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Bioaccumulation of Lead, Cadmium, and Lindane in Zebra Mussels (*Dreissena polymorpha*) and Associated Risk for Bioconcentration in Tufted Duck (*Aythia fuligula*)

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In Europe, Lake Geneva is as a major bird conservation area, especially with regards to colonial waterbirds. It is a very important place for migratory birds especially. Tufted ducks (*Aythia fuligula*) winter around the lake and in wetlands like the Rhône delta (Camargue) or in Corsica. They can be observed from November until mid-April, when they leave to fly north. Many birds are also sedentary in the northern part of France (Duquet *et al*, 1995). During their stay around Lake Geneva, lesser scaups primarily feed on zebra mussels. They can dive down to 7 m depth and usually consume shellfish around 1 to 1.5 cm length (Duquet *et al.*, 1995, Custer C. and Custer T., 1995, Tessier *et al.*, 1995). In the Netherlands, it has already been postulated that zebra mussels could constitute a threat to their predators in particularly contaminated areas, because of their high pollutant load (De Kock and Bowmer, 1993, Tessier *et al.*, 1995). It is also suggested, that zebra mussels are a low-energy diet for ducks and may, as such, impair the reproductive success of birds. Despite a high proportion of water (>90% fresh weight), the high lipid content of zebra mussels (12 % dry weight) may, however, compensate for their high water content (De Kock and Bowmer, 1993). Zebra mussels represent over 50% of the total biomass of eukaryots in Lake Geneva. The pollutants analyzed were selected on the basis of their presence in zebra mussels (Corvi *et al.*1998). Lead and cadmium appeared as the major metallic contaminants along the lakeshore, while lindane was present at all sampling locations. Until July 1998, lindane was a major insecticide used as a seed coating and as a wood-preservative agent, in this area of traditional wood dwelling. Other chlorinated hydrocarbons such as diphenyl dichloroethylene (DDT) derivatives and Polychlorinated biphenyls (PCBs') were not present at high concentrations in mussels, therefore they were not selected for further analysis around Lake Geneva (Corvi *et al.* 1998). The purpose of this study was to determine lead, cadmium and lindane accumulation in zebra mussels and in Tufted ducks feeding on them locally.

MATERIALS AND METHODS

Mussels were collected between 2 and 4 m depth at various locations along the south bank (see Figure 1). At each site, mussels were manually removed from stones and stored in local freshwater for 2 hours before freezing (-25°C).

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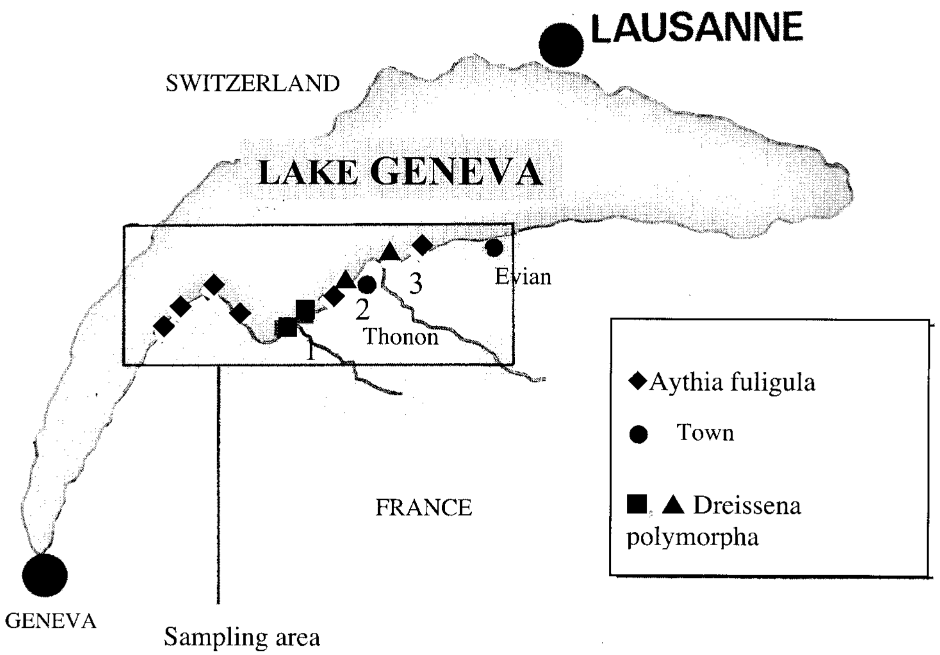

Figure 1. Sampling location of *Aythia fuligula* and *Dreissena polymorpha* along the south bank of Lake Geneva. *Dreissena sp* collected ■ in agricultural areas, ▲ in other areas 1 : Sechex area; 2. Thonon area; 3. Port Pinard area

Sampling occurred during spring and fall 1996, 1997 and 1998. Individuals between 1 and 1.5 cm represent the majority of the shellfish population and were selected for contaminant analysis. This size corresponds to adult mussels (about 2 years old) and is fairly consistent along Lake Geneva shore (Corvi *et al.* 1998)
At each site and time of sampling, 1 water sample was taken and kept in closed polyethylene containers. Containers were pre-washed with ethanol and acidified (HNO_3, 0.5%) ultra pure MilliQ® water. Water samples were analyzed shortly after collection and always kept at –25°C before analysis.

Liver samples were obtained from hunters and the Hunting Federation of Haute-Savoie on birds shot (kidneys could not be collected in all birds). The organs selected were kept frozen (-25°C) until analyzed. A total of 21 ducks were collected at various locations around the French (south) bank (Figure 1). Gender was recorded at the time of shooting and confirmed after necropsy (8F, 13M). For each bird, visual examination of the gizzard was performed. During this examination, the diet of all birds appeared to be mussels (soft tissue and shells). X-ray analysis was also conducted to detect any lead shot potentially ingested as part of the grit. It was necessary to remove all shells before taking X-rays, because shells absorbed X-rays. Only one radiograph revealed a lead shot in the gizzard, probably as a result of shooting, since the shot was deeply inserted in the gizzard mucosa with evidence of hemorrhage and no other tissue reaction. Duck samples were collected during winter 1996/1997 and 1997/1998 (mostly between December and January). Lead was analyzed by graphite furnace atomic absorption spectrophotometry (GF-AAS). All material was acid-washed and kept in closed conditions before use. All solvents and chemicals used in heavy metal extractions were of the highest purity available. After drying, mussel and duck liver samples were mineralized at 700°C for 10 hours, with progressive increase/decrease in temperature. Samples were then digested with concentrated HNO_3 (65%) and acid was eliminated by slow heating. The dry residue was dissolved in acidic water (0.5% HNO_3) and diluted as needed (based on the Pb or Cd concentration) with ultra pure MilliQ® water. Only soft tissue was analyzed. GF-AAS was performed with a Thermo-Optek (Unicam) apparatus, using Zeeman backgound correction, at 217 nm. Cadmium was analyzed on the same sample using a specific lamp at 228.8 nm. Linear regression was used for calibration purposes (lead). For Cadmium, quadratic regression was always used. Standard curves were accepted when correlation coefficients were >0.99. Certified mussel samples were used (CRM-278, Promochem, France) and analyzed as quality control samples. For better homogeneity, 12-15 mussels of each site were pooled for each analysis. Only 1.0 g duck liver or mussel tissue was mineralized. For each site and each sampling period, 4 independent measures were obtained in zebra mussels. Water samples were analyzed directly after acidification with HNO_3 (0.5%). Lindane was analyzed according to Berny *et al* (2002) on soft tissues of mussels. One g sample was extracted. For liver samples, 1.0 g was used. For water samples, 10 mL were used. Lindane standard (>99% purity) was obtained from CIL (Ste Foy la Grande, France). Analysis was performed on a gas chromatograph with a ^{63}Ni ECD (HP 5890 series II). The limit of detection (mean noise level + 3 SD) was 0.8 ng/g, the limit of quantification (mean noise level + 10 SD) was 1.2 ng/g in mussels and in duck liver. The Limit of detection was 0.1 μg/L for water. Spiked mussel and chicken liver samples were also used in order to determine the linearity of the procedure over the range 1 – 100 ng/g wet weight. The correlation coefficients were always found to be > 0.99 (five points). Repeatability was determined with 3 determinations of standards and spiked samples (water, mussels and duck liver) and CVs' were found in acceptable ranges (< 10%) both for standards and samples. Therefore, each sample was run only once afterwards.

Our data grossly followed log-normal distributions. Therefore, groups were compared by means of non-parametric tests (Mann-Whitney and Kruskall-Wallis). A p-value of 0.05 was selected as the significance threshold.

RESULTS AND DISCUSSION

The mean value measured in certified mussel samples was 1.92 µg/g for lead (certified value 2.0 ± 0.04 µg/g) and 0.040 µg/g for cadmium (certified value 0.0348 ± 0.007 µg/g). Coefficients of variation were computed for repeatability and < 15% in all cases. Lead, cadmium and lindane concentrations in zebra mussels are presented in Figure 2 for the 4 sampling periods and the three selected sites. Although spring samples generally appear less contaminated, the difference was not significant for Cd and Pb. There was a significant site effect for lindane ($p=0.02$), with Thonon (negative control site) less contaminated. The median lead concentration was 3.1 µg/g (dry weight) and the median cadmium concentration was 0.9 µg/g (dry weight). In water samples, lead (<5 µg/L), cadmium (<1 µg/L) and lindane (0.1 µg/L at the most) were below threshold values for drinking water. The concentrations of heavy metals found in zebra mussels were in the same order of magnitude as those measured in mussels collected in other rivers and lakes of France. For instance, Chevreuil et al (1996) measured values between between 1.4 and 3.7 µg/g dry weight for lead, and between 0.7 and 1.8 µg/g dry weight for cadmium in mussels collected in the river Seine. Values were in the same range in the Rhine and Meuse rivers (France, Germany) (Jan Hendriks *et al.* 1998). Our data were also very similar to or even slightly higher than those measured in *Dreissena polymorpha* in Northern Germany (Wiesner *et al.* 2001) and in Austria (Sures *et al.* 1999). These authors considered that no health effect was to be expected in mussels with such values. According to the 4-grade classification suggested by Mersch (1996), the concentrations of lead measured in zebra mussels were representative of an "intermediate pollution"(grade 2 : between 0.5 and 4 µg/g dry weight) situation, with occasionally (spring 1997) concentrations interpreted as "pollution" peaks (grade 3 : between 4 and 14 µg/g dry weight). For cadmium, values were always grade-2 (between 1 and 2.5 µg/g dry weight) (Corvi *et al.* 1998). Seasonal variation has inconsistently been observed with lead and cadmium and may reflect changes in abiotic as well as metabolic factors (spawning may decrease the overall metal content for instance). There does not seem to be a simple explanation for this variation (Wiesner *et al.* 2001). Residues of lead and cadmium in shells have been determined in few studies but found to be in the same order of magnitude as those found in soft tissues (Wiesner *et al.*, 2001). The median lindane concentration was 6.6 ng/g in this study. There was a significant site effect. Lindane concentrations measured in mussels from Thonon were much less elevated than concentrations detected in samples from the 2 other sites. There was no significant effect of season on lindane distribution in *Dreissena polymorpha*. With respect to lindane in zebra mussels, our values were similar to other results from France (Chevreuil *et al.* 1996), although quite elevated compared with other countries like the USA (Roper *et al.* 1996). Lindane concentrations in mussels were close to the accepted maximum residue limit in shellfish in France (for human beings) and not toxic to mussels (Berny *et al.* 2002). Lindane and lead concentrations in duck samples are given in Table 1. Cadmium concentrations were <0.04 µg/g (dry weight) in all birds.

In tufted ducks, heavy metal concentrations were low, especially for lead when compared with other results in France (Pain 1991), or when compared with Canvasbacks (*A. valisineria*) and scaups (*A. affinis*) from San Francisco Bay, USA (Hothem *et al.* 1998). Lead poisoning by ingestion of lead shots is a very common problem in many countries. For instance, Scheuhammer and Dickson (1996) consider that, in Canada, ingestion of lead shots is the primary source of lead poisoning and Mateo *et al.* (1998) found up to 87% of waterfowl with lead shots in the gizzard in Spain.

Table 1. Lead and lindane (HCH) concentrations in liver of tufted ducks

Gender	*Pb**	*HCH°*	*Gender*	*Pb**	*HCH°*
F	0.29	74.6	M	0.38	9.8
F	0.29	40.5	**M**	**0.31**	**18.0**
F	0.2	44.7	M	0.55	9.9
F	0.42	47.7	M	0.13	10.0
F	0.04	5.5	M	2.97	8.9
F	0.18	175.6	M	1.22	11.0
F	0.35	148.2	M	0.48	11.5
F	0.39	86.6	M	0.26	29.6
			M	0.16	52.2
			M	0.26	40.9
			M	0.35	35.4
			M	0.15	38.0
			M	0.05	26.8

* in µg/g dry weight ; ° in ng/g wet weight. **bold** : duck with embedded lead shot

Lake Geneva is a large wetland and the load of lead shots is spread over a wide area, therefore resulting in a lower risk of poisoning. Apart for the lead shot observed in one gizzard (muscle) as a result of shooting, we did not find any lead shot in the gizzard content analyzed (21 ducks). Based on these considerations, the major source of lead appeared to be via the ingestion of zebra mussels. Cadmium has been less extensively studied in waterfowl. In our study, cadmium concentrations in the liver were unusually low, although mussels were moderately contaminated. Unfortunately we could not analyze kidneys, which are considered to concentrate cadmium in many species, including avian species (Hothem *et al.* 1998). The median lead concentration was 0.29 µg/g (dry weight), and the median lindane concentration was 35.4 ng/g. There was a significant gender effect for lindane, with higher concentrations in females, but there was no gender effect with lead.

The only significant toxicant found in tufted ducks was lindane, at values close to MRL accepted in France. These values have not been associated with bird poisoning, however, and should not represent a threat either to tufted ducks or to hunters consuming these birds. It has been shown that migratory ducks feeding on zebra mussels are susceptible to short term contaminant exposure and that their contaminant load primarily reflects local exposure (Mazak *et al.* 1997). Steady –state concentrations of dieldrin and PCBs in muscle were achieved after 8 days of consumption, as reported by Mazak *et al.* (1997) for instance.

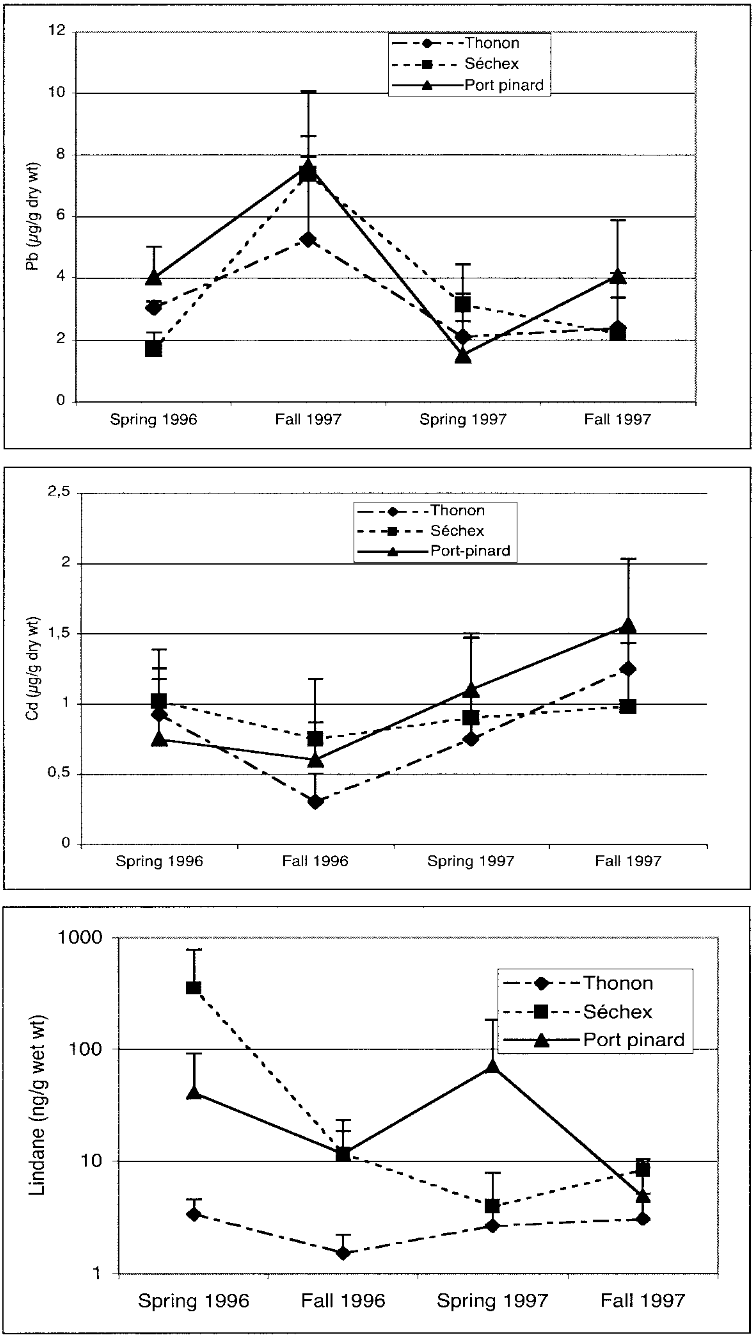

Figure 2. Lead, cadmium and lindane concentrations in *Dreissena sp*. collected at 3 sites along the south bank of lake Geneva from Spring 1996 to Fall 1997 (vertical bars represent standard errors of four pooled samples).

Therefore, the results analyzed here can be regarded as representative of the local situation, especially since birds hunted and analyzed in this study were killed several weeks after mass migration occurred (November). Furthermore, organic contaminants appear as the major threat to wintering waterfowl feeding on zebra mussels, while metals such as cadmium accumulate over longer periods of time and should be considered when long term exposure ($\geq$ 6 months) may occur, in non-migratory birds (De Kock and Bowmer 1993, Mazak *et al.* 1997). Therefore, based on values measured in zebra mussels and tufted ducks, we can conclude that food transfer of the heavy metals Pb and Cd is a minor problem in wintering ducks around Lake Geneva, while lindane, the only organic contaminant tested here, is more significant on a short-term basis.

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