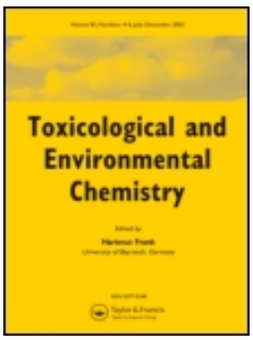

Toxicological & Environmental Chemistry

ISSN: 0277-2248 (Print) 1029-0486 (Online) Journal homepage: http://www.tandfonline.com/loi/gtec20

Analysis of the cyanotoxins anatoxin-a and microcystins in Lesser Flamingo feathers

J. S. Metcalf , L. F. Morrison , L. Krienitz , A. Ballot , E. Krause , K. Kotut , S. Pütz , C. Wiegand , S. Pflugmacher & G. A. Codd

To cite this article: J. S. Metcalf , L. F. Morrison , L. Krienitz , A. Ballot , E. Krause , K. Kotut , S. Pütz , C. Wiegand , S. Pflugmacher & G. A. Codd (2006) Analysis of the cyanotoxins anatoxin-a and microcystins in Lesser Flamingo feathers , Toxicological & Environmental Chemistry, 88:1, 159-167, DOI: 10.1080/02772240500491604

To link to this article: https://doi.org/10.1080/02772240500491604

Published online: 01 Feb 2007.

Toxicological & Environmental Chemistry, Jan.–Mar. 2006; 88(1): 159–167

Analysis of the cyanotoxins anatoxin-a and microcystins in Lesser Flamingo feathers†

J. S. METCALF[1], L. F. MORRISON[1], L. KRIENITZ[2], A. BALLOT[2], E. KRAUSE[3], K. KOTUT[4], S. PÜTZ[3], C. WIEGAND[3,5], S. PFLUGMACHER[3], & G. A. CODD[1]

[1]*University of Dundee, Dundee DD1 4HN, UK,* [2]*Leibniz Institute of Freshwater Ecology and Inland Fisheries, D-16775 Neuglobsow, Germany,* [3]*Leibniz Institute of Freshwater Ecology and Inland Fisheries, Mueggelseedamm, D-12587 Berlin, Germany,* [4]*Kenyatta University, Nairobi, Kenya, and* [5]*Humboldt-University at Berlin, Unter den Linden, 10099 Berlin, Germany*

(Received 16 March 2005; revised 9 October 2005; in final form 22 November 2005)

Abstract

Feathers from carcasses of the Lesser Flamingo (*Phoeniconaias minor*), which had died after ingesting cyanobacterial toxins (cyanotoxins) contained between 0.02 and 30.0 μg microcystin-LR equivalents per gram of feather according to HPLC and ELISA analysis of feather extracts. Anatoxin-a was detected less frequently in the Lesser Flamingo feathers, up to 0.8 μg anatoxin-a per gram of feather being recorded. When feathers from different body regions were analysed and compared for microcystins and anatoxin-a, wing feathers were found to contain the highest concentrations of these cyanotoxins, the order of concentration and frequency of analytical detection being wing > breast > head. Consistent with the presence of the microcystins and anatoxin-a in gut contents and the livers of the dead birds and negligible *in vitro* adsorption to feathers, the cyanotoxins associated with the feathers of the dead wild flamingos are inferred to be primarily of dietary origin.

Keywords: *Lesser Flamingo, cyanotoxins, deposition, feathers, microcystin, anatoxin-a*

Introduction

Cyanobacteria (blue-green algae) produce a wide range of compounds harmful to animal and plant systems [1,2]. These cyanotoxins include hepatotoxins, (microcystins, nodularins), cytotoxins (cylindrospermopsin) and neurotoxins (saxitoxins, anatoxin–a, anatoxin–a(s)). Since investigations into environmental cases involving cyanotoxin

Correspondence: J. S. Metcalf, Univ. Dundee, Dundee DD1 4HN, UK. Tel: +44-1382-344866. Fax: +44-1382-344275. E-mail: j.s.metcalf@dundee.ac.uk

†Dedicated to the memory of Ekkehard and Angelika Vareschi.

ISSN 0277-2248 print/ISSN 1029-0486 online/06/00000159–167 © 2006 Taylor & Francis
DOI: 10.1080/02772240500491604

intoxications are almost always retrospective, methods are necessary to analyse for the presence of cyanotoxins and their degradation products in post-mortem tissues. The methods developed have employed a wide range of physicochemical and biochemical techniques including liquid chromatography, mass spectrometric systems and the application of enzyme assays and immunoassays [3,4].

Recently, collected dead birds from filter-feeding populations of the Lesser Flamingo (*Phoeniconaias minor*) were shown to contain significant concentrations of anatoxin-a and microcystin variants (-RR and -LR) from analysis of their livers, stomach contents, intestines and faecal pellets [5]. These populations, in the East African Rift Valley lakes, have undergone episodic mass mortalities in recent years and it is likely that the cyanotoxins have been among several contributing causes [5]. In addition to cyanotoxins, a number of possible causes for the mass deaths of the flamingos have been suggested; including a *Mycobacterium avium* related epizootic, heavy metals, pesticides and other organic pollutants [6–8]. Cyanotoxins have also been suggested as initiators of avian botulism [9].

Several bird species have been shown to accumulate natural toxins and man-made toxic compounds within their feathers, via their diet. These have included heavy metals such as arsenic, cadmium, chromium, lead, manganese, mercury and selenium in a wide variety of marine birds [10,11], the sodium channel-blocking batrachotoxins in the Pitohui bird [12] and the protein phosphatase-inhibitor cantharidin in spur-winged geese [13]. The occurrence of toxins in bird species has been reviewed recently [14].

The purpose of this study was to analyse feathers taken from carcasses of the Lesser Flamingo which had been exposed *in vivo* to cyanotoxins and been found to have accumulated significant concentrations of these toxins (microcystins and anatoxin-a) in their body tissues [5]. The possibility that the cyanotoxins subsequently detected in extracts of the feathers from dead wild Lesser Flamingos were the result of adsorption due to contact with lake water was investigated using feathers from *P. minor* specimens which had not been previously exposed to cyanotoxins. Finally, the applicability of feather analysis as a non-invasive means of assessing exposure to- and ingestion of- cyanotoxins in flamingos and other birds is discussed.

Experimental

Extraction of cyanotoxins from feathers

Feathers from the breast, wing and head were removed from carcasses of the Lesser Flamingo (*P. minor*) known to have been exposed to the cyanotoxins, microcystins and anatoxin-a, at Lakes Bogoria and Nakuru, Kenya, and stored at −20°C. Control feathers from Lesser Flamingos which had not previously been exposed to cyanobacterial toxins were obtained from Suffolk Wildlife Park, Lowestoft, Suffolk, England and stored at −20°C. Feathers were examined under a binocular dissecting microscope (Nikon Labophot 2, Nikon, Japan) for the occurrence of adhered cyanobacterial colonies, filaments, single cells or cell fragments. For the analysis of environmentally exposed feathers and for the laboratory exposure trials, feathers were allowed to warm to room temperature and weighed. Multiple feathers from the head, breast and wing were cut into four sections, termed the basal, lower part, middle part and top sections. Each section was weighed, frozen in liquid nitrogen and then ground to a powder with a mortar and pestle. The powder was extracted with 70% methanol in water by standing for 24 h at −20°C. The resulting suspension was filtered through a GF/C disc and the residue extracted with 100% methanol for a further 24 h. After a second filtration, the filtrates were combined, dried

under a nitrogen stream at 50°C and redissolved in 500 μL 70% aqueous methanol for analysis.

To understand the distribution of cyanotoxins in terms of wing feather structure, feathers were sectioned into the barbs, rachis and quill [15] and placed into pre-weighed Eppendorf tubes. Feathers were extracted with 100% methanol [12] for 1 h at room temperature. The feathers were centrifuged (14,000*g*, 10 min) and the supernatants transferred to glass vials and dried under N_2 gas at 50°C. Once dried, the residue was resuspended in 1 mL 100% (v/v) methanol, mixed and 500 μL transferred to a second glass vial. Both vials were dried as described before storage at −20°C before analysis.

Analysis of cyanotoxins in feather extracts

Extracts from feathers were removed from storage and allowed to warm to room temperature. For the analysis of microcystins, one glass vial containing the residue was suspended in a minimal volume (200 μL) of 70% methanol in water and centrifuged as before to obtain a clear supernatant. This was analysed by high performance liquid chromatography with photodiode array detection (HPLC-PDA) for microcystins [16], and diluted with MilliQ water for analysis by protein phosphatase inhibition assay [17] and microcystin ELISA, using antibodies raised against microcystin-LR [18] and by the EnviroLogix microcystin ELISA kit according to the manufacturer's instructions. For anatoxin-a analysis, the second aliquot of extract was suspended in a minimal volume (200 μL) of MilliQ water and centrifuged to obtain a clear supernatant before analysis by HPLC-PDA [19] or HPLC with fluorescence detection (HPLC-FLD) [e.g. 20]. The samples were also analysed for the presence of cyanotoxins by MALDI-TOF mass spectrometry [21].

Adsorption of cyanotoxins to Lesser Flamingo feathers

Control Lesser Flamingo feathers (Suffolk Wildlife Park, England), from live birds which had not previously been exposed to cyanotoxins, were removed from storage and allowed to warm to room temperature and weighed before adsorption trials. MilliQ water (600 mL) containing purified microcystin-LR or anatoxin-a was prepared at concentrations of 10 and 100 $\mu g\,L^{-1}$. The feathers were submerged in these solutions and 1 mL water samples removed at 0, 0.5, 1, 2, 4, 8, 24 and 32 h for analysis by HPLC-PDA (microcystin-LR [16]; anatoxin-a [19]) and ELISA (microcystin-LR [18]). The feathers which had been exposed to these solutions for 32 h were mechanically separated [15], extracted with 100% methanol and analysed for cyanotoxins as described before.

Results and discussion

The phenomenon that birds are able to sequester toxic compounds into feathers is well documented. However, we are not aware of previous investigations of cyanotoxin-feather associations. As feather tissues are forming, toxic compounds in the blood stream, such as metals, can be deposited in feathers where they attach to sulfhydryl groups of keratin [10]. As the feather develops further, the blood supply is eventually cut off and the toxins are effectively removed from the blood stream. The feathers from the wild birds analysed in this study were from carcasses collected after mortalities (these birds were not a mass mortality with thousands of dead birds) of Lesser Flamingos in the African Rift Valley lakes. These birds are known to consume cyanobacteria as their principal diet and their stomach,

Table I. Cyanotoxin analysis by HPLC-PDA of feathers from Lesser Flamingo (*P. minor* Geoffroy) carcasses.

Feather source	Feather section	No. of feathers analysed	Minimum concentration	Maximum concentration	Mean concentration	Toxin (%)
HEAD	NA	9	0	0	0	0
Microcystin-LR ($\mu g\,g^{-1}$)						
Wing	Basal	4	0.425	1.013	0.715	100
	Lower	4	0.018	0.951	0.582	100
	Middle	4	0	0.083	0.038	50
	Top	4	0	0.428	0.107	50
Breast	Basal	5	0	1.256	0.341	60
	Lower	5	0	0.630	0.156	60
	Middle	5	0	0.058	0.044	60
	Top	5	0	0.070	0.023	40
Microcystin-RR ($\mu g\,g^{-1}$)						
Wing	Basal	4	0.485	1.349	0.933	100
	Lower	4	0.286	0.948	0.612	100
	Middle	4	0	0.066	0.03	50
	Top	4	0	0.162	0.04	25
Breast	Basal	5	0	0.789	0.357	80
	Lower	5	0	0.344	0.137	60
	Middle	5	0	0	0	0
	Top	5	0	0	0	0
Anatoxin-a ($\mu g\,g^{-1}$)						
Wing	Basal	4	0.137	0.8	0.427	100
	Lower	4	0	0.205	0.055	50
	Middle	4	0	0	0	0
	Top	4	0	0	0	0
Breast	Basal	5	0	0	0	0
	Lower	5	0	0	0	0
	Middle	5	0	0	0	0
	Top	5	0	0	0	0

intestine contents and excreta, contained microcystins and anatoxin-a [5]. Microscopic examination revealed occasional mineral particles adhering to the feathers, but no discernable attached, or trapped, cyanobacterial colonies, filaments, single cells or cell fragments. Analysis of feathers from various locations of the Lesser Flamingo carcasses for cyanotoxins was carried out by HPLC-PDA and HPLC-FLD (Table I). Two microcystin variants, microcystin-LR and -RR and anatoxin-a were found, with distributions and concentrations which differed between the sections of the breast, wing and head feathers examined. The head feathers were never found to contain detectable cyanotoxins, whereas the basal section of the wing feathers was always positive for all three cyanotoxins. In the case of microcystins, the middle section of the wing feather was positive for all feathers tested. The breast feathers were consistently negative for the neurotoxin anatoxin-a, but the basal and lower sections of these feathers included samples which were positive for microcystins -LR and -RR. Although microcystin-RR was not found in the middle and top part of the breast feathers, the more hydrophobic microcystin-LR variant was frequently found in these sections (Table I).

Table II. Summarised microcystin concentrations from analysis of single wing feathers from Lesser Flamingo carcasses at Lakes Bogoria and Nakuru, in comparison with unexposed control feathers and feathers exposed to microcystin-LR in the laboratory. Analysis of extracts was performed by high performance liquid chromatography, protein phosphatase inhibition assay and enzyme-linked immunosorbent assay.

	Microcystin-LR equivalents ($\mu g\,g^{-1}$) in feather tissues		
	Barbs	Rachis	Quill
Controls from captive birds	ND (ELISA, 0.08; HPLC, 0.64)	ND (ELISA, 0.014; HPLC, 1.13)	ND (ELISA, 0.052; HPLC, 4.14)
Controls from captive birds, after *in vitro* exposure (32 h)			
10 ($\mu g\,l^{-1}$)	<0.002–0.03	<0.003–0.01	≤0.02
100 ($\mu g\,l^{-1}$)	0.03–0.11	0.014–0.09	≤0.02
Wild, carcasses			
Lake Bogoria, Kenya	3.52–30.0	2.98–6.8	1.14–3.2
Lake Nakuru, Kenya	ND (ELISA, 0.027; HPLC, 2.2)	ND (ELISA, 0.144; HPLC, 11.54)	No sample

ND, not detected.
Figures in parentheses indicate the detection limit of the analytical method for the sample analysed when negative.

Differences in the distribution of other toxins in bird feathers have been demonstrated [12]. Analysis of skin and feathers of Pitohui and Ifrita birds by HPLC-MS/MS showed the highest concentrations of batrachotoxins (potent natural neurotoxins) in the contour feathers of the belly, breast and legs, with lesser amounts in the head, back, tail and wing feathers [12]. The differences in batrachotoxin distribution are thought to be due to a direct result of the diet and the possible ecological function of toxin-containing feathers to protect eggs and to help control pests within the nests of these birds [12]. However, the reasons for the differences in the distribution of cyanobacterial toxins in flamingo feather types are unclear.

The famous Lesser Flamingo pink plumage colour is due to the elimination into the feather of pigments obtained via the consumption of cyanobacteria [22]. Indeed the presence of dietary pigments in the feathers of birds is a common phenomenon, including in the Western Tanager (*Piranga ludoviciana* [23]) and the Greater Flamingo (*Phoenicopterus ruber* [24]). By analogy, it may therefore be expected that additional compounds of dietary origin may occur in flamingo feathers and the presence of heavy metals in the feathers of Lesser Flamingos has been documented [11].

Analysis of microcystins by ELISA and HPLC showed that feathers from Lesser Flamingos from Lake Nakuru were negative, whilst all parts of individual feathers from dead birds from Lake Bogoria were positive, although there were differences in quantification between these two methods (Table II). The HPLC-PDA analysis of a basal wing extract from a single feather revealed the presence of microcystin-LR and microcystin-RR (e.g. Figure 1). The MALDI-TOF analysis for microcystins showed m/z values of 995 (MC-LR) and 1038 (MC-RR), in addition to confirmatory analysis by microcystin ELISA and protein phosphatase inhibition assay in feather extracts. In a second extract from a basal wing feather, HPLC-FLD and MALDI-TOF analysis also confirmed the presence of anatoxin-a (Figure 2) with an m/z of 166 [5].

The possibility that the microcystins and anatoxin-a present in the feather extracts were due to adsorption of dissolved cyanotoxins, if present in the Kenyan lake waters, was

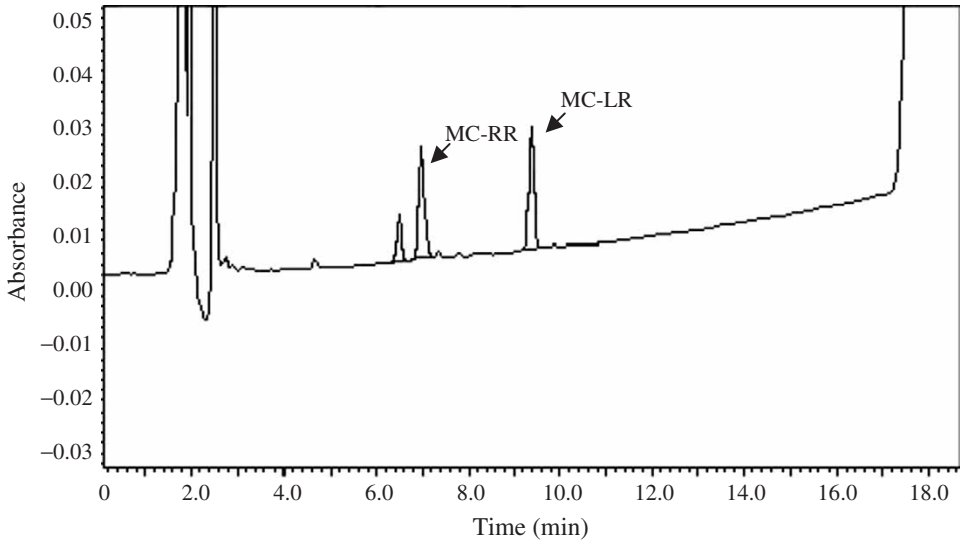

Figure 1. HPLC chromatogram of an extract of a basal wing feather from the carcass of a Lesser Flamingo (*P. minor* Geoffroy) showing the identification of microcystin-LR and -RR.

investigated by exposing feathers from captive birds which had not been previously exposed to cyanobacterial blooms or cyanotoxins. Such control feathers were exposed to the purified cyanotoxins in a bathing aqueous solution at environmentally-relevant concentrations of 10 and 100 $\mu g\,L^{-1}$ for up to 32 h. Analysis of these bathing solutions showed no significant decreases in cyanotoxin concentration throughout the exposure period (data not shown). The feathers that had been exposed to these solutions were analysed for cyanotoxins by HPLC-PDA (for microcystins, anatoxin-a) and by ELISA and protein phosphatase inhibition assay (for microcystins) (Table II). No detectable anatoxin-a was found in the feathers exposed to this cyanotoxin (minimum detection level, 0.5 $\mu g\,g^{-1}$; data not shown). Microcystin analysis of the feathers after incubation showed the presence of low toxin concentrations by all methods in the rachis, barbs and quill portions which had been incubated at 100 $\mu g\,L^{-1}$, with microcystin-LR equivalents up to 0.09 $\mu g\,g^{-1}$ for the rachis, and between 0.03 and 0.11 $\mu g\,g^{-1}$ for the barbs (Table II). The remainder of the samples showed very low positive concentrations by analysis with the EnviroLogix ELISA, but were below minimum detection limits for the other microcystin ELISA and protein phosphatase inhibition assay. These findings indicate that the adsorption of microcystins and anatoxin-a onto Lesser Flamingo feathers from aqueous solution is not a likely explanation for our findings of the higher concentrations of the toxins in the feathers of the dead birds. Indeed if cyanotoxins were likely to be adsorbed onto feathers, it may be hypothesised that the toxins would be particularly associated with the head feathers which are closest to the water when the birds feed. However, the head feathers from the carcasses were consistently negative for the cyanotoxins, in contrast to feathers from other parts of the birds (Table I). Furthermore, microcystins-LR and -RR are among the more hydrophilic of the over 70 microcystin variants [1,16]. These, together with the highly polar anatoxin-a, would be expected to be relatively easily removed by washing if feathers showed adsorptive capacities for cyanotoxins. As metals have been shown to bind to sulfhydryl groups of keratin in feathers, the possibility that the methyldehydroalanine moiety of microcystin [1] could also

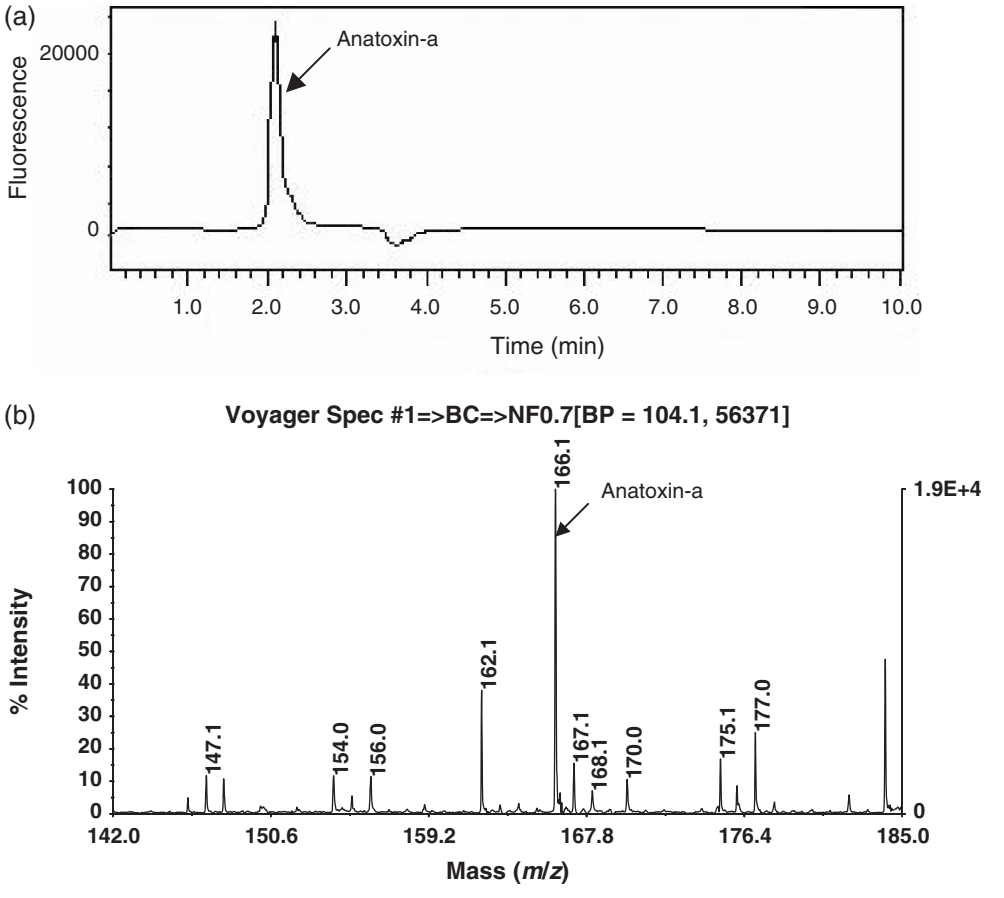

Figure 2. HPLC fluorescence analysis (a) and a MALDI-TOF MS spectrum (b) of anatoxin-a in an extract from a basal wing feather from a Kenyan Lesser Flamingo (*P. minor* Geoffroy) exposed to cyanobacterial toxins.

covalently attach to sulfhydryl groups should be assessed as this site is open to nucleophilic addition, resulting in a potential underestimation of the microcystin burden in flamingo feathers. Furthermore, detoxication of microcystins occurs by conjugation to glutathione as the first step, which also proceeds via the cysteine sulfhydryl group of glutathione and the methyldehydroalanine moiety of microcystin [25,26].

Mass mortalities of other populations of flamingos, in which microcystins have been indicated to be involved (if not solely responsible), have recently been reported in addition to those at the Kenyan lakes [5]. These include over half the population (579/943) of Greater Flamingos which died at a wetland in the Donana National Park, Spain [27]. Additional waterfowl species (mallard, black coot, purple swamphen and moorhen) also died at the same location in the Spanish event and feather analysis might prove useful for the assessment of these and other bird species. Ten captive adult Chilean Flamingos died at SeaWorld, Orlando, Florida, USA, their deaths being attributed to microcystins as a result of signs of poisoning, post-mortem examination and toxin analysis [28].

The analysis of feathers from flamingos and other water birds for cyanotoxins, in concert with the analysis of environmental blooms, water samples or other media via which the birds may be exposed to these toxins, may contribute usefully to the assessment of exposure to bird populations to these potent toxins. If the cyanotoxins associated with the feathers of exposed birds are of dietary origin, as indicated here, then feather analysis for cyanotoxins provides a potentially useful non-invasive method for assessing the exposure and responses of birds to cyanotoxins.

Conclusions

Cyanotoxins are a potential threat to water resources and to human and animal health. Although a great deal of research has investigated the effect of these toxins retrospectively, methods are required to analyse these toxins in susceptible populations. Feathers from carcasses of the Lesser Flamingo, collected after mass mortalities at Kenyan lakes, contain cyanobacterial hepatotoxins (microcystins) and a neurotoxin (anatoxin-a). These findings are consistent with the presence of the cyanotoxins in the birds' habitats and in the alimentary canal contents and excreta of these birds, which principally feed on cyanobacteria. Negligible adsorption of purified microcystin and anatoxin-a to Lesser Flamingo feathers indicates that the cyanotoxins present in the feathers of the dead birds from the Kenyan lakes were primarily of dietary origin. Multiple analytical techniques, namely HPLC-PDA, MALDI-TOF, protein phosphatase inhibition assay and enzyme-linked immunosorbent assays, all produced positive results for feathers from birds exposed to cyanotoxins. Although there were differences in the results obtained by some analytical methods, e.g. ELISA and HPLC-PDA, these are likely to be due to the principles involved in these analytical techniques. Methods developed for the extraction and analysis of cyanotoxins in feathers may provide a means to actively assess population fitness and health. Cyanotoxin analysis of feathers may provide a facile, non-invasive approach contributing to determining the exposure and responses of birds to cyanotoxins.

Acknowledgements

The Dundee laboratory thanks Suffolk Wildlife Park, England, for supplying unexposed *Phoenicanaias minor* feathers and Dr. J. Lindsay for useful discussions. The Nairobi and Neuglobsow Laboratories thank the Government of Kenya for providing research permission (No. MOEST 13/001/31 C90). This work was partly supported by the German Federal Ministry of Education and Research in the frame of BIOLOG (Biodiversity and Global Change) program (grant no. 01LC001). We also thank the County Councils of Koibatek and Baringo Districts and the Kenya Wildlife Service for granting free access to the Lake Bogoria Game Reserve and Lake Nakuru National Park, and for their collaboration and helpful assistance.

References

1. Codd GA, Bell SG, Kaya K, Ward CJ, Beattie KA, Metcalf JS. Eur. J. Phycol. 1999;34:405–415.
2. Kuiper-Goodman T, Falconer I, Fitzgerald J. In: Bartam J, Chorus I, editors. Toxic Cyanobacteria in Water. London: E & FN Spon; 1999. pp 113–153.
3. G. A. Codd, J. S. Metcalf, C. J. Ward, K. A. Beattie, S. G. Bell, K. Kaya, G. K. Poon. J. AOAC Int. 2001;84:1626–1635.

4. Metcalf JS, Codd GA. Chem. Res. Toxicol. 2003;16:103–112.
5. Krienitz L, Ballot A, Kotut K, Wiegand C, Pütz S, Metcalf JS, Codd GA, Pflugmacher S. FEMS Microbiol. Ecol. 2003;43:141–148.
6. Kock ND, Kock RA, Wambua J, Kamau GJ, Mohan K. J. Wildlife Dis. 1999;35:297–300.
7. Nelson YM, Thampy RJ, Motelin GK, Raini JK, DiSante CJ, Lion LW. Environ. Toxicol. Chem. 1998;17:2302–2309.
8. Greichus YA, Greichus A, Ammann BB, Hopcraft J. Bull. Environ. Contam. Toxicol. 1978;19:454–461.
9. Murphy T, Lawson A, Nalewajko C, Murkin H, Ross L, Oguma K, McIntyre T. Environ. Toxicol. 2000;15:558–567.
10. Gochfeld M, Gochfeld DJ, Minton D, Murray BG Jr, Pyle P, Seto N, Smith D, Burger J. Environ. Mon. Assess. 1999;59:343–358.
11. Burger J, Gochfeld M. Environ. Res. Sec. A 2000;82:207–221.
12. Dumbacher JP, Spande TF, Daly JW. PNAS 2000;97:12970–12975.
13. Dumbacher JP, Pruett-Jones S. Current Ornithol. 1996;13:137–174.
14. Bartram S, Boland W. Chembiochem. 2001;2:809–811.
15. Young JZ. The Life of Vertebrates. 2nd ed. Oxford, England: Oxford University Press; 1966.
16. Lawton LA, Edwards C, Codd GA. Analyst 1994;119:1525–1530.
17. Ward CJ, Beattie KA, Lee EYC, Codd GA. FEMS Microbiol. Lett. 1997;153:465–473.
18. Metcalf JS, Bell SG, Codd GA. Wat. Res. 2000;34:2761–2769.
19. Edwards C, Beattie KA, Scrimgeour CM, Codd GA. Toxicon 1992;30:1165–1175.
20. Furey A, Crowley J, Shuilleabhain AN, Skullberg OM, James KJ. Toxicon 2003;41:297–303.
21. Pflugmacher S, Wiegand C, Beattie KA, Krause E, Steinberg CEW, Codd GA. Environ. Toxicol. Chem. 2001;20:846–852.
22. Fox DL. In: Kear J, Duplaix-Hall N, editors. Flamingos. Berkhamstead: T. & A.D. Poyser; 1975. pp 163–182.
23. Fox DL, McBeth JW. Comp. Biochem. Physiol. 1970;34:707–713.
24. Lewis DC, Metallinos-Katsaras ES, Grivetti LE. J. Cult. Geography 1987;7:51–65.
25. Pflugmacher S, Wiegand C, Oberemm A, Beattie KA, Krause E, Codd GA, Steinberg CEW. Biochim. Biophys. Acta. 1998;1425:527–533.
26. Kondo F, Ikai Y, Oka H, Okumura M, Ishikawa N, Harada K-I, Matsuura K, Murata H, Suzuki M. Chem. Res. Toxicol. 1992;5:591–596.
27. Alonso-Andicoberry C, Garcia-Vilada L, Lopez-Rodas V, Costas E. Veterinary Record 2002;151:706–707.
28. Chittick E, Puschner B, Walsh M, Gearhart S, St. Leger J, Skocelas E, Branch S. Proceedings of the American Association of Zoo Veterinarians. Milwaukee, USA: American Association of Zoo Veterinarians; 2002. pp 115–116.