As, Cd, Cr, Cu, Hg, Pb; hereafter referred to as metals) in seabird guano as well as in soils, vegetation, and soil invertebrates from an active seabird colony, an abandoned colony, and an area that has never been occupied by seabirds to determine if marine-derived contaminants are accumulating and persisting in the terrestrial environment.

2. Materials and methods

2.1. Study area

Lord Howe Island (31°33'S, 159°05'E) is in the Tasman Sea, approximately 600 km east of mainland Australia. It is home to the world's largest population of Flesh-footed Shearwaters, with approximately 22,000 breeding pairs (Lavers et al., 2019). Flesh-footed Shearwaters ingest significant quantities of plastic debris, with 88.7% of necropsied individuals containing plastics (Lavers et al., 2021). These birds also have high contaminant loads, due in part to being apex predators (Bond & Lavers, 2011, 2020), but also from the sorption of contaminants to the plastics they consume (Lavers et al., 2014). There are six colonies spread across the northern half of the island (Ned's Beach, Clear Place, Little Muttonbird Ground, Middle Beach, Old Settlement Beach, and Steven's Point). In terms of vegetation, these are dominated by the endemic kentia palm (*Howea forsteriana*), and a few other species including the Lord Howe Island banyan (*Ficus macrophylla* subsp. *Columnaris*; Sheringham et al., 2016). We sampled across a gradient of seabird influence, including a currently occupied Flesh-footed Shearwater colony (Clear Place; ~13,400 breeding pairs; Lavers et al., 2019), a formerly occupied site that was abandoned in the

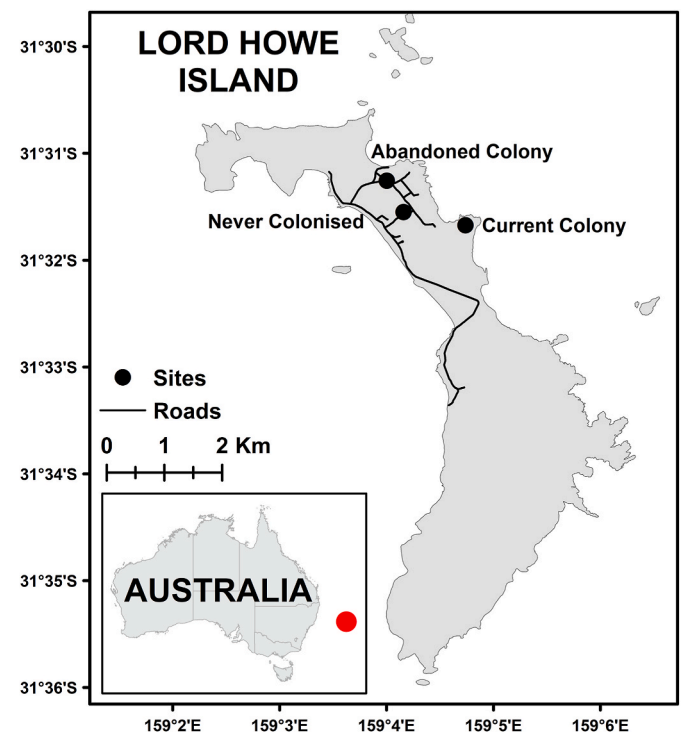


Fig. 1. Location of each site on Lord Howe Island (red circle in inset map). 'Current Colony' is in the Flesh-footed Shearwater (*Ardenna carneipes*) colony known as Clear Place and has an active breeding colony of approximately 13,400 pairs (Lavers et al., 2019). 'Abandoned colony' is in an area that had an active Flesh-footed Shearwater colony approximately twenty years ago but has since been abandoned. 'Never colonised' is in an area with similar forest and soil structure to the other two sites but has never had a colony of Flesh-footed Shearwaters, or any other seabirds, in recorded history (i.e., since human habitation commenced in 1833). Sealed roads are indicated by the black lines.

last twenty years (see Fig. 1; Lavers et al., 2019), and a site that has no recorded history of seabird occupation (i.e., in the 190 years since humans colonised the island in 1833; Paramonov 1960). All sites had similar substrates (sandy calcarenite soils) and vegetation communities, though the abandoned site and uncolonised site have a greater variety of woody vegetation (Sheringham et al., 2016), and were separated by at least 600 m (Fig. 1).

2.2. Sample collection

Most samples were collected in a two-week period between 26 April – May 10, 2019. Five 3 × 3 m quadrats were randomly selected at each site, and soil samples from depths of 0–5 cm and 25–30 cm were collected using a clean metal trowel from a single point within each quadrat (the trowel was cleaned with 80% ethanol (trace metal grade, Sigma-Aldrich) between each sample). These depths were chosen to represent soil that is most likely in contact with shearwaters and guano, with 0–5 cm representative of soil receiving guano directly from shearwaters as they traverse across the colony's surface and 25–30 cm reflecting soil that has been in contact with guano within burrows. Approximately 500 g of soil from each depth was placed in sterile, plastic snap-lock bags. Soil samples were air dried until a constant mass was achieved (approximately 5 days). From each quadrat, we also cut the youngest, fully expanded leaf from a kentia palm frond from the parent plant with clean scissors. Leaves were weighed to the nearest 0.1 g before being oven-dried at 60 °C until a constant mass was achieved (24–48 h). Leopard slugs (*Limax maximus*) were collected at night from each site using a spotlight. Leopard slugs were chosen because they have a small home range (i.e., we can be sure that slugs found in one site have only visited that site; Barker and McGhie, 1984), are soil invertebrates (spend a large portion of their lives in close proximity to soil and as they have no exoskeleton metals can be taken up via the integument from soil; Markert et al., 2003), and consume a variety of food including guano and detritus (Kavaliers et al., 1985). We attempted to collect a minimum of ten slugs from each site, however, only eight were found at the abandoned colony. Slugs were stored separately in plastic sample containers and left overnight to purge their gut contents before being frozen at –20 °C for a minimum of 4–8 h to euthanise them. Slugs were then weighed to the nearest 0.1 g before being oven-dried at 60 °C to a constant mass.

Ten guano samples were collected from Flesh-footed Shearwater chicks between 26 April – May 10, 2021 from birds euthanised after a failed fledging attempt as part of other research (Lavers et al., 2021). For these samples, the last 10 cm of the gastro-intestinal tract (including the cloaca) was removed, and the contents were expelled into an Eppendorf tube. A total of 16 adult guano samples were collected in October 2020 from live birds by holding a shearwater in a sterile box until defecation, or until the holding time reached a maximum of 10 min. If defecation was successful, the bird was released, and the guano was collected into an Eppendorf tube using a clean metal spatula (the spatula was cleaned with 80% ethanol (trace metal grade; Sigma-Aldrich) between each sample). If defecation was not successful, then the bird was released. All guano samples (chicks and adults) were frozen at –20 °C until analysis.

2.3. Sample analysis

Guano, palm leaves, and whole slugs were freeze dried until a constant mass was achieved (48–88 h). Leopard slugs were crushed with a ceramic mortar and pestle until homogenised. Kentia palm leaves were chopped with scissors into small lengths (~1–2 cm) before being homogenised in a hammer mill. Guano was homogenised in the sample containers with clean plastic spatulas. All guano samples were below the minimum mass required for analysis (0.4 g), therefore, depending on initial mass, between 2 and 4 individual guano samples from the same age class were pooled until the threshold mass was reached (3 × chick and 5 × adult guano samples).

Samples were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for As, Hg, Cu, Cr, Cd and Pb. To digest soil samples, approximately 0.4 g of each sample was digested using an aqua regia solution (7.5 ml HCl:2.5 ml HNO₃; RCI Labscan brand trace metal grade) on a heat block for 1 h at 120 °C. Two blanks and a standard reference material (SRM; Sandy loam 2 CRM020) were digested with the samples, and duplicates were performed for every tenth sample. For all other samples (guano, kentia leaf, slugs), approximately 0.4 g of sample was digested with 10 ml of concentrated HNO₃ (70%; RCI Labscan) on a heat block for 1 h at 120 °C. We also prepared two blanks and two SRMs (Dogfish liver DOLT-5, Peach leaves NIST-1547). Recoveries of SRMs are presented in Table S1. All these samples were small, thus there was insufficient sample mass to perform duplicate analysis of samples. Following the digestion of all sample types, samples were made up to a final volume of 25 ml using ultrapure water (>18.2 MΩ cm), allowed to settle, and then diluted to a final volume of 250 ml with ultrapure water.

ICP-MS analysis was conducted on a PerkinElmer NexION 2000B ICP-MS, using a power of 1600 W, 0.9 L/min nebuliser gas flow, and 15 L/min plasma gas flow. To reduce molecular interferences some elements were analysed in Kinetic Energy Discrimination mode using helium (99.9995% purity) as the collision gas at 4 ml/min. Values are reported as mg/kg dry weight (dw). Sample preparation (digestions) and analysis by ICP-MS were undertaken by the Environmental Analysis Laboratory at Southern Cross University under ISO/IEC 17025 accreditation (no. 14960) and strict quality assurance and quality control (QA/QC) measures were applied.

2.4. Statistical analysis

All statistical analysis was complete in R v.4.2.0 (R Core Team, 2022). When metal concentrations were below the limit of quantification (LOQ), a number equal to 0.5 × LOD was used to replace the value/s (Mikkonen et al., 2018). If values were <LOQ for >50% of the values for a given metal in each sample medium, summary statistics were calculated using regression on order statistics. This was done only for Hg in soil samples from the currently occupied site and abandoned site samples but could not be done for soil samples from the uncolonised site because all the values were <LOQ. To test for normality, we used the Shapiro-Wilk significance test. When $p \geq 0.05$ the variance in the data were considered to be normally distributed and parametric tests were used to assess differences between treatments. When $p < 0.05$, the variance in the data were considered to be not normally distributed, thus non-parametric tests were used. For normally distributed data, we used general linear models to test for differences in metal concentrations in sample mediums across our three sites and used Tukey's post hoc tests to determine treatment differences. For all other data, we used non-parametric Kruskal-Wallis tests with post hoc Bonferroni-corrected Dunn tests. Mercury concentrations in soils were only compared between the currently occupied site and the abandoned site because all samples from the control site were <LOD. A general linear model was used to test if there were significant differences in the concentrations of metals between adult and chick guano. Differences were considered significant when $p < 0.05$. Data are reported as mean ± standard deviation (SD) throughout.

3. Results

The concentrations of metals in Flesh-footed Shearwater guano varied between chicks and adults. Arsenic concentrations were significantly higher in adults (mean ± SD; 14.5 ± 5.3 mg/kg) compared to chicks (6.0 ± 2.5 mg/kg; $F_{1,6} = 6.58$, $p = 0.043$), whereas the concentration of Hg was significantly higher in chicks (0.33 ± 0.13 mg/kg) than in adults (0.11 ± 0.03; $H_1 = 5.00$, $p = 0.025$; Table 1). The concentrations of all other metals (Pb, Cd, Cr, Cu) were not significantly different between adult and chick guano. The highest concentration of all metals was Cu with a mean of 18.9 ± 7.2 mg/kg (mean for adult and

Table 1

Flesh-footed Shearwater (*Ardenna carneipes*) chick and adult guano contain varying concentrations of potentially toxic metals. Mean \pm standard deviation (SD) and range of As, Cd, Cr, Cu, Pb, and Hg, measured in mg/kg dry weight, are shown for chicks, adults, and overall. General linear models (F) were used to test for differences between chick and adult guano, Pb and Hg were not normally distributed thus Kruskal-Wallis tests (H) were used instead. P values are reported in full unless $p < 0.001$.

Variable	Chick (n = 3)		Adult (n = 5)		Overall (n = 8)		Test Statistics		
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	F/H	df	p value
Arsenic	6.0 \pm 2.5 ^a	3.3–8.3	14.5 \pm 5.3 ^b	10.3–23.7	11.3 \pm 6.1	3.3–23.7	6.58	1,6	0.043
Cadmium	17.7 \pm 3.0 ^a	14.2–19.7	11.9 \pm 4.2 ^a	7.3–15.9	14.0 \pm 4.6	7.3–19.7	4.31	1,6	0.083
Chromium	2.8 \pm 1.9 ^a	0.7–4.2	5.9 \pm 3.9 ^a	1.7–9.8	4.8 \pm 3.5	0.7–9.8	1.65	1,6	0.247
Copper	18.7 \pm 4.3 ^a	14.1–22.6	19.1 \pm 9.1 ^a	9.5–30.7	18.9 \pm 7.2	9.5–30.7	0.00	1,6	0.952
Lead	0.8 \pm 1.3 ^a	0.1–2.3	0.3 \pm 0.1 ^a	0.2–0.3	0.5 \pm 0.7	0.1–2.3	0.56	1	0.456
Mercury	0.33 \pm 0.13 ^b	0.2–0.5	0.11 \pm 0.03 ^a	0.10–0.16	0.19 \pm 0.13	0.10–0.47	5.00	1	0.025

chick guano combined; Table 1).

Soils from the current colony were enriched in metals compared to soils from the abandoned colony and uncolonised site. The concentrations of all metals, except for Pb and Hg, were significantly higher in active colony soils than those from abandoned and uncolonised areas ($p < 0.05$; Hg only compared against the abandoned site; Table 2). However, there was no significant difference in the concentration of As, Cd or Cu in soil between abandoned and uncolonised sites (Table 2). The concentration of Cr was the highest in soil from the current colony (123.0 ± 75.3 mg/kg; $H_2 = 18.44$, $p < 0.001$) and was 4.2 \times greater than Cr levels in soil samples from the uncolonised site (28.6 ± 6.7 mg/kg), while Cu concentrations were highest in current colony soils (33.3 ± 15.2 mg/kg; $H_2 = 19.37$, $p < 0.001$) and were 7 \times greater than levels in soils from abandoned and uncolonised sites (Fig. 2; Table 2).

Site differences in elemental concentrations of leopard slugs and kentia palm leaves were not as defined as those for soils (Table 2). In many instances, metal concentrations did not differ significantly across sites, however, the concentration of Cd was significantly greater in kentia palm leaves from the currently occupied site (0.2 ± 0.2 mg/kg; $H_2 = 6.72$, $p = 0.035$) as was Cu (9.7 ± 0.7 mg/kg; $F_{2,12} = 3.38$, $p = 0.047$; Fig. 2; Table 2). In contrast, leopard slug samples from the

Table 2

Soil, kentia palm (*Howea forsteriana*) leaf, and leopard slug (*Limax maximus*) samples from three distinct areas on Lord Howe Island were compared to determine if there were statistically significant differences. The three sampling sites were spread across a gradient of seabird influence: a currently occupied Flesh-footed Shearwater (*Ardenna carneipes*) colony, an abandoned colony, and an area that has never had an active shearwater colony (control site). Kruskal-Wallis tests (H) and post-hoc Bonferroni-adjusted Dunn tests were used to determine where differences lie. P-values are reported in full unless $p < 0.001$. Five variables (Hg in slugs, and As, Cu, Pb and Hg in kentia palm leaves) were normally distributed, thus, general linear models were used to test for differences. For these variables, the value in the H column are F-values. Mercury concentrations in soil could only be compared between the abandoned site and currently occupied site because all samples from the control site were <LOD.

Sample	Variable	H	df	p value
Soil	As	22.72	2	<0.001
	Cd	12.03	2	0.002
	Cu	19.37	2	<0.001
	Cr	18.44	2	<0.001
	Pb	9.76	2	0.008
	Hg	1.47	1	0.226
Kentia	As (GLM)	5.27	2, 12	0.228
	Cd	6.72	2	0.035
	Cu (GLM)	4.00	2, 12	0.047
	Cr	3.38	2	0.185
	Pb (GLM)	1.45	2, 12	0.274
	Hg (GLM)	1.91	2, 12	0.191
Slugs	As	4.43	2	0.109
	Cd	12.71	2	0.002
	Cu	6.65	2	0.036
	Cr	5.64	2	0.060
	Pb	7.88	2	0.019
	Hg (GLM)	1.15	2, 28	0.330

uncolonised site had significantly higher concentrations of Cu and Pb, while the greatest concentration of Cd in leopard slugs was from the abandoned colony (Fig. 2).

Leopard slugs had comparatively high concentrations of Cd and Cu relative to the same elements in soil samples for each site (Fig. 2, Table S2). In contrast, kentia palm leaves had generally lower metal levels than soil samples from the same sites (Fig. 2, Table S2).

4. Discussion

Seabirds are well-known vectors for the movement of nutrients and contaminants from marine to terrestrial environments (Mallory et al., 2015; De La Peña-Lastra, 2021) with data available for a wide range of seabird species (Grant et al., 2021), but few available for declining species or those that ingest considerable quantities of plastics, including Flesh-footed Shearwaters. We suggest that Flesh-footed Shearwaters are vectors for metal contaminants with their guano contributing to significantly higher metal concentrations in soils within the colonies on Lord Howe Island compared to areas on the island without shearwater colonies. While differences in metal concentrations between soil samples across sites were substantial, differences in the same metals in other environmental samples collected (leopard slugs, kentia palm leaves) were less pronounced and with few significant differences observed (Fig. 2; Table S2). It is possible that metals are bioaccumulating in other organisms within shearwater breeding colonies that were not measured in this study, and there is scope for future studies to test additional factors (e.g., other species of invertebrates).

4.1. Metals in guano

While this is the first study that examined the guano of Flesh-footed Shearwaters for metal concentrations, previous studies have explored metal concentrations in other biological samples from this species. Bond and Lavers (2011) sampled feathers for 17 elements, including five of the metals examined in this study (Cu, As, Cd, Hg, Pb), and found that shearwaters from Lord Howe Island had elevated concentrations of Hg and Pb. While feathers and guano are not directly comparable, concentrations of Cu, As, and Cd were considerably higher in adult guano collected in 2022 than adults feathers ($2 \times$, $60 \times$, and $134 \times$ higher, respectively; Table 1), whereas feathers had much higher concentrations of Hg compared to guano (feathers $102 \times$ higher; Bond and Lavers, 2011). Overall, the Bond and Lavers (2011) study and the current study indicate that Flesh-footed Shearwaters continue to be exposed to elevated metal concentrations.

Flesh-footed Shearwaters likely obtain at least a portion of the metal measured in this study through their diet, as these metals bioaccumulate in marine organisms (Rahman et al., 2012), and as the shearwaters annually migrates between Lord Howe Island and the Sea of Japan, it is possible that a fraction of the metals excreted via the guano of adult birds has come from their foraging grounds in the Northern Hemisphere (Marchant and Higgins, 1990). The diet of Flesh-footed Shearwaters consists predominately of mackerel and squid, with neon flying squid

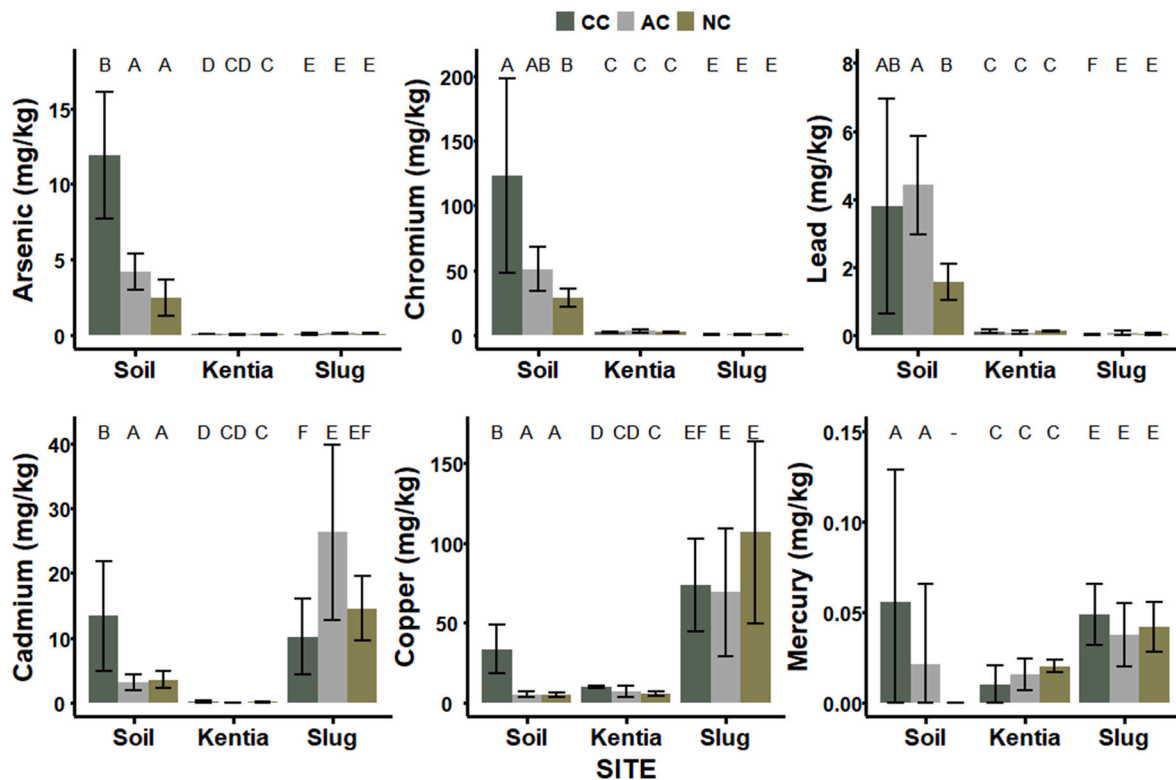


Fig. 2. Concentrations (mean \pm standard deviation (SD); mg/kg dry weight) of As, Cr, Pb, Cd, Cu, and Hg in soil, leopard slugs, and kentia palm leaves from a currently occupied Flesh-footed Shearwater (*Ardenna carneipes*) colony (CC), an abandoned colony (AC), and a site that has never hosted a shearwater colony (NC) on Lord Howe Island, Australia. Letters above bars indicate significant differences between sites for each sample type; sites that do not have the same letter are significantly different. Mercury concentrations in soil samples from site NC were all < LOQ. For exact values of certain samples, see Table S2.

(*Ommastrephes bartramii*) commonly ingested (Reid et al., 2013; Bond and Lavers, 2020). Neon flying squid have previously been reported to contain high Cd, Cu, Cr, As, and Pb concentrations (Martin and Flegal, 1975; Jiao et al., 2018), as well as Hg, including the highest concentration of Hg reported for ommastrephid squid species (García Barcia et al., 2021). While the metal concentrations in guano indicate that the shearwaters can excrete some of the metal burden from their bodies, as they are able to do with their feathers (Bond and Lavers, 2011), we do not know what proportion remains within the individuals (e.g., accumulated in tissues). However, studies in chickens suggest that $\sim 10\%$ of metals obtained from their diet are retained, though this varies across metals (Mohanna and Nys, 1998). Chronic exposure to metals at elevated bioavailable concentrations can lead to toxic effects and negative consequences for individuals. For example, Hg and Pb contamination have been associated with adverse effects for reproduction (Chastel et al., 2022) and Cd has been linked with eggshell thinning and suppression of egg production (Burger and Gochfeld, 2000).

The concentrations of metals in guano from Flesh-footed Shearwaters in this study were higher than those reported for guano in other studies of the order Procellariiformes (see a review of studies in Table 4; Grant et al., 2022) for the metals As, Cu, Cd, Pb (Hg and Cr were not included in the review). Metal loads in seabirds differ for a variety of reasons and can be influenced by prey choice and trophic level (Elliott and Scheuhammer, 1997), environmental contamination (e.g., penguins feeding in a semi enclosed bay adjacent to a large city had high levels of metal contamination; Finger et al., 2017), or from the ingestion of plastics (e.g., certain metals are added to plastics during production and can also adsorb to the surface from seawater; Turner, 2016). Thus, guano concentrations for seabirds are the combined outcome of multiple environmental and species factors and cannot be compared easily without an understanding of the relative contribution of each factor to the concentration in the guano.

Sampling guano is an excellent non-invasive method for obtaining an understanding of metal burden within the individual being sampled. However, similar to other non-invasive methods using feathers or eggshells, the concentration of metals may not accurately reflect the concentrations of metals within the body, for example, the source of metals in feathers can come from both internal and surface contamination (Clapp et al., 2012). Furthermore, different sample types can provide information on different exposure and fate pathways, for example, metal concentration in eggs can be helpful in determining maternal transfer (Morganti et al., 2021). Thus, future studies should take care when choosing a sample (e.g., eggs, feathers, guano, blood, tissue) to ensure that the sample will effectively answer the aims of the study (see Pacyna-Kuchta, 2023 for a detail detailed review on this topic), noting that some studies would benefit from multiple matrices to fully explore metal burden and fate pathways within the individual. In the case of our study, we chose guano as it has been likened to fertiliser and we were interested in the flow of metals from Flesh-footed Shearwaters to their terrestrial environment.

4.2. Transfer of metals to soil, vegetation, and invertebrates

Metal concentrations in soil samples collected in Flesh-footed Shearwater colonies on Lord Howe Island were generally higher than those from abandoned colonies and never colonised sites (Fig. 2; except for Pb). This suggests that these metals have been deposited by seabirds and are likely marine in origin (e.g., see Blais et al., 2005; Signa et al., 2013; Celis et al., 2015). Given that Flesh-footed Shearwaters are the only seabird species breeding in the areas studied, it is likely that they are responsible for elevating the concentrations of metals in the soil of their colonies above background levels (i.e., those observed at the uncolonised site).

Metal concentrations of soil from the abandoned colony were

significantly lower than soil from the active colony for As, Cd and Cu, which can be attributed to the absence of Flesh-footed Shearwaters and their guano since the site was abandoned ~20 years ago. The concentrations of these metals were not significantly higher than soil from the control site, despite these metals being persistent contaminants. Metal export and leaching from soils has been found to increase in areas with large quantities of rain and frequent precipitation (Meite et al., 2018). It is likely that the high amount and frequency of precipitation that Lord Howe Island receives (Auld et al., 2010) has resulted in these contaminants leaching from the soil in the abandoned colony.

Interestingly, Pb was significantly higher in soil from the abandoned colony than soil from the control site and was higher than soil from the active colony (though this difference was not significant; Fig. 2). Pb is one of the least mobile metal contaminants in soils, with a study by Kim et al. (2008) on the movement of Cd, Cu, and Pb through soil by rainfall finding Pb to be retained in all soils tested (i.e., Pb was not found in leachate). It is possible that the concentration of Pb in soil from the abandoned site remains despite the frequent precipitation that the island receives. Furthermore, Pb concentrations in Flesh-footed Shearwater feathers have decreased by 2% since 1900 (Bond and Lavers, 2020), such a decrease is likely to be reflected in the birds' guano as well. It is possible that this decrease in Pb burden in the shearwaters is associated with the absence of significant difference in soil Pb concentrations between the active colony and the abandoned colony.

Metal concentrations in soil samples from the Flesh-footed Shearwater colony were compared to Australian and Canadian Soil Quality Guidelines ([SQG]; CCME; Canadian Council of Ministers for the Environment, 1999; NEPC; National Environmental Protection Council, 2013) to determine if the shearwaters are contaminating the soil to a level that exceeds the concentration-based thresholds (Australian SQG only cover As, Cr, Cu, and Pb, hence why Canadian SQG were included here too). In Australia, the soil quality guidelines indicate that when levels are exceeded, a risk assessment should be completed to determine if the given contaminant poses an unacceptable risk for the environment or human health. The concentration of Cu, Hg, and Pb in soil samples from the currently occupied colony did not exceed the SQGs (Table S3), while only 10% ($n = 1$) of samples exceeded the SQG for As in the Canadian guidelines. Metals of current concern in soils of the Flesh-footed Shearwater colonies are Cd and Cr, with >50% of samples exceeding the Australian and Canadian SQGs (100% of samples surpassed the Australian SQG for Cr; Table S3). This indicates that the Clear Place colony is contaminated with Cd and Cr but the current concentrations of these elements in kentia palms and leopard slugs are not of toxicological concern (de Vaufleury et al., 2006; Abass et al., 2018). Compared with other studies that have also examined Cd and Cr in colony soils, our results are much higher (Cd: 1.8 mg/kg, Cr: 6.1 mg/kg, Otero, 1998; e.g., Cd: 4.8 mg/kg, Cr: 29.6 mg/kg, Ziólek et al., 2017). Recent research by Bond and Lavers (2020) indicates that Cd concentrations in Flesh-footed Shearwater feathers have increased by 1.5% per year since 1900, thus further research is warranted to understand the risks associated with Cd, as well as Cr, contamination on the Lord Howe Island environment to elucidate any possible negative consequences in the future.

While soil samples from the occupied colony had higher metal concentrations than soil samples from the active colony and area never occupied by shearwaters (Fig. 2), differences in metals in kentia palm and leopard slug samples were less pronounced. There were no clear patterns for any particular metal, and in some cases the concentration of metals was lowest in samples from the current colony compared with the abandoned and uncolonised site samples (e.g., Cr; Fig. 2). This may be due to the relative bioavailability of these metals once they have been deposited on the colony surface. Bioavailability is affected by soil pH, in general, a decrease in metal bioavailability is associated with an increase in soil pH. The soils in the Flesh-footed Shearwater colony studied here are calcareous and have a high pH (pH of ~8–9; Savolainen et al., 2006; Osborne et al., 2019). It is likely that this reduces the

bioavailability of metals found in shearwater guano, thus limiting the uptake of these metals by biota such as the kentia palm and leopard slugs. However, the apparent low bioavailability of colony soils should not be used to assume a low level of risk. It highlights the importance of doing risk assessments to ascertain if the elevated concentrations of Cd and Cr in colony soils are likely to be having a broader environmental impact to all biota within these sites.

4.3. Plastics as a source of metal contamination

Plastics in the marine environment can adsorb contaminants from ambient seawater, while also containing compounds that are included as additives during the manufacturing process (Tanaka et al., 2013). Such additives include colourants, UV and heat stabilisers, fillers, flame retardants, and antimicrobial agents, and As, Cd, Pb, Cu, Cr, and Hg have all been used in the past (and in some cases are still used) for these purposes (Turner, 2016). Once marine plastics have been ingested by seabirds, contaminants or additives can be released into animals through digestive processes (Tanaka et al., 2015). In the case of the Flesh-footed Shearwater population on Lord Howe Island, considerable quantities of marine plastics are ingested (Lavers et al., 2021), with metal loads in feathers associated with plastic exposure and reduced bird body size (Lavers et al., 2014). Therefore, it is possible that the reason the guano examined in this study had higher concentrations of metals than the concentrations of those same metals in the guano of other Procellariiformes reviewed in Grant et al., 2022 could be due to the large amounts of plastics the Flesh-footed Shearwaters from Lord Howe Island are consuming.

Seabirds that ingest plastics can deposit debris within breeding colonies through regurgitations and decomposition of carcasses (Buxton et al., 2013), and Grant et al., 2022 found that Flesh-footed Shearwaters on Lord Howe Island can deposit approximately 688,480 pieces of meso- (5–20 mm) and macro-plastics (<20 mm; Barnes et al., 2009) every breeding season. These deposited plastics potentially could leach metals into the surrounding soil (Xu et al., 2020), particularly as older plastics (e.g., marine plastics) have been shown to leach metals faster than virgin plastics (Meng et al., 2021). Furthermore, Keys et al. (2023) found that Flesh-footed Shearwater guano contains extremely high amounts of nano- (1 nm–1 μ m) and ultrafine-plastics (1 μ m–1 mm; mean of 350.7 pieces/mg; compared with Provencher et al., 2018 who calculated 12.8 pcs/g in Northern Fulmars (*Fulmarus glacialis*), however the authors used a different method and measured plastics >100 μ m), which due to their higher surface area could also leach metals into soil once excreted.

5. Conclusion

We conclude that Flesh-footed Shearwater guano contains potentially toxic metals (As, Cd, Cr, Cu, Hg, Pb) and this coincides with increased concentrations of these same metals in soils of the breeding ground site in comparison with control sites. It is likely that the Flesh-footed Shearwaters are responsible for increasing these metals within the soil of their breeding grounds. While concentrations of contamination for most metals are not presently a concern, except for Cd and Cr which are already above Australian and Canadian soil quality guidelines, it is possible that this may become a problem in the future. As a World Heritage site, Lord Howe Island is safe guarded from many human activities, but this listing does nothing to protect the island from the influx of contaminants via Flesh-footed Shearwaters, other seabird species, nor by sea or air. Thus, current protections may be inadequate considering increasing levels of highly mobile pollutants.

The transport of contaminants is not restricted to Flesh-footed Shearwaters nor to Lord Howe Island. This is a global phenomenon seabirds unknowingly contaminating their breeding locations on islands and mainlands with potentially negative consequences for these ecosystems. This is concerning given many seabird breeding islands are refugia for other wild species, including many island endemics and

threatened species. As conservation managers, we must account for non-human vectors of pollution (e.g., seabirds) so that pressures can be addressed holistically, with island habitats protected for decades to come.

CRedit authorship contribution statement

Megan L. Grant: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Alexander L. Bond:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Suzie M. Reichman:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. **Jennifer L. Lavers:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The dataset supporting this study is freely available from the Institute for Marine and Antarctic Studies (IMAS) Data Portal: <https://doi.org/10.25959/56N1-EC97> (Grant, 2024).

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Appendix A. Supplementary data

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