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The Determination of Mercury Levels in Aquatic Avian Populations Using Atomic Spectroscopy Techniques

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The Determination of Mercury Levels in Aquatic Avian Populations Using Atomic Spectroscopy Techniques

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Abstract

Mercury contamination has been shown to have a negative impact in fish-eating birds, including impacts on behavior, reproductive success, and their overall survival. Higher than normal mercury levels have been detected in birds who depend on fish for their food. High levels of mercury have been detected in the bird's blood and feathers using analytical techniques. The present accepted method of determining levels of mercury in feathers and blood is the use of atomic spectroscopy. The amount of mercury in feathers appears to depend on the growth of the feathers with more growth associated with lower levels of mercury contamination. In this review paper, I will review the role of atomic spectroscopy in determining mercury levels in bird blood and the feathers of birds.

Introduction

Mercury (Hg) is a persistent substance that comes from natural and anthropogenic sources. Bacteria in water convert inorganic Hg that enters aquatic systems into methylmercury, bioaccumulating in aquatic food webs. As a consequence, birds and other wildlife are exposed to Hg, primarily through the consumption of contaminated fish. Because they are highly visible top predators, seabirds may be used to monitor spatial and temporal patterns of Hg contamination in marine ecosystems. Several studies in a variety of seabirds have shown that 70% of the Hg body burden is contained in the plumage. Seabirds usually renew their plumage every year after breeding, although some species have summer and winter plumages and therefore have two more or less complete molts per year.

Feather samples have been used to assess Hg body burdens in several seabird species. These studies have demonstrated large differences among species in the Hg content of feathers, even among co-occurring species. Furthermore, high Hg levels have been reported from seabirds inhabiting remote islands where anthropogenic releases of Hg are low.

One of the most frequently used methods for determining Hg concentrations in biological samples is cold vapor atomic absorption spectrometry (CV-AAS). Widely used in the biomonitoring of environmental pollution, this method gives the total amount of mercury in a sample. The cold-vapor technique is an atomization method limited to only the determination of mercury, due to it being the only metallic element to have a large

enough vapor pressure at ambient temperature. Because of this, it has an important use in determining organic mercury compounds in samples and their distribution in the environment. Detection limits for this technique are in the parts-per-billion range making it an excellent mercury detection atomization method.

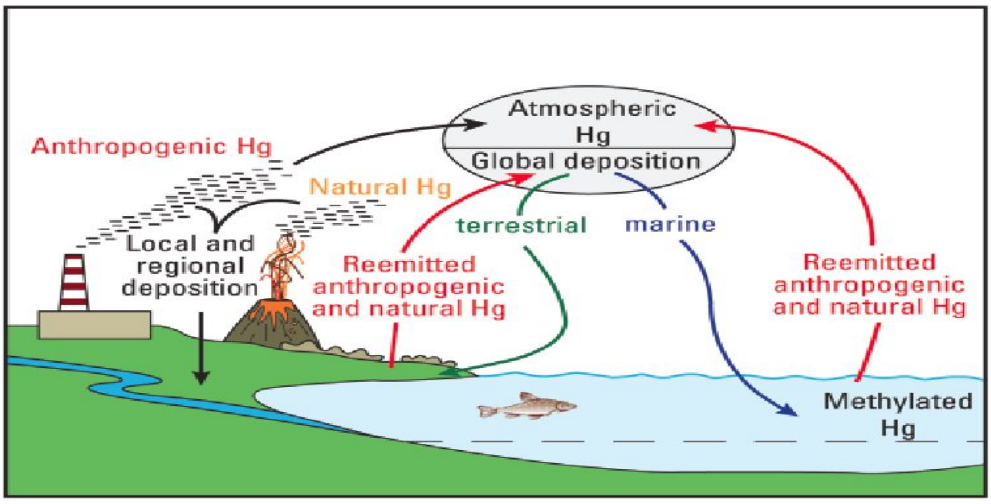
The purpose of this paper is to review the route of transmission of mercury in the aquatic environment and to review the techniques currently used to detect the trace amounts of mercury in aquatic birds.

Sources of Mercury in Aquatic Environments

Anthropogenic sources has accounted for mercury contamination in water bodies worldwide. Restrictions put in place in North America have limited emissions of Hg but global recycling of previous pollution continues in many areas [1]. Marsh and wetland areas are often areas of high mercury methylation and bioavailability due to the hydrology, acid-base status, and sediment characteristics present [2]. Much of the mercury found in aqueous environments is due to discharges from chlor-alkali manufacturing plants [3] These type of plants produce Cl_2 and NaOH which are both important for the chemical industry. They are both produced by the electrolysis of aqueous sodium chloride. A carbon anode is used to generate chlorine and a mercury pool cathode collects metallic sodium as a mercury amalgam. The metallic sodium is then reacted with water in a separate compartment to produce sodium hydroxide. The two-stage process in the production of NaOH is used to keep the product free of the NaCl starting material. Discharges of the mercury amalgam have caused serious

mercury contamination in the water ways. Although, Chlor-alkali plants can be retrofitted to reduce the mercury discharges, most of the installations are being phased out. Aquatic organisms have been seen to have elevated levels of mercury even in areas that are remote from a mercury source. It has been found that mercury is transported over long distances in aquatic environments [3]. Other discharge sources of mercury into the environment are from manufacturing plants that use mercury in various applications such as in manufacturing batteries, lamps, and other electrical equipment. Also, mercury is used to extract gold and silver from ores in Brazil [3]. **(See Figure 1)**

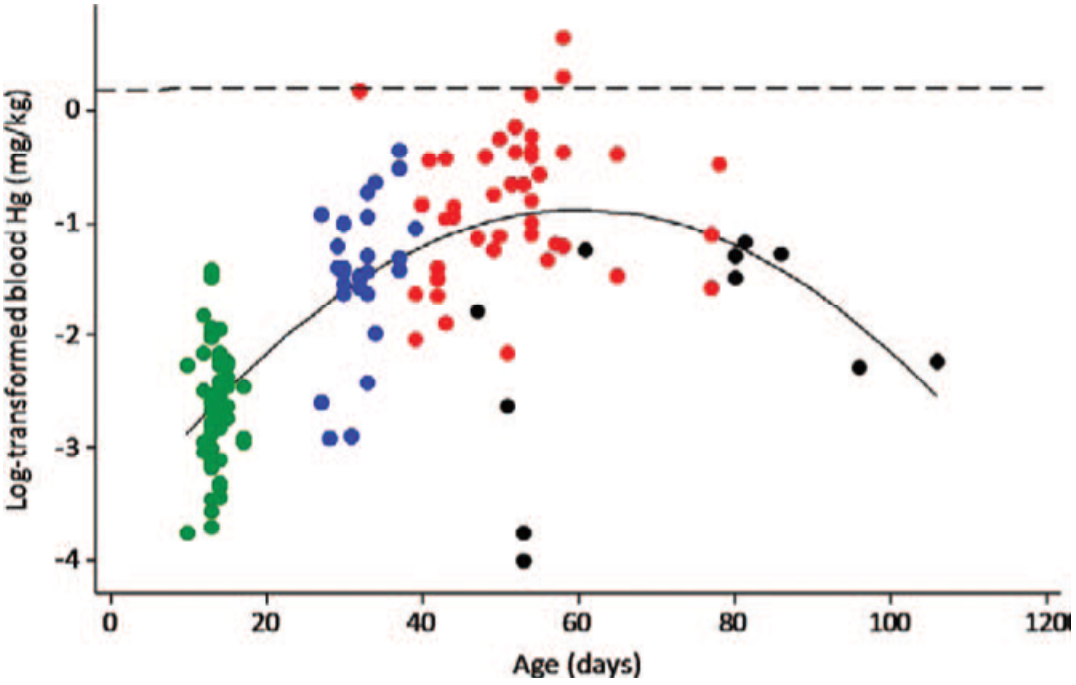
Figure 1: Mercury cycle in Aquatic environments [5]



Mercury Poisoning in Aquatic Birds

Mercury contamination in the environment has been included among the most toxic of compounds detected [4]. Mercury is considered more of a problem when contrasted with other heavy metal contaminants due to its ability to transform into more toxic compounds like methyl mercury. Mercury also has a strong tendency to bioaccumulate to high levels in aquatic food chains making it a toxin of global concern [5]. Methylmercury contamination can have serious effects in fish-eating birds. Birds may have effects on their behavior, reproductive success, and survival when exposed to methylmercury [6]. Birds that are contaminated with methylmercury, appear to have several mechanisms to reduce their levels of mercury. Growing feathers in birds may serve as an elimination route for mercury but is supported with indirect evidence. Methylmercury has a high affinity for free thiol groups (-SH) that are abundant in the keratin found in bird feathers [7].It has been determined that a large proportion (50-93%) of body burden mercury is found in the feathers of birds.[8]. Laboratory studies have suggested that turnover of mercury levels in aquatic birds is faster during feather growth as compared to after feather growth ceases in adults [9].(**See Figure 2).** Mercury dynamics is an important factor in assessing lifetime risk. Net annual bioaccumulation occurs in tissues when the annual intake of mercury is greater than the elimination capacity of the organism [10].

Figure 2: Blood mercury plotted against age of fledgling. Colors represent different feather growth stages. Green represents nestling; blue represents waning; red represents none; black represents molt. [14]



Monitoring and Collection of Mercury Samples

Aquatic birds are useful for indicating changes in contaminant levels in an aquatic environment [11]. Several recommendations have been made in regard to the kinds of specimens that should be collected and monitored [12]. The species and tissue selected for study should bioaccumulate contaminants in concentrations that can be measured in small amounts but large enough to allow multiple analyses. The species and tissue chosen should be easy and relatively inexpensive to sample allowing for constant access for more samples to be obtained. The tissue samples must be collected in a manner that eliminates extraneous contamination or changes in the contaminants of interest. Finally, the species and tissue chosen for the project must be representative of the area being studied. Eggs from aquatic bird species have been identified as a key

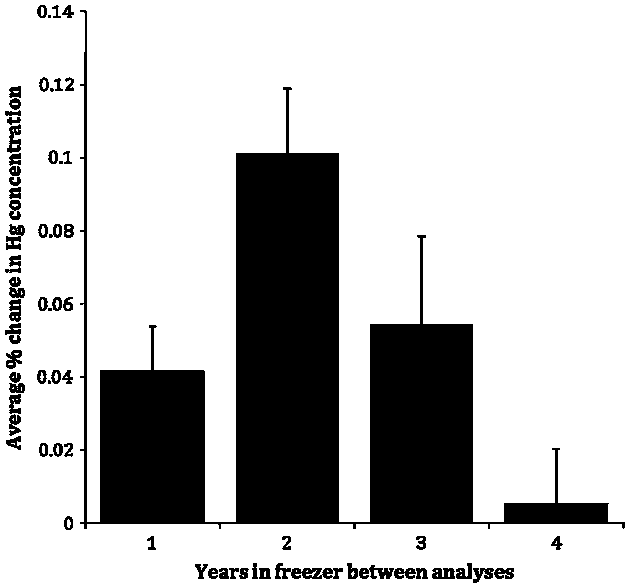
tissue for measuring and monitoring persistent toxic pollutants in the environment. Measuring accumulation of contaminants in aquatic birds is easier if the birds are at or near the top of the food web. Bioaccumulation of contaminants are higher for organisms at the top of the food web and are therefore easier to measure. Eggs of aquatic birds are easy to collect and represent a complete, distinct unit. The egg contents are protected from extraneous contamination by the eggshell and membrane [13]. Another method of measuring contaminants in aquatic birds is by collecting samples of blood. Blood sampling is usually done with nestlings because of the ease at which to obtain samples. Nestling blood has been used as a biomarker because it contains contaminants that are localized spatially and temporally [14]. With this method of collecting samples, birds must be trapped repeatedly and blood samples taken with each capture.

Storing and Banking of Biological Samples

Specimen banking is distributed worldwide and are used to store samples collected from a wide variety of sources and for a variety of purposes. Specimen banking and storage has been used on a small scale for the storage of aquatic bird tissues that have been contaminated. Private research collections are the largest collection of samples but they may not be as stringent to avoid contamination [13]. One advantage to having banked samples is the reduction of differences arising from inter-laboratory quality assurance. This allows for a better comparability of results between different labs. Having banked samples also allows for long-term studies such as with global climate change and its effect on contamination patterns [15]. The use of banked samples has also been used to examine the effects of changing analytical techniques [16]. Re-testing

using more current techniques may be done on older samples that were not tested regularly or done with poor analytical techniques. Storage techniques are in need of standardization. Questions regarding techniques used to store samples have arisen regarding possible contamination of samples [17]. Mercury levels have been seen to change owith the number of years in storage. (**See Figure 3)** Standardized storage techniques and procedures would reduce or eliminate extraneous contamination of samples.

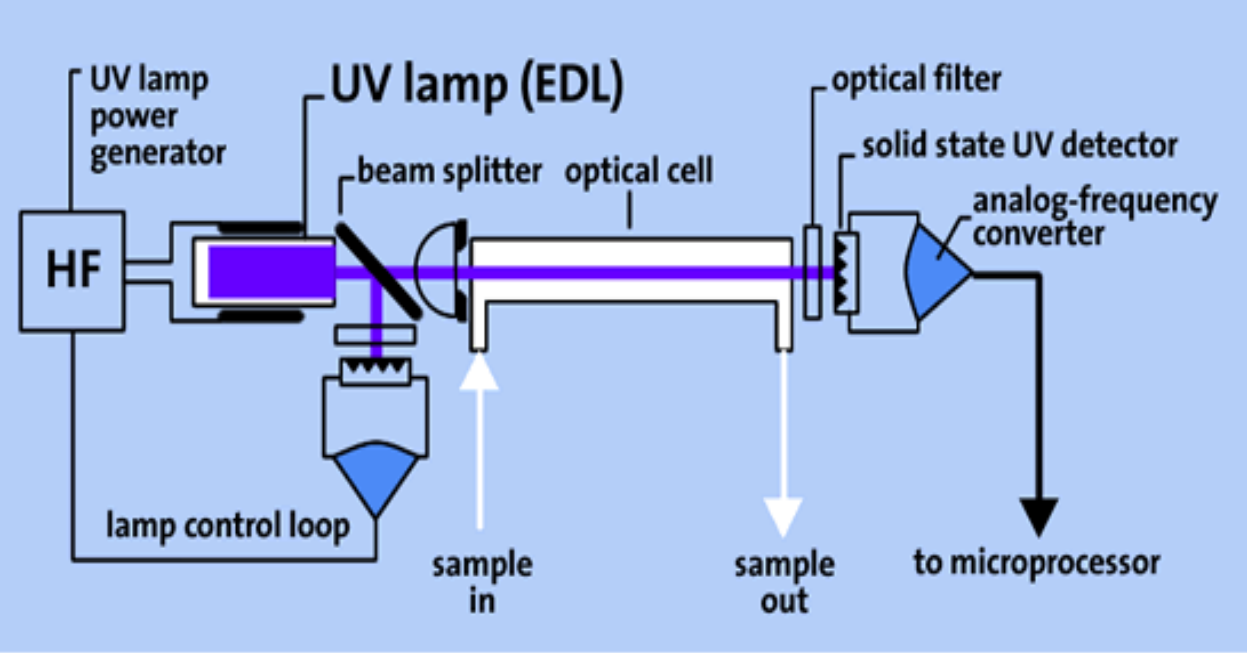
Figure 3 Average change in mercury by number of years in storage. *Error bars* are one standard error [26]



Analytical Methods for Mercury Determination in Biological Samples

Cold vapor atomic absorption spectroscopy (CV-AAS) is one of the most common analytical approaches for determining mercury levels in environmental samples because of its simplicity, high sensitivity and relatively low operating costs [18] Commonly used when measuring trace amounts of volatile heavy metals such as mercury, CV-AAS makes use of the unique properties of mercury that allows vapor measurement at room temperature. The method initiates by converting mercury into Hg^{2+} by oxidation from nitric and sulfuric acids, followed by a reduction of Hg^{2+} with tin(II) chloride. The mercury is then excited by a collimated ultraviolet light source at a wavelength of 253.7 nm. The sample is then swept into a long-pass absorption tube by adding a stream of inert gas through the reaction mixture. The sample is swept into the path of an atomic absorption detector consisting of a multichannel peristaltic pump for controlling liquid flow, a device for drying the carrier gas, thermally stable double beam optics , and ultra stable mercury vapor lamp and detector capable of measuring atomic absorbance at 253.7 nm (See Figure 4) [19,20].

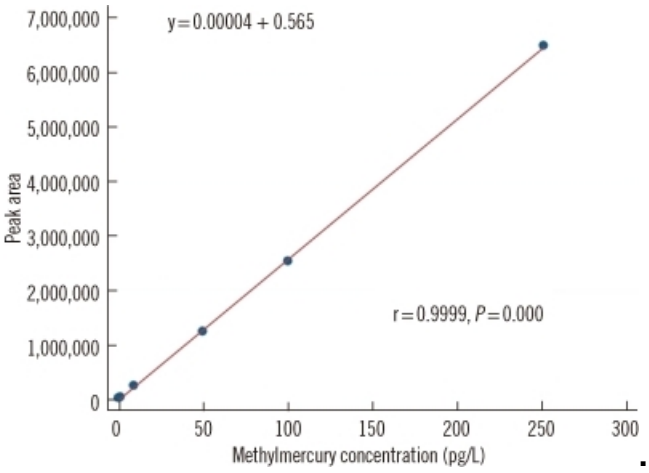
Figure 4: Schematic diagram of the AAS measuring-cell unit [Mercury Instruments]



Because of the very low mercury concentrations in biological samples, a preconcentration step needs to be combined with the cold vapor atomic absorption technique. For the quantification of very low mercury levels, amalgamation for generated Hg(0) vapor on silver, iridium, gold or platinum traps have been suggested for use[21]. The gold trap consists of gold wire wound on a pyrolite graphite platform. This platform is inserted into the graphite tube prior to analysis. Besides the gold trap providing an increased sensitivity to the trace mercury, the preconcentration step will also reduce the interferences in the atomization step. In order to determine accurately the amount of mercury by CV-AAS in organisms, the mercury must be converted to mercury (II) prior to its reduction to elemental mercury. This step requires the decomposition of the samples prior to the reduction step. The most efficient method for the decomposition of the sample is using microwave oven digestion for preparing the

environmental and biological samples when determining the total mercury by CV-AAS. The microwave digestion method takes the dried sample and treats it with an aqua solution of HCl/HNO_3. The sample if then heated in the oven for a predetermined amount of time. When the sample is removed, it is filtered and stored until analysis [21]. The microwave digestion method is efficient because it minimizes the loss of mercury and reduces the digestion time significantly [22]. Prior to analyzing the samples, the CV-AAS instrument must be standardized using a standard blank and standard solutions of known concentrations. From the measurements with the standard solutions, a standard curve can be determined. **(See Figure 5)** Once the instrument is standardized (correlation coefficient is less than or equal to 0.998), then the sample can be analyzed with confidence [20]. If the sample concentration is above or below the working range concentrations of the standards, then the analytical sample will need to be diluted or concentrated to fall within the range. The analyte concentration can be determined using a standard additions technique. The slope of standard additions curve for analytical samples is + or – 50% of the slope of standard additions curve for a standard blank [20].

Figure 5 Calibration curve of methylmercury concencentration vs. peak area. [20]



Data Analysis of CV-AAS Analysis of Biological Samples

The calculation of the mercury content is done on the basis of the calibration curve parameters. The peak height of the sample is determined from the recorder chart and the mercury level is determined based on the calibration curve determined prior to the sample being analyzed. The operating conditions in this analytical method should be optimized by improving the performance. One way of improving the performance is to conduct a multivariate approach, which accounts for the possible interaction between all the variables [23]. A composite design can be applied to study the effect of hydrochloric acid, reductant ($NaBH_4$ and $SnCl_2$) concentration and the sample flow on the Hg(0) signal. The multivariate optimization of the Hg(0) signal allows the researcher to find the best analytical conditions with a reduced number of experiments [24**].**

Reliability and Validation Standards for CV-AAS and Mercury Detection

A basic feature of any measurement is reliability: the numerical value representing a measured property can only be considered a measurement if it is reliable. The analytical tools used in measuring must be reliable in order to be relevant. Every procedure should be characterized as precisely as possible (validated) in order that highly reliable measurements can be obtained. Validation of any method is important to ensure the accuracy of results [19]. Validation parameters for the CV-AAS analysis (linearity, repeatability, LOD, LOQ, range, trueness, accuracy, and uncertainty) are determined prior to analysis. CV-AAS has a high level of selectivity because the amalgamation is a selective reaction for mercury and absorption is measured using a wavelength characteristic of mercury. A high linear procedure is demonstrated when a high regression coefficient is produced with the calibration curve. The measurement range is a concentration range from the LOQ section to the maximum standard solution concentration. This range is equal to 0.29-1,000 ng. [20]. The range of sample concentration should be within the standard range. LOD (limit of detection) and LOQ (limit of quantification) are determined from a series of measurements of standard solutions with the three lowest mercury levels. The LOD for mercury detection with CV-AAS is 0.096 ng and the LOQ is 0.29 ng [19, 20]. Repeatability is determined with three independent measures of standard solutions used for calibration with different mercury levels. An uncertainty value is determined with the maximum values for each measure compared. Trueness is based on the results of three samples of certified reference materials and is shown by recovery values of the mercury. Uncertainty is a result of the uncertainty of the calibration curve, the unrepeatability of results, and the determination

of trueness. The combined uncertainty value is calculated and compared to a standard for the method (<15% for mercury detection) [20]. If the uncertainty is below this level then the method is compliant.

Issues Related to Detection of Mercury in Aquatic Birds

When researching mercury contamination in aquatic birds, the results obtained may be complicated to interpret. Mercury can disrupt reproduction for many aquatic birds preventing their eggs from hatching and increasing mortality in chicks [25] Birds living in wetlands suffer from mercury contamination because bacteria in the water convert inorganic mercury into methylmercury, which birds absorb more readily. The fish also take up the methylmercury and when the birds eat the fish more contaminant is transferred. To measure mercury levels in birds, scientists have measured mercury in the blood, feathers, and eggs of birds. Scientists will usually test one of the three tissues due to collection difficulties and measuring limits. When researchers have tested all three tissue types from the same birds, they found no correlation in mercury levels between the three types of tissues [26]. These results suggest that assumptions on mercury levels cannot be made when only a single tissue is tested. The fact that there is no detectable mercury level in the blood doesn't mean there isn't mercury contamination in the eggs of the birds. Other factors that may affect the results of a study include the time between measurements and also long-term storage of blood samples, which may cause a change in mercury levels [25].

Conclusions

The accumulation of mercury in aquatic bird tissues can influence growth and cause reproduction and breeding related problems. Because of mercury's recognized toxicity and its largely documented presence in the aquatic food web, data obtained by atomic spectroscopy is valuable in gathering more information on the presence of such risks in birds. The results of studies using these methods will lead to a better understanding of the effects of mercury on aquatic birds and may contribute to the initiation of actions to control the emissions and help protect and conserve biodiversity.

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