Determination of total mercury in muscle, intestines, liver and kidney tissues of cormorant *(Phalacrocorax carbo)*, great crested grebe *(Podiceps cristatus)* and Eurasian buzzard (*Buteo buteo)*

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ABSTRACT: The total mercury concentrations in four tissues (muscle, intestines, liver and kidney) of aquatic birds (cormorant – *Phalacrocorax carbo* and great crested grebe – *Podiceps cristatus*) and Eurasian buzzard (*Buteo buteo*) were determined by cold vapour atomic absorption spectrometry (CV-AAS) using an Advanced Mercury Analyser AMA 254. The results of the direct CV-AAS analyses of homogenised solid samples were in very good agreement with those obtained by CV-AFS and CV-AAS analyses after acid digestion. Mercury concentrations in the tested tissues of adult populations of great crested grebe and cormorant were nearly twice as high as in the Eurasian buzzard. Significantly higher mercury concentrations were found in the liver and kidney of the cormorant (7-times and 2-times, respectively) compared to great crested grebe. The highest mercury concentration (39.2 mg/kg DM) was found in liver of adult population of cormorant while the content of mercury in younger cormorants was approx. 6-times lower (5.8 mg/kg DM). The total mercury concentration in liver was 6-times higher (2–3-times in muscle and kidney) but 13-times lower then those of the cormorant population living in Japan (Tokyo, Lake Biwa) and in the United States (Nevada, Carson River), respectively.

Keywords: total mercury; atomic spectrometry; bird tissues; muscle; intestines; kidney; liver; cormorant (*Phalacrocorax carbo*); great crested grebe (*Podiceps cristatus*); Eurasian buzzard (*Buteo buteo*)

Mercury and its compounds are included among the most toxic substances found in the environment (Renner, 1997; Wheatley and Wyzga, 1997). The hazardous effect of mercury accumulated in living organisms (fishes, seafood etc.), however, was not well recognised before the Minamata tragedy in Japan (1953–1960). After this disaster began, environmental scientists, legislators, politicians and the public have become aware of mercury pollution in the global environment (Gerbersmann et al., 1997; Cai, 2000; Queuvallier et al., 2000). The Czech Republic faces increasing risks of environmental pollution caused by the emissions of mercury into the environment from a number of natural, as well as anthropogenic sources.

In contrast with most of other heavy metals, mercury and most of its compounds behave exceptionally in the environment due to their volatility and capability for alkylation. Its transformation to more toxic compounds like methyl mercury and strong tendency to bioaccumulation in the aquatic food chains has motivated intensive research on mercury as a pollutant of a global concern. Mercury takes part in a number of complex environmental cycles, i.e., aquatic-biological and atmospheric cycles. Environmental cycling of mercury can be

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described as series of processes where chemical and physical transformations are the governing factors for the distribution of mercury in, and between different compartments of the environment (Gerbersmann et al., 1997; Downs et al., 1998; Cai, 2000; Ebinghauser et al., 2000; Queuvallier et al., 2000; Lambertsson et al., 2001).

Mercury exists in a large number of different physical and chemical forms with a wide range of physical, chemical, and ecotoxicological properties that are of fundamental importance to its environmental behaviour (Downs et al., 1998; Ebinghauser et al., 2000). The three most important inorganic chemical forms of mercury (elemental mercury – Hg^0, mercurous – Hg_2^{2+} and mercuric – Hg^{2+} ions) are far more soluble and have a strong affinity for many inorganic and organic ligands, especially those containing sulfur. In aquatic environments part of total mercury content (T-Hg) is transformed into the form of mono-/di-alkylated (methylated Me-Hg^+, ethylated Et-Hg^+, etc.) and/or arylated forms of mercury. Those forms are strongly accumulated in aquatic organisms and also concentrated in aquatic food chains i.e., fish, birds etc. (Downs et al., 1998; Ebinghauser et al., 2000).

Fishes in the surface water serve as bioindicators of pollutant loads (Teh et al., 1997). Their increasing pollution is becoming a major problem for aquatic birds. Consuming contaminated fish and plants has caused mercury levels to become very high in some birds (O´Brien et al., 1995; Monteiro and Furness, 1997, 2001; Heinz and Hoffmann, 1998; Saeki et al., 2000; Henny et al., 2002; Heinz and Hoffmann, 2004). Because they are at the top of the food chain, tiny amounts of mercury are magnified in the fish-consuming birds.

The main aim of the paper was to determine the content of mercury in the first stage of the mercury cycle in the region of Hana (central part of Moravia). We selected aquatic birds, i.e., great crested grebe and cormorant (*Podiceps cristatus* and *Phalacrocorax carbo*) and Eurasian buzzard (*Buteo buteo*) of different age and gender as one model.

MATERIAL AND METHODS

Instrumentation

A Grindomix GM 200 (Retsch GmbH & Co. KG, Germany) mill was used for homogenisation of lyophilised samples. A high-pressure microwave digestion unit Ethos SE (Milestone, Italy) was applied for wet digestion of homogenised samples.

A PSA Millenium Merlin automated mercury analyser controlled by Avion software (P. S. Analytical Ltd., Orpington, GB) was used for the flow injection atomic fluorescence spectrometric (FIA-CV-AFS) determination of total mercury concentrations. The sample digests were injected (100 µl) into a flow injection analysis (FIA) manifold, merged with a stream of acidified bromide/bromate mixed solution and then passed through a UV cracking reactor. All organomercury species were converted to inorganic mercury. The inorganic species were reduced by reaction with $SnCl_2$ to elemental mercury. The elemental mercury cold vapours were purged with an argon stream, dried in PermaPure® membrane unit and detected by AFS at 253.65 nm in a quartz cell.

An Advanced Mercury Analyser AMA 254 (Altec, Prague, Czech Republic) controlled by a WinAMA software was used for the determination of total mercury concentrations. The instrument was suitable for the direct analysis of solid and liquid samples without the need for any sample pre-treatment. The homogenised solid samples were directly weighed (100 mg ± 0.1 mg) into pre-cleaned combustion boats and automatically inserted into the AMA 254 analyser. The sample was dried at 120°C for 90 s and thermally decomposed at 550°C for 180 s under an oxygen flow. Decomposition products were carried by the oxygen flow to an Au-amalgamator. Selectively trapped mercury was released from the amalgamator by a brief heat-up and finally quantified (measuring cycle, 60 s) as Hg^0 by cold-vapour AAS technique at 253.65 nm. The sample digests were injected (100 µl) into pre-cleaned combustion boats and treated as above.

The mercury analysers were regularly calibrated using two sets of standard solutions of 0.05, 0.1, 0.2, 0.3, 0.4 mg/l and 1, 2, 3, 4 and 5 mg/l of mercury for the first and second calibration interval, respectively. The calibration solutions were prepared daily in 0.1% (m/v) $K_2Cr_2O_7$ and 0.6 % (v/v) HNO_3 to improve their stability by dilution of a primary calibration standard at concentration 1.000 ± 0.002 g/l Hg in 2% (v/v) HNO_3 (Czech Metrological Institute, Prague, Czech Republic). Calibration curves were non-linear for both calibration ranges (0.05–0.4 and 1–5 mg/l) for AMA 254 analyser. The accuracy of the results was controlled by the analysis of standard reference material (SRM) of dogfish mus-

cle DORM-2 with content of Hg 4.64 ± 0.25 mg per kg (Institute for National Measurement Standard, National Research Council, Canada). The relative standard deviation (RSD) and limit of detection (3.S/N) was 2.06% (at 4.64 ± 0.25 mg/kg, n = 10) and 0.05 µg/kg, respectively.

Chemicals and samples

The bioaccumulation of mercury was evaluated in selected tissues (muscle, cleaned intestines, liver and kidney) of cormorant *(Phalacrocorax carbo)*, great crested grebe *(Podiceps cristatus)* and Eurasian buzzard (*Buteo buteo)* collected in the region of central Moravia. Birds nesting near the water reservoir of Zahlinice near Prerov were collected for the study. The birds (10 pcs. of Eurasian buzzard, 20 pcs. of great crested grebe – including 7 females and 20 pcs. of cormorant – including 10 pcs. of juveniles approx. 1 year olds, plumage was used) were collected on March 26th 2003. ANOVA statistical treatment of the data (P = 0.001) was applied.

Methods

All selected tissues were deeply frozen, lyophilised and homogenised in Grindomix GM 200. The homogenised samples were directly weighed into the combustion boats and determined by cold-vapour AAS technique at 253.65 nm in triplicate. Another two portions of the homogenised samples were mineralised by high-pressure acid digestion (in 10 ml of HNO_3, conc.) in a microwave oven Ethos SE (Milestone, Italy) in triplicate. One portion of the digest was directly introduced into pre-cleaned combustion boats and automatically inserted into the AMA 254 analyser and treated as above. The second portion of digest was analysed by hydride generation FIA-AFS using the PSA Millenium Merlin automated mercury analyser.

RESULTS AND DISCUSSION

Optimisation of FIA-CV-AFS conditions

Content of organic solvents (methanol, acetonitrile etc.) needed for complete dissolution of organometallic part of mercury negatively influenced the analytical signal of FIA-CV-AFS (Figure 1). Also presence of extraction agents (thioacetic acid, citric acid, cysteine, mercaptoethanole) and chloride ions (NaCl, HCl etc.) decreased the signal. For example, the AFS signal was reduced 6-times in 1% thioacetic acid and 3 mol/l HCl compare to 6 mol/l HCl and 0.1 mol/l NaCl or HNO_3 at the concentration of 20 ppb Hg. In addition, the signal was divided into two peaks in 1% thioacetic acid and 3 mol/l HCl.

Flow rate ratio of $SnCl_2$ and BrO_3^-/Br^- influenced the height, area and width of fluorescence signals of 10 ppb Hg in the presence of 6 mol/l HCl. The peak width was decreased and peak height was increased with increasing flow rate of one agent (Figure 2) when the flow rate of the other agent was kept constant at 2.5 ml/min. The peak shape was seriously improved. The sensitivity of the FIA-CV-AFS procedure reached 8.6 ppb, LOD = 0.4 ppb and LOQ = 1.3 ppb with reliability R = 0.9996 when 100 µl of

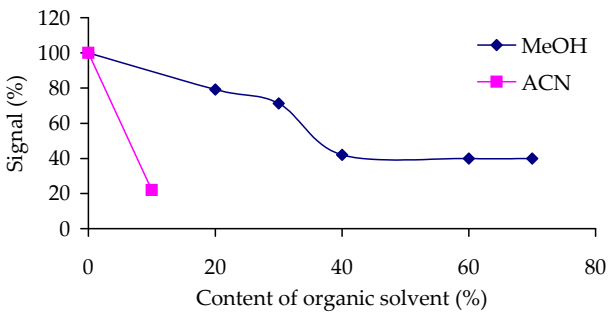

Figure 1. Dependence of an analytical signal of Hg on content of organic solvent (acetonitrile, methanol in %) in FIA-CV-AFS procedure at 10 ppb Hg in the presence of 6 mol/l HCl. The flow rate of both agents was 2.5 ml per min

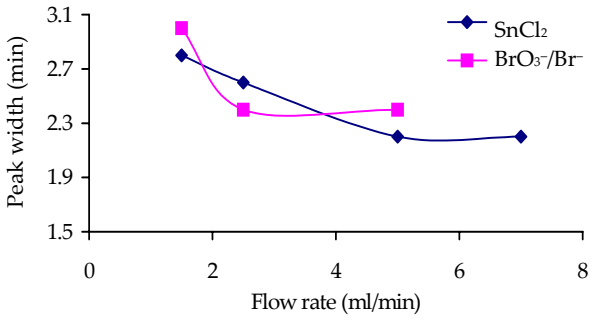

Figure 2. Dependence of an analytical signal of Hg on flow rate ratio of $SnCl_2$ and BrO_3^-/Br^- streams in FIA-CV-AFS procedure at 10 ppb Hg in the presence of 6 mol/l HCl. The flow rate of one agent was kept constant at 2.5 ml/min

Table 1. Comparison of the FIA-AFS method (AFS) and the CV.AAS method after acid digestion (AMA digest) and direct CV-AAS method (AMA solid)

Tissues	AFS (mg/kg)	AMA digest (mg/kg)	Δ[a] AFS-AMA (%)	AMA solid (mg/kg)	Δ[a] AMA solid – digest (%)
Cormorant (n = 20)					
Muscle	0.916	0.923	0.80	1.074	14
Intestines	0.741	0.749	1.10	0.825	9
Liver	5.106	4.906	4.10	5.23	6
Kidney	2.315	2.346	1.30	2.642	11
Great crested grebe (n = 20)					
Muscle	0.802	0.85	5.70	0.96	11
Intestines	1.509	1.568	3.80	1.815	14
Liver	2.23	2.234	0.20	2.538	12
Kidney	1.086	1.15	5.60	1.362	15
Mean			2.83%		12%

[a]difference in %; n – number of samples

the sample in 30% methanol was injected. Absolute limit of detection was estimated as 40 pg.

Comparison of FIA-AFS and CV-AAS

The homogenised solid samples and one portion of digests of muscle, intestines, liver and kidney of cormorant and great crested grebe were directly analysed by the AMA 254 analyser in triplicate. One portion of the same mineralised samples was analysed by hydride generation FIA-AFS (n = 3–5). The results were compared using t- and F-tests. All mean values obtained by the three methods were identical. Also variances for digests analysed by FIA-AFS and AAS were identical. On the other hand, the variances for digests and solid samples analysed by AAS differed for both sets of tissues. The results of direct AAS analyses were 6–15% rel. higher than the results of analyses of digests (Table 1). Due to the simplicity, better repeatability, sufficient sensitivity and higher throughput, the

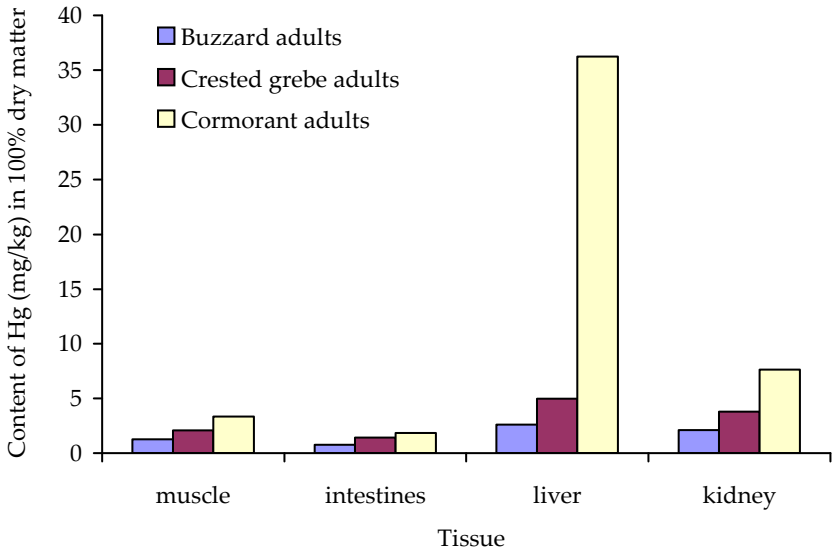

Figure 3. Content of total mercury (mg/kg DW) in the tissues of adult birds

direct analysis of homogenised solid samples by CV-AAS using AMA 254 analyser was used for the determination of total mercury in the tissues.

Analyses of real samples

Concentrations of total mercury (n = 3–5) were determined in four tissues (muscle, intestines, liver and kidney) of adult birds including cormorant *(Phalacrocorax carbo)*, great crested grebe *(Podiceps cristatus)* and Eurasian buzzard (*Buteo buteo)* nesting near the water reservoir of Zahlinice. Two of them (cormorant and great crested grebe) are predominantly fish predators. Small fishes (perch – *Perca fluviatilis*, jack – *Esox lucius*, carp – *Cyprinus carpio*, approximately 8 cm long), insects, amphibians and molluscs form the main part of diet of great crested grebes in contrary to cormorant that prefer mainly largest fishes (approx. 20–25 cm long). The Eurasian buzzard population preferably consumes field-mice – *Microtus arvalis* (70%) and was selected as a control population. Mammals (89.1%) form the main part of rest of their feed, in addition to birds (7.2%) and vertebrates (3.7%).

Approximately twice higher concentrations of mercury were found in all tested tissues of adult

Table 2. Concentration of total mercury (in mg/kg in dry matter – DM) in muscle, intestines, liver and kidney of birds nesting near water reservoir Zahlinice (Prerov region)

	Tissue	Median	SD	CV	Max	Min
Eurasian buzzard (*Buteo buteo*) Adults (n = 10)	muscle	1.27	0.27	1.01–1.53	1.69	0.60
	intestines	0.77	0.38	0.45–1.08	1.17	0.44
	liver	2.61	1.81	1.52–3.70	4.50	0.78
	kidney	2.09	0.44	1.70–2.50	2.65	1.09
Great crested grebe (*Podiceps cristatus*) Adults (n = 20)	muscle	2.07	0.54	1.63–2.51	3.50	1.40
	intestines	1.42	0.39	1.08–1.76	2.30	0.66
	liver	4.97	2.47	0.00–10.39	20.38	3.63
	kidney	3.78	1.95	1.79–5.77	12.11	2.67
Cormorant (*Phalacrocorax carbo*) Adults (n = 10)	muscle	3.34	0.84	2.24–4.43	4.81	2.14
	intestines	1.85	1.05	0.69–3.01	3.12	0.87
	liver	36.24	30.56	5.85–66.62	58.22	13.32
	kidney	7.61	5.79	2.33–12.91	11.36	4.13
Cormorant (*Phalacrocorax carbo*) Juveniles (n = 10)	muscle	2.59	1.06	0.37–4.81	3.64	1.20
	intestines	1.05	0.94	0.00–2.90	2.14	0.40
	liver	5.77	3.08	0.00–17.41	14.68	3.94
	kidney	4.61	0.67	1.26–7.96	5.26	1.43
Great crested grebe (*Podiceps cristatus*) Male (n = 13)	muscle	2.06	0.30	1.72–2.39	2.38	1.40
	intestines	1.42	0.39	0.96–1.88	2.30	0.66
	liver	3.87	0.44	0.00–8.72	20.35	3.63
	kidney	3.94	2.11	0.71–7.17	12.11	2.67
Great crested grebe (*Podiceps cristatus*) Female (n = 7)	muscle	3.12	0.72	0.26–5.97	3.50	1.79
	intestines	1.11	0.23	0.19–2.03	1.54	0.99
	liver	6.82	4.28	0.00–33.32	19.82	4.51
	kidney	4.10	2.58	0.00–17.46	10.54	2.71

SD – standard deviation; CV – coefficient of variation; Max, Min – extreme values; n – number of samples

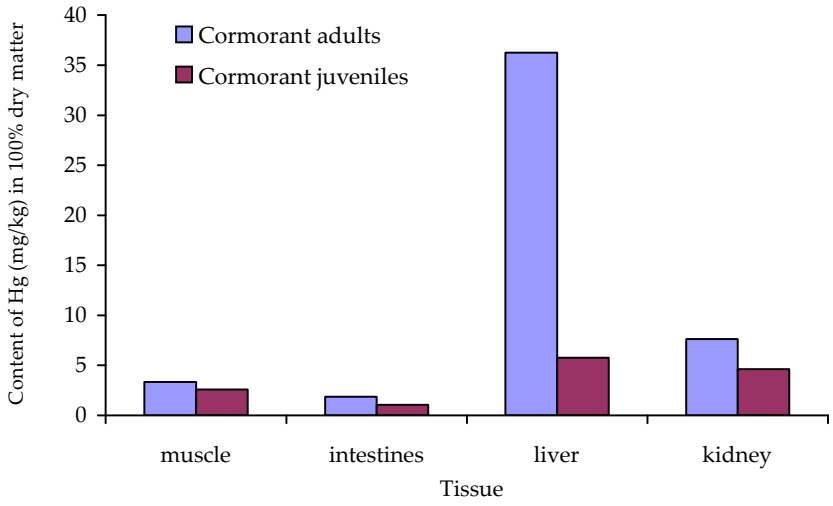

Figure 4. Content of total mercury (mg/kg DW) in the tissues of adult and young cormorants

populations of great crested grebe and cormorant (Table 1, Figure 3) compare to Eurasian buzzard (1.6-times in muscle, 1.9-times in intestines and liver and 1.8-times in kidney) due to the different diet. Previous studies have shown that total mercury contents exceeded 0.3 to 0.4 mg/kg in the great crested grebe's preys (fish 8 cm long). Such concentrations were found in fish of certain water reservoirs but clear relations remain to be established between these data and the breeding success of birds.

Significantly higher content of mercury was observed in liver and kidney of cormorant (7.3-times in liver and 2-times in kidney) compared to great crested grebe by ANOVA analysis. The highest content of total mercury (39.2 mg/kg DM) was observed in liver of adult population of cormorant. Thus the higher content of Hg could be related to the higher amount of Hg accumulated from the longer fishes eaten by older cormorant species during longer period.

The influence of age of the cormorant population (Figure 4) was also approved by ANOVA analysis. Since the exact age of bird is difficult to determine, the whole population was divided into two groups on the basis of plumage and the colour of feathers – to juveniles (younger then 1 year) and adults. The content of mercury in liver of juveniles was approx. 6-times lower then in liver of older population.

Generally, the highest concentrations of mercury were determined in liver of all three population of interest since the birds demethylate mercury in tissues like liver and kidney to inorganic forms and those forms are accumulated in the tissues. The total mercury levels in great crested grebes

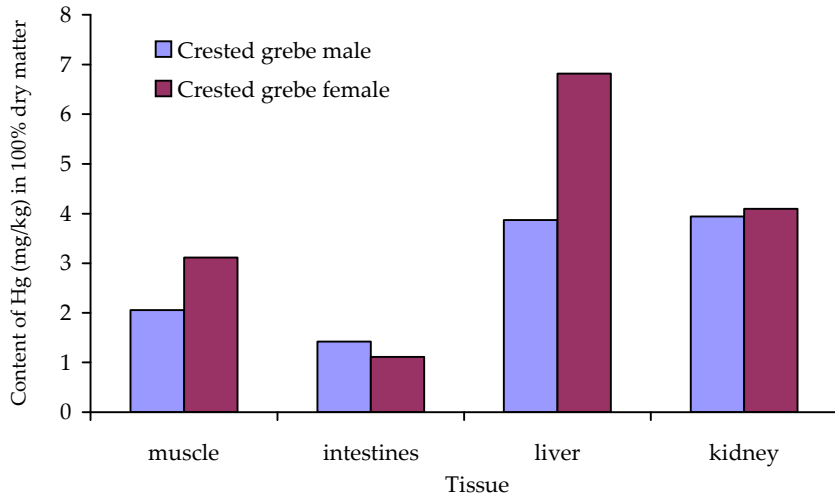

Figure 5. Content of total mercury (mg/kg DW) in the tissues of adult great crested grebes (male and female)

Table 3. Content of total mercury (in mg/kg of fresh matter) in liver of cormorant population (n = 20) nesting near Zahlinice (Czech Republic), in Japan and Nevada (Carson River)

	Muscle (mg/kg) FM	Liver (mg/kg) FM	Kidney (mg/kg) FM	Reference
Tokyo (Japan)	0.3 ± 0.2	1.2 ± 0.5	0.9 ± 0.7	Saeki et al. (2000)
Lake Biwa (Japan)	0.5 ± 0.2	1.7 ± 0.8	1.5 ± 1.0	Saeki et al. (2000)
Carson River, Nevada	–	134.8	69.4	Henny et al. (2002)
Our results	1.0 ± 0.3	10.0 ± 8.3	2.7 ± 2.1	This paper

FM – fresh matter

and cormorant tissues depend on the composition of bird's feed. Since the cormorant populations predominantly consume the larger amounts of the highly Hg contaminated (and of course also older) fishes than great crested grebe populations, the total content of mercury is much higher in liver and kidney of cormorants. The mercury concentrations were 1.8-times higher in liver of female population than in male great crested grebe population (Figure 5).

Our results show that the concentrations of total mercury in the tested tissues of the cormorant population living near Zahlinice (Czech Republic) are much higher (Table 2) then those in tissues of cormorant (Saeki et al., 2000) living in Japan (Tokyo, Lake Biwa), but one order of magnitude lower then those found in tissues of cormorants nesting along the lower Carson River, Nevada, USA (Henny et al., 2002). Concentration of mercury in liver was 6-times higher then those from Japan but 13-times lower then those from Nevada.

CONCLUSIONS

The accumulation of mercury in animal tissues can influence growth and can cause reproduction and breeding related problems, therefore endangering the survival of affected organisms. Because of this element's recognised toxicity and its largely documented presence in the ichthyological fauna (fish, seafood etc.), our data could be useful to gather more information on the presence of such risks to these birds. The results of this study will provide a better understanding of the effects of mercury on the fauna and contribute to the adjustment of actions in the control of emissions and the protection and conservation of biodiversity.

REFERENCES

Cai Y. (2000): Speciation and analysis of mercury, arsenic and selenium by atomic fluorescence spectrometry. TrAC Trends in Analytical Chemistry, 19, 62–66.

Downs S.G., Macleod C.L., Lester J.N. (1998): Mercury in precipitation and its relations to bioaccumulation in fish: A literature review. Water, Air and Soil Pollution, 108, 149–187.

Ebinghauser R., Kock R.R., Schmolke S.R. (2000): Measurements of atmospheric mercury with high time resolution. Recent applications in environmental research and monitoring. Fressenius Journal of Analytical Chemistry, 371, 806–815.

Gerbersmann C., Heisterkamp M., Adams F.C., Broekaert J.A.C. (1997): Two methods for the speciation analysis of mercury in fish involving microwave-assisted digestion and gas chromatography-atomic emission spectrometry. Analytica Chimica Acta, 350, 273–285.

Heinz G.H., Hoffmann D.J. (1998): Mercury chloride and selenomethionine interactions on health and reproduction in mallard eggs. Environment Toxicological Chemistry, 17, 139–145.

Heinz G.H., Hoffmann D.J., (2004): Mercury accumulation and loss in mallards. Environment Toxicological Chemistry, 23, 222–224.

Henny C.J., Hill, E.F., Hoffmann D.J., Spalding M.G., Grove R.A. (2002): Nineteenth century mercury: Hazard to wading birds and cormorants of the Carson River, Nevada. Ecotoxicology, 11, 213–231.

Lambertsson L., Lundberg E., Nilsson M., Frech W. (2001): Applications of enriched stable isotope tracers in combination with isotope dilution GC-ICP-MS to study mercury species transformation in sea sediments during in situ ethylation and determination. Journal of Analytical Atomic Spectroscopy, 16, 296–301.

Monteiro L.R., Furness R.W. (1997): Accelerated increase in mercury contamination in north Atlantic mesopelagic

food chains as indicated by time series of seabird feathers. Environment Toxicological Chemistry, 16, 2489–2493.

Monteiro L.R., Furness R.W. (2001): Kinetics, dose response, excretion and toxicity of methylmercury in free-living Cory's sherwater chicks. Environment Toxicological Chemistry 20, 1816–1823.

O´Brien D.J., Poppenga R.H., Ramm C.W. (1995): An exploratory analysis of liver element relationships in a case series of common loons (*Gavia immer*). Preventive Veterinary Medicine, 25, 37–49.

Queuvallier P., Filippelli M., Horvat M. (2000): Method performance evaluation for methylmercury determination in fish and sediment. TrAC Trends in Analytical Chemistry, 19, 157–166.

Renner R. (1997): Rethinking water quality standards for metal toxicity. Environmental Science and Technology, 31, 466A–468A.

Saeki K., Okabe Y., Kim E-Y., Tanabe S., Fukuda M., Tatsukawa R. (2000): Mercury and cadmium in common cormorants (*Phalacrocorax carbo*). Environmental Pollution, 108, 249–255.

Teh S.J., Adams S.M., Hinton D.E. (1997): Histopathologic biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. Aquatic Toxicology, 37, 51–70.

Wheatley B., Wyzga R. (1997): Mercury as a global pollutant – human health issues. Water, Air and Soil Pollution, 97, 5–7.

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