

Monitoring Potential Cadmium Levels in Avian Tissues Associated with the Savannah Harbor Expansion Project: Year 3 of Monitoring Annual Report

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INTRODUCTION

The Savannah Harbor Expansion Project (SHEP) involves dredging the Savannah River and will eventually result in deposition of sediment containing potentially high concentrations of Cadmium (Cd) into dredged material containment areas (DMCAs) near Savannah. Cadmium is a toxic metal with both natural and anthropogenic sources and no known nutritional value (Wayland and Scheuhammer 2011). Effects of toxic concentrations of Cd on birds range from organ damage to behavioral changes. The SHEP DMCAs contain upland and aquatic habitats and are bounded by salt marsh and riverine habitats, which tend to attract a wide range of avifauna. Dredged material deposition may be a potential Cd exposure route for birds attracted to these impoundments, although USACE is developing a plan to limit avifauna exposure to Cd-laden sediments. The Environmental Assessment and FONSI can be found at <http://www.sas.usace.army.mil/About/Divisions-and-Offices/Planning-Division/Plans-and-Reports/> As part of the monitoring program to determine potential environmental impacts associated with the SHEP, we examined Cd uptake by avian species associated with containment areas in 2014/2015 and 2015/2016, both sampling periods prior to deposition of dredged materials containing the Cd. Inner harbor dredging also did not occur in 2016/2017 (Year 3, this report) and samples collected in this period are included as an additional year of pre-construction monitoring. This monitoring will continue during the construction (placement of Cd-laden sediments) and for three years post construction.

The strategy to examine potential Cd risk to avifauna using the DMCAs was to document potential Cd uptake in a set of sentinel avian species. These sentinel avian species, which were monitored during two seasons (summer and winter), were determined by USACE and the US Fish and Wildlife Service (USFWS) and based on long-term monitoring of avian occurrences at the DMCAs. Suites of available sentinel species were influenced by the hydrologic condition (wet vs dry) of the DMCAs. In the third year of this monitoring, as in the first two years, we attempted to monitor all sentinel species to provide a baseline for future monitoring. However, discussions prior to the completion of 3rd year of sampling resulted in a reduction in sentinel species (see Table 1). Monitoring in subsequent years, during potential Cd deposition and bioavailability, will likely focus on a further reduced number of sentinel species, but with a greater number of samples per species.

The primary approach to assess Cd risk, as requested in the SOW, was to analyze blood for Cd from the sentinel resident and migratory species because concentrations of Cd in blood are indicative of recent uptake in adult birds. Cadmium can be removed from blood as quickly as 48-hours (Sell 1975). The use of blood from live-captured birds for metals analysis is a nonlethal technique that has little impact on avian populations. We determined during the two previous sampling years that certain species (e.g., Mottled Ducks, Northern Shovelers, American Avocets, Mourning Doves) could not be live-captured effectively and, after discussions with USACE, acquired appropriate federal and state permits for lethal take of these species. In addition to the blood samples collected from the lethal takes, this method allowed us to examine blood concentrations relative to other tissues of interest (e.g., kidney and liver), tissues that are more indicative of long-term exposure. As optional monitoring, we opportunistically collected avian prey base samples including invertebrates (e.g., aquatic and terrestrial insects) and seeds/fruit from the DMCA's and analyzed them for Cd to provide insights into potential transmission routes of Cd into sentinel and other non-sentinel wildlife.

Our goal in the third year of this project was to continue capturing sentinel avian species and collecting blood samples for chemical (primarily Cd) analyses during the Summer (2016) and Winter (2016/2017) seasons, as well as the concentrations in associated tissues and potential prey base. Here we report our live captures, lethal takes, and the contaminant findings within the avian blood and tissue and potential prey base.

METHODS

Study Area

The DMCA's targeted to receive the sediments potentially contaminated with Cd are 14A and 14B, so our avian collections were originally to be focused within and adjacent to these areas. They are bordered on the north by salt marsh and tidal creeks and to the south by the Savannah River. Dikes around the perimeter will maintain standing water within the DMCA, but the perimeter may still support shrubs and small trees, providing habitat for additional species (Figure 1).

However, construction activities at DMCA 14B during the first and second year of monitoring prevented us from accessing or collecting any samples from that area. In the third year, 14B was available for sampling but 14A and portions of other DMCA's were not available due to construction activities. In addition, the impacts of heavy rainfall and large equipment used on the DMCA's occasionally rendered several of the access roads impassable for short periods, which also influenced avian sampling. Therefore, given these conditions and that this was another a "control"/pre-Cd deposition year, we attempted to focus sampling effort on 14B and 13B but agreed to include captures from five DMCA's (12A, 13A, 13B, 14A, 14B) within the Savannah Harbor Navigation Project.

Sentinel Species & Potential Alternative Species

The sentinel species collection SOW (provided by USACE) targeted collecting blood from a minimum of 10 individuals of each sentinel species during each season (summer vs winter) (see Table 1). To the extent possible, the within-season collections were to be split between early and late periods: Summer – April & August; Winter – October/November & March/April. However, the availability of certain target species was influenced by existing hydrological conditions and/or the behavior of the species. As mentioned above, we had varied success capturing the project sentinel species. Therefore we banded and collected blood samples from some potential alternative avian species in case capturing the sentinel species proved problematic. In Year 3 this potential “alternate” species were limited to Northern Cardinals, only. This species was often easier to capture than the sentinel species and we include their Cd concentrations in this report for comparison and additional background information.

Mid-year discussions between USACE and SREL pertaining to reducing the number of sentinel species to allow increased sample size resulted in dropping three original sentinel species: Brown Thrashers, Eastern Towhees, and Mourning Doves. We included the Cd findings of the three dropped species within this report. Blue-winged Teal were added as a winter sentinel species due to concerns that the preferred sample rate for American Avocets might not be obtainable.

Avian Capture/Blood & Tissue Collection Methods

We employed multiple techniques for capturing DMCA avifauna. Many of the sentinel species (Table 1) were passerines and were captured with mist nets. These nets (30-mm mesh, 2.4m tall X 12m long) were typically deployed in cleared lanes either adjacent to shrubby habitat or through forested habitat during the morning/early afternoon period. Playback equipment broadcasting specific bird songs were often used to attract and capture specific species. Black-necked stilts were captured by two methods. We employed nest traps and remotely-triggered bownets to capture nesting adult stilts (in May/early June), which involved flushing an attending parent off a nest with eggs and then covering the nest with a “funnel trap” made of light-weight fencing (1m tall, 1.2m diameter) or placing the bownet next to the nest. After deploying the trap/net, the parent would return to the nest and either be enclosed in the trap or netted under the bownet. A second method for the stilts, later in the summer, was to hand-capture and collect samples from pre-flight age stilt nestlings

Due to poor success live-capturing four sentinel species (Mourning Dove (summer species), Mottled Ducks (summer species), Northern Shovelers (winter species), and American Avocets (winter species)) in the two initial years of this project despite multiple efforts employing multiple methodologies, we received permission from USACE and scientific collecting permits from USFWS/SCDNR to collect these species lethally (e.g., shotgun).

Avian Blood/Tissue Collections

For all live-captures, we collected blood samples from either the jugular, brachial (wing) or femoral (leg) vein with a syringe. Volumes of blood collected ranged from < 0.05 ml to 0.80 ml, depending on bird condition, size, and method (live vs lethal) of collection (we were permitted to collect blood volume NTE 1% of the bird's weight for birds to be released). Blood samples were archived (frozen) prior to chemical analysis. Captured individuals received appropriately sized USGS aluminum leg bands prior to release to allow potential observations of marked birds and/or recaptures (and resampling) of known individuals. Resampling (blood) from recaptured birds, although not common, allows for between/among year comparisons. These blood collection/banding activities were conducted under SREL's bird banding permit (BBL permit # 22002).

As mentioned above, select species were collected by lethal methods (shotgun). In addition, a small number of birds (N=3) died during the handling process. The lethally-collected birds were immediately dissected to access the heart and blood was drawn from the organ with a syringe and archived as with the other blood live-capture collections. The carcasses were stored in ice and eventually frozen. After returning to SREL, kidney, liver, and gizzard contents were removed from the carcasses for analyses for contaminants and comparison to the blood concentrations. Lethal take activities were conducted under SREL's federal migratory bird scientific collecting permit (MB65214A-0) as well as state of South Carolina scientific collecting permits.

Optional Prey Base Collections

Preliminary collections of potential prey base (for avian species) samples within and/or adjacent to the DMCAs occurred again in Year 3. Collections of aquatic and terrestrial insects and other potential prey involved use of sweep nets and other devices. Seeds and fruits were collected by hand. Vegetation (leaves) of selected plants was collected to determine if they were the source of Cd for the insects. Due to the small size/mass of many potential prey items, we combined many items into composite samples prior to analyses.

Chemical Analyses

Biological samples (e.g., blood, tissues, prey) were analyzed for Cd as well as a suite of other metals (As, Cr, Cu, Ni, Pb, Se, Sr, Zn) at SREL. Samples were digested in multiple batches of 12, which included two sample replicates, a reagent blank, and a certified standard reference material, following EPA method 3052. After digestion, trace element analyses were performed according to EPA method 6020, utilizing an ICP-MS NexION 300X, (PerkinElmer Inc., Toronto, Canada). Concentrations of elements were reported in ppm ($\mu\text{g/g}$) wet weight for blood and ppm ($\mu\text{g/g}$) dry weight for avian tissues and potential prey items.

The average method detection limit (MDLs – Cd concentrations below these levels could not be accurately determined given the instrumentation, volumes of samples, and Cd concentrations within the samples) was 0.006 Cd ppm wet weight for avian blood, and was 0.013 $\mu\text{g/g}$ dry

weight for avian tissues and potential prey items. Lethal collections allowed for larger blood volumes to be analyzed than with live captures, and the resulting MDL for these samples was 0.001 Cd ppm. Regardless, MDLs determined for avian blood and tissues in this study were far below the Cd concentrations listed as potential levels resulting in toxic effects (see Discussion).

Concentrations of elements other than Cd in blood, avian tissues, and potential prey are documented in Appendices 1, 2, and 3.

RESULTS

Blood Concentrations

In the third year of sampling, 88 blood samples were collected from birds associated with the Savannah DMCA, including 81 (total) from the 11 sentinel species and 7 from the alternative species. Twenty-two of these sentinel species samples (~27%) were above the MDLs (0.001 – 0.006 ppm wet wt) for Cd (Table 2). The majority of these above MDL samples (14 of 22, ~64%) were collected during the Summer sampling period (April-August). The summer avian species with the highest percentage of >MDL Cd blood concentrations were “terrestrial” species: Brown Thrasher (100%, but only 2 collected), and Mourning Dove (70%). Of the four “aquatic” sentinel species, Northern Shovelers and American Avocets, both winter species, had the highest percentage (50 and 40%, respectively) of > MDL blood concentrations, although sample sizes were low (≤ 5). The maximum blood Cd concentration observed in the third year of monitoring was 0.12 ppm (wet wt) in a Savannah Sparrow, far below the concentration (0.26 ppm wet wt.) associated with possible toxic effects for Cd (see Discussion).

Within-season comparisons (early vs late) for those species with sufficient sample sizes are shown in Table 3. Only Mourning Doves had enough > MDL Cd levels for coarse statistical comparison, which was not significantly different (Wilcoxon Two-sample Test; $PR > |Z| = 0.0570$), but suggested that late season samples might have higher Cd concentrations than early season samples.

A single bird (Savannah Sparrow) banded in Year 2 was recaptured in Year 3. Its blood Cd concentration was < MDL in Years 2 and 3.

Tissue Comparisons

We lethally collected 5 American Avocets, 11 Mottled Ducks, 11 Mourning Doves, 4 Northern Shovelers, and had three capture/handling mortalities (one each: Black-necked Stilt nestling, Song Sparrow, and Northern Cardinal) which allowed us to examine Cd concentrations among other tissues and gizzard contents. Kidney and liver are the primary storage organs for Cd, and we analyzed gizzard contents as an indicator of recent dietary intake. Unfortunately, blood Cd concentrations of most of these collected birds were <MDL, which precluded comparisons of

these tissues with blood levels for all species except Mourning Doves (N=11). There was no significant correlation between blood and gizzard content concentrations (Spearman Rank Correlation, $r_s = 0.1793$, Prob > $|r| = 0.5978$). Kidney concentrations were 1.5-20 times higher than liver concentrations among all species (Table 4). None of the kidney and liver concentrations of Cd were greater than the concentrations reported to impact avian wildlife (see Discussion). Assessment of the relatedness of kidney and liver tissues varied considerably by species (Table 5) although the tissues were significantly related overall (N=31, Spearman Rank, $r_s = 0.7790$, Prob > $|r| = 0.0001$). Gizzard contents averaged < 0.3 ppm Cd dry wt. and varied considerably among species (Table 4).

Potential Prey Base

Cadmium concentrations in potential prey items collected on the DMCA's remained as highly variable in the third year of monitoring (Table 6) as they were in the first two years. Potato beetles (Family Chrysomelidae) again had the highest average Cd concentration (> 11 and 2 ppm dry wt, for adults and larvae, respectively) among potential prey items. These insects typically foraged on leaves of Tropical Soda Apple (*Solanum viarum*), which averaged a Cd concentration of ~4 ppm Cd dry wt. Fruit of Blackberry (*Rubus* sp.) averaged ~1.4 ppm Cd whereas other plant seeds, foliage, and other insects all averaged < 1.0 ppm Cd dry wt.

DISCUSSION

Cadmium concentrations in birds vary widely according to ecosystem use, diet, age, and physiological status (Wayland and Scheuhammer 2011). Relative to ecosystem use, seabirds typically have > Cd than coastal birds, which typically have > Cd than freshwater wetland birds, which typically have > Cd than terrestrial birds. Dietary exposure to Cd tends to be greatest with insectivores, which includes several monitored seabird species such as albatrosses, petrels, and storm petrels (Wayland and Scheuhammer 2011), as opposed to piscivores or scavengers, but this varies. Cadmium does not “biomagnify” throughout trophic levels like some elements (e.g., mercury), but can biomagnify in lower trophic levels (e.g., water or sediment to lower invertebrates) (Eisler 1985, Scheuhammer 1987). Insects and their larvae, which are often more closely associated with potentially contaminated sediments, may provide the more likely pathway for Cd exposure to avifauna. Incidental sediment ingestion during normal foraging is another potential exposure pathway.

Within avian tissues, concentrations of Cd in kidney and liver reflect chronic exposure whereas blood concentrations reflect recent dietary exposure. The majority of Cd in birds is found in the kidney and liver, which typically accounts for 67-97% of the total body burden of Cd. Kidneys are the long-term storage organ for Cd, whereas liver concentrations are generally only 10-70% of kidney concentrations. Cadmium concentrations in blood are generally very low ($\leq 0.50 \mu\text{g/g}$ Cd wet wt). Age and diet are the two most important factors influencing Cd accumulation, with

older/adult birds typically having considerably higher Cd concentrations than younger, developing birds.

Avian tissue concentrations of Cd associated with potential toxic effects (e.g., organ damage, reduced breeding success, altered behaviors, etc.) are largely based on laboratory uptake studies and vary widely among species. Kidney concentrations $> 65 \mu\text{g/g}$ Cd (wet wt) are associated with a 50% likelihood of negative effects, although $100 \mu\text{g/g}$ Cd (wet wt) is a more liberal level of potential effects (Wayland and Scheuhammer 2011). Liver concentrations greater than $45\text{-}70 \mu\text{g/g}$ Cd (wet wt) and blood concentrations $\geq 0.26 \mu\text{g/g}$ Cd (wet wt) are suggested to be associated with toxic effects.

Concentrations of Cd in blood of avian sentinel species from the Savannah Harbor DMCA in Year 3 remained generally low, with approximately 73% being below the MDL range of $0.001\text{-}0.006 \mu\text{g/g}$ Cd (wet wt), and no blood samples were close to or greater than the concentration $0.26 \mu\text{g/g}$ Cd (wet wt) suggested to be associated with toxic effects. Some of the reduction in the percentage of samples exceeding the MDL in Year 3 (73% in Year 3 vs 71-72% in Years 1 and 2; Rhodes *et al.* 2015, 2016) may be due in part to the reduction in sentinel species, particularly Brown Thrashers. As in previous years, more terrestrial birds (64%) than aquatic birds (36%) had concentrations of blood Cd $>$ than the MDLs. This remains contrary to expectations that birds utilizing aquatic habitats should have higher Cd concentrations than those utilizing terrestrial habitats (Wayland and Scheuhammer 2011). Analyses of a limited set of potential avian prey collected on-site in Year 3 again also documented highly variable Cd bioavailability in the existing DMCA environment. The potato beetle samples (composite adult and average larvae) had the highest Cd concentrations (11 and 2 ppm Cd dry wt, respectively) again in Year 3 (27 and 15 ppm Cd average for larvae in Years 1 and 2, respectively), as did Tropical Soda Apple leaves, an invasive species where the larvae were collected. Other terrestrial vegetation and fruit had Cd concentrations < 1.5 ppm Cd (dry wt). Variability in bioaccumulation of Cd by plants has been documented (Eisler 1985, Rodrigue *et al.* 2007).

Analyses of potential within-season (early vs. late) and between season (summer vs. winter) differences in blood Cd levels was limited by detection levels and sample sizes. So few of the blood samples were above the MDLs that statistical comparison within and between seasons were not feasible. General within-season comparisons of three summer sentinel species (Mottled Ducks and Black-necked Stilts) did not suggest differences in Cd concentrations or percentages of $>$ MDL. Mourning Doves Cd concentrations suggested a possible seasonal difference (late $>$ early), but was not significantly different (see Results, Table 3). Numbers and percentages of above MDL sample concentrations (all species) suggests greater exposure to Cd in the summer months, although general comparisons like this are confounded by a lack of similar species, and numbers of samples of similar species, between seasons. The greater percentage during the summer season may be linked to greater insect abundance and/or larvae emergence during the spring/summer months. These within and between season comparisons may be more informative in the future sampling when the number of sentinel species are reduced to two per

season and the number of individuals per species per season increases from a maximum of 10 to 30.

Lethal collection of those species (Mottled Ducks, Northern Shovelers, American Avocets, and Mourning Doves) that had not been prone to live capture was generally effective during sample Year 3, especially for the summer species (Mottled Ducks, Mourning Doves). Collection of shovelers and avocets in the later portion of the winter season proved problematic as it was unseasonably warm during this period, numbers were lower than normal, and neither species was observed in accessible habitats during multiple site visits. We are exploring new approaches to allow greater access to the shallow habitats. However, normal facility operations on the active DMCAAs will likely to continue to influence collections of certain species (e.g., American Avocets).

Analyses of Cd concentrations among lethally-collected avifauna blood and gizzard content samples were not feasible for most species due to $< \text{MDL}$ Cd concentrations for avian blood. Dove blood and gizzard content concentrations, both as indicators of recent Cd uptake, were not correlated, possibly due to Cd absorption prior to ingested materials reaching the gizzard, retention time of contents within the gizzard, and/or simply low sample sizes. Regardless, detectable Cd in both blood and gizzard content suggest bioavailable Cd in the habitats used by these species. Concentrations of Cd in other tissues indicated accumulation primarily in kidneys, as expected. Liver concentrations were also all above detection limits, but considerably lower than kidney concentrations. As with the blood sampling effort, these findings will be enhanced by increased sample sizes and analysis of a greater number of potential prey items.

The findings of the three combined years of avian monitoring (Table 7) are similar to those of the individual year findings and continue to build a suitable baseline for comparison to samples when the Cd-containing sediments are added to the DMCAAs. For the pre-construction monitoring, terrestrial birds exhibited a higher percentage of $> \text{MDL}$ blood Cd concentrations than aquatic species and, among terrestrial species, summer samples exhibited a higher percentage of $> \text{MDL}$ blood concentrations than winter samples. Presumably the latter is due to greater insect availability as prey during this season.

The Savannah DMCA system is very dynamic, particularly relative to habitat availability and likely associated Cd bioavailability. The site constantly receives sediment from on-going maintenance dredging and these actions result in: (1) fluctuating water levels within active DMCAAs, which influences avian habitat availability, numbers and types of birds using them, and our ability to sample/collect sentinel species, and (2) potential legacy chemical inputs into the DMCAAs from harbor sediments. Construction related to the Savannah Harbor Expansion Project has also resulted in limited access to certain areas within the Savannah DMCA system for safety reasons.

An inherent component of the initial years of avian monitoring was the determination of which set of sentinel species (both within and between habitats and seasons) would be appropriate for the focused sampling (~30 samples per each of two species per season) when the Cd-contaminated sediment is added to the DMCA. The avian capture results through the initial two years of monitoring led to discussions and resulted in the recent reduction in number (-3) of sentinel species. Selection of the focal species during the Cd deposition period will also be dependent upon the landscape/habitat availability in the area of the active DMCA, hydrologic condition (wet or dry), as well as species occurrence and potential hazing activities on the DMCA (USACE 2016).

SUMMARY

The majority of avian blood samples (~73%) collected from the Savannah Harbor DMCA in the third year of monitoring was below the range of instrument detection levels (0.001-0.006 µg/g Cd wet wt). This finding is slightly lower but still similar to those of Years 1 and 2. No blood sample was found to be above the level of concern for potential toxic effects. The majority of the above-MDL samples were collected during the summer months, possibly due to the greater availability of certain species of insects (a food source) that bioaccumulate Cd during that season. This trend is also apparent in the cumulative avian blood samples (Years 1 - 3) presented in Table 6, which also indicates capture success rates between seasons. Cadmium found in blood, gizzard contents (from lethal collections) and on-site potential prey indicate that Cd is currently bioavailable in the DMCA system and/or that these birds forage at nearby sites with bioavailable Cd. Analyses of kidney and liver tissues from lethally-collected avifauna on the DMCA indicate that these species are accumulating Cd and that they are also likely accumulating Cd from other locations utilized in their history.

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Table 1. Sentinel^a and Alternative Birds Captured and Sampled on the Savannah Dredge Material Containment Areas (DMCA) in the Summer (S) and Winter (W) Periods of Year 3 of Monitoring.

Sentinel Species	Sentinel		
	Season	Spring-Summer	Fall-Winter
American Avocet ^b	W	-	5
Black-necked Stilt ^b	S	8	-
Brown Thrasher ^c	Both	2	0
Eastern Towhee ^c	Both	2	0
Mottled Duck ^a	S	12	-
Mourning Dove ^c	S	10	-
Northern Mockingbird	Both	3	1
Northern Shoveler ^a	W	-	4
Red-winged Blackbird	S	9	
Savannah Sparrow	W	-	16
Song Sparrow	W	-	9
<u>Alternative Species</u>			
Northern Cardinal	Both	7	0

^aThe list of sentinel species was reduced in mid-year after discussions between USACE and UGA-SREL.

^bThese species are typically associated with wet conditions/aquatic habitats, although all species utilize edge habitat between aquatic and terrestrial habitats.

^cThis species is no longer a sentinel species.

Table 2. Avian Blood Concentrations ($\mu\text{g/g}$ wet) of Cadmium from Birds Captured on the Savannah Harbor DMCA's Relative to the Method Detection Limits in 2016-2017 (Year 3 of Monitoring).

SPECIES	SEASON	N	# (%) < MDL	Max	MDL
<u>Sentinel^a</u>					
American Avocet	Winter	5	3(60%)	0.004	0.001
Black-necked Stilt	Summer	8	6 (75%)	0.042	0.006
Brown Thrasher ^a	Summer	2	0 (0%)	-	0.006
Eastern Towhee ^a	Summer	2	2 (100%)	-	0.006
Mottled Duck	Summer	12	10 (83%)	0.034	0.001
Mourning Dove ^a	Summer	10	3 (30%)	0.030	0.001
Northern Mockingbird	Summer	3	2 (67%)	0.040	0.006
	Winter	1	0 (0%)	-	0.006
Northern Shoveler	Winter	4	2 (50%)	0.012	0.001
Red-winged Blackbird	Summer	9	9 (100%)	-	0.006
Savannah Sparrow	Winter	16	14 (87%)	0.123	0.006
Song Sparrow	Winter	9	8 (89%)	0.024	0.006
<u>Alternate^b</u>					
Northern Cardinal	Summer	7	7 (100%)	-	0.006

^aSelected species were removed from the list of sentinel species in mid-season.

^bAlternate species were captured/sampled in case other sentinel species proved difficult to capture.

Table 3. Within-season Comparison of Avian Blood Concentrations ($\mu\text{g/g}$ wet) of Cadmium from Birds Captured on the Savannah Harbor DMCA in 2016-2017 (Year 3 of Monitoring).

SPECIES	Early Season		Late Season	
	N	# (%) < MDL	N	# (%) < MDL
<u>Sentinel</u>				
Black-necked Stilt	5	4 (80%)	3	2 (67%)
Mottled Duck	6	5 (83%)	5	4 (80%)
Mourning Dove ^a	5	2 (40%)	5	0 (0%)
Savannah Sparrow	9	7 (78%)	7	7 (100%)

^aWithin-season comparison of Cd concentrations was almost significantly different ($P < 0.06$). Two < MDL Cd concentrations were replaced with $\frac{1}{2}$ the MDL (MDL = 0.006 ppm) prior to comparison.

Table 4. Cadmium Concentrations ($\mu\text{g/g}$ dry wt) in Tissue Samples and Gizzard Contents of Sentinel Species Lethally Collected on the Savannah Harbor DMCAs in 2016/2017 (Year 3 of Monitoring).

Species (# Samples)	Cd Concentrations ($\mu\text{g/g}$)		
	Kidney	Liver	Gut Content
	Mean \pm St Dev (Max)	Mean \pm St Dev (Max)	Mean \pm St Dev (Max)
American Avocet (5)	10.892 \pm 6.493 (20.851)	0.948 \pm 0.597 (1.987)	0.542 \pm 0.183 (0.684)
Mourning Dove (11)	31.040 \pm 36.964 (108.758)	5.546 \pm 5.446 (17.286)	0.440 \pm 0.250 (0.831)
Northern Shoveler (4)	7.846 \pm 8.089 (18.579)	1.097 \pm 0.875 (2.188)	0.120 \pm 0.163 (0.358)
Mottled Duck (11)	2.509 \pm 3.726 (13.535)	0.403 \pm 0.270 (0.882)	0.261 \pm 0.418 (1.253)
Black-necked Stilt (1)	0.131	0.052	2.296
Song Sparrow (1)	1.721	1.377	0.732
Northern Cardinal (1)	110.741	5.077	1.189

^aThe Black-necked Stilt, Savannah Sparrow and Song Sparrow were capture mortalities, all others were collected via shotgun.

Table 5. Correlations^a of Kidney and Liver Cd Concentrations in Selected Species of Avifauna Collected on the Savannah Harbor DMCA in 2016/2017 (Year 3 of Monitoring).

Species	N	<i>r</i>	Prob > <i>r</i>
American Avocet	5	0.3000	0.6238
Mourning Dove	10	0.9393	<0.0001 ^a
Mottled Duck	11	0.3566	0.2551
Northern Shoveler	4	1.0000	<0.0001 ^a

^aExamined with Spearman Rank correlations. Significant differences at $P < 0.05$.

Table 6. Concentrations ($\mu\text{g/g}$ Cd dry wt) of Cadmium Found in Potential Avian Prey Items Collected on the Savannah Harbor DMCAs in 2016/2017 (Year 3 of Monitoring).

Group/ Species (N) ^a	Cd Concentrations ($\mu\text{g/g}$) Mean \pm St Dev (Max)
<u>Plant Material</u>	
<i>Sesbania</i> Leaves (3)	0.337 \pm 0.142 (0.454)
Soda Apple (<i>Solanum</i>) Leaves (3)	4.109 \pm 4.110 (8.652)
<u>Seeds/Fruit</u>	
Blackberry (<i>Rubus</i> spp.) (3)	1.381 \pm 1.100 (2.483)
<i>Sesbania</i> seeds (2)	0.390 \pm 0.305 (0.606)
Cheat Grass (<i>Bromus</i> spp) seeds (7)	0.248 \pm 0.102 (0.365)
Scotch Broom (<i>Cytisus</i> spp) seed (3)	0.225 \pm 0.035 (0.264)
<u>Insects</u>	
Potato Beetle Larvae (<i>Leptinotarsa</i>) (3)	2.215 \pm 1.955 (4.244)
Potato Beetle Adult (<i>Leptinotarsa</i>) (1)	11.468
<i>Sesbania</i> Beetle (<i>Neodiplogrannus</i> spp) (2)	0.369 \pm 0.018 (0.382)

^aN=number of composite samples analyzed for Cd.

Table 7. Cumulative Blood Samples from Sentinel and Alternative Species in Years 1-3 on the Savannah Dredge Material Containment Areas (DMCA) in the Summer (S) and Winter (W) Periods.

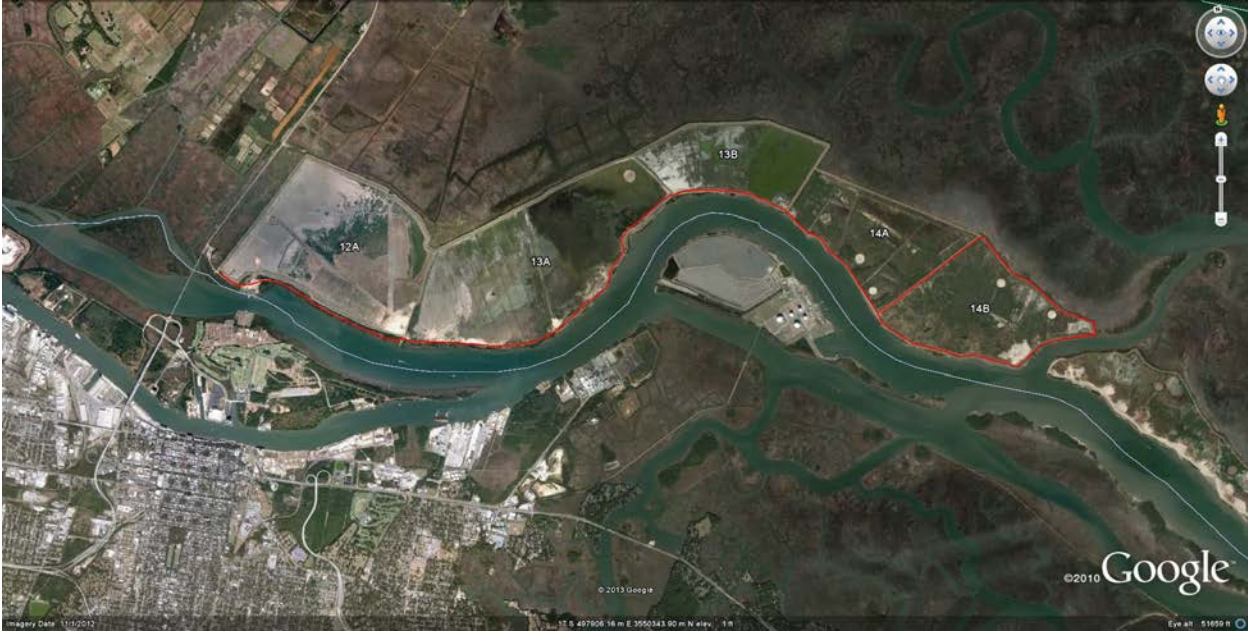
<u>Sentinel Species</u>	<u>Season</u>	<u>N</u>	<u># (%) < MDL</u>	<u>MDL Range^a</u>
American Avocet	W	11	8 (73%)	0.001-0.018
Black-necked Stilt	S	27	22 (81%)	0.003-0.034
Brown Thrasher ^b	S	17	2 (12%)	0.001-0.014
	W	3	1 (33%)	0.007-0.026
Eastern Towhee ^b	S	11	3 (27%)	0.001-0.010
	W	8	4 (50%)	0.006-0.072
Mottled Duck	S	16	14 (87%)	0.001-0.011
Mourning Dove ^b	S	29	14 (48%)	0.001-0.011
Northern Mockingbird	S	22	15 (68%)	0.001-0.020
	W	6	1 (17%)	0.005-0.018
Northern Shoveler	W	20	18 (90%)	0.001-0.003
Red-winged Blackbird	S	29	26 (90%)	0.001-0.015
	W	1 ^c	1 (100%)	0.004
Savannah Sparrow	S	9 ^c	7 (78%)	0.001-0.013
	W	41	39 (95%)	0.001-0.021
Song Sparrow	W	32	31 (97%)	0.005-0.021
<u>Alternative Species</u>				
Northern Cardinal	S	16	15 (94%)	0.001-0.022
	W	17	17 (100%)	0.007-0.014

^aSample-specific Method Detection Limits (MDLs) factoring in sample volume, etc.

^bThese species will not be considered sentinel species in future surveys and reports.

^cIndividuals were captured in their “off-season”, generally near the transition period between seasons.

Figure 1. Dredge material containment areas (DMCAs) associated with the Savannah Harbor Expansion Project.



APPENDIX 1. Metal Concentrations (ppm wet wt) in Avian Blood Samples Collected on the Savannah Harbor DMCA in Year 3 (Summer 2016-Winter 2017).

Species	Season	N		Cr	Ni	Cu	Zn	As	Se
<u>SENTINEL SPECIES</u>									
American Avocet	Winter	5	Mean	<MDL	<MDL	0.43	4.41	0.16	1.58
			SD	<MDL	<MDL	0.17	0.45	0.07	0.53
Black-necked Stilt	Summer	8	Mean	<MDL	<MDL	0.24	4.01	<MDL	0.83
			SD	<MDL	<MDL	0.10	1.37	<MDL	1.02
Brown Thrasher	Summer	2	Mean	<MDL	<MDL	0.61	4.88	<MDL	<MDL
			SD	<MDL	<MDL	0.42	1.02	<MDL	<MDL
Eastern Towhee	Summer	2	Mean	<MDL	<MDL	0.26	3.72	<MDL	0.52
			SD	<MDL	<MDL	0.07	0.20	<MDL	0.32
Mourning Dove	Summer	10	Mean	<MDL	<MDL	0.27	5.08	<MDL	0.31
			SD	<MDL	<MDL	0.06	0.85	<MDL	0.16
Mottled Duck	Summer	12	Mean	<MDL	<MDL	0.36	4.46	0.06	0.65
			SD	<MDL	<MDL	0.13	1.40	0.04	0.35
Northern Mockingbird	Summer	3	Mean	0.27	0.19	0.67	7.63	0.01	0.70
			SD	0.01	0.18	0.32	1.30	0.00	0.40
	Winter	1	Mean	0.03	<MDL	0.80	4.73	<MDL	0.51
			SD	-	-	-	-	-	-
Northern Shoveler	Winter	4	Mean	0.02	<MDL	0.53	4.08	0.12	0.99

			SD	0.02	<MDL	0.21	0.44	0.06	0.30
Species	Season	N		Cr	Ni	Cu	Zn	As	Se
SENTINEL SPECIES (cont'd)									
Red-winged Blackbird	Summer	9	Mean	0.03	<MDL	0.30	4.11	<MDL	0.87
			SD	0.02	<MDL	0.12	0.94	<MDL	0.74
Savannah Sparrow	Winter	16	Mean	0.16	<MDL	0.86	6.23	<MDL	<MDL
			SD	0.33	<MDL	0.98	3.30	<MDL	<MDL
Song Sparrow	Winter	9	Mean	<MDL	<MDL	0.29	4.27	<MDL	<MDL
			SD	<MDL	<MDL	0.16	2.35	<MDL	<MDL
<u>ALTERNATE SPECIES</u>									
Northern Cardinal	Summer	7	Mean	<MDL	<MDL	0.25	3.91	<MDL	<MDL
			SD	<MDL	<MDL	0.041	0.50	<MDL	<MDL
Method Detection Limits (range of MDLs in ppm)				Cr	Ni	Cu	Zn	As	Se
				0.029-0.236	0.017-0.341	0.213-0.716	0.093-0.413	0.018-0.097	0.341-0.977

APPENDIX 2. Metal Concentrations (ppm dry wt) in Tissues and Gut Contents from Lethally-collected Avian Species on Savannah harbor DMCA's in Year 3 (Summer 2016 – Winter 2017).

Species	N	Tissue		Cr	Ni	Cu	Zn	As	Se
Am. Avocet	5	Liver	Mean	0.06	0.04	35.72	111.14	1.15	11.60
			SD	0.03	0.03	20.06	15.95	0.52	3.70
		Kidney	Mean	<MDL	0.05	26.90	120.90	0.96	9.55
			SD	-	0.02	5.75	11.27	0.40	3.01
		Gut Content	Mean	15.21	5.55	22.59	86.80	17.63	5.66
			SD	5.67	2.22	4.49	17.84	6.05	1.18
Mottled Duck	12	Liver	Mean	<MDL	<MDL	30.19	134.53	0.49	5.20
			SD	-	-	24.71	62.26	0.41	1.95
		Kidney	Mean	<MDL	0.08	17.19	94.03	0.73	5.96
			SD	-	0.07	7.25	12.85	0.62	2.01
		Gut Content	Mean	4.09	1.08	3.92	20.48	4.54	<MDL
			SD	6.78	0.87	2.09	17.90	5.33	-
Mourn. Dove	10	Liver	Mean	<MDL	<MDL	15.15	93.00	<MDL	1.73
			SD	-	-	2.13	16.71	-	1.33
		Kidney	Mean	<MDL	0.11	14.39	122.86	<MDL	2.51
			SD	-	0.09	1.58	27.68	<MDL	1.59
		Gut Content	Mean	0.40	1.33	5.87	19.58	0.27	<MDL
			SD	0.52	1.12	1.92	12.03	0.44	-
N. Shoveler	4	Liver	Mean	0.08	0.10	130.24	122.55	0.94	9.44
			SD	0.11	0.04	122.30	20.58	0.59	1.27
		Kidney	Mean	0.04	0.07	18.95	85.14	1.21	7.07
			SD	0.04	0.03	2.74	8.95	0.61	0.93
		Gut Content	Mean	4.09	0.96	5.08	25.22	3.73	<MDL
			SD	3.64	0.35	2.61	14.96	2.01	-

APPENDIX 2 (cont'd)

Species	N	Tissue		Cr	Ni	Cu	Zn	As	Se
Bl-necked Stilt	1	Liver	Conc	<MDL	0.06	11.92	77.81	0.27	2.62
		Kidney	Conc	<MDL	0.13	10.60	75.30	0.44	6.36
		Gut Content	Conc	2.59	3.96	10.03	110.74	25.41	3.55
Song Sparrow	1	Liver	Conc	<MDL	0.08	14.35	69.06	<MDL	2.79
		Kidney	Conc	<MDL	<MDL	10.66	80.95	<MDL	<MDL
		Gut Content	Conc	0.72	0.50	9.43	49.72	<MDL	<MDL
N. Cardinal	1	Liver	Conc	<MDL	0.14	14.02	101.93	<MDL	2.96
		Kidney	Conc	<MDL	0.19	18.08	114.24	<MDL	3.02
		Gut Content	Conc	0.25	0.88	50.59	161.91	<MDL	<MDL
Method Detection Limits (MDL in ppm)				0.032	0.031	0.040	0.128	0.055	1.012

APPENDIX 3. Metal Concentrations (ppm dry wt) in Potential Avian Prey Items Collected From the Savannah Harbor DMCA in Year 3 (Summer 2016-Winter 2017).

Tissue	N		Cr	Ni	Cu	Zn	As	Se
Sesbania beetle	2	Mean	0.07	0.83	17.89	90.74	0.21	<MDL
		SD	0.04	0.21	5.02	20.85	0.02	-
Potato Beetle larvae	3	Mean	0.57	1.13	48.28	73.95	0.19	<MDL
		SD	0.25	0.17	37.67	14.89	0.04	-
Potato beetle adults	1	Mean	0.35	3.45	95.19	68.19	0.23	<MDL
		SD	-	-	-	-	-	-
<i>Rubus</i> berries	8	Mean	0.15	3.42	8.27	21.40	<MDL	<MDL
		SD	0.03	1.36	0.91	3.47	-	-
Scotch Broom seeds	3	Mean	0.48	2.07	6.74	51.37	<MDL	<MDL
		SD	0.75	1.28	1.35	12.61	-	-
Cheat Grass seeds	7	Mean	0.29	0.97	5.72	34.56	<MDL	<MDL
		SD	0.21	0.79	1.03	12.64	-	-
Sesbania leaves	3	Mean	0.17	7.29	3.61	34.75	<MDL	<MDL
		SD	0.13	0.81	1.90	2.13	-	-
Sesbania seeds	2	Mean	0.17	7.58	4.11	64.58	<MDL	<MDL
		SD	0.16	3.76	2.02	46.09	-	-
Soda apple leaves	3	Mean	0.53	2.29	21.76	66.20	0.09	<MDL
		SD	0.15	1.51	11.42	34.89	0.06	-
MDL			0.13	0.10	0.07	0.10	0.05	0.52