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ENVIRONMENTAL CONTAMINANTS ASSOCIATED WITH A SWINE CONCENTRATED ANIMAL FEEDING OPERATION AND IMPLICATIONS FOR MCMURTREY NATIONAL WILDLIFE REFUGE.



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ABSTRACT

Waste generated by concentrated animal feeding operations (CAFOs) may contain a variety of contaminants including nutrients, pathogens, trace elements, antibiotics, and hormones. In 2000, the U.S. Fish and Wildlife Service began to characterize CAFO contaminants in lagoons, canals, and created wetlands operated by Hastings Pork, a large swine CAFO adjacent to McMurtrey National Wildlife Refuge (McMurtrey) in Clay County, Nebraska. The created wetlands were designed to attract waterfowl; therefore, the primary purpose of this research was to evaluate whether migratory waterfowl were likely exposed to CAFO contaminants. A secondary research objective was to determine if created wetland water was suitable as a supplementary water source for McMurtrey. Wetlands created from swine wastewater effluent had 5-50 fold greater concentrations of phosphorus, ammonia, and total nitrogen and 2-3 fold greater salinity compared to control sites. Cyanobacteria (*Microcystis* spp.) were abundant in the created wetlands and microcystin toxins were detected in concentrated water samples. Tetracycline, macrolide, and diterpene antibiotics were detected in lagoon and canal sediment and water samples; however, in the created wetlands only oxytetracycline was detected (once in sediment at 41 nanograms per gram). Concentrations of 17-β estradiol and testosterone in CAFO wastewater (n=4) exceeded toxicity thresholds for aquatic life. Fecal coliform and streptococci counts in water (n=38) generally exhibited a decreasing gradient with lagoons > canals > created wetlands > McMurtrey. Bacteria (*Salmonella* spp. and *Yersinia enterocolitica*) were recovered in the created wetlands but not McMurtrey. Created wetland invertebrate communities were dominated by chironomid species and had lower taxa diversity when compared to McMurtrey. Eutrophication of created wetlands may represent the greatest health threat to waterfowl by creating an environment conducive to cyanobacteria blooms and outbreaks of avian botulism and avian cholera. Trace elements from swine waste will likely continue to accumulate in the created wetlands over time, leading to an increased risk of exposure to wetland biota. Research is ongoing and includes use of sentinel mallards (*Anas platyrhynchos*) to further evaluate the need to decrease concentrations of CAFO contaminants in swine wastewater before it is used to create waterfowl habitat.

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INTRODUCTION

Nature and Scope of the Problem

The trend in livestock operations in the U.S., with fewer operations and increased numbers of animals per operation, has created a concern that the animal wastes from these facilities may represent an increased risk to the environment. According to the U.S. Environmental Protection Agency (USEPA), the number of animal feeding operations for hogs in the top ten production states in the U.S. decreased from over 300,000 to under 150,000 from 1978 to 1992; however, during this same period, the average number of hogs per operation increased by 134 percent (USEPA, 2001). A similar trend is occurring in Nebraska where, according to the Nebraska Agricultural Statistics Service (NASS), the average number of hogs per farm more than doubled from 368 to 853 in 1992 and 2001, respectively (NASS, 1996 and 2002). This concentration of animals and their waste is not without consequence to the environment. According to the Nebraska Department of Environmental Quality (NDEQ), the number of fish kills attributed to livestock wastes in Nebraska increased from 5 in 1998 and 1999 to 11 in 2002 and 2003 (NDEQ, unpublished data).

A "large" swine concentrated animal feeding operation (CAFO) is defined by USEPA as a facility with more than 2,500 swine, each weighing over 25 kilograms (kg), in confinement (USEPA, 2003). Pollutants associated with these facilities include trace elements, salts, nutrients, cyanobacteria toxins, bacterial pathogens, hormones, and antibiotics (USEPA, 2003). These pollutants can enter rivers, streams, and wetlands by spills, lagoon ruptures, field run-off, and contaminated groundwater. Adverse effects of swine lagoon breaches to fish and wildlife are well documented (Mallin et al., 1997; Burkholder et al., 1997; Williams, 1998; Denn, 1999). A 25 million gallon swine wastewater spill in 1995 from a CAFO in North Carolina killed 10 million fish and closed 364,000 acres of coastal wetlands to shellfishing (Williams, 1998). In Missouri, swine CAFOs were designated as the biggest culprit in polluting 150 miles of Missouri's streams, causing 61 fish kills and killing more than 500,000 fish (Denn, 1999). Land

application of animal manure also can lead to the accumulation of heavy metals and phosphorus in soil. When soil adsorption sites become limited, the ability to bind excess phosphorus and metals decreases and the soil changes from a sink to a source for the transport of these elements to surface run-off (Sharpley et al., 1999). Buffalo Lake National Wildlife Refuge in Texas experienced this in the 1960's and 1970's when large fish kills on the refuge were attributed to field run-off and discharges from cattle feedlots within the refuge's watershed (Baker et al., 1998). Water quality degradation eventually led to the draining of Buffalo Lake. Wetlands at the refuge now receive well water to compensate for the nutrient loading from run-off (Baker et al., 1998).

The Rainwater Basin (RWB), named for its abundance of natural wetlands, is increasingly at risk from CAFO run-off. The RWB region encompasses more than 4,200 square miles within 17 counties of south-central Nebraska and is recognized as the focal point of a spring migration corridor used by millions waterfowl and shorebirds annually (RWBJV, 2000; Figure 1). During the spring waterfowl migration, the RWB hosts approximately 90 percent of the mid-continent population of greater white-fronted geese (*Ansu albiforns*), 50 percent of the mid-continent population of mallards *(Anas platyrhynchos*), and 30 percent of the continent population of northern pintails (*Anas acuta*) (Benning 1987; Bortner et al., 1991). Threatened and endangered species including the whooping crane (*Grus americana*), bald eagle (*Haliaeetus leucocephalus*), and piping plover (*Charadrius melodus*) also have been observed at RWB wetlands (Wally Jobman, U.S. Fish and Wildlife Service Wildlife Biologist, pers. comm., 2004). The U.S. Fish and Wildlife Service (Service) Rainwater Basin Wetland Management District (RWB-WMD) manages 61 Waterfowl Production Areas (WPAs) that range in size from 38 to 1,989 acres (USFWS, 2001). Between 131 to 166 CAFOs are in operation within the RWB-WMD, and at least five WPAs have CAFOs in their watershed (Nebraska Department of Environmental Quality, unpublished data, 2002).

In 1991, the RWB was identified by the North American Waterfowl Management Plan as waterfowl habitat of major concern in North America and received Joint Venture status. The overall goal of the Rainwater Basin Joint Venture (RWBJV) is to restore and

maintain wetland habitat within the RWB. The RWBJV promotes sustainable agricultural practices to reduce the level of certain chemicals and other environmental contaminants from entering wetlands (Gersib et al., 1992).

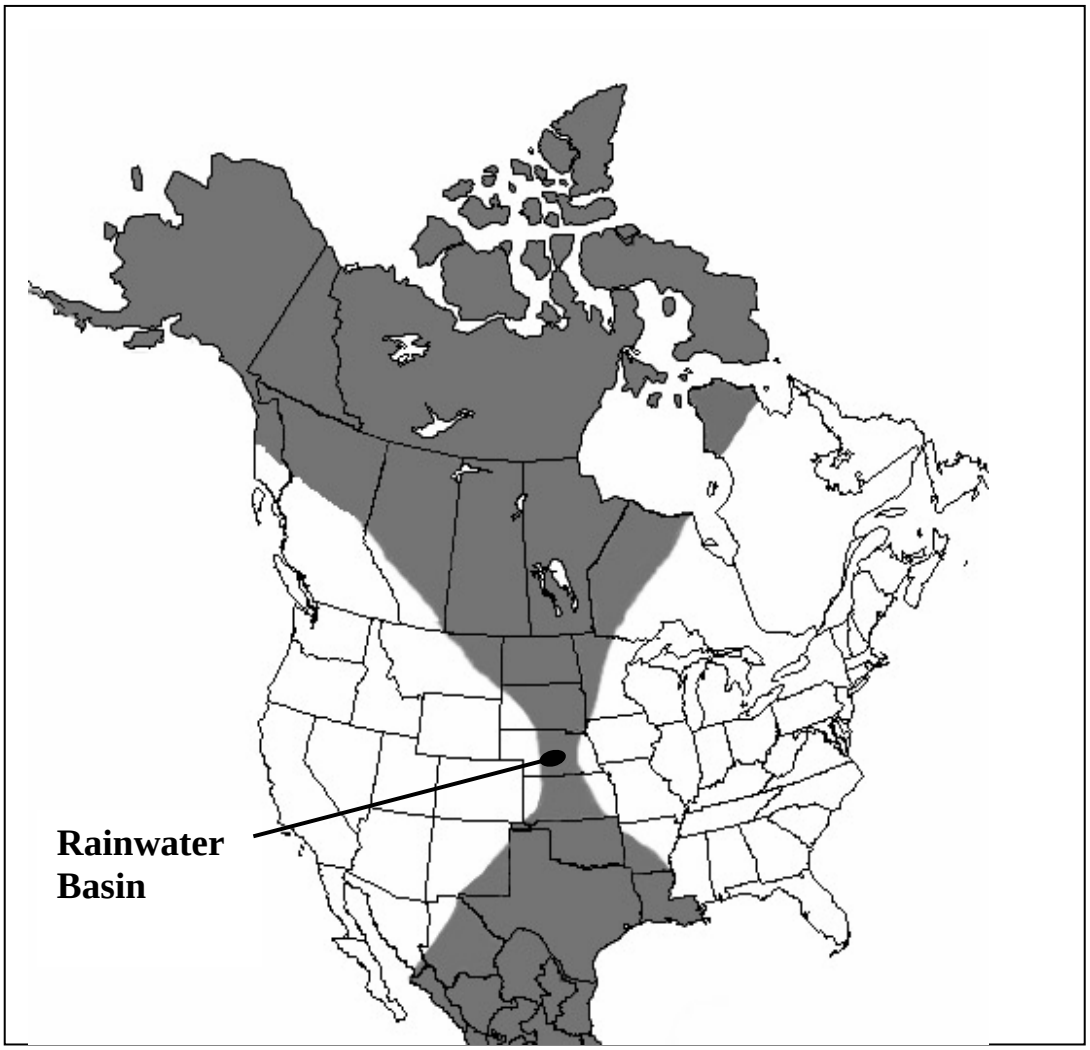


Figure 1. Location of the Rainwater Basin Region at the focal point of a spring migration corridor used by millions of ducks, geese and other migratory birds annually. Note: figure taken from the Rainwater Basin Joint Venture Evaluation Plan (RWBJV, 2000).

Site Description and History

The study site was located in Clay County, Nebraska, within the RWB (Figure 2) and included Hastings Pork and McMurtrey National Wildlife Refuge (NWR). Hastings Pork is located on what was formerly a Naval Ammunition Depot. Since the 1960s, Hastings Pork has utilized the area for livestock and crop production. About 260 bunkers that were formerly used by the Navy to store munitions are now used for swine production. These bunkers house approximately 64,000 swine and around 1.5 million liters of water per day is used to flush them out. In addition, an estimated 325,000 liters of swine urine and manure slurry are generated daily (based on calculations for swine between 36-55 kg of body weight; Fraser 1991), for a total of 1.8 million liters of wastewater per day. In an effort to utilize this wastewater, a partnership between Hastings Pork and the RWBJV resulted in the creation of seven wetlands (known as the Hayden Thompson wetlands and referred to herein as the created wetlands) totaling 17 acres on Hastings Pork property. These wetlands receive swine wastewater effluent from lagoons by a canal system, with a distance of delivery ranging from less than a mile to 5 miles. The created wetlands were designed to provide waterfowl habitat and were not intended to treat swine-waste effluent; therefore, the Service and Hastings Pork formed a partnership to evaluate whether migratory birds attracted to the created wetlands may be exposed to contaminants and disease pathogens. The Service is also concerned that waterfowl may transmit disease pathogens from Hastings Pork to nearby habitats. McMurtrey Marsh is located within McMurtrey NWR and is located approximately 1 mile east of the created wetlands (Figure 3). McMurtrey NWR contains 650 acres of wetlands and 400 acres of upland habitat and is managed primarily for migratory waterfowl.

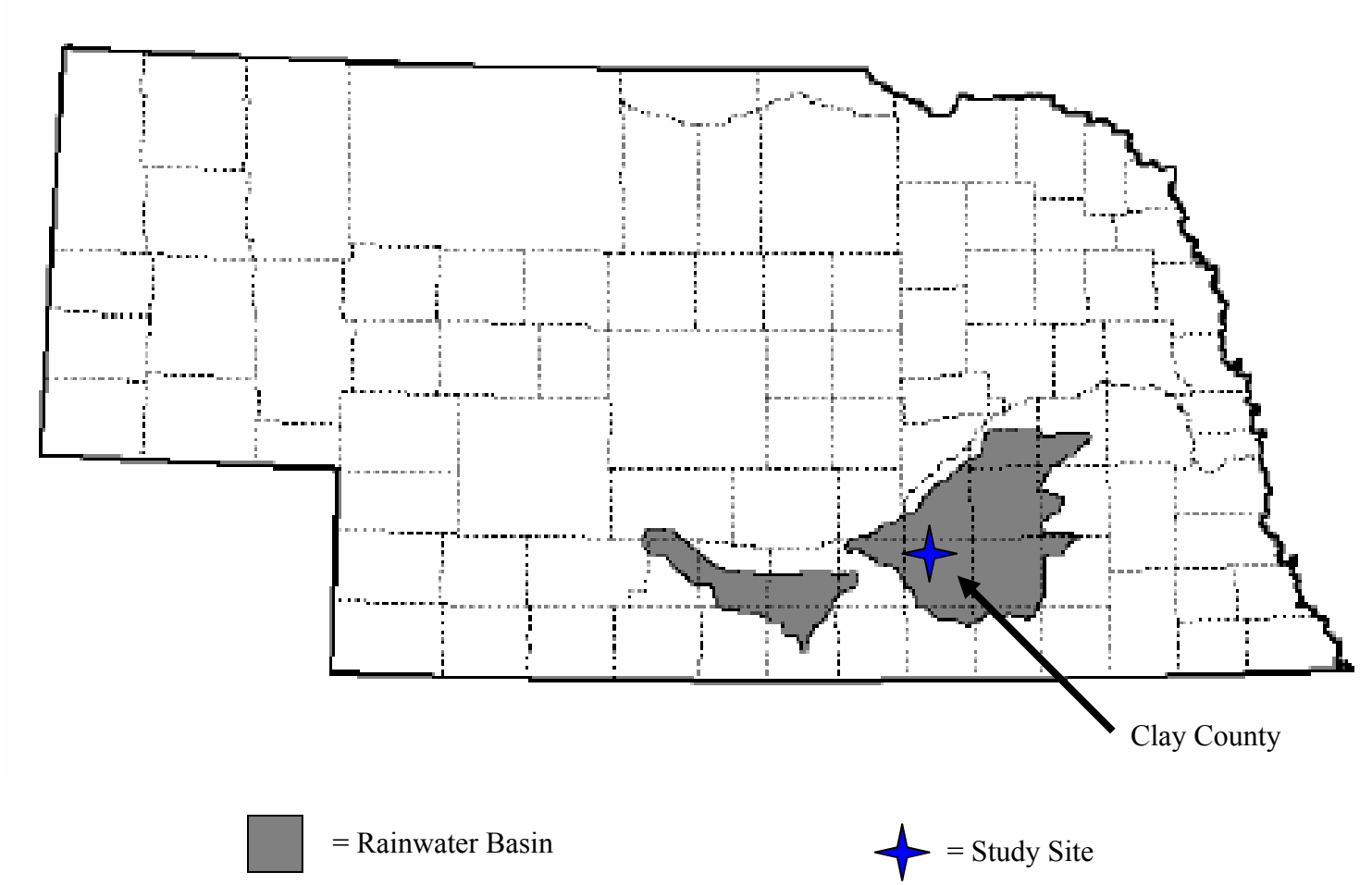
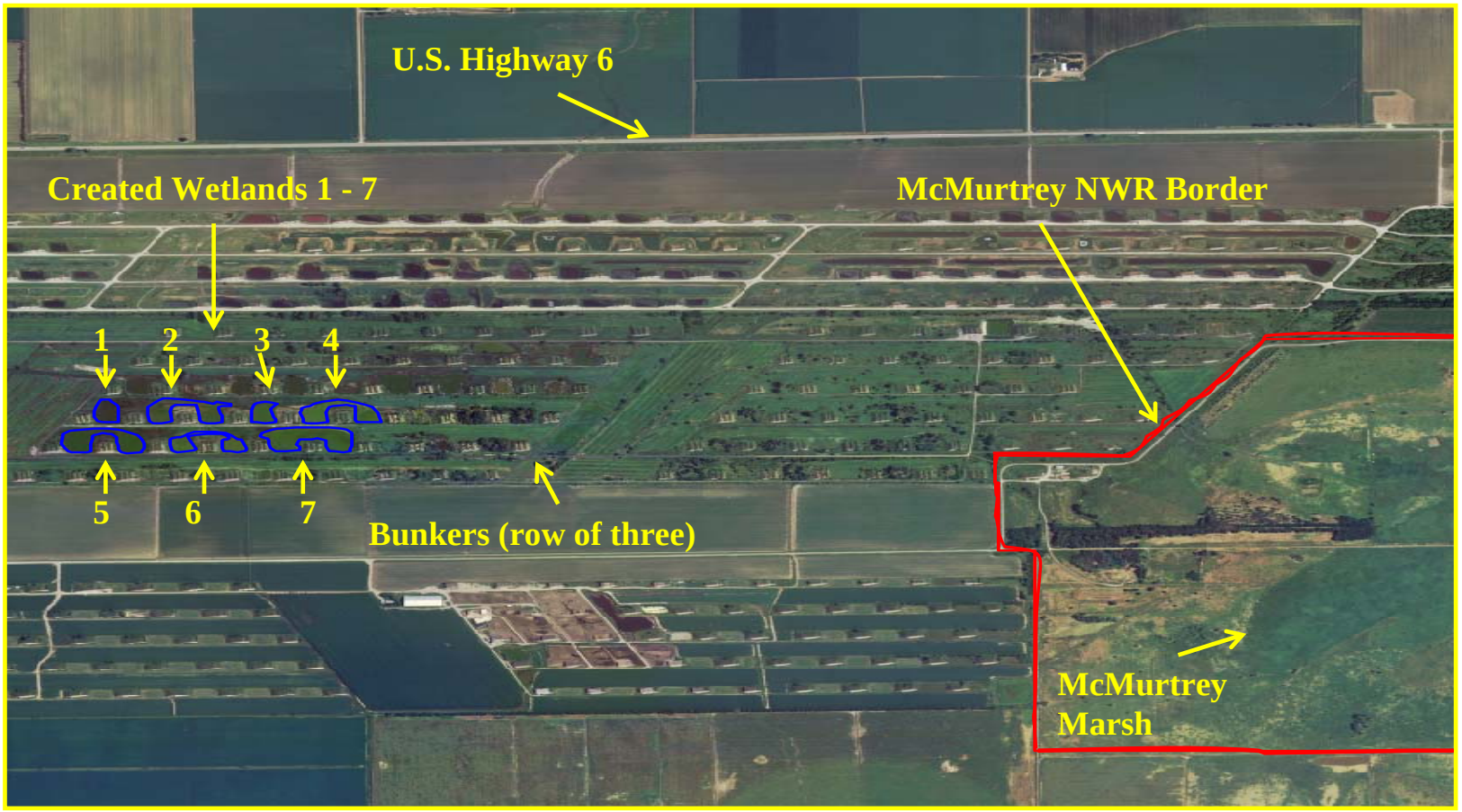


Figure 2. Location of the Nebraska Rainwater Basin and the study site in Clay County, Nebraska.



Scale: |----------|
1 mile

Figure 3. Location of the Hastings Pork created wetlands, McMurtrey National Wildlife Refuge (NWR), and McMurtrey Marsh. Note: See title page for a picture of the created wetlands and bunkers.

Research Justification and Objectives

To our knowledge, there have been no studies that have evaluated risk to waterfowl that utilize wetlands created from primary treated CAFO wastewater. However, many contaminants associated with CAFOs are known to be potentially toxic to waterfowl. For example, selenium or boron are directly toxic to waterfowl and can cause reproductive failure (USDI, 1998). Other contaminants in swine waste may not be very toxic to waterfowl directly (e.g., ammonia and nutrients), but have the potential to adversely affect waterfowl populations indirectly through habitat modification. Excessive nutrient loading can cause wetland eutrophication and may result in an environment with less foraging potential (Gaiser and Lang, 1998) or an environment more conducive to harmful cyanobacterial blooms or disease pathogens (Crowder and Bristow, 1988; USGS, 1999a).

The purpose of this research was to evaluate whether migratory waterfowl that utilize the created wetlands and McMurtrey NWR are likely exposed to CAFO contaminants including trace elements, nutrients, salts, cyanobacterial toxins, bacterial pathogens, hormones, and antibiotics. Sampling was performed to evaluate movement of contaminants from lagoons through canals and to the created wetlands. Contaminants were tested in water and sediments from a control site (McMurtrey Marsh), and in invertebrates, water, and sediments from the created wetlands, canals, and lagoons. In addition to the exposure assessment, taxa richness of invertebrate communities among McMurtrey Marsh and the created wetlands were compared to evaluate the effect of swine waste on the wetlands' function as waterfowl habitat.

Contaminants Associated with CAFOs

Trace elements, salts, nutrients, cyanobacterial toxins, bacterial pathogens, hormones, and antibiotics are swine CAFO pollutants of concern that may adversely affect natural ecosystems (USEPA, 2003). Background information on the toxicity of swine CAFO contaminants and their potential to adversely affect waterfowl and their habitat (e.g., plant and invertebrate food items) is presented below.

Trace elements

Trace elements in hog manure of environmental concern include aluminum, arsenic, boron, cadmium, copper, iron, lead, manganese, molybdenum, nickel, selenium, and zinc (USEPA, 2003). Many of these trace elements (e.g., selenium, copper, and zinc) are ingredients in hog feed. Copper (Cu) is added to swine feed to promote growth and control disease, and zinc (Zn) is commonly added to Cu-enriched rations to ameliorate Cu toxicity to swine (Payne et al., 1988). Trace elements from CAFOs may accumulate in sediments, water, and biota to concentrations that are toxic to plants and lead to reproductive impairment, poor body condition, and immune system dysfunction in animals (Stubbs and Cathey, 1999).

Aluminum (Al) toxicity and bioavailability to aquatic biota is largely dependent on its solubility and generally increases as pH decreases (Gensemer and Playle, 1999). Aluminum bioavailability and toxicity at a pH greater than 7.0 is largely unknown; however, at a pH below 5.5, Al can be toxic to many plant species (Sparling et al., 1997). Concentrations of 400 to 500 micrograms per liter (μg/L) of Al in water within a pH range of 4.0 to 4.3 had negligible effects on mortality in amphipods, snails, or insect larvae (Sparling et al., 1997). Waterfowl are most likely exposed to Al by dietary uptake and dietary concentrations less than (<) 1000 mg/kg are not considered harmful (Sparling et al., 1997). There is no evidence of aluminum bioaccumulation in aquatic invertebrates (Gensemer and Playle, 1999); however, there is a potential for food items including invertebrates, tadpoles, and a few species of plants, to have sufficient concentrations of Al to be toxic to avian species (Sparling et al., 1997).

Arsenic (As) toxicity and bioavailability depends on its chemical speciation, with trivalent (As^{+3}) generally being more toxic than pentevalent (As^{+5}). Pentevalent As predominates in most aquatic environments where it is relatively persistent and may bioaccumulate in aquatic organisms (USDI, 1998). Concentrations of As^{+5} in water ranging from 1 to 15.2 μg/L have been reported to disrupt aquatic ecosystems by inhibiting the growth of certain aquatic plants (Sanders and Cibik, 1985 cited in Eisler, 1988a). Mallard ducklings fed As^{+5} at 30 mg/kg dry weight (dw) for 10 weeks exhibited

a reduced growth rate (Camardese et al., 1990). Adult mallards exhibited reduced body and liver mass, delayed onset of egg laying, decreased whole egg mass, and egg shell thinning when fed sodium arsenate at 400 mg/kg dw (Stanley et al., 1994).

Boron (B) toxicity is most often observed in plants where it functions as an essential trace element for growth and development (USDI, 1998). Boron in soil can cause toxic effects to plants at concentrations > 5 mg/L (Gupta et al., 1985 cited in USDI, 1998). Studies on the toxicity of B to aquatic invertebrates are lacking (USDI, 1998); however, adverse reproductive effects in the water flea (*Daphnia magna*) can occur at B concentrations of 13.6 mg/L (Gerisch, 1984). Waterborne concentrations of B greater than 450 mg/L can cause adverse reproductive effects in mallards and concentrations ranging from 30 mg/L to 300 mg/L can cause reduced weight gain in mallard ducklings (USDI, 1998).

Cadmium (Cd) is a known teratogen and mutagen, and can cause severe deleterious effects to fish and wildlife (Eisler, 1985). Freshwater biota is the most sensitive group. Concentrations of 0.8 to 9.9 mg/L Cd in water are lethal to several species of aquatic insects and crustaceans (Eisler, 1985). The kidney is the critical organ in avian species chronically exposed to cadmium, and young birds may be more susceptible to kidney damage than adults (Furness, 1996). Mallard ducklings given 20 mg/kg Cd for 90 days developed kidney damage (Cain et al., 1983) whereas adult mallards exhibited kidney damage at 200 mg/kg Cd (White et al., 1978).

Copper (Cu) toxicity mechanisms include free radical production, alteration in activities of several enzymes, and interference with metallothionein synthesis. Excess cellular copper leads to lipid peroxidation as superoxide radicals are created during the oxidation of Cu^{+1} to Cu^{+2} at the cellular membrane (Eisler, 1998a). Copper concentrations of 34 mg/kg in sediment rarely impair benthic invertebrate survival or reproduction, whereas concentrations above 270 mg/kg generally do (Long et al., 1995; Hull and Suter, 1994; USDI, 1998). Copper concentrations in soil greater than 25 to 50 mg/kg are toxic to sensitive plants (Demayo et al., 1982a). Copper is relatively nontoxic

to birds and mammals in comparison to aquatic invertebrates, plants, and fish as copper homeostasis in birds and mammals is well regulated by metallothionein (USDI, 1998).

Iron (Fe) can disrupt aquatic ecosystems by decreasing species diversity and abundance of periphyton and benthic invertebrates (Vuori, 1995: Dickman and Rygiel, 1998). Macroinvertebrates exposed to high levels of Fe, manganese, and concurrent blooms of iron-depositing bacteria (*Leptothrix ochracea*) exhibited varying responses including mortality by direct toxic effects and/or smothering, behavioral avoidance of bacterial-coated substrates, and an inability to feed on the bacteria (Wellnitz et al., 1994). Mallards ingesting eight BB size tungsten-iron shot over a 30-day period did not exhibit any adverse health effects (Kelly et al., 1998). No apparent adverse effects were observed in Mute Swans (*Cygnus olor*) after they received a diet containing moderately polluted sediments with high concentrations of Al, Fe, vanadium (V), and barium (Ba) for six weeks (Beyer et al., 2000).

Lead (Pb) targets kidney, bone, the central nervous system, and the hematopoietic system and can cause adverse biochemical, histopathological, neuropsychological, fetotoxic, teratogenic, immunotoxic, and reproductive effects (Eisler, 1988b, Goyer and Clarkson, 2001). *Daphnia magna* exhibit decreased reproduction in water with Pb concentrations as low as 1 µg/L (Vighi, 1981). Freshwater algae can bioconcentrate Pb to high levels (Eisler, 1988b). Algae exposed to Pb concentrations of 5 µg/L in water exhibited a bioconcentration factor of 92,000 (Demayo et al. 1982b). Ingestion of spent Pb shot has caused considerable mortality of migratory waterfowl and other birds, including raptors that eat waterfowl killed or wounded by hunters (Eisler, 1988b). Exposure to Pb in ways other than ingestion of Pb shot (or ingestible Pb objects such as sinkers) are unlikely to cause clinical signs of Pb poisoning in birds (Eisler, 1988b). Mallard hatchlings are apparently tolerant to Pb, as they do not exhibit adverse effects on growth at dietary levels of 500 mg Pb/kg, or survival at 2,000 mg Pb/kg (Eisler, 1988b).

Manganese (Mn) is considered the least toxic of the trace elements for poultry and mammals (Pais and Jones, 1997). However, the cycling of manganese between Mn^{+2} and

Mn^{+3} may be potentially deleterious to biological systems because it can involve the generation of free radicals (Goyer and Clarkson, 2001).

Molybdenum (Mo) is relatively nontoxic to aquatic organisms and plants (USDI, 1998). Molybdenum toxicity is largely dependent on interactions with copper. A low copper-to-molybdenum ratio (<2), rather than the dietary Mo concentration, is the primary determinant of an organism's susceptibility to Mo poisoning (USDI, 1998). Aquatic organisms and plants generally do not exhibit adverse effects on growth or survival at water Mo concentrations < 50 mg/L; however, the same aquatic plants bioconcentrate Mo to levels potentially toxic to organisms that feed on them (USDI, 1998). Bioconcentration factors of 628; 3,300; and 3,570 have been reported for freshwater algae, cyanobacteria, and periphyton, respectively (USDI, 1998). Literature on Mo toxicity to wild birds is lacking; however, adverse effects from Mo exposure to domestic chickens include reduced growth at dietary concentrations of 200 to 300 mg/kg, decreased egg production at 500 mg/kg, and decreased survival at 6,000 mg/kg (Eisler, 1989).

Nickel (Ni) toxicity mechanisms include oxidative damage to DNA and proteins and the inhibition of cellular antioxidant defenses (Rodriguez et al., 1996 cited in Eisler, 1998b). Sensitive species of aquatic organisms are adversely affected at nominal waterborne concentrations of 11-113 mg Ni^{2+}/L (Eisler, 1998b). Nickel compounds typically have a low hazard when administered orally (NAS, 1975 cited in Eisler 1998b; USEPA, 1980). Mallards fed diets containing 800 mg Ni/kg ration for 90 days exhibited metabolic upset and altered bone densities and mallards fed 1,200 mg Ni/kg exhibited reduced growth and survival (Cain and Pafford 1981; Eastin and O'Shea 1981).

Selenium (Se) is one of the most toxic trace elements with a narrow margin of safety between toxicity and dietary deficiency. Nutritionally optimal dietary Se exposure is generally reported as 0.1 to 0.3 mg/kg dw whereas thresholds for dietary toxicity in animals range from 2 to 5 mg/ kg dw (USDI, 1998). The toxic effects of both Se deficiency and excess are similar and include reproductive depression, anemia, weight loss, and immune dysfunction (Koller and Exon, 1986; USDI, 1998). Vertebrates are

generally much more susceptible to Se toxicity than are most plants and invertebrates; therefore, the direct toxic effects of consuming Se-contaminated plants are believed to be more important than indirect ecological effects from changes in plant communities (USDI, 1998). Reproductive impairment (e.g. reduced hatchability and teratogenesis) can result in birds with diets containing as little as 3 to 8 mg Se per kg (Wilber, 1980; Heinz, 1996; USDI, 1998). Dabbling duck species are among the most Se sensitive waterbird species (USDI, 1998). The concentration of Se in duck eggs estimated to cause teratogenesis in duck eggs is 23 mg/kg dw (Skorupa 1998, USDI, 1998). The potential for Se to bioaccumulate and adversely affect sensitive species (including waterfowl) at waterborne concentrations less than 5 µg/L has resulted in the Service's request for the USEPA to develop a new chronic aquatic life water quality criterion of 2 µg/L.

Zinc is an essential element for all living organisms and is generally more toxic to aquatic invertebrates and plants than birds and mammals due to homeostatic control by metallothionein (USDI, 1998). Decreased growth rate in invertebrates has been reported for Zn concentrations > 10 µg/L and increased mortality at concentrations > 80 µg/L (Hatakeyama, 1989 cited in USDI, 1998; Eisler, 1993). Zinc poisoning in birds is indicated when liver concentrations exceed 2,100 mg/kg dw (Eisler, 1993). Mallards exposed to dietary Zn concentrations of 3,000 mg/kg dw for 30 days exhibited leg paralysis and decreased food consumption (Eisler, 1993).

Salts

Salinity is a measure of the mass of dissolved salts in a given mass of solution (USDI, 1998), and can be determined by measuring conductivity and then converting to parts per thousand (ppt). Salinity is acutely toxic to amphipods at a concentration of 22 ppt and to daphnia at 8 to 11 ppt (USDI, 1998). Waterfowl hatched in moderate to high saline environments and without access to fresh drinking water exhibit decreased growth, development, and survival rates (Stolley et al., 1999). Saline-induced mortality of ducklings or goslings generally happens before day 6 of life, after which the nasal salt glands are functional (Stolley et al., 1999). Salinity > 20 ppt is uniformly fatal to 48-

hour-old mallard and black duck ducklings (Barnes and Nudds, 1991). Salt glands collected from fully grown mallard and black ducks increased in size with increasing age and salinity, and hypertrophied to a maximum size at 1 percent NaCl, indicating a failure to regulate salts at a salinity > 10 ppt (Barnes and Nudds, 1991).

Nutrients

The primary nutrients released from hog manure are nitrogen, phosphorus, and potassium (USEPA, 2003). Nitrogen and phosphorus are high in livestock manure and can cause eutrophication in aquatic ecosystems. The average feeder hog will excrete 11 kg of nitrogen and 6 kg of phosphorus in one year (Fraser, 1991). Documented adverse effects to aquatic ecosystems that may develop following eutrophication include increased biomass of phytoplankton, shifts in the phytoplankton community to bloom-forming species that are toxic or inedible, decreased invertebrate and plant species diversity, and oxygen depletion (USEPA, 2003). Increased algal growth can disrupt aquatic ecosystems by consuming dissolved carbon dioxide and increasing pH (USEPA, 2003). Amphibian declines have been attributed, in part, to nitrite (NO_2^-) toxicity. Adverse effects of nitrate (NO_3^-) and nitrite exposure to five species of amphibian larvae included reduced feeding activity, less vigorous swimming, disequilibrium, paralysis, abnormalities, edemas, and death (Marco et al., 1999). These adverse effects increase with dose and, although sensitivity is different among species, all species showed 15-day LC50s (i.e., the concentration in water that is lethal to 50 percent of the test species in 15 days) lower than 2 mg nitrite per liter (Marco et al., 1999). Cascades frog tadpoles (*Rana cascadae*) exposed to 3.5 mg nitrite per liter exhibited retarded development and emerged at an earlier developmental stage (Marco and Blaustein, 1999). There are no known cases of acute toxicity to waterfowl from exposure to aqueous nitrogen or phosphorus; however, a die-off of 250 herring gulls (*Larus argentatus*) and ring-billed gulls (*Larus delawarensis*) in 1984 was attributed to ingestion of fertilizer waste containing 1,730 parts per million (ppm) nitrite (Ley, 1986).

Algal Toxins

Toxins produced by cyanobacteria (blue-green algae) have caused mortality among a variety of wildlife populations including amphibians, fish, snakes, waterfowl, raptors, deer, muskrats, fox, squirrels, skunks, mink, bats, and bees (Carmichael, 1992). A common class of toxins produced by cyanobacteria is the hepatotoxic microcystins. There are 52 microcystin variants, all of which share a similar acute toxic mechanism (Carmichael, 1997). Microcystins inhibit the protein phosphatases needed to control liver blood circulation, resulting in extensive hemorrhaging in the liver (Sivonen, 1996). Microcystin-LR (MC-LR) is one of the most common microcystin variants and rodent toxicity tests indicate that it is also one of the most toxic (Carmichael, 1997). The acute lethal dose to 50 percent of the treated population (LD50) for mice given an intraperitoneal injection of MC-LR is 50 μg/kg body weight (bw); whereas mice LD50s for other microcystin variants range from 50 to >1,200 μg/kg bw (Carmichael, 1997). Chronic *Microcystis* exposure to laboratory mice resulted in liver injury, increased incidence of pneumonia, decreased survival, reduced brain size of neonates, and skin tumor promotion (Falconer et al., 1988; Falconer, 1991).

Microcystins have been related to many accidental animal poisonings. In Japan, 20 spot-billed ducks (*Anas paecilorhyncha haringtoni*) died from acute exposure to microcystins in a pond that became eutrophic from an influx of untreated sewage (Matsunaga, 1999). In England, ingestion of water from a waste storage reservoir containing a bloom of *Microcystis* was linked to the death of 20 lambs from an adjacent farm and 15 neighborhood dogs (Falconer, 1991). In addition, algal toxins may also initiate avian botulism outbreaks (Murphy et. al., 2000).

Bacterial Pathogens

Pasteurella multocida, *Yersinia* ssp., *Salmonella* spp., *Erysipelothrix* spp., *Clostridium botulinum*, and *Esherichia coli* are bacteria associated with animal waste. The U.S. Geological Survey (USGS) National Wildlife Health Center (NWHC) has

identified these organisms as known or suspected waterfowl pathogens (USGS, 1999a and 2001).

Pasteurella multocida is highly infectious and can cause avian cholera in waterfowl. Infections of *P. multocida* in waterfowl are usually acute, often resulting in death within 6 to 12 hours, but also can be carried latently by birds and result in disease only under conditions of animal stress (USGS, 1999a). Environmental endurance of *P. multocida* may contribute to the length of time a seasonal outbreak persists (Rosen and Bischoff, 1950; Price and Brand, 1984) but is unlikely a source of outbreaks from one year to the next. Many avian cholera outbreaks have historically occurred among migrating waterfowl populations in the RWB and have resulted in substantial waterfowl mortality events (Zinkl et al., 1977; Price and Brand, 1984).

Yersiniosis in animals is characterized by gastroenteritis and diarrhea. *Yersinia* spp. are known to have established pathogen potential in animals, and swine are an especially important reservoir (Aleksic and Bockemühl, 1999). The pathogenic potential of *Y. intermedia* has not been completely determined, especially in relation to wildlife (Aleksic and Bockemühl, 1999).

Salmonella spp. are divided into six subgroups and there are over 2000 different serotypes recognized (Popoff and Minor, 1997). The natural reservoir for salmonellae is the intestinal tract of warm-blooded and cold-blooded animals. The majority of infected animals are apparently sub-clinically ill animals that harbor and shed the pathogen. *Salmonella* can survive in the environment for up to nine months or more, increasing dissemination potential (Quinn et al., 1994). In wild birds, particularly songbirds, gulls, and terns, salmonellosis can cause massive mortality events (USGS, 1999a). Avian salmonellosis (*Salmonella typhimurium*) was first diagnosed as a major cause of avian disease within the Salton Sea ecosystem in 1989, which resulted in the death of an estimated 4,515 cattle egrets (*Bubulcus ibis*) (Friend, 2002).

Erysipelothrix spp. have widespread environmental distribution in soil and water. Although primarily considered a swine pathogen, the organism has been isolated from many mammalian, avian, and amphibian species (Quinn et al., 1994). The distribution of

Erysipelothrix is probably under-reported. The association of *Erysipelothrix* with wildlife and fish, including major mortality events in eared grebes (*Podiceps nigricollis*) at the Great Salt Lake (Jensen and Cotter, 1976) suggests its inclusion in pathogen screens for waterfowl exposed to swine wastewater (USGS, 2001).

Bacteria of the genus *Clostridium* cause more wild avian mortalities than any other pathogen (USGS, 1999a). Avian botulism is a food poisoning caused by the ingestion of type C toxins produced by the bacterium *Clostridium botulinum* (USGS, 1999a). *Clostridium botulinum* spores are resistant to environmental extremes (Smith et al., 1982) and are widely distributed in wetland sediments (Smith and Sugiyama, 1988 as cited in Rocke and Samuel, 1999); however, botulism outbreaks in birds is dependent on several ecological factors including optimal environmental conditions for spore germinantion and bacterial growth, suitable material or substrates that provide energy for bacterial replication, and a mechanism of toxin transfer to birds (USGS, 1999a). Wetlands that tend to have botulism outbreaks have a greater percent organic matter in sediment, negative redox potential in the water, water pH between 7.5 and 9, water temperature above 20 ^{o}C, and salinity below 2 ppt (Rocke and Samuel, 1999).

Escherichia coli often infect the respiratory tracts of birds, resulting in colibacillosis, a chronic respiratory disease (USGS, 1999a). Lesions common to colibacillosis include pericarditis (i.e., an inflammation of the transparent membrane that encloses the heart), and perihepatitis (i.e., an inflammation of the peritoneal covering of the liver)(USGS, 1999a). Acute mortality to young waterfowl from *E. coli* infections has been reported in unhygienic hatcheries (USGS, 1999a).

Antibiotics

Hastings Pork regularly administers three tetracyline antibiotics (tetracycline, chlortetracycline, and oxytetracycline), two macrolide antibiotics (lincomycin and tylosin) and one diterpene antibiotic (tiamulin) to protect swine from disease and promote growth (Owen Nelson, Hastings Pork Farm Manager, pers. comm., 1999).

Antibiotics have become environmental contaminants of concern as they are designed to be biologically active, are generally water soluble, and they often have a low biodegradability (Wollenberger et al., 2000). The environmental fate of tetracycline antibiotics and their concentrations in CAFO wastewater is largely unknown (Zhu et al., 2001). The few studies that have examined the potential adverse effects from trace levels of antibiotics in the environment have focused on human health concerns associated with antibiotic resistance (DuPont and Steele, 1987; Guardabassi et al., 1998; Goñi-Urriza et al., 2000). Although the benefits from growth-promoting properties of antibiotics in animal feed have been known since the late 1940s, there has been little research on the mechanisms of antibiotics and potential effects to wildlife from chronic exposure to antibiotics (DuPont and Steele, 1987; Halling-Sørensen et al., 1997).

Tetracycline antibiotics are widely distributed in the body and sequestered particularly in liver, kidney, bone, and dentine (EAEM, 1995). There is no evidence of tetracycline antibiotics having the potential to cause reproductive or developmental toxicity, or carcinogenic or genotoxic effects (EAEM, 1995). Tetracycline and oxytetracycline are not acutely toxic to the freshwater crustacean *Daphnia magna* at environmentally relevant concentrations (Wollenberger et al., 2000). However, chronic toxicity tests on reproduction with *D. magna* indicated 50 percent of the population exhibited decreased reproductive output at concentrations of 46.2 mg/L and 44.8 mg/L for oxytetracycline and tetracycline, respectively (Wollenberger et al., 2000). Domestic rams receiving 20 mg/kg bw oxytetracycline for 6 or 10 days exhibited a decrease in spermatozoa motility and live spermatozoa count; however, these effects discontinued within 60 days following cessation of the treatment (HSDB, 1998). Tetracycline administered to pregnant rats resulted in neonates with discolored lens, cornea and sclera (HSDB, 1998).

Tiamulin inhibits protein synthesis at the ribosomal level (EAEM, 1995). Tiamulin chronic toxicity testing with *D. magna* resulted in decreased reproductive output for 50 percent of the population at 5.4 mg/L (Wollenberger et al., 2000). An oral dose of 50 mg/kg bw tiamulin to rats resulted in decreased testosterone-stimulated growth

of seminal vesicles with a no observed effect level (NOEL) of 15 mg/kg bw; however, dose levels up to 20 times the NOEL did not produce effects on reproductive performance, fertility, mass of gonads, or pathology (EAEM, 1995). Another study focused on reproductive effects to rats indicated no effects on fertility, growth, and survival of offspring at an oral dose of 100 mg/kg bw/day for 71 days prior to mating (EAEM, 1995).

Human treatment with lincomycin causes diarrhea in approximately 10 percent of patients and colitis (i.e., inflammation of the colon) in 1 percent of patients (Kelly et al., 1994). The antibiotic not only works against pathogenic bacteria but also against bacteria that are part of the digestive-tract flora of a healthy individual. When not kept in check by beneficial bacteria, microorganisms such as clostridia have the opportunity to grow excessively and release toxins resulting in colitis (Silva and Fekety, 1981). Less frequent toxic effects from lincomycin exposure in humans include cardiac arrhythmias, dermatitis, nephrotoxicity, hepatotoxicity, and various hematological abnormalities (HSDB, 1998).

Hormones

Run-off from CAFO wastes has been identified as an important source of synthetic and natural hormones to aquatic ecosystems (Shore, 1995; Kolpin et al., 2002; Burnison et al., 2003; Soto et al., 2004). Unlike the cattle industry, the swine industry does not use synthetic hormones (USEPA, 2003). Although synthetic hormones can be more potent and persistent than natural hormones, natural hormones can still exert effects on wildlife at low concentrations (Schiewer et al., 2001; Atienzar et al., 2002; Gross et al., 2003) and also may be more prevalent in the environment (Desbrow et al., 1998). Natural hormones associated with swine waste include testosterone, 17-β estradiol (E_2), estrone (E_1), and the phytoestrogen equol.

Risk to wildlife exposed to elevated levels of natural hormones is largely unknown due to a lack of data on environmental transport and fate in different environmental media (Kolpin et al., 2002; Ying et al., 2002). In sewage-treatment plant

effluent, E_2 is quickly metabolized to the less estrogenic E_1 by bacteria in sewage sludge, and the risk of extensive accumulation of natural estrogens in the environment is believed to be small (Lee and Liu, 2002). However, natural estrogens originating from swine waste were only degraded up to 20 percent after 12 weeks of storage at 20-23 ^{o}C (Lange et al., 2002), indicating a potential for concentrations to increase, especially under chronic exposure scenarios.

Most research on hormone contamination of natural waters has focused on sewage-treatment plants and exposure to fish (Routledge et al., 1998; Harries et al., 1999; Rogers-Gray et al., 2000; Sole et al., 2000) and there is a lack of information on the potential exposure and effects to other species (Kolpin, 2002; Lange et al., 2002). There are no studies that have evaluated waterfowl or shorebird exposure to natural hormones from ingestion of contaminated water or food items. However, in human health risk assessment there is a concern that ingestion of natural hormone residues in meat could result in neurobiological, developmental, reproductive, immunological, mutagenic and carcinogenic effects (European Commission, 1999).

METHODS

Sample collections for this study were aimed at comparing CAFO contaminants in sediments, water, and biota from the canals and created wetlands, which receive wastewater from the lagoons, and McMurtrey Marsh, a site adjacent to Hastings Pork that does not receive swine wastewater effluent. McMurtrey Marsh was often too dry to sample; therefore, data collected from other studies also were used for comparisons. A more detailed description of the methods for each analysis is provided below.

Algal Toxins

Service personnel from the Nebraska Ecological Services Field Office (NEFO) examined the created wetlands for cyanobacteria blooms on a total of 17 occasions during the spring, summer, and fall of 2000. Phytoplankton samples were collected and viewed under a light microscope to determine presence or absence of cyanobacteria. If the algal community appeared to be dominated by cyanobacteria with the potential to form toxic blooms (e.g. *Anabaena*, *Aphanizomennon*, *Nodularia*, *Nostoc, Oscillatoria*, *and Microcystis*), then a concentrated sample was collected using a 67 micro-meter (μm) phytoplankton tow net. Concentrated samples were placed in 125 milliliter (ml) brown plastic containers and immediately shipped on dry ice to the U.S. Geological Survey Biological Resource Division's Columbia Environmental Research Center (CERC). Concentrations of hepatotoxins (i.e., microcystins reported as total microcystin LR) were determined by enzyme linked immunosorbent assay (ELISA). Microcystin and nodularian toxin variants were identified, to further characterize toxicity, by high-pressure liquid chromatography (HPLC). A more detailed description of the ELISA and HPLC methods used to analyze water samples collected for this study is available in Echols (2001).

Antibiotics

In March, June, and October, 2000, Service personnel collected water and sediment samples for pharmaceuticals analysis from lagoons, canals, created wetlands, and McMurtrey Marsh. Water samples were placed in pre-cleaned 500 ml amber glass bottles and stored on ice or chilled until extraction. Sediment samples were stored in 160 ml glass jars and kept frozen. The University of Nebraska-Lincoln Water Sciences Laboratory performed a tetracycline scan and a macrolide scan to analyze water and sediment samples for tetracycline, oxytetracycline, chlortetracycline, tiamulin, lincomycin, and tylson. Tetracycline antibiotic concentrations were determined by solid phase extraction followed by detection with liquid chromatography and ion-trap electrospray ionization mass spectrometry as described by Zhu et al. (2001). Macrolide antibiotics were measured using solid phase extraction and liquid chromatography-tandem mass spectrometry according to procedures developed by Snow et al. (2003).

Bacteria Pathogens

Sediment and water samples were tested for microbial pathogens likely to occur in swine (i.e., *Clostridium botulinum* type C, *Salmonella* spp., *Pasteurella multocida*, *Yersinia* spp, *Erysipelothrix* spp, fecal coliforms, and fecal streptococci). Personnel from the NWHC and NEFO collected samples in April, June, and October 2000, from 14 stations at the study site (Figure 4). Water samples were collected in 1 L sterilized bottles and top sediment (i.e., < 10 centimeters) was placed in 125 ml sterilized containers. Water samples were kept on ice and processed within 6 hours of collection. Sediment samples (~0.25 grams) were placed into microcentrifuge tubes and stored frozen until analyzed. All samples were analyzed at the NWHC. Fecal coliform and fecal streptococci counts were obtained by membrane filtration (Clesceri et al., 1998). Samples with fecal coliform or fecal streptococci colony forming units that were too numerous to count (i.e., the agar plates were too overgrown to distinguish colony units) were not included for statistical analysis. The presence of *P. multocida*, *Salmonella*,

Yersinia and *Erysipelothrix* was verified biochemically by either the API-20E or Vitek systems (bioMerieux, St. Louis, Missouri). The presence of Botulism type C spores was determined using a DNA isolation kit (UltraCleanTM, MoBio Laboratories, Inc., Solana Beach, CA). Although not part of the original study plan, *Salmonella* and *Escherichia coli* isolates were tested for antibiotic resistance using standardized materials. A more detailed description of the methods used to determine antibiotic resistance, pathogen counts, and isolates is provided by USGS (2001).

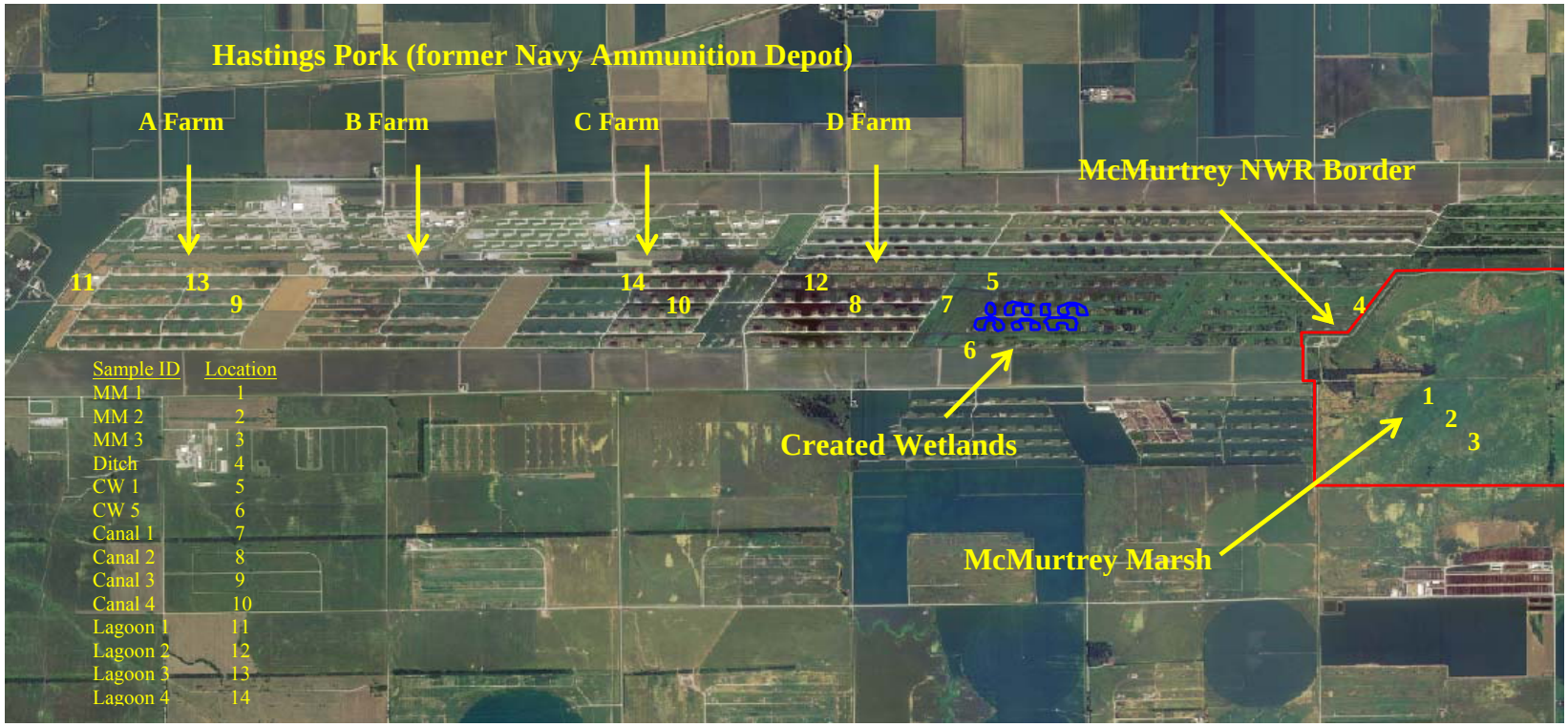


Figure 4. Locations of samples collected for identification of bacteria pathogens at Hastings Pork and McMurtrey NWR, Clay County, Nebraska, 2000.

Hormones

Determining concentrations of hormones in water was not part of the original study plan; however, funding allowed for a limited analysis. Water samples were collected in 50 ml Falcon tubes, kept on ice, and shipped to the USGS Florida Caribbean Science Center where they were analyzed directly (i.e., without preparing an extract) for 17-β estradiol and testosterone using ELISA methods.

Waterfowl Use

The Rainwater Basin Wetland Management District performed waterfowl brood surveys in and around the created wetlands to determine waterfowl use. Surveys were conducted in July and September of 2000 and July and August of 1999. The number of broods, species, age class, and number of ducklings were recorded.

Invertebrate Diversity

Wetland benthic invertebrates were sampled three times during the year in spring, summer, and fall. Invertebrates were sampled using a 20 centimeter (cm) diameter stovepipe sampling core. Water within the pipe was decanted through a 297 um sieve and sediments were collected until firm ground was reached. For each created wetland and McMurtrey Marsh, a composite sample from six locations on each wetland was preserved in 10 percent formalin. There was a total of nine samples, with each sample representing one of three seasons (spring, summer, fall) for one of three sites, McMurtrey Marsh, created wetland 1 (CW1) and created wetland 5 (CW5). Invertebrates were randomly sub-sampled following standard USEPA rapid assessment procedures (Barbour et al., 1999) by picking all individuals within four randomly selected 6 by 6 cm grids. Analysis of invertebrates focused on taxa richness as our methods did not allow for density estimates. EcoAnalysts, Inc. (Moscow, Idaho) identified (usually to genus) all invertebrates in each subsample.

Water Quality

Temperature, dissolved oxygen, conductivity, salinity (YSI model 85) and pH (Accument® AP61) measurements were taken every 2 weeks on all created wetlands and McMurtrey Marsh from March 14 to November 07, 2000. In addition, water samples were collected monthly from McMurtrey Marsh, CW1, and CW5 from March to October 2000, and analyzed for total Kjeldahl nitrogen, ammonia, nitrates, and total phosphorus (Servi Tech Laboratories, Hastings, Nebraska). Water quality from the created wetlands and McMurtrey Marsh were compared to water quality data collected by the NDEQ for 30 stations at 15 RWB wetlands (NDEQ, 1997).

Trace Elements

Water, sediment, and invertebrate samples were submitted to the Service's Patuxent Analytical Control Facility (PACF) for trace metal analysis (Table 1). Samples were collected in USEPA certified clean glass containers and sampling equipment was decontaminated between sites. Water samples were collected in 500 ml containers and 2 ml of nitric acid were added to each sample to obtain a pH near 2. Sediment samples were collected into 1,500 ml containers with a stainless steel spoon in all areas except the lagoons where samples were collected using a ponar dredge. Forceps were used to collect chironomids from sediments filtered by a 1.1 mm mesh size sieve. Care was taken to select chironomids of all sizes as chironomid size can alter metal uptake (Krantzberg, 1989).

Inductively coupled plasma atomic emission spectrometry was used to determine concentrations of Al, B, Ba, beryllium (Be), Cd, chromium (Cr), Cu, Fe, magnesium (Mg), Mn, Mo, Ni, Pb, strontium (Sr), V, and Zn. Mercury (Hg) concentrations were determined by cold vapor atomic absorption. Graphite furnace atomic absorption was used to measure As, Se, and small concentrations of Pb and Cd.

Concentrations of trace elements in sediment were compared among Hastings Pork lagoons, canals, created wetlands, and Rainwater Basin wetlands. Data from water and sediment samples collected at McMurtrey Marsh during this study were pooled with

data on trace element concentrations from other studies on Rainwater Basin wetlands to increase the sample size of the "control wetlands" for comparison with the created wetlands, canals and lagoons. The RWB wetland sediment data set included 10 samples collected in 1993 and 1994 from RWB wetlands at McMurtrey NWR, Harvard WPA, Massie WPA, Eckhart WPA, and Smith WPA (PACF catalog numbers 6050045 and 6050027). The RWB wetland water data set included 16 water samples collected by the Service in 1991 and 1993 (catalogs 6050017 and 6050045, respectively). Not all samples collected were analyzed for the same trace elements; therefore, sample sizes for RWB wetland sediment and water data sets varied from 2 to 18 (Appendix, Table A.1).

Data on trace element concentrations in chironomids from wetlands in Nebraska is lacking; therefore, trace element concentrations in chironomids collected from the Hastings Pork created wetlands and canals were compared to those in chironomids collected at two National Irrigation and Water Quality Program (NIWQP) sites, namely, the Stillwater NWR in west-central NV (Tuttle et al., 1996) and the Sun River Irrigation Project Area in west-central Montana (NIWQP, 2003). Data on trace element concentrations in chironomids from these two areas were obtained through PACF and included catalogs 1070005, and 1070009 to 1070012 for Stillwater NWR and 6070002, 6070006, 6070010, 6070019, 6070029, 6070030, 6070033, 6070041, 6070042, 6070044, 6070049, 6070051 for sites within the Sun River Irrigation Project. Concentrations of Al, As, B, Hg, and Zn in biota, sediment and water samples collected from Stillwater NWR frequently exceeded concentrations associated with adverse biological effects (Tuttle et al., 1996); therefore, Stillwater NWR data represented a contaminated site. The Sun River Irrigation Project Area contained concentrations of Se in biota, sediment and water samples above toxicity thresholds (Palawski et al., 1991), but most other trace elements were typically below levels of concern (Bill Olsen, U.S. Fish and Wildlife Service Contaminants Specialist, pers. comm., 2003). Therefore, with the exception of Se, concentrations of trace elements in chironomids from this site represented an uncontaminated site.

Statistical Analyses

Statistical calculations were performed in software from the Statistical Analysis System (SAS) Institute (either JMP® Version 5 or SAS® Version 8.2). Data were typically nonparametric; therefore, a Kruskal-Wallis nonparametric one-way analysis of variance was used to test significance among three groups and a Wilcoxon rank sums test was used to test significance between groups. Differences among three or more groups were analyzed using PROC MIXED in SAS (SAS Institute, 2001). If more than 50 percent of the samples analyzed were above the detection limit for a particular trace element, then half the detection limit was used in place of those below the detection limit for statistical analyses, unless otherwise noted. Trace elements below the detection limit for more than 50 percent of the samples were not analyzed statistically.

Table 1. Summary of samples collected for trace element analyses at Hastings Pork and McMurtrey Marsh, 2000 to 2001.

Date Collected	PACF Catalog ID	Site	Laboratory	Number of Samples per Matrix		
				Sediments	Water	Invertebrates
March, 2000	6050062	CW1	RTI	1	1	0
		CW5		1	1	0
		Canal		1	1	0
		MM		1	1	0
June, 2000	6050065	CW1	MRI	1	1	2
		CW5		1	1	2
		Canal		1	1	0
October, 2000	6050066	CW1	RTI	1	1	1
		CW5		1	1	1
		Canal		1	1	0
		MM		1	1	0
July, 2001	6050091	CW1	GERG	2	0	0
		CW2		2	0	1
		CW3		2	0	1
		CW4		2	0	1
		CW5		2	0	1
		CW6		2	0	1
		CW7		2	0	1
		Lagoon		10	0	0
		Canal		4	0	2
		MM		0	0	0
			TOTAL	39	11	14

Note: CW = created wetland; MM = McMurtrey Marsh; PACF = Patuxent Analytical Control Facility, Laurel, MD; ID = identification number; Laboratory = the laboratory that performed the analytical analysis (i.e., RTI = Research Triangle Institute, MRI = Midwest Research Institute, GREG = Geochemical & Environmental Research Group, Texas A&M).

RESULTS

Algal Toxins

Cyanobacteria genera (i.e., *Microcystis*, *Anabaena*, *Hapalosiphon*, *Nodularia*, and *Oscillatoria* were identified in four of seven created wetlands sampled at Hastings Pork in 2000 (created wetlands 3, 4, 6, and 7). Algal blooms dominated by *Microcystis* were observed in three created wetlands (CW 4, 6, and 7) on October 2 and 18, 2000. Six samples were collected from these blooms. All six samples contained microcystins reported as total microcystin-LR. The mean total microcystin-LR concentration determined by ELISA methods was 81 nanograms (ng) per mg (standard error = 24 ng/mg). Analysis by HPLC indicated microcystin-RR was the dominant microcystin variant in all six samples with microcystin-LR detected at concentrations ranging from 0.1 to 1.6 ng/mg (Table 2).

Table 2. Total microcystin (MC) concentrations in algal blooms from the Hastings Pork created wetlands, Clay County, Nebraska, 2000.

Sample ID and date collected	ELISA Total MC-LR	HPLC MC-LR	HPLC MC-RR	HPLC MC-YR	HPLC MC-LA
CW4 10/2/2000	200	ND	120	ND	ND
CW6 10/2/2000	60	0.1	0.6	ND	ND
CW7 10/2/2000	73	0.3	19	ND	ND
CW4 10/18/2000	61	ND	22	ND	ND
CW6 10/18/2000	50	1.6	4.9	ND	ND
CW7 10/18/2000	41	ND	4.2	ND	ND

Note: all microcystin concentrations are in ng/mg dry weight. ND=non-detect.

Antibiotics

The total number of water samples (n) collected for determining concentrations of antibiotics in water was 32 and included collections from lagoons (n=14), canals (n=10), created wetlands (n=6), and McMurtrey Marsh (n=2). In addition, nine sediment samples were collected in October from the lagoons (n=3), canals (n=3), created wetlands (n=2), and McMurtrey Marsh (n=1). Tetracycline, oxytetracycline, chlortetracycline, tiamulin, lincomycin, and tylosin were detected in either water or sediment samples collected from Hastings Pork lagoons and canals. In the created wetlands, only oxytetracycline was detected in sediments (41 ng/g); however, sample size for created wetlands was small (n = 2 and 6 for sediments and water, respectively). No antibiotics were detected in McMurtrey Marsh.

Tetracycline, oxytertracycline, chlortetracycline and tiamulin were frequently detected in sediments or water from the Hastings Pork lagoons and canals (Appendix, Table A.7). Oxytetracycline was detected in all water samples collected from the lagoons and in all sediment samples collected from the lagoons and canals. Average concentrations of oxytetracycline were more than 60 times greater in sediments than water (Figures 5 and 6). Tiamulin was detected in all water samples collected from the lagoons and in 2 of 3 sediment samples from the lagoons. In the Hastings Pork canals, tiamulin was detected in 80 percent and 30 percent of the water samples and sediment samples collected, respectively. Chlortetracycline was detected in all 6 sediment samples from lagoons and canals. Concentrations of chlortetracycline in sediments ranged from 311 to 6,430 ng/g in the lagoons and from 108 to 1,800 ng/g in the canals. Tetracycline was detected in 5 of 6 sediment samples and concentrations ranged from 119 to 1,328 ng/g in the lagoons and from 50 to 98 ng/g in the canals. Tetracycline and chlortetracycline in water samples were generally below the detection limit of 10 µg/L. In lagoon water samples (n=11), lincomycin was only detected twice (780 µg/L and 53 µg/L) and tylosin was only detected once (11.4 µg/L). Lincomycin and tylosin were not detected in lagoon sediments (n = 6).

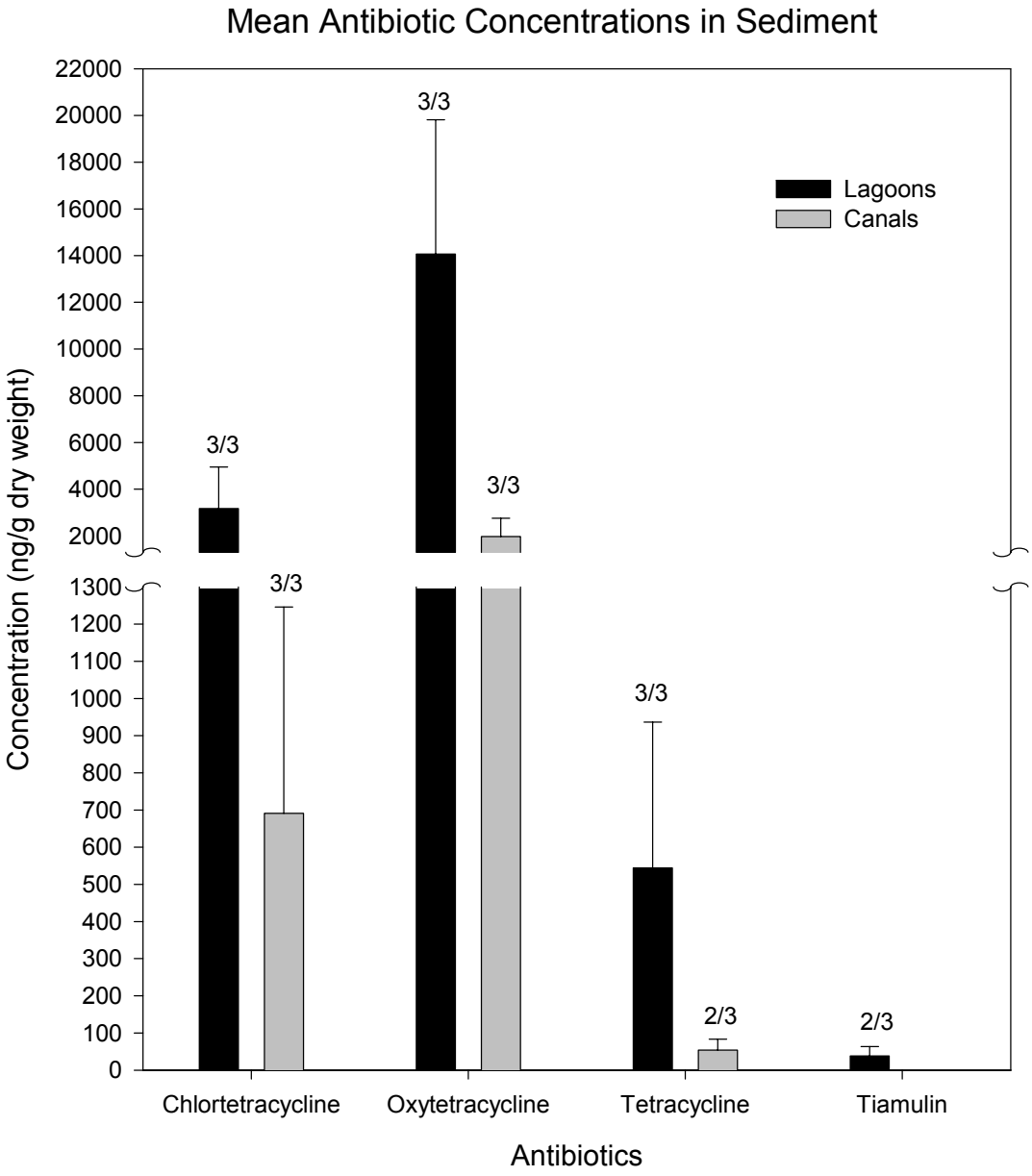


Figure 5. Mean (± S.E.) concentrations of antibiotics in sediments from lagoons and canals at Hastings Pork, Clay County, Nebraska, 2000. The number of detects per number of samples analyzed is displayed above each standard error bar. Note: tiamulin was detected once in canal sediments at 29 ng/g (data not shown).

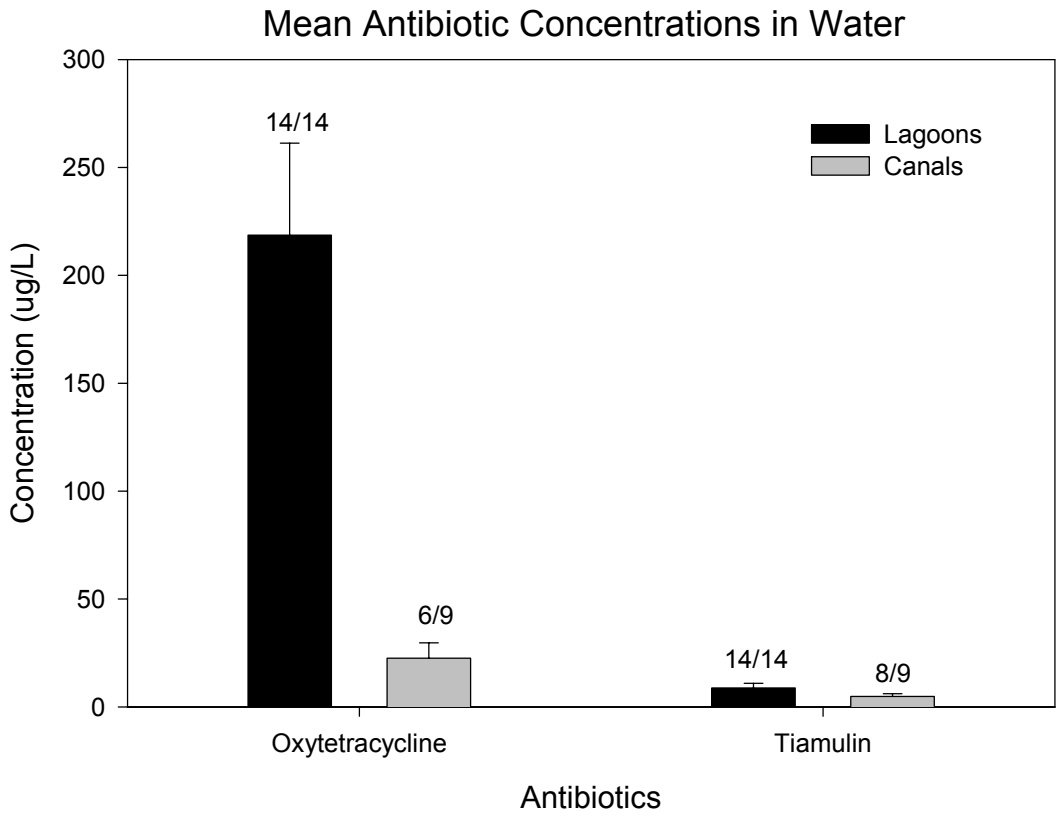


Figure 6. Mean (± S.E.) concentrations of antibiotics in water from lagoons and canals at Hastings Pork, Clay County, Nebraska, 2000. The number of detects per number of samples analyzed is displayed above each standard error bar.

Bacterial Pathogens

Bacterial results indicated the occurrence of fecal coliforms, fecal streptococci, *Salmonella* spp., *Yersinia* spp., and *Clostridium botulinum* type C in the created wetlands, lagoons and canals. Fecal coliform and fecal streptococci colony forming units (cfu) were counted in samples collected from the lagoons (n = 12), canals (n=12), created wetlands (n=6), a drainage ditch leading into McMurtrey NWR (n=3), and McMurtrey Marsh (n=6)(See Figure 4 for site locations). Fecal streptococci and fecal coliform colonies were too overgrown to distinguish colonies (too numerous to count) on 12 occasions from samples collected at the created wetlands, canals, and lagoons (Appendix, Table A.2). Annual mean counts of fecal coliform and fecal streptococci were highest in the lagoons and lowest in McMurtrey Marsh and the drainage ditch (Table 3). Fecal coliform and streptococci cfu per 100 ml often varied considerably within sites and by season (Figure 7). Although this variation precluded significant differences in mean counts of cfu/100ml between many of the sites, the created wetlands had a significantly ($P < 0.05$) greater mean number of fecal streptococci cfu/100 ml when compared to McMurtrey Marsh (Table 3).

Table 3. Mean annual fecal coliforms and fecal streptococci colony forming units per 100 ml of water from sites at Hastings Pork and McMurtrey Marsh, Clay County, Nebraska, 2000.

Pathogen	Site	n	Mean ± S.E.	min	max	Significance*
Fecal Coliforms	Lagoon	9	164,894 ± 81,990	4,000	770,000	A
	Canal	12	10,145 ± 7,297	100	90,000	B
	CW	6	1,277 ± 523	100	3,340	B
	Ditch	2	247 ± 168	79	415	B
	MM	6	1,208 ± 495	50	2,650	B
Fecal Streptococci	Lagoon	10	46,280 ± 15,452	3,250	143,500	A
	Canal	11	4,768 ± 1,067	600	12,100	B
	CW	5	27,472 ± 24,432	200	125,000	AB
	Ditch	2	147 ± 112	35	258	BC
	MM	6	82 ± 46	5	300	C

*Different letters indicate significance ($p<0.05$) as determined by a Kruskal-Wallis test followed by pairwise Wilcoxon rank sums tests. CW = created Wetland, MM = McMurtrey Marsh, n = sample size and does not include samples in which fecal coliforms or streptococci were too overgrown to distinguish colonies (see Appendix table A. 2).

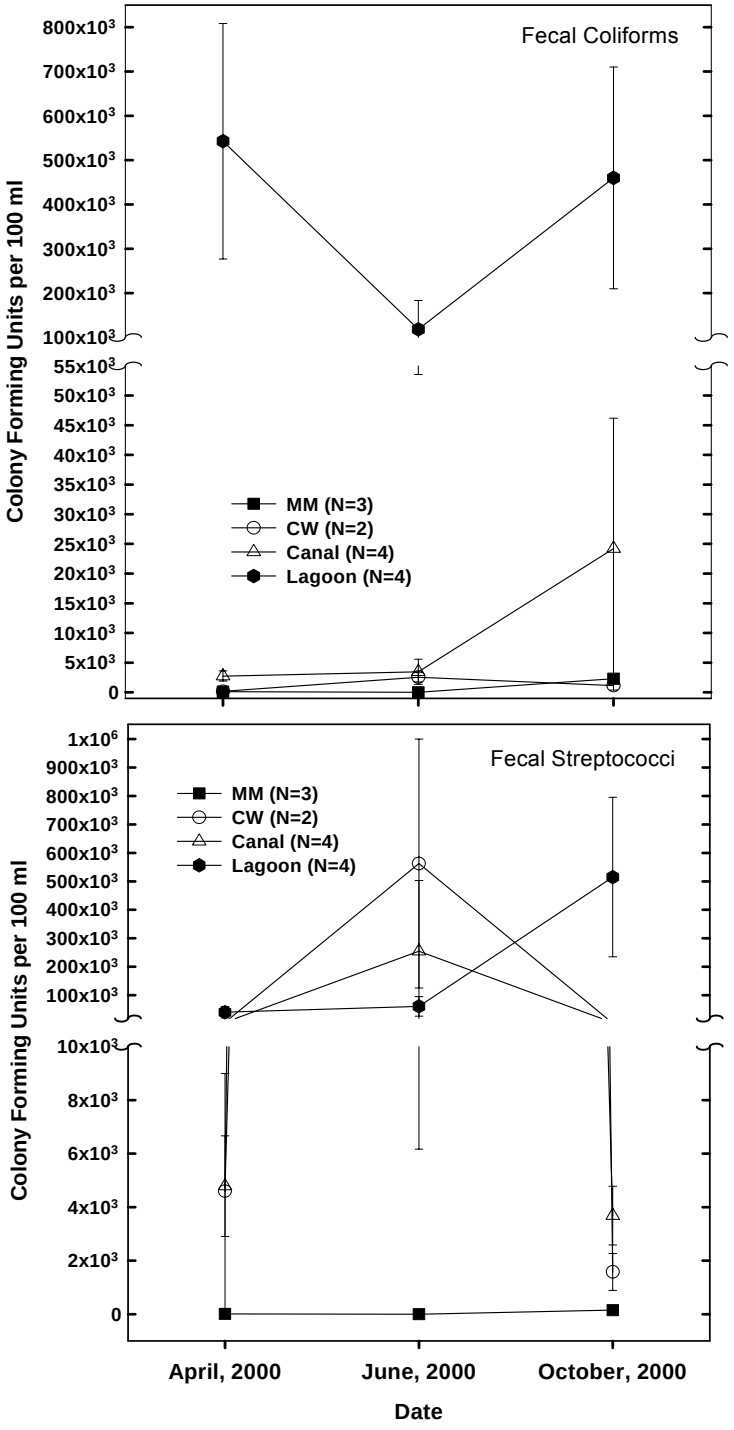


Figure 7. Mean (± S.E.) fecal coliform and fecal streptococci counts for water samples collected from created wetlands (CW), canals, lagoons, and McMurtrey Marsh (MM) in Clay County, Nebraska, 2000. N = the number of samples analyzed for each site per season; however, samples were not collected from MM in June of 2000 due to dry conditions.

Sixteen isolates of *Salmonella* spp. and 24 isolates of *Yersinia* spp. were recovered during this study (Appendix, Tables A.3 and A.4). All *Salmonella* spp. isolates were recovered from water samples, no isolates were obtained from sediments. *Salmonella* isolates were recovered from the Hastings Pork lagoons, canals, created wetlands, and a ditch leading into McMurtrey NWR, but were not recovered from McMurtrey Marsh (Figure 8). Salmonella serotypes isolated included Newport, Infantis, Muenchen, and Typhimurium (Copenhagen) (Appendix, Tables A.3 and A.4). *Yersinia* spp. isolates were recovered from both water and sediment samples with water being the primary source. The most common isolate was *Yersinia intermedia,* accounting for 14 of the 24 isolates. *Yersinia enterocolitica* accounted for 4 of 24 isolates.

Bacterial resistance to multiple antibiotics was detected for *Salmonella* spp and *E. coli* isolates. Two of 16 *Salmonella* spp. isolates exhibited resistance to multiple antibiotics. Out of 31 *E. coli* isolates tested; 28 were resistant to at least one antibiotic, 12 were resistant to two or more antibiotics, 9 were resistant to four or more antibiotics and 1 was resistant to eight antibiotics tested. Resistance to tetracycline was most common for both *Salmonella* spp. (20 percent of isolates tested) and *E coli* (> 90 percent tested). A more detailed description of the antibiotic resistance results are provided by USGS (2001).

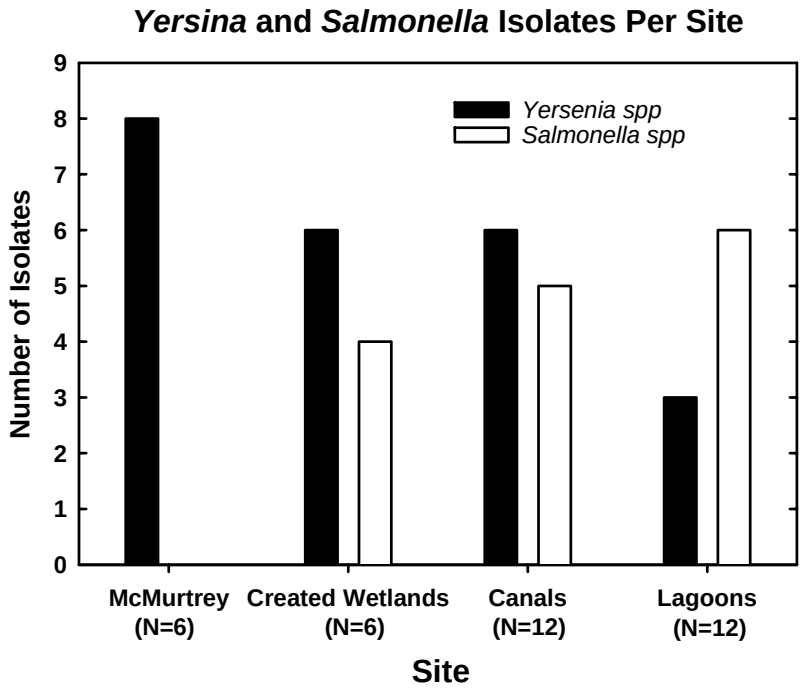


Figure 8. Number of isolates of *Yersenia* and *Salmonella* species from McMurtrey Marsh and Hastings Pork created wetlands, canals and lagoons, Clay County, Nebraska, 2000. N = the total number of samples analyzed for each site.

Hormones

Concentrations of testosterone and E_2 in water from the created wetlands, lagoons and canals (n = 4 total) were greater than detected at McMurtrey Marsh (n=1) and were similar to concentrations from contaminated sites as reported by others (Table 4). 17 beta estradiol was detected in 4 out of 5 samples and concentrations ranged from 49 pico-grams per militer (pg/ml) at Created Wetland 1 to below detection limits (< 5 pg/ml) at McMurtrey Marsh. Testosterone was detected in all 5 samples and concentrations ranged from 206 pg/ml in the canal to 23 pg/ml at McMurtrey Marsh.

Table 4. Concentrations of 17-β estradiol and testosterone in water samples collected from McMurtrey Marsh and Hastings Pork created wetlands, canals and lagoons in Clay County, Nebraska, 2000, compared to those published by other studies.

Hormone	Site	Measure	Con.(ppt)	Units	Method	Suspected Source	Citation
17-β estradiol	Lagoon	N=1	21	pg/ml	RIA	Swine CAFO	This Study
	Created Wetland 5	N=1	7	pg/ml	RIA	Swine CAFO	This Study
	Canal	N=1	31	pg/ml	RIA	Swine CAFO	This Study
	Created Wetland 1	N=1	45	pg/ml	RIA	Swine CAFO	This Study
	McMurtrey Marsh	N=1	0	pg/ml	RIA	Uncontaminated	This Study
	British Rivers	Max	50	ng/L	Yeast bioassay	Sewage	Desbrow et al., 1998
	Farm pond	Max	7.4	ng/L	RIA	Cattle Farm	Irwin et al., 2001
	Grassland run-off	Range	50 to 150	ng/L	ELISA	Poultry farm	Finlay-Moore et al., 2000
	U.S. Streams	Median*	9	ng/L	GC/MS	Industry, sewage, agricultural	Kolpin et al., 2002
	U.S. Streams	Max (n=70)	93	ng/L	GC/MS	Industry, sewage, agricultural	Kolpin et al., 2002
Testosterone	Lagoon	N=1	131	pg/ml	RIA	Swine CAFO	This Study
	Created Wetland 5	N=1	76	pg/ml	RIA	Swine CAFO	This Study
	Canal	N=1	206	pg/ml	RIA	Swine CAFO	This Study
	Created Wetland 1	N=1	176	pg/ml	RIA	Swine CAFO	This Study
	McMurtrey Marsh	N=1	23	pg/ml	RIA	Uncontaminated	This Study
	Grassland run-off	Range	15 to 125	ng/L	ELISA	Poultry farm	Finlay-Moore et al., 2000.
	U.S. Streams	Median*	116	ng/L	GC/MS	Industry, sewage, agricultural	Kolpin et al., 2002
	U.S. Streams	Max (n=70)	214	ng/L	GC/MS	Industry, sewage, agricultural	Kolpin et al., 2002

Note: Con. (ppt) = Concentration in parts per trillion; GC/MS = gas chromatography/mass spectroscopy. Full citations provided in the References section.

Waterfowl Use

Hastings Pork and Service personnel observed heavy use of the created wetlands by waterfowl and shorebird species in 1999, 2000, and 2001. Species observed included: mallard, green-winged teal (*Anas crecca*), blue-winged teal (*Anas discors*), wood duck (*Aix sponsa*), redhead (*Aythya americana*), gadwall (*Anas strepera*), scaups (*Aythya* spp.), bufflehead (*Bucephala albeola*), American wigeon (*Anas americana*), northern shoveler (*Anas clypeata*), northern pintail, ruddy duck (*Oxyura jamaicensis*), Wilson's phalarope (*Phalaropus tricolor*), spotted sandpiper (*Actitis macularia*), dowitchers (*Limnodromus* spp.), and killdeer (*Charadrius vociferus*). Broods of mallard, gadwall, wood duck, redhead, and teal were observed in surveys performed in August of 1999, and July and September of 2000, with mallards being most frequently observed. Waterfowl also were observed loafing in the lagoons and canals, although these areas appeared to be utilized less often and by fewer numbers compared to the created wetlands.

Invertebrate Analysis

Invertebrate abundance and diversity were markedly different between McMurtrey Marsh and created wetland sites evaluated (Table 5). Invertebrates of the order Trichoptera, Odonata, Copepoda, Arhynchobdellida and members of the class Ostracoda and Oligochaeta were present at McMurtrey Marsh and absent from the created wetlands. Benthic invertebrate communities in the created wetlands were dominated by Chironomidae (93 to 100 percent) during all three seasons. In comparison, chironomids made up 0 to 15 percent of the benthic invertebrate community at McMurtrey Marsh. The invertebrate community at McMurtrey Marsh appeared to be comprised mainly of Cladocera in the spring and summer and Odonata in the fall. Taxa diversity was greatest at McMurtrey Marsh during the spring, summer, and fall when compared to the created wetland sites (Figure 9). Chironomids of the genus *Tanypus* was the dominant taxa in created wetlands for all seasons sampled with the exception of CW1 in the spring when the chrionomid genus *Glyptotendipes* was dominant.

Table 5. Total number of individuals by invertebrate order found in the spring, summer, and fall of 2000 from sites at McMurtrey Marsh and created wetlands 1 and 5.

		McMurtrey NWR			Created Wetland 1			Created Wetland 5		
Order-Family or (Class)	Genus	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall
Odonata	Coenagrion and Enallagma	-	-	22	-	-	-	-	-	-
	Libellulidae	-	-	2	-	-	-	-	-	-
Hemiptera	Buenoa sp.	-	-	-	-	-	-	-	-	15
	Corisella sp.	-	1	2	-	-	4	1-	6	32
	Sigara sp.	-	-	-	-	-	-	-	5	-
Coleoptera	Berosus sp.	-	-	2	-	-	-	-	-	-
	Coleoptera	-	-	-	-	-	2	-	-	-
	Ilybius sp.	1	-	-	-	-	-	-	-	-
Trichoptera	Oecetis sp.	-	-	1	-	-	-	-	-	-
Diptera	Ceratopogon sp.	-	-	7	-	-	-	7	7	6
	Mallochohelea sp.	-	-	-	-	-	-	1	-	-
	Muscidae	-	-	-	-	-	2	-	-	2
	Sphaeromias sp.	-	-	1	-	-	-	-	7	-
Diptera-Chironomidae	Chironomus sp.	-	-	-	2	4	41	25	47	142
	Clinotanypus sp.	-	-	4	-	-	-	-	-	-
	Glyptotendipes sp.	-	-	-	104	20	4	58	53	133
	Tanypus sp.	-	-	5	24	646	373	542	328	499
Arhynchobdellida	Erpobdellidae	2	4	2	-	-	-	-	-	-
Lumbricina	Oligochaeta	10	8	6	-	-	-	-	-	-
Cladocera		21	21	-	-	6	-	-	-	-
Ostracoda (Class)		2	1	-	-	-	-	-	-	-
Copepoda (Class)	Calanoida	8	1	4	-	-	-	-	-	-
	Total Number of Individuals	44	36	58	130	676	426	643	453	829
	Percent Chironomids	0%	0%	16%	100%	99%	98%	97%	94%	93%

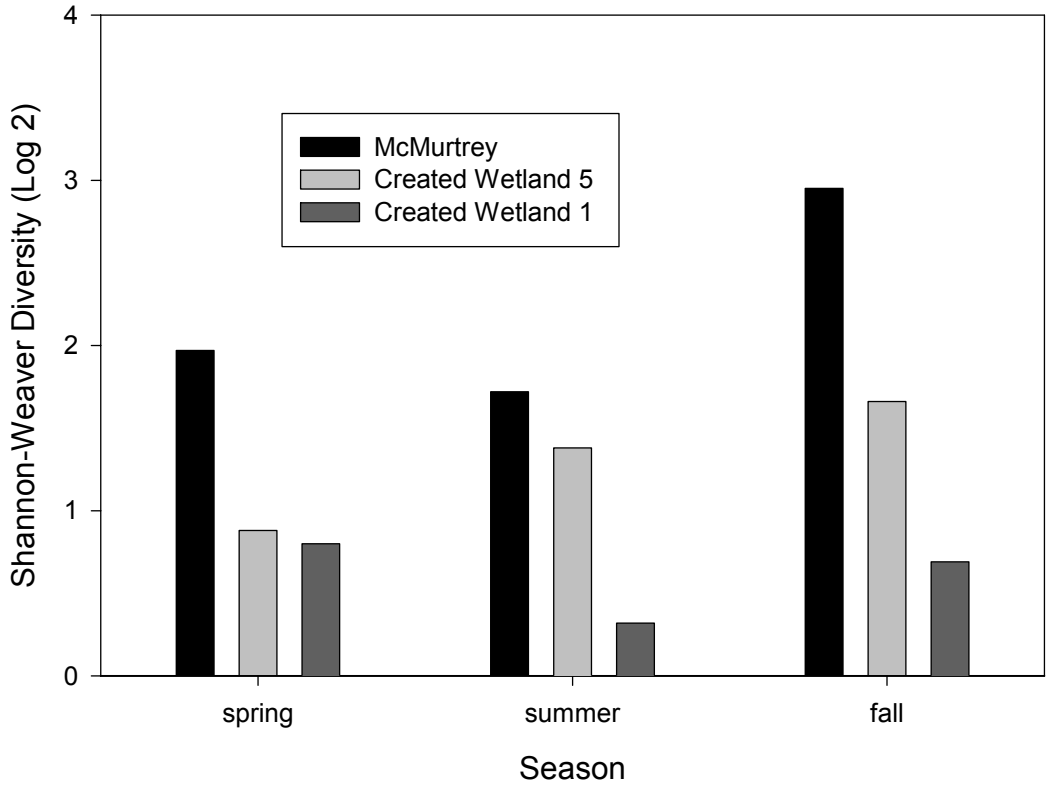


Figure 9. Shannon-Weaver taxa diversity for benthic invertebrates collected in the spring, summer, and fall from McMurtrey Marsh, Created Wetland 1, and Created Wetland 5 in Clay County, Nebraska, 2000.

Water Quality

Dissolved oxygen, specific conductivity, and pH were significantly greater ($p > 0.05$) in created wetlands compared to McMurtrey Marsh and the RWB wetlands sampled by NDEQ (Figure 10). Salinity also was significantly greater ($p< 0.05$) in created wetlands (mean = 0.72 ± 0.04) compared to McMurtrey Marsh (mean = 0.13 ± 0.02). There was no significant difference in water temperature between McMurtrey Marsh (mean = 16.6 ± 2.5) and the created wetlands (mean = 17.2 ± 1.0). The created wetlands had significantly greater concentrations ($p<0.05$) of total phosphorous, ammonia, and total nitrogen when compared to McMurtrey Marsh and RWB wetlands (Figure 11).

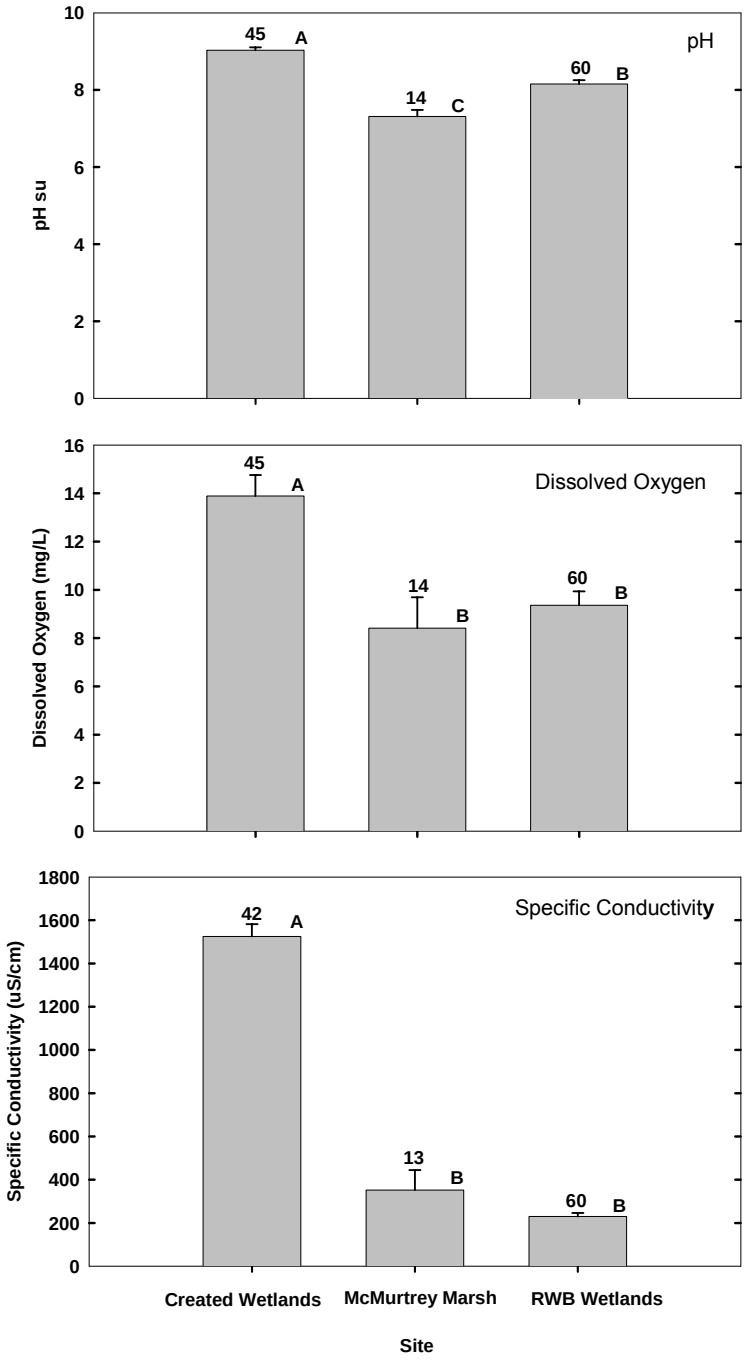


Figure 10. Mean (± SE) measurements of pH, dissolved oxygen, and specific conductivity in the Hastings Pork created wetlands, McMurtrey Marsh, and Rainwater Basin (RWB) wetlands in Clay County, Nebraska, 2000. Measurements were generally taken between 10:00 and 14:00. The sample size is given above each standard error bar. Different letters indicate significance ($p<0.05$) as determined by a Kruskal-Wallis test followed by pairwise Wilcoxon rank sums tests.

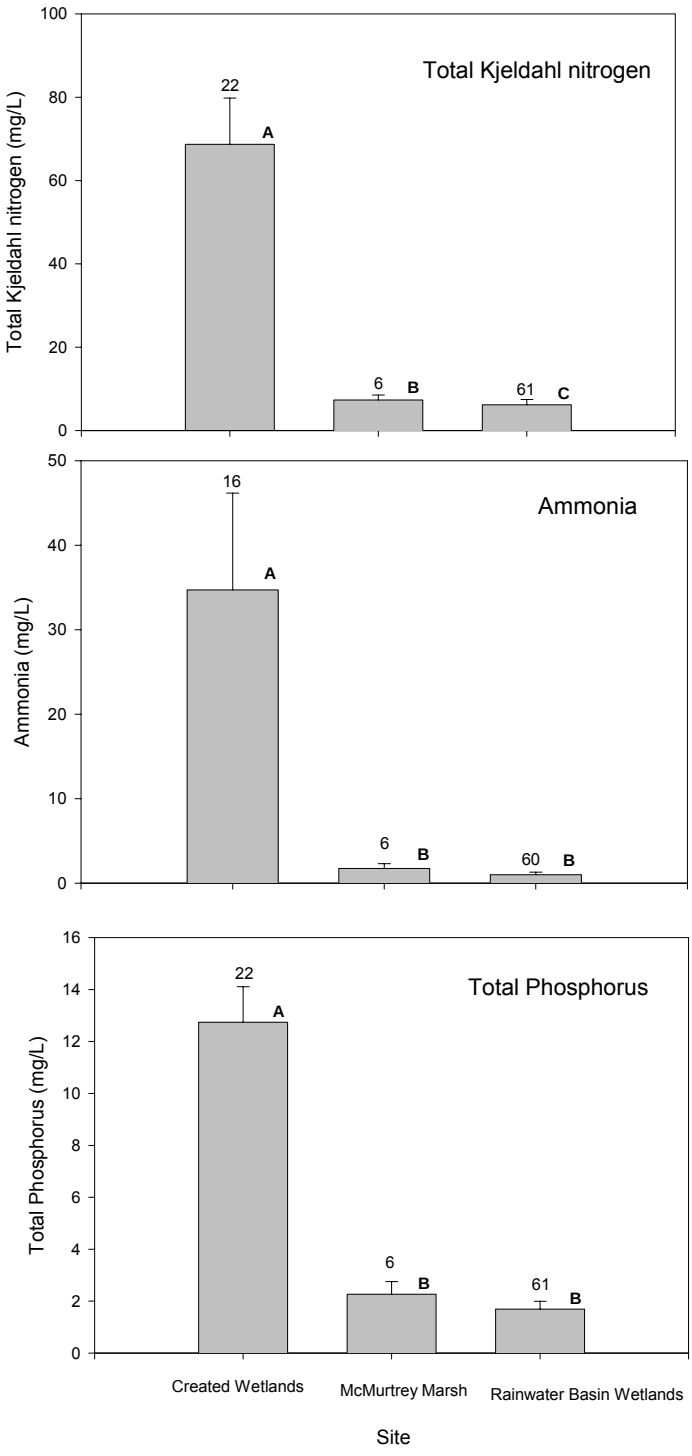


Figure 11. Mean (± SE) concentrations of ammonia, total Kjeldahl nitrogen, and total phosphorus in created wetlands, McMurtrey Marsh, and Rainwater Basin wetlands in Clay County, Nebraska, 2000. The sample size is given above each standard error bar. Different letters indicate significance ($p<0.05$) as determined by a Kruskal-Wallis test followed by pairwise Wilcoxon rank sums tests.

Trace Elements

Sediment. Concentrations of Cd, Cr, Cu, Mg, Mn, Ni, Se and Zn in sediment were significantly ($p<0.05$) greater in the lagoons compared to the canals, created wetlands, and RWB wetlands (Table 6). In addition, Mo was only found in samples collected from lagoons, where it was detected in 8 of 10 samples (Appendix, Table A.8). Created wetlands had significantly ($p<0.05$) greater concentrations of Al, B, Be, Cr, Mg, Mn, Sr, and V when compared to RWB wetlands (Table 6); whereas, RWB wetlands had significantly ($p<0.05$) greater concentrations of Pb than lagoons, canals, and created wetlands (Figure 12). However, mean trace element concentrations in created wetlands and RWB wetlands were similar or less than background concentrations and did not exceed sediment quality guidelines or literature established toxicity thresholds (Table 7). Sediment toxicity thresholds and quality guidelines/benchmarks were exceeded in lagoon sediments for Cd, Cu, Ni, Mn, Se, and Zn. Trace element concentrations in canal sediments exceeded quality guidelines for Cu and Zn.

Water. Concentrations of trace elements in water from the created wetlands and canals were generally similar to those at McMurtrey Marsh; however, only 2 to 3 samples were analyzed for each site (Table 8). When data were pooled for the canals and created wetlands, these sites had significantly greater concentrations of B, Mg, and Mn compared to pooled data from the RWB sites (Figure 13). Concentrations of Se in water were detected in 3 of 9 samples from the Hastings Pork canals and created wetlands but Se was not detected in two water samples from McMurtrey Marsh. However, the detection limits for Se and Cd in water samples collected for this study exceeded their water quality criteria and/or their effects thresholds (Table 8). Concentrations of Zn in water samples from all sites generally exceeded the 0.03 mg/L "level of concern" at which toxic effects may occur in sensitive phytoplankton and invertebrate species (Suter and Tsao, 1996; USDI, 1998). Water quality criteria for Nebraska wetlands were exceeded for Al, Cd, Cu, Fe, and Pb in the created wetlands, canals, and McMurtrey Marsh and for As and Ni in the created wetlands (Table 8). Concentrations of Cu in water from the canals and

created wetlands exceeded Nebraska Cu aquatic life water quality criteria in all nine samples; whereas, samples from RWB wetlands exceeded Cu criteria concentrations in 2 of 11 samples (Table 8 and Appendix, Table A.5)

Invertebrates. Concentrations of trace elements in invertebrates from the created wetlands and canals, with the exception of Se, did not exceed any known toxicity thresholds for invertebrates or avian dietary items. Chironomids from the canals and created wetlands had concentrations of Se that were typically within the normal background (i.e., 0.4 to 4.5 mg/kg dw) for aquatic invertebrates (USDI, 1998). However, reproductive impairment in avian species can result from diets containing 3 to 8 mg/kg (USDI, 1998). Chironomids from the created wetlands exceeded 3 mg/kg Se in 4 of 10 samples analyzed. Concentrations of trace elements in chironomids from Stillwater NWR were generally significantly greater than those from Hastings Pork, with the exception of Cr, Ni, Se and Zn (Table 9). Chironoimids from the Sun River Irrigation Project Area had significantly lower concentrations of Al, Ba, Cr, Cu, Fe, Mn, Pb, and V compared to chironomids from the created wetlands and significantly greater concentrations of Se compared to chironomids from Stillwater NWR or the created wetlands (Table 9).

Table 6. Results from Proc Mixed in the Statistical Analysis System (SAS) for trace elements in sediments from Rainwater Basin wetlands and Hastings Pork lagoons, canals, and created wetlands in Clay County, Nebraska.

Trace Elements	p values	Results from PROC MIXED in SAS			
Al	<0.0050	CW ^{A}	Canals ^{B}	Lagoons ^{B}	RWB ^{B}
B	<0.0001	CW ^{A}	Lagoons ^{AB}	Canals ^{BC}	RWB ^{C}
Be	<0.0024	CW ^{A}	Lagoons ^{A}	Canals ^{AB}	RWB ^{B}
Cd	<0.0001	Lagoons ^{A}	Canals ^{B}	RWB ^{B}	CW ^{B}
Cr	<0.0001	Lagoons ^{A}	CW ^{B}	Canals ^{BC}	RWB ^{BC}
Cu	<0.0001	Lagoons ^{A}	Canals ^{B}	CW ^{C}	RWB ^{C}
Mg	<0.0001	Lagoons ^{A}	Canals ^{B}	CW ^{B}	RWB ^{C}
Mn	<0.0001	Lagoons ^{A}	CW ^{B}	Canals ^{BC}	RWB ^{C}
Ni	<0.0001	Lagoons ^{A}	Canals ^{B}	CW ^{BC}	RWB ^{C}
Pb	<0.0001	RWB ^{A}	CW ^{B}	Canals ^{C}	Lagoons ^{D}
Se	<0.0001	Lagoons ^{A}	Canals ^{B}	BDL	BDL
Sr	<0.0001	Lagoons ^{A}	Canals ^{AB}	CW ^{B}	RWB ^{C}
V	<0.0001	Lagoons ^{A}	CW ^{A}	Canals ^{B}	RWB ^{C}
Zn	<0.0001	Lagoons ^{A}	Canals ^{B}	CW ^{C}	RWB ^{C}

Note: CW = created wetlands, RWB = Rainwater Basin Wetlands. Different superscript letters indicate significant differences among sites. Sites are listed from left to right in decreasing order of mean concentration for each trace element. Trace elements tested that were not statistically different among sites include As, Ba, and Fe. Mo was only detected in lagoons. BDL = over 50 percent of samples tested were below the detection limit.

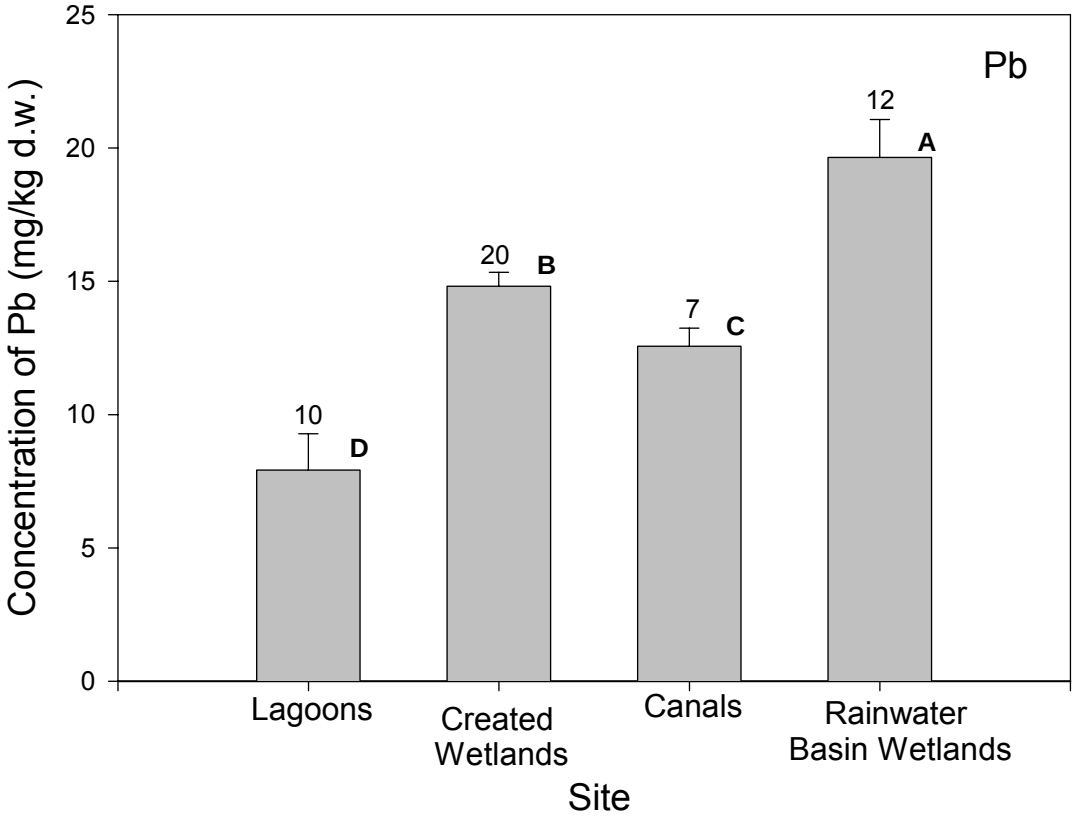


Figure 12. Mean (±SE) concentrations of lead (Pb) in sediments from Rainwater Basin wetlands and Hastings Pork created wetlands, canals, and lagoons, Clay County, Nebraska, 2000. The number of samples analyzed is displayed above each standard error bar. Different letters indicate significance ($p<0.05$) as determined by "proc mixed" in SAS®

Table 7. Mean (± SE) trace element concentrations in sediments from the Hastings Pork created wetlands and Rainwater Basin Wetlands compared to western background concentrations and effects thresholds.

Trace Element	Lagoons (n=20)	Canals (n=7)	Created Wetlands (n=20)	Rainwater Basin Wetlands[1] (n=12)	Western U.S. Background[2]	Effects Thresholds
Al	10,690 ± 1551	16,735 ± 1167	17,267 ± 1119	11,565 ± 963	74,000	58,030[A]
As	4.8 ± 0.5	4.3 ± 0.3	4.5 ± 0.3	3.6 ± 0.3	7.0	9.79[B], 12.10[C]
B	13 ± 1.1	8.9 ± 2	15.9 ± 1.3	5.2 ± 1.3	NA	No criterion
Ba	195 ± 5.9	220 ± 23	215 ± 15	202 ± 23	670	No criterion
Be	1.08 ± 0.07	0.85 ± 0.11	1.09 ± 0.08	0.64 ± 0.01	0.97	No criterion
Cd	**2.4** ± 0.4	0.5 ± 0.1	0.3 ± 0.04	0.4 ± 0.1	NA	0.59[C], 0.99[B]
Cr	42.6 ± 2.5	21.3 ± 3.5	21.3 ± 1.5	14.6 ± 1.0	56	43.4[B], 56.0[C]
Cu	**325** ± 50	**39.3** ± 9.3	16.3 ± 1.1	15 ± 1.0	27	31.6[B], 7.77[A], 270[D]
Fe	13,586 ± 1884	14,291 ± 752	17,101 ± 892	15,099 ± 2,154	26,000	No criterion
Hg	<0.2	<0.2	<0.2	0 ± 0	NA	No criterion
Mg	15,012 ± 2280	6728 ± 710	5773 ± 321	2702 ± 236	NA	No criterion
Mn	**1025** ± 134	436 ± 102	450 ± 49	311 ± 40	480	819.0[E]; 1,081[A]; 1,673[C]
Mo	12 ± 2.1	BDL	BDL	BDL	1.1	No criterion
Ni	**26.8** ± 1.6	18.6 ± 1.2	17.4 ± 0.9	14.4 ± 1.2	19	22.7[B]
Pb	7.9 ± 1.4	12.6 ± 0.7	14.8 ± 0.5	19.7 ± 1.4	20	34.2[C], 35.8[B]
Se	**6.1** ± 0.8	1.4 ± 0.4	<1.0	<1.0	0.34	4.0[E]
Sr	236 ± 39	158 ± 55	65 ± 7.0	33.7 ± 2.3	NA	No criterion
V	33.5 ± 1.4	26.4 ± 2.2	33.4 ± 2.1	18.0 ± 1.4	88	No criterion
Zn	**2,134** ± 407	**189** ± 45	72.1 ± 5.8	60.3 ± 2.8	65	121[B], 159[C]; 1,532[A]

Note: SE = standard error, n = sample size, NA = not applicable, < = all samples were below the detection limit (value equals the greatest detection limit for all catalogs). Bold values indicate that the mean exceeded an effects threshold.

[1] USFWS, unpublished data (see Methods text for description)

[2] Background soil concentrations for the Western U.S. (Shakette and Boerngen, 1984).

[A] = Probable Effects Concentration benchmark (Jones et al., 1997).

[B] Sediment quality guideline threshold effects concentration below which harmful effects are unlikely to be observed (MacDonald et al., 2000).

[C]= Toxic Effects Concentration benchmark below which effects are rarely expected to occur (Jones el al., 1997).

[D]= No Effects Concentration benchmark (Jones et al., 1997).

[E] = Toxicity threshold at which adverse effects on some fish and wildlife species may occur (USDI, 1998).

Table 8. Total recoverable concentrations (mg/L) of trace elements in water samples collected from McMurtrey Marsh and Hastings Pork canals and created wetlands, Clay County, Nebraska.

Trace Element	Site and Dates (month/year) of Sample Collection											Effects Threshold (mg/L)
	Canals			Created Wetland 1			Created Wetland 5			McMurtrey Marsh		
	3/00	6/00	10/00	3/00	6/00	10/00	3/00	6/00	10/00	3/00	10/00	
Al	**0.321**	**0.879**	**0.154**	**3.610**	**2.640**	**0.173**	**14.700**	**8.710**	**<0.111**	**0.657**	**11.900**	0.087[A], 0.750[B]
As	<0.0056	0.005	0.008	0.008	**0.017**	0.010	**0.017**	**0.029**	**0.019**	<0.0056	0.012	0.016[A], 0.340[B]
B	0.37	0.55	0.41	0.25	0.55	0.43	0.20	0.71	0.38	0.05	*0.111	6.0[C]
Ba	0.051	0.033	0.033	0.122	0.109	0.044	0.335	0.278	0.073	0.083	0.590	1.0[D]
Be	<0.0011	<0.0003	<0.0006	<0.0011	<0.0003	<0.0006	<0.0011	0.001	<0.0006	<0.0011	0.002	0.005[A], 0.130[B]
Cd	**0.0013**	<0.0004	<0.0006	<0.0011	<0.0004	<0.0006	**0.0014**	<0.0004	<0.0006	<0.0011	**0.0014**	*0.00025[A], *0.00590[B]
Cr	<0.0056	0.003	<0.0056	<0.0056	0.003	<0.0056	0.014	0.008	<0.0056	<0.0056	0.011	*0.077[A], *0.592[B]
Cu	**0.106**	**0.023**	**0.033**	**0.020**	**0.016**	**0.021**	**0.029**	**0.028**	**0.017**	0.006	**0.030**	*0.009[A], *0.013[B]
Fe	**1.18**	0.80	0.26	**3.36**	**1.91**	0.20	**13.70**	**5.15**	0.17	0.40	**12.20**	1.0[A]
Hg	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	0.0005[A], 0.0014[B]
Mg	31.60	51.40	50.80	25.20	50.90	52.30	31.40	55.80	35.00	9.46	11.50	82.0[E]
Mn	0.31	0.11	0.10	0.29	0.24	0.09	0.87	0.56	0.13	0.01	0.85	1.0[A], 1.10[E]
Mo	0.017	0.005	<0.056	0.007	<0.004	<0.056	<0.006	0.014	<0.056	<0.006	<0.056	10.0[F]
Ni	0.034	0.044	0.036	0.023	0.051	0.029	0.032	**0.074**	0.027	<0.0056	0.017	*0.052[A], *0.468[B]
Pb	**0.009**	0.001	<0.0111	**0.010**	**0.003**	<0.0111	**0.025**	**0.009**	<0.0111	<0.0056	**0.045**	*0.0025[A], *0.0646[B]
Se	<0.0056	**0.004**	<0.0044	<0.0056	**0.003**	<0.0044	<0.0056	**0.007**	<0.0044	<0.0056	<0.0044	0.002[G], 0.005[A]
Sr	0.442	0.261	0.220	0.244	0.256	0.188	0.261	0.366	0.182	0.190	0.226	42.0[E]
V	0.006	0.017	0.006	0.017	0.019	0.009	0.046	0.040	0.012	<0.0056	0.026	0.08[E]
Zn	**0.566**	**0.101**	**0.103**	**0.066**	**0.058**	**0.040**	**0.094**	**0.067**	0.013	<0.0011	**0.088**	0.03[G], *117.00[B]

Note: > Indicates the sample was below the detection limit (value = detection limit); Date Col. = approximate date of sample collection; CW = created wetland; * indicates the criterion value based on a water hardness of 100 mg/L. Thresholds listed below are for dissolved concentrations unless stated otherwise. Bold numbers indicate that one or more of the following water quality criteria were exceeded:

[A] = chronic aquatic life water quality criterion for wetlands (NDEQ, 2002).

[B] = acute aquatic life water quality criterion for wetlands (NDEQ, 2002).

[C] = level of concern threshold for aquatic invertebrates (USDI, 1998).

[D] = Canadian Council of Ministers of the Environment (CCME) water quality guideline for the protection of aquatic life (CCME, 2002)

[E] = The lowest chronic value benchmark above which toxic effects may occur in sensitive species (Suter and Tsao, 1996).

[F] = concentration associated with frequent molybdenosis in sensitive species (Eisler, 1989).

[G] = total recoverable Se toxicity threshold for apparent adverse effects to wildlife (USDI, 1998).

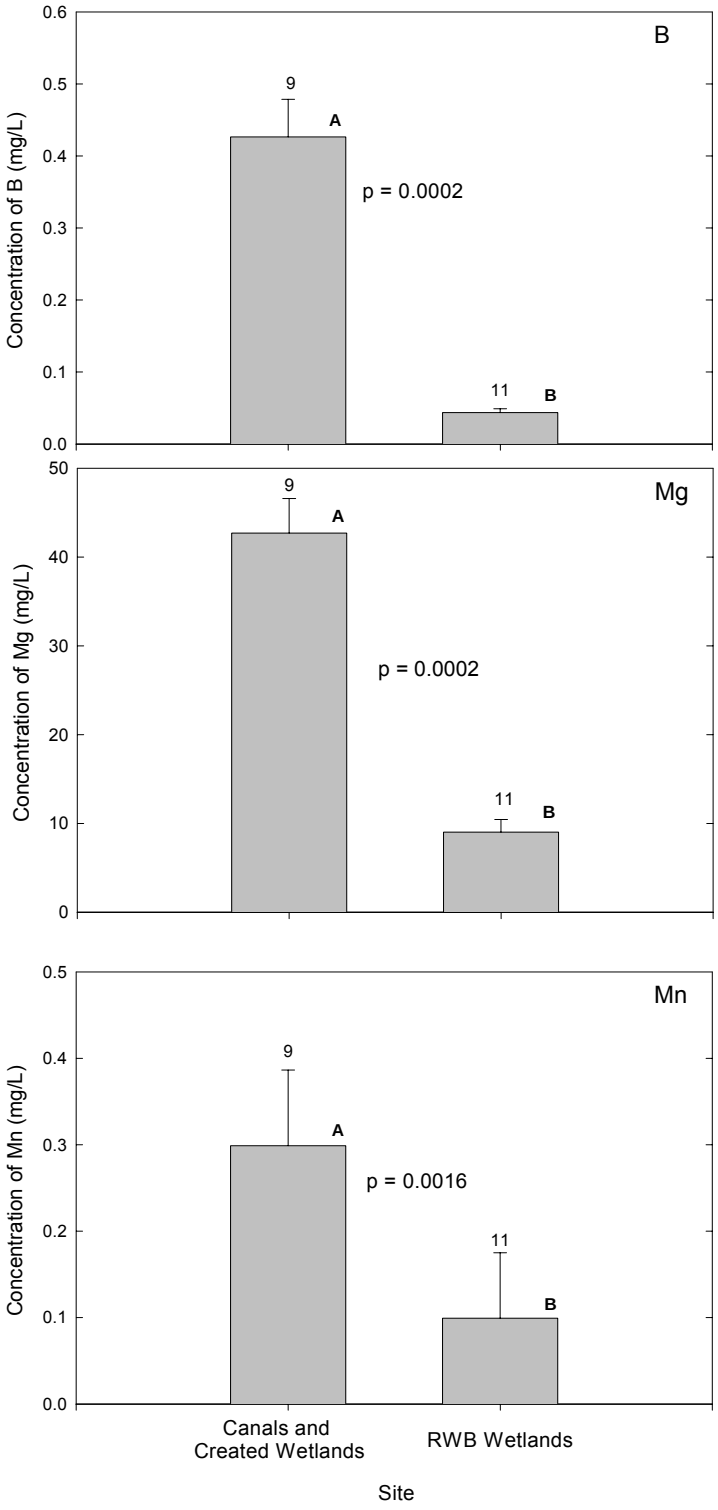


Figure 13. Mean (±SE) concentrations of boron (B), magnesium (Mg) and manganese (Mn) in water from Rainwater Basin wetlands and Hastings Pork created wetlands and canals, Clay County, Nebraska, 2000. The number of samples analyzed is displayed above each standard error bar. Different letters indicate significance as determined by a Wilcoxon rank sums test.

Table 9. Results from Proc Mixed in the Statistical Analysis System (SAS) for trace elements in chironomids from Hastings Pork, Nebraska; Stillwater National Wildlife Refuge (NWR), Nevada; and the Sun River Irrigation Project Area, Montana.

Trace Element	p values	Results of PROC MIXED in SAS and (mean ± standard error)		
Al	<0.0001	Stillwater NWR [A] (9756 ± 768)	Hastings Pork [B] (5167 ± 525)	Sun River [C] (2285 ± 229)
As	<0.0001	Stillwater NWR [A] (15.2 ± 0.9)	Sun River [B] (2.4 ± 0.2)	Hastings Pork [C] (1.5 ± 0.1)
B	<0.0001	Stillwater NWR [A] (114.2 ± 9.2)	Sun River [B] (8.7 ± 0.9)	Hastings Pork [B] (7.7 ± 0.9)
Ba	<0.0001	Stillwater NWR [A] (99 ± 7)	Hastings Pork [B] (64.7 ± 5)	Sun River [C] (36.2 ± 3)
Be	<0.0003	Stillwater NWR [A] (0.41 ± 0.04)	Hastings Pork [B] (0.26 ± 0.04)	Sun River [B] (0.16 ± 0.03)
Cd	<0.0001	Stillwater NWR [A] (1.13 ± 0.1)	Hastings Pork [B] (0.44 ± 0.1)	Sun River [B] (0.39 ± 0.1)
Cr	<0.0001	Hastings Pork [A] (7.2 ± 0.7)	Stillwater NWR [A] (6.9 ± 0.5)	Sun River [B] (2.9 ± 0.3)
Cu	<0.0001	Stillwater NWR [A] (30.0 ± 1.6)	Hastings Pork [B] (19.5 ± 1.7)	Sun River [C] (14.4 ± 0.6)
Fe	<0.0001	Stillwater NWR [A] (10487 ± 677)	Hastings Pork [B] (4429 ± 463)	Sun River [C] (2682 ± 228)
Mg	<0.0001	Stillwater NWR [A] (7951 ± 514)	Sun River [B] (3696 ± 290)	Hastings Pork [C] (2646 ± 158)
Mn	<0.0001	Stillwater NWR [A] (295 ± 20.3)	Hastings Pork [B] (130 ± 12.9)	Sun River [C] (81 ± 7.5)
Ni	0..0889	Hastings Pork (10 ± 2)	Stillwater NWR (8 ± 1)	Sun River (6 ± 1)
Pb	<0.0001	Stillwater NWR [A] (17.0 ± 2.1)	Hastings Pork [B] (5.0 ± 0.7)	Sun River [B] (3.0 ± 0.3)
Se	<0.0005	Sun River [A] (10.4 ± 0.6)	Hastings Pork [B] (2.8 ± 0.4)	Stillwater NWR [C] (1.6 ± 0.2)
Sr	<0.0001	Stillwater NWR [A] (206 ± 15)	Sun River [B] (41 ± 5)	Hastings Pork [B] (29 ± 4)
V	<0.0001	Stillwater NWR [A] (34 ± 2)	Hastings Pork [B] (10 ± 1)	Sun River [C] (5 ± 0.5)
Zn	0..1785	Hastings Pork (91 ± 7)	Stillwater NWR (76 ± 4)	Sun River (76 ± 41)

Note: Different superscript letters indicate significant differences among sites. Sites are listed from left to right in decreasing order of mean concentration (mg/kg dry weight) for each trace element. Mean Zn and Ni concentrations were not statistically different among sites.

DISCUSSION

Environmental pollution associated with CAFOs is a national issue (USDA and USEPA, 2003). Another national issue is the need to create and restore wetland habitat to remedy the perpetual decline of waterfowl and shorebird populations (North American Waterfowl Management Plan, 2003; Ducks Unlimited, 2003). The use of swine wastewater effluent from Hastings Pork to create waterfowl habitat is an attempt to simultaneously accommodate the need of CAFO managers to store and treat wastewater and the need for wildlife managers to provide habitat for waterfowl and shorebirds during the spring migration. However, wetland habitat created with swine wastewater may put these species at risk, if the water quality of these created wetlands is not suitable.

Although there are no known studies that specifically investigate risk to waterfowl exposed to swine wastewater, many of the constituents or environmental factors associated with swine waste are potentially harmful to waterfowl including Se (Heinz, 1996; USDI, 1998); cyanobacteria toxins (Matsunaga et al., 1999); *Salmonella* (Friend, 2002), eutrophication (Gaiser and Lang, 1998); and increased water conductivity (Mitcham and Wobeser, 1988; USDI, 1998). However, there also are many unknowns regarding swine waste exposure and effects to waterfowl and their habitat such as chronic exposure to antibiotics, natural hormones, and microcystin toxins.

The purpose of this study was to evaluate whether migratory waterfowl that utilize wetlands created from primary treated swine wastewater are likely exposed to contaminants including trace elements, salts, nutrients, cyanobacteria toxins, bacterial pathogens, and antibiotics. Waterfowl were chosen as the species of concern as the created wetlands were designed specifically to attract them. A CAFO contaminant exposure pathway from the lagoons, through the canals, and to the created wetlands was evaluated by collecting sediment, water, and invertebrate samples. In addition to the exposure assessment, habitat variables (water quality and invertebrate assemblages) were compared between the created wetlands and Federal wetlands managed for waterfowl habitat.

Results of this study indicate that many CAFO contaminants such as disease pathogens, nutrients, and some trace elements are passed through primary treatment lagoons and into the canals and created wetlands; whereas, many other trace elements and antibiotics appeared to be mainly trapped in lagoons. Trace element concentrations in sediment exceeded toxicity thresholds in lagoons and canals but not in created wetlands or RWB wetlands. Antibiotics also were frequently detected in lagoon sediments and water, but not in the created wetlands. When compared to McMurtrey Marsh and other RWB wetlands, created wetlands exhibited eutrophication; increased species richness of disease pathogens, pH, specific conductivity; and higher concentrations of nutrients and some trace elements.

Trace Elements

The comparison of trace element concentrations in sediment samples between RWB sites and Hastings Pork lagoons, identified B, Cd, Cr, Cu, Mg, Mn, Mo, Ni, Se, Sr, V, and Zn as CAFO contaminants. These trace elements are frequently detected in hog manure (Racz and Fitzgerald, 2001) and many of them (e.g., Cu, Cr, Se, and Zn) are supplied in feeds as nutritional supplements for disease suppression and growth promotion (Sims 1995 as cited by USEPA, 1998). The tendency for concentrations of trace elements to decrease in waterbodies down-gradient from the lagoons may indicate an effectiveness of the primary treatment in limiting their movement. However, concentrations of B, Cr, Cu, Mg, Mn, Ni, Se, Sr, V, and Zn in sediments and/or water from the created wetlands and canals appears to indicate that these trace elements are moving out of the lagoons. The transport of these trace elements to the created wetlands is likely due to the water solubility of the excreted trace element compounds. As much as 95 percent of dietary Cu in hog feed is subsequently excreted and much of it is in a readily soluble form (Payne et al., 1988). Boron compounds, especially from sewage and laundry products, also have high water solubility and conventional sewage treatment removes little or no boron (USEPA 1975 cited in Eisler, 1990).

Concentrations of Al, As, Cd, Cu, Fe, Ni, Pb, Se, and Zn in water from the created wetlands exceeded Nebraska aquatic life water quality criteria or literature established toxicity thresholds. However, the same criteria also were exceeded in McMurtrey Marsh for all these elements except As, Ni, and Se. In addition, the comparison of trace element concentrations detected in this study with Nebraska water quality criteria may not accurately evaluate whether wetland plant and invertebrate species are at risk. Recoverable metals were measured in this study, whereas Nebraska water quality criteria are generally based on measurements of dissolved metals. Water samples are filtered before an analysis for dissolved metals, whereas total recoverable analysis includes microorganisms and suspended particulates that are not filtered and thus result in higher concentrations. The total recoverable method was used in this study due to the highly productive nature of wetland systems where nutrients and toxins are quickly taken up by biota, leaving decreased concentrations in water (USDI, 1998). Concentrations of Al, Cd, and Fe in water were similar between created wetlands and McMurtrey Marsh and may reflect naturally high background concentrations (NDEQ, 1997). The greater concentrations of Pb in sediments and water from RWB wetlands compared to the created wetlands may reflect the presence of Pb shot from public hunting, even though steel or non-toxic shot has been required for all waterfowl hunting on Service RWB wetlands since 1980. Arsenic was detected at greater concentrations in water from the created wetlands compared to RWB wetlands indicating possible As contamination from swine waste. However, concentrations of As in sediments were similar across all sites and did not exceed sediment toxicity thresholds. Concentrations of Se in water from the created wetlands were generally below detection; however, sample sizes were small and the detection limits exceeded a 2 μg/L total recoverable Se toxicity threshold (USDI, 1998). Cu and Zn concentrations frequently exceeded Nebraska wetland water quality criteria and conditions; however, the high pH of the created wetlands likely limits their bioavailability and toxicity to wetland plants and invertebrates. Wetland macrophytes and algae were not analyzed for trace element concentrations; however, some wetland plant species can bioaccumulate copper to high concentrations (Buckley, 1994; Eisler,

1998a) and many studies have reported trace element accumulation in soil and terrestrial plants exposed to applications of swine slurry (Christie and Beattie, 1989; Arzul and Maguer 1990).

Chironomids were selected as a potential food item for waterfowl and shorebirds as they appeared to be the most abundant benthic invertebrate at the created wetlands and are food items of major importance to blue-winged teal, northern pintail, mallard, gadwall, and redhead hens (Eldridge, 1990). Concentrations of trace elements in the chironomids did not exceed literature established toxicity thresholds and were generally significantly less than those found in chironomids from areas of known metal contamination. Trace element concentrations in chironomids at the created wetlands do not appear to represent a risk to waterfowl; however, when compared to concentrations in chironomids from Stillwater NWR (a contaminated site) or the Sun River Irrigation Project Area (a site with elevated Se), chironomids from the created wetlands appear to be accumulating some of the trace elements that were detected at high concentrations in lagoon sediments (e.g., Cr, Cu, Mg, Mn, Se, V, and Zn). Background concentrations of trace elements for chironomids from RWB wetlands are needed to better evaluate trace element bioaccumulation by chironomids in the created wetlands.

Antibiotics

The distribution of tetracycline antibiotics between sediment and water in the lagoon and canals is consistent with their high sorption coefficient in soil/sediment (K_d). The K_d for tetracycline and oxytetracycline ranges from > 400 to 1,600 L/kg soil (Tolls, 2001). The detection of tetracycline antibiotics in the canals suggests that even highly sorptive antibiotics are being transported away from the lagoons (Dr. Daniel Snow, Environmental Geochemist at University of Nebraska Water Sciences Laboratory, pers. comm., 2004).

Antibiotic exposure to waterfowl and shorebirds at the created wetlands appeared to be essentially negligible; however, detection limits may have been too high. All six water samples collected from the created wetlands had oxytetracycline concentrations

below the 10 µg/L detection limit. A national survey that consisted of 189 water samples from 13 fish hatcheries reported that oxytetracycline was detected in 27 samples at a median concentration less than 0.05 mg/L and a one-time maximum concentration of 10 µg/L (Thurman et al., 2002). The same study concluded that the source of oxytetracycline was fish hatchery feed; therefore, the similar addition of oxytetracycline to hog feed would likely lead to its presence in the created wetlands.

There are no known studies that evaluate low-level chronic antibiotic exposure to avian wildlife; however, chronic toxicity tests on reproduction with *Daphnia magna* found 50 percent of the population exhibited decreased reproductive output at concentrations of 46.2 mg/L oxytetracycline (Wollenberger et al., 2000). Differences in wildlife sensitivity to environmental contaminants often occur between species and especially between classes (Calabrese and Baldwin, 1993). Avian specific toxicity evaluations would be needed to adequately evaluate potential adverse effects to migratory birds chronically exposed to low concentrations of oxytetracycline.

Bacterial Pathogens

Swine wastewater from Hastings Pork is apparently a source for some disease pathogens including fecal coliforms, fecal streptococci, *Salmonella* spp., and *Yersinia enterocolitica*; however, *Erysipelothrix* spp. and *P. multocida* were not detected. The lack of *Erysipelothrix* recoveries was unexpected because swine have been considered an important reservoir for this pathogen (Dr. Mark Wolcott, Microbiologist, pers. comm., 2002). Improvements to the *Erysipelothrix* recovery protocol may be needed to insure there are truly no *Erysipelothrix* spp. present in the study area (USGS, 2001). The absence of *P. multocida* in sediment and water samples collected for this study is not too surprising, as carrier animals are believed to be the most important reservoir and the bacteria are not believed to persist more than 2-4 weeks after carcasses are removed from a die-off event (Dr. Michael Samuel, Researcher at NWHC, pers. comm., 2004). There are no other known studies that have attempted to recover *P. multocida* from swine or cattle wastewater or sediments, although Smith (et al., 1989) looked at adjacent land and

found no association between cattle feed lots and wetlands with frequent avian cholera outbreaks.

Avian cholera outbreaks at the created wetlands may still be a concern as disease transmission from carrier birds in the area has occurred historically and previous research is inconclusive regarding whether water quality characteristics might increase risk to avian cholera outbreaks due to increased survival of *P. multocida.* The RWB is one of four major U.S. enzootic areas for avian cholera in waterfowl (USGS, 1999a) and previous outbreaks of avian cholera have occurred at McMurtrey NWR and Harvard WPA located within two miles of the created wetlands (Smith et al., 1989). Created wetland water quality characteristics that enhance survival of *P. multocida* in laboratory studies include warmer water temperature, increased protein material (Bredy and Botzler, 1989), and high concentrations of Ca and Mg (Price et al., 1992). In addition, the created wetlands exhibit high conductivity, a condition associated with avian cholera outbreaks in Nebraska wetlands (Windingstad et al., 1984; Gordon, 1989). However, more recent analysis by USGS (1999b) found no associations between risk of avian cholera outbreaks and Ca, Mg, specific conductance, protein, or the abundance of *P. multocida*. Mallard ducks exposed to sewage sludge in their diet did not exhibit increased susceptibility to avian cholera, but exhibited increased cadmium concentrations in liver (Goldberg and Yuill, 1989).

Salmonella appears to be associated with the swine wastewater as it was frequently isolated from samples collected on sites that receive swine wastewater effluent. Salmonellosis has caused die-offs of several captive-reared avian species including waterfowl; although, large-scale die-offs of free ranging wild birds other than songbirds and colonial nesting birds, have rarely been reported (Dr. Kathy Converse, Wildlife Disease Specialist at NWHC, pers. comm., 2004).

Recoveries of *Yersinia intermedia* from all sites indicates that the pathogen is not likely specific to swine wastewater; however, *Yersinia enterocolitica* recoveries only occurred in areas associated with swine waste. The disease potential of *Y. intermedia* in wildlife species is not well understood (Aleksic and Bockemühl, 1999) and yersiniosis in

wild waterfowl or shorebirds is not commonly reported to the NWHC (Dr. Kathy Converse, Wildlife Disease Specialist at NWHC, pers. comm., 2004). Potential sources for the *Yersinia* recoveries at McMurtrey Marsh are warm-blooded mammals, such as cattle that graze the area, as well as non-point source agricultural runoff from Hastings Pork or other swine and cattle feeding operations in McMurtrey NWR's watershed. Further research and pathogen fingerprinting techniques would be needed to determine whether disease pathogens are being transferred to McMurtrey NWR from Hastings Pork and whether the exposure pathways include waterfowl pathogen carriers or sediment and water from run-off.

Bacterial antibiotic resistance was not included in the original study design; however, the finding of multiple antibiotic resistances in *Salmonella* spp. and *E. coli* isolates recovered from the Hastings Pork lagoons, canals, and created wetlands raises a concern. Antibiotic resistance may not present a direct threat to wildlife. Nonetheless, human health concerns related to the spreading of antibiotic resistant bacteria by waterfowl may dictate how these species are managed (Dr. Mark Wolcott, Microbiologist, pers. comm., 2002).

Hormones

Hormone exposure to waterfowl at the created wetlands needs to be further evaluated. The created wetlands appear to be contaminated with testosterone and E_2 from the CAFO waste; however, only a limited number (n=5) of water samples were analyzed and samples were run directly. The limited sampling indicates that the lagoons do not appear to limit concentrations of E_2 and testosterone in the created wetlands and these hormones may lead to adverse effects to wetland wildlife. Concentrations of E_2 in three of four samples from Hastings pork exceeded 10 ng/L, a concentration likely to exert significant adverse effects on wildlife reproduction (Witters et al., 2000). Amphibians may be the species at greatest risk to hormone exposure at the created wetlands. Amphibian exposure to E_2 results in vitellogenin induction in adults (Palmer and Palmer, 1995) and sexual differentiation in larvae (Hayes, 1998). There are no known studies that

have evaluated waterfowl or shorebird exposure to natural hormones from ingestion of contaminated water or food items.

Water Quality

Created wetland nutrients, pH, and specific conductivity were significantly greater than those measured on RWB wetlands and, although they did not exceed any known toxicity thresholds for waterfowl, they are resulting in eutrophication. Eutrophication of this system may represent the greatest health risks to waterfowl that utilize the area as previous research indicates wetland eutrophication can adversely affect waterfowl by decreasing their foraging potential (Gaiser and Lang, 1998) or by creating an environment conducive to disease pathogens and toxin-producing algal blooms (USGS, 1999a; Carmichael, 1997). Eutrophication of the created wetlands is likely limiting their potential as quality habitat for waterfowl and shorebirds by altering natural wetland invertebrate and plant communities. Eutrophication in wetland systems is characterized by decreased invertebrate species density and richness, dominance by few nutrient tolerant taxa, and loss of endemic and characteristic species (Bedford et al., 1999). Anoxic sediments from eutrophication are likely the cause for chironomid dominance in the benthic invertebrate communities of the created wetlands and the decreased Shannon-Wiener diversity index. Decreased taxa diversity and dominance of the invertebrate community by chironomids is common in areas of degraded water and sediment quality (Dickman and Rygiel, 1996 and 1998; Chow-Fraser, 1998; Victor and Onomivbori, 1999; Nelson et al., 2000). Wetland eutrophication among prairie pothole wetlands in northwest Iowa also was associated with limited abundance and composition of microcrustaceans (Gaiser and Lang, 1998). Although flying insect abundance was not measured in this study, wetland eutrophication tends to decrease abundance of flying insects when compared to oligotrophic wetlands and may contribute to relatively poor foraging conditions for young waterfowl (King and Brazner, 1999).

Differences in invertebrate communities between the created wetlands and McMurtrey Marsh also may be due to differences in water regimes. McMurtrey Marsh functions as a seasonal wetland, whereas the created wetlands function as open permanent wetlands creating an environment more conducive to chironomid species. In North Dakota, chironomids were reported to be the dominant fauna of semi-permanent prairie wetlands and comprised a smaller percentage in seasonal wetlands (Nelson, 1990). Advanced eutrophication can result in bare mud substrates with anoxic sediments, an environment that results in botulism-related mortality of birds (Crowder and Bristow, 1988). Avian botulism also is associated with sewage and other wastewater discharges into marshes (USGS, 1999a). Created wetland water was frequently between a pH of 8 to 10, greater than 20^{o}C, and less than 2 parts per thousand salinity; all conditions that tend to favor avian botulism outbreaks (Rocke and Samuel, 1999).

The environment created by the eutrophication of the created wetlands also is suitable for cyanobacteria blooms and results from this study indicate that waterfowl and shorebirds are likely exposed to cyanobacteria toxins. Cyanobacteria toxins can be lethal to foraging dabbling ducks (Carmichael, 1992; Matsunaga, 1999). In Japan, 20 spot billed ducks (*Anas paecilorhyncha*) died from acute exposure to microcystins in a pond following eutrophication caused by an influx of untreated sewage (Matsunaga, 1999). The Nebraska Game and Parks Commission (NGPC) also have attributed waterfowl die-offs in eastern Nebraska to cyanobacteria blooms (NGPC, 1992). In August and September of 1992, three separate duck die-off events occurred in a small lake at Fontenelle Park in Omaha, Nebraska (NGPC, 1992). After the third die-off occurred, it was confirmed that water samples contained an abundance of *Microcystis* spp. and *Anabaena circinalis*, two species that produce microcystin toxins (NGPC, 1992).

Uncertainty Analysis and Data Gaps

Ongoing research

The effects of CAFO contaminants on waterfowl that utilize the Hastings Pork created wetlands are currently being investigated in a separate study titled "Post-

Remediation Evaluation Using Mallard Sentinels at the Hastings Pork Confined Animal Feeding Operation and Implications for Water Quality at McMurtrey NWR." Waterfowl enclosures were constructed on two of the created wetlands (CW4 and CW6) and on two control RWB wetlands located within three miles of Hastings Pork (McMurtrey NWR and Harvard WPA). This research will provide further data on water quality parameters for the created wetlands and will measure disease pathogens and concentrations of trace elements and microcystins in mallard sentinels. Concentrations of Se in sentinel mallard eggs and liver, in conjunction with measurements of Se in sediments, water, and invertebrates obtained from this study, will allow for an aquatic hazard assessment of Se as described by Lemly (1995). At the time of this report, field sampling for this project has been completed; however, sample analysis and data interpretation are ongoing.

Future Research Needs

Trace element concentrations in created wetland plants, sediments, water, and invertebrates may continue to accumulate over time as the created wetlands have no outlet and thus serve as a sink for contaminants that enter from the lagoons. In addition, clay soils have a greater capacity to adsorb metals and phosphorus than other soil types and negative effects may develop only after adsorption sites are exhausted after long periods of continued application (Ap Dewi, 1994). Land application of swine waste and subsequent field run-off also can lead to trace element contamination of surface waters (Eisler, 1990). The use of swine waste as a fertilizer can lead to increased inputs of phosphorus in run-off as the soil's capacity to adsorb phosphorus diminishes over time (Racz and Fitzgerald, 2001). Future monitoring will be needed to evaluate these potential delayed effects on trace element accumulation in created wetland biota, sediments, and plants. It is recommended that the Service conduct an aquatic hazard assessment for Se, as described by Lemly (1995), within 5 to 10 years from this report.

Further monitoring is needed to determine if natural hormone concentrations in the created wetlands are high enough to adversely affect wetland species. In addition to E_2 and testosterone, equol (a phytoestrogen) also may be a concern and should be

included in any future monitoring of natural hormones at the site. Concentrations of equol as high as 16.6 ppm have been reported in swine manure (Burnison et al., 2002). Although equol is between 200 and 1,000 times less estrogenic than E_2 (Burnison et al., 2002); it may still be an important contributor to the total estrogen exposure.

Future pathogen screening at the site should include *Trichomonas* spp. Swine manure can be a source of trichomoniasis, a disease caused by the protozoan parasite *Trichomonas suis* (Ap Dewi, 1994). Although it is rare in free-ranging waterfowl, it has caused major die-offs in doves and pigeons as well as less visible chronic losses (USGS, 1999a). This may be of importance, as morning doves (*Zenaida macroura*) appear to be attracted to the study area.

The potential for contaminants in swine wastewater to modify RWB wetland invertebrate communities needs to be further researched. Only benthic invertebrates from McMurtery Marsh, a temporary wetland, were compared to those from the created wetlands. Invertebrate community structures from other RWB wetlands, especially those that function more as permanent wetlands (e.g., Smith WPA), also should be compared to the created wetlands and RWB wetlands with animal feeding operations in their watershed.

Lagoon water may be an important exposure pathway for CAFO contaminants to waterfowl. Although lagoon water was analyzed for antibiotics and disease pathogens, trace elements concentrations in lagoon water were not determined during this study and should be included in future site evaluations.

Recommendations

In April of 2001, NEFO personnel met with Hastings Pork and the RWBJV to discuss preliminary results. Four management recommendations were agreed upon: 1) water from Hastings Pork would not be used as a supplemental water source for McMurtrey NWR; 2) Phase II of the Hastings Pork/RWBJV partnership, to create additional wetlands in the summer of 2001, would be delayed; 3) water quality would be improved by implementing a comprehensive nutrient management plan that applies “Best

Management Practices" including remediation of the effluent delivery system; and 4) a post-remediation evaluation of contaminants at the site and an assessment of contaminant exposure and effects to waterfowl would be produced before construction of additional created wetlands would be supported by the RWBJV.

Remediation work to improve water quality (recommendation # 3 above) scheduled for the summer of 2001 was not completed by Hastings Pork, as they decided to halt any further development of created wetlands or the remediation of existing created wetlands until all current Service research investigations were completed. Consequently, recommended management actions to Hastings Pork will be included in the final report for the mallard enclosure study. These recommendations will likely focus on the use of remediation measures, such as the use of constructed wetlands that are designed specifically to treat domestic sewage to improve the quality of the influent to the created wetland habitat. The treatment of CAFO wastewater is essential in protecting Federal trust resources at McMurtrey NWR as the movement of this effluent into the refuge during heavy rain events remains a concern.

Conclusions

Although lagoon sediments typically contained the highest concentrations of CAFO contaminants and toxicity thresholds for metals were frequently exceeded in lagoons, waterfowl and shorebirds are most likely exposed to CAFO contaminants while foraging in the created wetlands.

Sediment toxicity thresholds for trace elements were exceeded only in lagoons and canals; however, many trace element that are associated with hog-manure appear to be reaching the created wetlands. Accumulation of these contaminants may increase with time possibly resulting in detrimental effects to wetland plants and invertebrates as well as waterfowl and shorebird species.

Antibiotic exposure to waterfowl and shorebirds in the created wetlands appeared to be essentially negligible, but the number of samples from created wetlands that were analyzed for antibiotics was small and the detection limits may have been too high.

Swine wastewater from Hastings Pork does not appear to be a source for *Erysipelothrix* spp. or *P. multocida*. Bacteria pathogens that appear to be associated with the Hastings Pork swine waste effluent and may be of concern include *Salmonella* spp. and *Yersinia enterocolitica*.

When compared to McMurtrey Marsh and other RWB wetlands, the created wetlands exhibited increased pH, specific conductivity, and salinity. Eutrophication of the created wetlands may represent the greatest health threat to waterfowl that utilize these wetlands by creating an environment that is conducive to cyanobacteria blooms and outbreaks of avian botulism and avian cholera. Large concentrations of waterfowl may be attracted to the created wetlands during drought years when other wetlands in the area are dry, potentially resulting in large-scale waterfowl or shorebird mortality events.

Created wetland invertebrate communities were dominated by abundant populations of pollutant tolerant chironomid species and were less diverse than those in McMurtrey Marsh. Eutrophication also may be degrading wetland habitat quality by limiting plant community diversity.

On-going research will further evaluate CAFO contaminant exposure and effects to waterfowl that utilize the created wetlands. Future research needs are to monitor the accumulation of trace elements in the created wetlands over time to determine if adsorption capacities in sediments and soils that receive swine waste are depleted and to evaluate the adverse effects that natural hormones from swine may have on waterfowl and/or shorebirds that utilize these wetlands.

Recommended remediation strategies will be included in the final report for the follow-up mallard enclosure study, and will likely focus on the use of constructed wetlands to improve water quality before it is used to create waterfowl habitat in the created wetlands. Further treatment is needed to decrease concentrations of contaminants in Hastings Pork swine wastewater before it is used to create waterfowl habitat as "High quality habitat is the key to healthy waterfowl populations" (Friend, 1992).

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APPENDIX: ADDITIONAL TABLES

Table A.1. Summary statistics for concentrations of trace elements in water samples from Rainwater Basin wetlands and Hastings Pork created wetlands and canals, Clay County, Nebraska.

Trace Element	MDL	Canals			Created Wetlands			Rainwater Basin Wetlands		
		N_D/N_A	Mean ± S.E.	Range	N_D/N_A	Mean ± S.E.	Range	N_D/N_A	Mean ± S.E.	Range
Al	<0.1	3/3	0.4513 ± 0.219	0.154 - 0.879	5/6	5.9666 ± 2.588	0.173 - 14.7	11/11	2.1073 ± 1.2051	0.091 - 11.9
As	<0.0056	2/3	0.0063 ± 0.001	0.005 - 0.008	6/6	0.0167 ± 0.003	0.0082 - 0.029	9/18	0.0035 ± 0.0013	0.0006 - 0.0119
B	<0.111	3/3	0.443 ± 0.054	0.373 - 0.549	6/6	0.4182 ± 0.077	0.204 - 0.707	10/11	0.0425 ± 0.0057	0.005 - 0.0718
Ba	<0.001	3/3	0.039 ± 0.006	0.033 - 0.051	6/6	0.1601 ± 0.048	0.0438 - 0.335	11/11	0.1952 ± 0.0443	0.0478 - 0.59
Be	<0.0011	0/3	NA	NA	1/6	0.000691	NA	4/11	0.0022 ± 0.0004	0.0012 - 0.0032
Cd	<0.0011	1/3	0.0013	NA	1/6	0.0014	NA	4/11	0.002 ± 0.0006	0.0009 - 0.0036
Cr	<0.0056	1/3	0.00266	NA	3/6	0.0086 ± 0.003	0.0034 - 0.014	4/11	0.0082 ± 0.0013	0.005 - 0.0113
Cu	<0.005	3/3	0.054 ± 0.026	0.023 - 0.106	6/6	0.0218 ± 0.002	0.016 - 0.029	5/11	0.0216 ± 0.01	0.0062 - 0.0572
Fe	<0.02	3/3	0.7457 ± 0.266	0.261 - 1.18	6/6	4.0815 ± 2.076	0.171 - 13.7	10/11	2.1403 ± 1.2434	0.0457 - 12.2
Hg	<0.005	0/3	NA	NA	0/6	NA	NA	0/18	NA	NA
Mg	<0.02	3/3	44.6 ± 6.502	31.6 - 51.4	6/6	41.767 ± 5.225	25.2 - 55.8	11/11	9.02 ± 1.4111	2.01 - 18.9
Mn	<0.0056	3/3	0.1707 ± 0.068	0.095 - 0.307	6/6	0.3629 ± 0.122	0.0856 - 0.874	9/11	0.121 ± 0.0918	0.002 - 0.85
Mo	<0.002	2/3	0.011 ± 0.006	0.005 - 0.017	2/6	0.0109 ± 0.004	0.0073 - 0.014	3/11	0.0078 ± 0.0009	0.0062 - 0.0094
Ni	<0.0056	3/3	0.0378 ± 0.003	0.034 - 0.044	6/6	0.0394 ± 0.008	0.0233 - 0.074	4/11	0.0138 ± 0.0028	0.0056 - 0.0172
Pb	<0.01	2/3	0.0048 ± 0.004	0.001 - 0.009	4/6	0.0115 ± 0.005	0.003 - 0.025	5/11	0.0167 ± 0.0074	0.0053 - 0.0448
Se	<0.0056	1/3	0.00351	NA	2/6	0.0048 ± 0.002	0.0027 - 0.007	14/18	0.006 ± 0.0014	0.0013 - 0.0199
Sr	<0.002	3/3	0.3077 ± 0.068	0.22 - 0.442	6/6	0.2495 ± 0.027	0.182 - 0.366	11/11	0.2572 ± 0.0441	0.042 - 0.542
V	<0.0056	3/3	0.0095 ± 0.004	0.006 - 0.017	6/6	0.0236 ± 0.006	0.0086 - 0.046	8/11	0.0133 ± 0.0026	0.0054 - 0.026
Zn	<0.0111	3/3	0.2567 ± 0.155	0.101 - 0.566	6/6	0.0565 ± 0.011	0.013 - 0.094	3/11	0.0471 ± 0.0205	0.0258 - 0.0881

Note: MDL = the minimum detection limit; N_D/N_A = the number of samples with detected concentrations over the number of samples analyzed; and NA = not applicable. A single value in the "Mean ± S.E." column indicates the concentration detected in one sample. The summary statistics are for samples that were above detection limits only.

Table A.2. Monthly counts of fecal coliforms and fecal streptococci colony forming units per 100 ml of water from sites at Hastings Pork and McMurtrey Marsh, Clay County, Nebraska, 2000.

		Monthly count per 100 ml		
Pathogen	Site	April	June	October
Fecal Coliforms	Lagoon 1	TNTC	209,500	770,000
	Lagoon 2	10,550	10,000	11,000
	Lagoon 3	160,000	4,000	59,000
	Lagoon 4	TNTC	250,000	TNTC
	Canal 1	2450	1022	3866
	Canal 2	400	2181	100
	Canal 3	3900	9733	90,000
	Canal 4	4250	933	2900
	CW 1	100	1766	1875
	CW 2	200	3340	380
	Ditch	ND	79	415
	MM 1	50	ND	2200
	MM 2	150	ND	2050
	MM 3	150	ND	2650
Fecal Streptococci	Lagoon 1	50,000	90,500	54,000
	Lagoon 2	9200	5000	TNTC
	Lagoon 3	9350	3250	TNTC
	Lagoon 4	92,000	143,500	6000
	Canal 1	1800	4800	4250
	Canal 2	1700	12,100	600
	Canal 3	9500	TNTC	5800
	Canal 4	6150	1650	4100
	Wetland 1	200	TNTC	2270
	Wetland 2	9000	125,000	890
	Ditch	ND	258	35
	MM 1	27	ND	300
	MM 2	5	ND	50
	MM 3	8	ND	100

Note: CW = created wetland, MM = McMurtrey Marsh, Ditch = ditch that drains runoff from Hastings Pork into McMurtrey Marsh, ND = work not done due to dry conditions, TNTC = too numerous to count (i.e., the agar plates were too overgrown to distinguish seperate colony units). See figure 3 for the locations of these sites.

Table A.3. *Salmonella* species (serotype) recovered from Hastings Pork lagoons, created wetlands and canals, Clay County, Nebraska, 2000.

Site	Date	Source	*Salmonella* species (serotype) identification
Lagoon 2	October 2000	water	Newport
Lagoon 2	October 2000	water	Newport
Lagoon 2	October 2000	water	Newport
Lagoon 3	October 2000	water	Newport
Lagoon 3	October 2000	water	Typhimurium (Copenhagen)
Lagoon 3	October 2000	water	Typhimurium (Copenhagen)
Canal 3	October 2000	water	Derby
Canal 3	April 2000	water	Infantis
Canal 3	October 2000	water	Infantis
Canal 1	October 2000	water	Newport
Canal 1	October 2000	water	Newport
CW 5	June 2000	water	Muenchen
CW 1	October 2000	water	Newport
CW 1	October 2000	water	Newport
CW 2	October 2000	water	Newport
Ditch	October 2000	water	Muenchen

Note: CW = Hastings Pork created wetland, The "Ditch" drains runoff from Hastings Pork into MM Marsh.

Table A.4. *Yersinia* species recovered from McMurtrey Marsh and Hastings Pork created wetlands and canals, Clay County, Nebraska, 2000.

Site	Date	Source	*Yersinia* species identification
Lagoon 3	April 2000	sediment	*Yersinia enterocolitica*
Lagoon 1	October 2000	sediment	*Yersinia* sp.
Lagoon 4	April 2000	water	*Yersinia* sp.
Canal 2	April 2000	sediment	*Yersinia intermedia*
Canal 2	April 2000	sediment	*Yersinia intermedia*
Canal 1	April 2000	sediment	*Yersinia* sp.
Canal 1	April 2000	water	*Yersinia enterocolitica*
Canal 4	April 2000	water	*Yersinia intermedia*
Canal 1	April 2000	water	*Yersinia intermedia*
CW 2	April 2000	sediment	*Yersinia intermedia*
CW 1	April 2000	water	*Yersinia enterocolitica*
CW 1	April 2000	water	*Yersinia enterocolitica*
CW 2	April 2000	water	*Yersinia intermedia*
CW 2	April 2000	water	*Yersinia intermedia*
CW 2	April 2000	water	*Yersinia* sp /*Aeromonas* sp.
MM 4	June 2000	water	*Yersinia* sp.
MM 1	April 2000	sediment	*Yersinia intermedia*
MM 1	April 2000	sediment	*Yersinia intermedia*
MM 2	April 2000	sediment	*Yersinia intermedia*
MM 3	April 2000	sediment	*Yersinia intermedia*
MM 3	April 2000	sediment	*Yersinia* sp.
MM 1	April 2000	water	*Yersinia intermedia*
MM 1	April 2000	water	*Yersinia intermedia*
MM 3	April 2000	water	*Yersinia intermedia*

Note: CW = Hastings Pork created wetland, MM = MM Marsh.

Table A.5. Concentrations (mg/L) of trace elements in water samples collected from Waterfowl Production Areas (federally managed wetlands) in the Rainwater Basin, Clay County, Nebraska, 1992-2001.

			Date Collected		Trace Element Concentration mg/L wet weight						
Catalog No.	General Location	Sample ID	Month	Year	Al	As	B	Ba	Be	Cd	Cr
6050045	Harvard WPA	M1M93	August	1993	8.04	<0.0056	0.0361	0.0776	<0.0005	<0.0005	<0.005
6050045	McMurtrey NWR	M6M93	August	1993	1.24	<0.0056	0.0292	0.0478	<0.0005	<0.0005	<0.005
6050045	Massie WPA	W10M93	August	1993	0.119	<0.0056	0.0577	0.252	<0.0005	<0.0005	<0.005
6050045	Harvard WPA	W1M93	August	1993	0.154	<0.0056	0.005	0.15	<0.0005	<0.0005	<0.005
6050045	Harvard WPA	W4M93	August	1993	0.168	<0.0056	0.0458	0.124	<0.0005	<0.0005	<0.005
6050045	McMurtrey NWR	W5M93	August	1993	0.183	<0.0056	0.0718	0.19	<0.0005	<0.0005	<0.005
6050045	McMurtrey NWR	W6M93	August	1993	0.119	<0.0055	0.0412	0.223	0.0023	0.0019	0.0089
6050045	McMurtrey NWR	W7M93	August	1993	0.091	<0.0056	0.0375	0.218	0.0012	0.0009	0.005
6050045	Massie WPA	W9M93	August	1993	0.509	0.0088	0.0536	0.192	0.0032	0.0036	0.0075
6050017	Harvard or McMurtrey	M1M	October	1991	NC	0.00202	NC	NC	NC	NC	NC
6050017	Harvard or McMurtrey	M6M	October	1991	NC	0.00258	NC	NC	NC	NC	NC
6050017	Harvard or McMurtrey	W1M	October	1991	NC	0.000619	NC	NC	NC	NC	NC
6050017	Harvard or McMurtrey	W4M	October	1991	NC	0.00146	NC	NC	NC	NC	NC
6050017	Harvard or McMurtrey	W5M	October	1991	NC	0.00126	NC	NC	NC	NC	NC
6050017	Harvard or McMurtrey	W6M	October	1991	NC	0.00154	NC	NC	NC	NC	NC
6050017	Harvard or McMurtrey	W7M	October	1991	NC	0.00134	NC	NC	NC	NC	NC
6050062	McMurtrey NWR	320ME1	March	2000	0.657	<0.0056	0.047	0.0827	<0.0011	<0.0011	<0.0056
6050066	McMurtrey NWR	MW	October	2000	11.9	0.0119	<0.111	0.59	0.0019	0.0014	0.0113

					Cu	Fe	Hg	Mg	Mn	Mo
6050045	Harvard WPA	M1M93	August	1993	<0.005	<0.02	<0.002	2.46	<0.002	<0.004
6050045	McMurtrey NWR	M6M93	August	1993	<0.005	5.81	<0.002	2.01	<0.002	<0.004
6050045	Massie WPA	W10M93	August	1993	<0.005	1.18	<0.002	7.85	0.0817	<0.004
6050045	Harvard WPA	W1M93	August	1993	<0.005	0.301	<0.002	12.2	0.085	<0.004
6050045	Harvard WPA	W4M93	August	1993	<0.005	0.0654	<0.002	8.69	0.002	<0.004
6050045	McMurtrey NWR	W5M93	August	1993	<0.005	0.345	<0.002	18.9	0.0048	<0.004
6050045	McMurtrey NWR	W6M93	August	1993	0.0064	0.0457	<0.002	8.42	0.0024	0.0094
6050045	McMurtrey NWR	W7M93	August	1993	0.0572	0.398	<0.002	6.53	0.0076	0.0062
6050045	Massie WPA	W9M93	August	1993	0.008	0.66	<0.002	11.2	0.0457	0.0079
6050017	Harvard or McMurtrey	M1M	October	1991	NC	NC	<0.00498	NC	NC	NC
6050017	Harvard or McMurtrey	M6M	October	1991	NC	NC	<0.00497	NC	NC	NC
6050017	Harvard or McMurtrey	W1M	October	1991	NC	NC	<0.00497	NC	NC	NC
6050017	Harvard or McMurtrey	W4M	October	1991	NC	NC	<0.00498	NC	NC	NC
6050017	Harvard or McMurtrey	W5M	October	1991	NC	NC	<0.00497	NC	NC	NC
6050017	Harvard or McMurtrey	W6M	October	1991	NC	NC	<0.00498	NC	NC	NC
6050017	Harvard or McMurtrey	W7M	October	1991	NC	NC	<0.00499	NC	NC	NC
6050062	McMurtrey NWR	320ME1	March	2000	0.0062	0.398	<0.0002	9.46	0.01	<0.0056
6050066	McMurtrey NWR	MW	October	2000	0.0302	12.2	<0.0002	11.5	0.85	<0.0556

					Ni	Pb	Se	Sr	V	Zn
6050045	Harvard WPA	M1M93	August	1993	<0.005	<0.005	<0.0056	0.047	<0.004	<0.01
6050045	McMurtrey NWR	M6M93	August	1993	<0.005	<0.005	<0.0056	0.042	<0.004	<0.01
6050045	Massie WPA	W10M93	August	1993	<0.005	<0.005	0.0057	0.242	0.0138	<0.01
6050045	Harvard WPA	W1M93	August	1993	<0.005	<0.005	0.0071	0.429	0.0092	<0.01
6050045	Harvard WPA	W4M93	August	1993	<0.005	<0.005	0.0073	0.282	0.0054	<0.01
6050045	McMurtrey NWR	W5M93	August	1993	<0.005	0.0056	0.0199	0.542	0.0195	<0.01
6050045	McMurtrey NWR	W6M93	August	1993	0.0154	0.0053	0.0125	0.286	0.0081	<0.01
6050045	McMurtrey NWR	W7M93	August	1993	0.0056	0.0101	0.0075	0.221	0.0064	0.0274
6050045	Massie WPA	W9M93	August	1993	0.0172	0.0175	0.0062	0.322	0.0183	0.0258
6050017	Harvard or McMurtrey	M1M	October	1991	NC	NC	0.00222	NC	NC	NC
6050017	Harvard or McMurtrey	M6M	October	1991	NC	NC	0.00166	NC	NC	NC
6050017	Harvard or McMurtrey	W1M	October	1991	NC	NC	0.00212	NC	NC	NC
6050017	Harvard or McMurtrey	W4M	October	1991	NC	NC	0.00282	NC	NC	NC
6050017	Harvard or McMurtrey	W5M	October	1991	NC	NC	0.0057	NC	NC	NC
6050017	Harvard or McMurtrey	W6M	October	1991	NC	NC	0.00218	NC	NC	NC
6050017	Harvard or McMurtrey	W7M	October	1991	NC	NC	0.0013	NC	NC	NC
6050062	McMurtrey NWR	320ME1	March	2000	<0.0056	<0.0056	<0.0056	0.19	<0.0056	<0.0111
6050066	McMurtrey NWR	MW	October	2000	0.017	0.0448	<0.0044	0.226	0.026	0.0881

Note: < indicates the sample was below the detection limit (value = detection limit) and NC = not collected.

Table A.6. Concentrations (mg/kg) of trace elements in sediment samples collected from Waterfowl Production Areas (federally managed wetlands) in the Rainwater Basin, Clay County, Nebraska, 1992-2001.

			Date Collected		Trace Element Concentration in mg/kg dry weight						
Catalog ID	General Location	Sample ID	Month	Year	Al	As	B	Ba	Be	Cd	Cr
6050045	Harvard WPA	HVSM193	August	1993	10970	3.19	< 4.97	175	0.73	< 0.198	12.90
6050045	Harvard WPA	HVSM293	August	1993	14360	4.57	< 4.95	277	0.84	< 0.198	19.20
6050045	Harvard WPA	HVSM493	August	1993	12310	3.14	< 4.96	259	0.51	< 0.198	14.40
6050045	McMurtrey NWR	MCSM593	August	1993	8386	1.81	< 4.91	132	0.22	0.21	12.20
6050045	McMurtrey NWR	MCSM693	August	1993	10330	4.35	12.20	275	0.34	0.38	14.20
6050045	McMurtrey NWR	MCSM893	August	1993	7804	2.05	7.59	114	0.92	0.62	9.56
6050027	Eckhart WPA	RWB_E_S	June	1992	11084	4.67	NC	NC	NC	0.68	16.01
6050027	Harvard WPA	RWB_H_SM	June	1992	16343	4.94	NC	NC	NC	0.57	26.02
6050027	Harvard WPA	RWB_H_SS	June	1992	10811	3.26	NC	NC	NC	0.25	13.15
6050027	Smith WPA	RWB_S_S	June	1992	17788	4.74	NC	NC	NC	0.26	14.82
6050066	McMurtrey NWR	MS	October	2000	6539	2.07	3.58	227	1.00	1.32	11.70
6050062	McMurtrey NWR	320ME1S	March	2000	12050	4.41	8.15	158	0.58	0.75	11.80

					Cu	Fe	Hg	Mg	Mn	Mo
6050045	Harvard WPA	HVSM193	August	1993	12.90	9850	< .0994	2359	400	< 4.97
6050045	Harvard WPA	HVSM293	August	1993	16.00	13920	< .0982	3481	675	< 4.91
6050045	Harvard WPA	HVSM493	August	1993	13.60	11640	< .0990	3246	310	< 4.95
6050045	McMurtrey NWR	MCSM593	August	1993	11.70	7546	< .0992	2180	179	< 4.96
6050045	McMurtrey NWR	MCSM693	August	1993	13.90	12980	< .0982	3695	296	< 4.91
6050045	McMurtrey NWR	MCSM893	August	1993	9.54	7407	< .0988	1945	271	< 4.94
6050027	Eckhart WPA	RWB_E_S	June	1992	20.33	24495	<0.1	NC	345	NC
6050027	Harvard WPA	RWB_H_SM	June	1992	22.82	30274	<0.1	NC	316	NC
6050027	Harvard WPA	RWB_H_SS	June	1992	14.41	19713	<0.1	NC	240	NC
6050027	Smith WPA	RWB_S_S	June	1992	13.24	22971	<0.1	NC	358	NC
6050066	McMurtrey NWR	MS	October	2000	16.20	9543	0.05	2428	209	*5.09
6050062	McMurtrey NWR	320ME1S	March	2000	14.80	10850	0.04	2278	133	*5.42

					Ni	Pb	Se	Sr	V	Zn
6050045	Harvard WPA	HVSM193	August	1993	13.40	23.60	< .497	27.70	17.30	42.50
6050045	Harvard WPA	HVSM293	August	1993	17.50	21.60	< .491	35.50	20.70	52.20
6050045	Harvard WPA	HVSM493	August	1993	13.00	17.00	< .495	33.90	16.40	43.00
6050045	McMurtrey NWR	MCSM593	August	1993	8.54	14.20	< .491	31.00	12.20	36.60
6050045	McMurtrey NWR	MCSM693	August	1993	14.60	13.10	< .494	36.60	16.90	50.40
6050045	McMurtrey NWR	MCSM893	August	1993	11.60	17.40	0.87	25.20	15.20	29.30
6050027	Eckhart WPA	RWB_E_S	June	1992	11.06	22.91	<1	NC	NC	101.5
6050027	Harvard WPA	RWB_H_SM	June	1992	22.99	30.52	1.47	NC	NC	125
6050027	Harvard WPA	RWB_H_SS	June	1992	19.48	17.45	<1	NC	NC	73.36
6050027	Smith WPA	RWB_S_S	June	1992	18.06	15.33	<1	NC	NC	77.27
6050066	McMurtrey NWR	MS	October	2000	11.80	22.40	1.22	47.10	25.20	63.00
6050062	McMurtrey NWR	320ME1S	March	2000	10.90	20.30	1.33	32.90	19.90	57.50

Note: < indicates the sample was below the detection limit (value = detection limit) and NC = not collected.

Table A.7. Antibiotic concentrations in sediment (ng/g) and water (µg/g) from Hastings Pork created wetlands, canals and lagoons, Clay County, Nebraska, 2000.

				Antibiotic concentration (ng/g for sediments and ug/L for water)						
Sample ID	Date Col.	Site	Sample Type	Tetracycline	Chlortetracycline	Oxytetracycline	Lincomycin	Tylosin	Erythromycin	Tiamulin
A127	000314	Lagoon	Water	<10	<10	13	<2	<2	<2	4.9
A63	000314	Lagoon	Water	<10	<10	317	<2	<2	<2	17.7
C124	000314	Lagoon	Water	10	11	318	<2	<2	<2	32.6
C416	000314	Lagoon	Water	<10	10	241	<2	<2	<2	13.5
D327	000314	Lagoon	Water	<10	<10	90	<2	<2	<2	7.6
D415	000314	Lagoon	Water	<10	<10	282	<2	<2	<2	7
D618	000314	Lagoon	Water	<10	<10	77	<2	<2	<2	4.9
A127	000613	Lagoon	Water	<10	<10	18.1	<2	<2	<2	2.7
A21	000613	Lagoon	Water	10	12	426	53.4	<2	<2	5.1
C124	000613	Lagoon	Water	15	18	533	780.1	11.4	<2	3.4
D327	000613	Lagoon	Water	<10	<10	265	<2	<2	<2	6.7
A130	001003	Lagoon	Sediment	186	2787	7708	<20	<20	<20	78
C124	001003	Lagoon	Sediment	1328	6430	25549	<20	<20	<20	27
D327	001003	Lagoon	Sediment	119	311	8943	<20	<20	<20	<20
A130	001003	Lagoon	Water	<10	<10	13	<2	<2	<2	3.4
C124	001003	Lagoon	Water	<10	17	259	<2	<2	<2	6.8
D327	001003	Lagoon	Water	<10	<10	208	<2	<2	<2	6.5
A127	000314	Canal	Water	<10	<10	31	<2	<2	<2	9.8
C124	000314	Canal	Water	<10	<10	10	<2	<2	<2	3.5
D327	000314	Canal	Water	<10	<10	63	<2	<2	<2	2.1
A127	000613	Canal	Water	<10	<10	26	<2	<2	<2	8.6
C124	000613	Canal	Water	<10	<10	15	<2	<2	<2	2.6
D327	000613	Canal	Water	<10	<10	<10	<2	<2	<2	2.8
A130	001003	Canal	Sediment	98	1800	1568	<20	<20	<20	29
C124	001003	Canal	Sediment	<30	165	851	<20	<20	<20	<20
D327	001003	Canal	Sediment	50	108	3494	<20	<20	<20	<20
A130	001003	Canal	Water	<10	<10	28	<2	<2	<2	10.9
C124	001003	Canal	Water	<10	<10	<10	<2	<2	<2	<2
D327	001003	Canal	Water	<10	<10	<10	<2	<2	<2	2.1
D227	000613	Feeder ditch	Water	<10	<10	<10	<2	<2	<2	<2
CW1	000314	CW	Water	<10	<10	<10	<2	<2	<2	<2
CW5	000314	CW	Water	<10	<10	<10	<2	<2	<2	<2
CW1	000613	CW	Water	<10	<10	<10	<2	<2	<2	<2
CW5	000613	CW	Water	<10	<10	<10	<2	<2	<2	<2
CW1	001003	CW	Sediment	<30	<30	41	<20	<20	<20	<20
CW5	001003	CW	Sediment	<30	<30	<30	<20	<20	<20	<20
CW1	001003	CW	Water	<10	<10	<10	<2	<2	<2	<2
CW5	001003	CW	Water	<10	<10	<10	<2	<2	<2	<2
MM	000314	McMurtrey Marsh	Water	<10	<10	<10	<2	<2	<2	<2
MM	001003	McMurtrey Marsh	Sediment	<30	<30	<30	<20	<20	<20	<20
MM	001003	McMurtrey Marsh	Water	<10	<10	<10	<2	<2	<2	<2

Note: Date Col. = the date of sample collection; < indicates below the detection limit (the value is the detection limit) and CW = created wetland.

Table A.8. Concentrations (µg/g) of trace elements in invertebrate samples collected from Hastings Pork created wetlands and canals, Clay County, Nebraska, 2000 – 2001.

				Al		As		B		Ba		Be		Cd		Cr	
Date Col.	Site	Sample ID	Family	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.
07/01	CANAL	C118CI	Chironomidae	3553	443	0.935	0.117	7.14	0.89	53.30	6.65	0.18	0.02	0.16	0.02	7.87	0.98
07/01	CANAL	D230CI	Chironomidae	3578	384	1.14	0.122	9.38	1.01	40.20	4.32	0.16	0.02	0.11	0.01	5.78	0.62
06/00	CW1	601CW1B	Corixidae	1296	407	0.298	0.0936	3.74	1.18	14.40	4.51	0.13	0.04	0.15	0.05	2.01	0.63
06/00	CW1	601CW1C	Chironomidae	5283	893	1.42	0.24	10.40	1.76	63.80	10.80	0.43	0.07	0.25	0.04	6.78	1.15
10/00	CW1	CW1C	Chironomidae	6170	932	1.46	0.22	11.20	1.69	73.00	11.00	0.31	0.05	0.32	0.05	5.08	0.77
07/01	CW2	CW2I	Chironomidae	3998	497	1.1	0.137	8.17	1.02	65.30	8.11	0.20	0.02	0.51	0.06	5.29	0.66
07/01	CW3	CW3I	Chironomidae	7040	1070	1.84	0.28	9.35	1.42	66.00	10.00	0.30	0.05	0.73	0.11	8.81	1.34
07/01	CW4	CW4I	Chironomidae	5485	615	1.88	0.211	6.51	0.73	92.20	10.30	0.26	0.03	0.63	0.07	8.00	0.90
06/00	CW5	602CW5B	Corixidae	2265	695	0.438	0.134	4.75	1.46	35.70	11.00	0.21	0.06	0.23	0.07	3.58	1.10
06/00	CW5	602CW5C	Chironomidae	7314	1485	2.32	0.471	12.00	2.44	81.70	16.60	0.51	0.10	0.47	0.09	7.92	1.61
10/00	CW5	CW5C	Chironomidae	4619	845	1.46	0.267	<4.05	<0.741	71.60	13.10	<0.202	<0.037	0.26	0.05	12.50	2.29
07/01	CW5	CW5I	Chironomidae	3373	426	1.08	0.136	3.56	0.45	41.60	5.25	0.17	0.02	0.35	0.04	4.85	0.61
07/01	CW6	CW6I	Chironomidae	8650	1220	1.8	0.253	8.84	1.25	85.60	12.10	0.39	0.05	0.48	0.07	9.82	1.39
07/01	CW7	CW7I	Chironomidae	2948	379	1.23	0.158	3.54	0.46	42.10	5.41	0.14	0.02	1.04	0.13	3.97	0.51

				Cu		Fe		Hg		Mg		Mn		Mo	
Date Col.	Site	Sample ID	Family	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.
07/01	CANAL	C118CI	Chironomidae	25.90	3.24	2574	321	<0.2	<0.025	2628	328	121.00	15.10	<2.00	<0.25
07/01	CANAL	D230CI	Chironomidae	19.50	2.10	3219	345	<0.2	<0.0215	2090	224	62.70	6.72	<2.00	<0.215
06/00	CW1	601CW1B	Corixidae	18.70	5.89	1154	362	<0.0977	<0.0307	1382	434	30.30	9.50	<0.48	<0.151
06/00	CW1	601CW1C	Chironomidae	19.70	3.33	5788	978	<0.0995	<0.0168	2862	484	126.00	21.40	<0.48	<0.0812
10/00	CW1	CW1C	Chironomidae	23.00	3.47	6718	1014	<0.0408	<0.00616	3268	493	142.00	21.50	<1.02	<0.154
07/01	CW2	CW2I	Chironomidae	15.50	1.92	3026	376	<0.2	<0.0248	2098	261	143.00	17.80	<2.00	<0.248
07/01	CW3	CW3I	Chironomidae	16.60	2.52	4750	722	<0.2	<0.0304	2998	456	114.00	17.30	<2.00	<0.304
07/01	CW4	CW4I	Chironomidae	12.70	1.43	4257	478	<0.2	<0.0224	3113	349	222.00	24.90	<2.00	<0.224
06/00	CW5	602CW5B	Corixidae	22.10	6.80	2012	618	<0.0964	<0.0296	1708	524	87.40	26.80	<0.50	<0.153
06/00	CW5	602CW5C	Chironomidae	22.80	4.64	7021	1425	<0.096	<0.0195	3183	646	151.00	30.80	<0.48	<0.0969
10/00	CW5	CW5C	Chironomidae	15.50	2.84	5216	955	0.05	0.01	2359	432	178.00	32.50	<1.01	<0.185
07/01	CW5	CW5I	Chironomidae	31.90	4.03	2853	360	<0.2	<0.0253	1897	240	87.00	11.00	<2.00	<0.253
07/01	CW6	CW6I	Chironomidae	18.20	2.57	5222	737	<0.2	<0.0282	3321	469	145.00	20.50	<2.00	<0.282
07/01	CW7	CW7I	Chironomidae	12.10	1.55	2503	322	<0.2	<0.0257	1930	248	72.30	9.30	<2.00	<0.257

				Ni		Pb		Se		Sr		V		Zn	
Date Col.	Site	Sample ID	Family	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.
07/01	CANAL	C118CI	Chironomidae	7.20	0.90	1.86	0.23	2.96	0.37	68.70	8.58	7.58	0.95	159.00	19.90
07/01	CANAL	D230CI	Chironomidae	6.67	0.72	2.07	0.22	4.27	0.46	24.60	2.64	8.48	0.91	104.00	11.20
06/00	CW1	601CW1B	Corixidae	2.39	0.75	2.05	0.64	1.08	0.34	6.62	2.08	2.40	0.75	124.00	39.00
06/00	CW1	601CW1C	Chironomidae	18.00	3.05	9.61	1.62	0.96	0.16	19.90	3.37	11.90	2.01	85.00	14.40
10/00	CW1	CW1C	Chironomidae	9.81	1.48	6.86	1.04	3.30	0.50	29.00	4.38	11.00	1.67	94.90	14.30
07/01	CW2	CW2I	Chironomidae	5.50	0.68	4.05	0.50	3.34	0.41	33.60	4.17	8.11	1.01	79.80	9.91
07/01	CW3	CW3I	Chironomidae	9.11	1.39	4.89	0.74	2.74	0.42	24.70	3.75	13.00	1.98	75.30	11.40
07/01	CW4	CW4I	Chironomidae	5.87	0.66	4.23	0.48	2.72	0.31	39.70	4.45	10.00	1.13	69.80	7.83
06/00	CW5	602CW5B	Corixidae	4.50	1.38	2.28	0.70	1.08	0.33	13.40	4.13	4.28	1.32	174.00	53.40
06/00	CW5	602CW5C	Chironomidae	20.30	4.12	9.05	1.84	0.57	0.12	25.90	5.25	16.20	3.30	80.80	16.40
10/00	CW5	CW5C	Chironomidae	8.44	1.54	5.49	1.00	2.28	0.42	21.20	3.87	9.88	1.81	75.30	13.80
07/01	CW5	CW5I	Chironomidae	17.40	2.20	4.83	0.61	4.87	0.62	17.20	2.18	5.34	0.67	94.80	12.00
07/01	CW6	CW6I	Chironomidae	8.72	1.23	4.82	0.68	3.78	0.53	26.60	3.75	14.60	2.06	106.00	14.90
07/01	CW7	CW7I	Chironomidae	3.41	0.44	2.51	0.32	2.30	0.30	15.00	1.94	5.36	0.69	67.30	8.66

< Indicates the sample was below the detection limit (value = detection limit). Date Col. = approximate date of sample collection, CW = created wetland, D.W. = dry weigh, and W.W. = wet weight.

Table A.9. Concentrations (µg/g) of trace elements in sediment samples collected from MM Marsh and Hastings Pork created wetlands and canals, Clay County, Nebraska, 2000.

			Al		As		B		Ba		Be		Cd		Cr	
Date Col.	Site	Sample ID	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.
07/01	LAGOON	A127LSa	7451	1562	3.52	0.738	11	2.3	232	48.7	0.987	0.207	3.21	0.674	48.6	10.2
07/01	LAGOON	A127LSb	8999	1745	4.75	0.92	14.5	2.8	211	40.8	1.17	0.228	4.47	0.867	54.5	10.6
07/01	LAGOON	A63LSa	14808	6587	8.38	3.73	<10	<4.45	206	91.8	1.03	0.457	1.64	0.728	43	19.1
07/01	LAGOON	A63LSb	13353	6253	6.39	2.99	13.8	6.45	200	93.7	0.685	0.321	0.606	0.284	56.2	26.3
07/01	LAGOON	C124LSa	7146	1168	3.42	0.558	11.9	1.94	179	29.2	0.912	0.149	2.88	0.47	38	6.22
07/01	LAGOON	C124LSb	5209	686	2.97	0.392	11.7	1.55	174	23	0.892	0.117	3.43	0.452	40.2	5.3
07/01	LAGOON	D327LSa	20763	7219	5.16	1.79	14.3	4.97	171	59.5	1.38	0.478	1.11	0.385	33	11.5
07/01	LAGOON	D327LSb	14362	3377	5.38	1.27	15.4	3.62	191	45	1.43	0.336	1.99	0.468	36.1	8.49
07/01	LAGOON	D415LSa	7620	1282	4.49	0.756	16.9	2.85	194	32.6	1.25	0.21	2.7	0.455	39.4	6.62
07/01	LAGOON	D415LSb	7193	1414	3.65	0.717	16.6	3.27	188	36.9	1.08	0.213	2.16	0.424	37.4	7.36
03/00	CANAL	320CC1S	9710	5020	2.19	1.13	4.35	2.25	216	112	0.717	0.371	0.621	0.321	12.8	6.61
07/01	CANAL	C118CSa	14896	6827	5.07	2.32	12	5.51	239	110	1.05	0.481	0.842	0.386	33.6	15.4
07/01	CANAL	C118CSb	14736	8084	4.83	2.65	12.6	6.91	347	191	0.964	0.529	0.802	0.44	33.6	18.4
06/00	CANAL	607DC2S	14213	5941	2.62	1.09	7.43	3.11	174	72.7	0.907	0.379	0.214	0.0895	14.3	5.99
10/00	CANAL	D230CS	12070	4756	3.44	1.36	14.3	5.63	167	65.8	0.279	0.11	0.544	0.214	12.2	4.82
07/01	CANAL	D230CSa	15054	7799	4.07	2.11	11.3	5.88	197	102	1.11	0.578	0.392	0.203	23.5	12.2
07/01	CANAL	D230CSb	13834	7126	4.19	2.16	<10	<5.15	197	102	0.932	0.48	0.282	0.145	19.3	9.92
03/00	CW1	320CW11S	7396	3062	2.22	0.919	5.22	2.16	191	79.2	0.786	0.325	0.617	0.255	13.9	5.75
06/00	CW1	607CW12S	16506	7791	2.5	1.18	8.83	4.17	209	98.4	0.997	0.471	0.31	0.146	15.6	7.38
10/00	CW1	CW1S	11550	6895	5.17	3.09	16.4	9.79	126	75.2	0.401	0.239	0.464	0.277	11.6	6.94
07/01	CW1	CW1Sa	21082	20207	5.67	5.43	22.9	21.9	226	217	1.31	1.26	0.33	0.316	26.7	25.6
07/01	CW1	CW1Sb	19492	18073	5.48	5.08	19.9	18.4	268	249	1.36	1.26	0.477	0.442	26.7	24.7
07/01	CW2	CW2Sa	15208	5660	4.43	1.65	11.4	4.26	144	53.6	1.04	0.386	0.242	0.0901	19.1	7.13
07/01	CW2	CW2Sb	20088	7555	5	1.88	20.4	7.66	143	53.9	1.18	0.443	<0.2	<0.0752	23.6	8.89
07/01	CW3	CW3Sa	21021	8585	5.76	2.35	20.1	8.2	279	114	1.35	0.55	0.244	0.0996	26.6	10.9
07/01	CW3	CW3Sb	19651	8004	5.37	2.19	18.7	7.62	293	120	1.35	0.548	0.335	0.136	26.2	10.7
07/01	CW4	CW4Sa	17304	6531	4.54	1.71	19.7	7.45	197	74.4	1.01	0.382	<0.2	<0.0755	22.4	8.47
07/01	CW4	CW4Sb	19916	7552	4.98	1.89	19.1	7.25	296	112	1.51	0.572	0.281	0.107	27.2	10.3
03/00	CW5	320CW51S	7920	3453	2.42	1.06	3.13	1.36	207	90.2	0.851	0.371	0.808	0.352	15	6.53
06/00	CW5	607CW52S	11279	5313	1.75	0.822	9.14	4.3	154	72.3	0.748	0.352	0.255	0.12	11.7	5.5
10/00	CW5	CW5S	8997	5173	4.05	2.33	13.6	7.82	123	70.8	0.257	0.148	0.536	0.308	9.83	5.65
07/01	CW5	CW5Sa	23280	8264	5.27	1.87	22.8	8.1	285	101	1.55	0.551	0.366	0.13	29.7	10.5
07/01	CW5	CW5Sb	22761	9280	5.17	2.11	21.2	8.65	357	146	1.51	0.615	0.543	0.221	29.5	12
07/01	CW6	CW6Sa	21811	7859	4.42	1.59	18	6.5	250	90.2	1.45	0.523	0.333	0.12	27.5	9.9
07/01	CW6	CW6Sb	22373	7453	5.22	1.74	18.1	6.03	263	87.5	1.49	0.497	0.277	0.0923	26.9	8.96
07/01	CW7	CW7Sa	12601	7077	2.91	1.63	12.7	7.14	143	80.2	0.886	0.497	<0.2	<0.112	17.7	9.92
07/01	CW7	CW7Sb	14467	7273	3.63	1.83	17.3	8.67	139	70.1	0.887	0.446	<0.2	<0.101	19.2	9.64
03/00	McMurtrey	320ME1S	6539	2570	2.07	0.814	3.58	1.41	227	89.1	0.995	0.391	1.32	0.517	11.7	4.61
10/00	McMurtrey	MS	12050	6266	4.41	2.29	8.15	4.24	158	81.9	0.581	0.302	0.748	0.389	11.8	6.11

Table A.9. Continued.

			Cu		Fe		Hg		Mg		Mn		Mo	
Date Col.	Site	Sample ID	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.
07/01	LAGOON	A127LSa	372	78	8437	1768	<0.2	<0.0419	18181	3811	1241	260	10.5	2.21
07/01	LAGOON	A127LSb	406	78.7	10717	2078	<0.2	<0.0388	21473	4164	926	180	11.2	2.17
07/01	LAGOON	A63LSa	109	48.7	27752	12344	<0.2	<0.089	4364	1941	968	431	<5.0	<2.22
07/01	LAGOON	A63LSb	70	32.8	12085	5659	<0.2	<0.0937	6675	3126	2105	986	<5.0	<2.34
07/01	LAGOON	C124LSa	301	49.1	9607	1570	<0.2	<0.0327	9799	1601	933	152	13.9	2.27
07/01	LAGOON	C124LSb	351	46.2	8302	1093	<0.2	<0.0263	10992	1448	922	121	17.8	2.35
07/01	LAGOON	D327LSa	204	70.9	18912	6576	<0.2	<0.0695	12200	4242	503	175	6.05	2.1
07/01	LAGOON	D327LSb	439	103	15667	3683	<0.2	<0.047	17803	4186	728	171	14.1	3.32
07/01	LAGOON	D415LSa	598	101	12992	2187	<0.2	<0.0337	23574	3967	924	156	22	3.7
07/01	LAGOON	D415LSb	398	78.3	11393	2240	<0.2	<0.0393	25057	4926	997	196	16.8	3.3
03/00	CANAL	320CC1S	25.1	13	10340	5346	<0.0198	<0.0102	7139	3691	294	152	<4.95	<2.56
07/01	CANAL	C118CSa	76.7	35.2	14134	6477	<0.2	<0.0917	8970	4111	624	286	<5.0	<2.29
07/01	CANAL	C118CSb	68.7	37.7	15297	8392	<0.2	<0.11	8664	4753	963	528	<5.0	<2.74
06/00	CANAL	607DC2S	18.9	7.88	14014	5858	<0.0986	<0.0412	4817	2013	181	75.5	<0.465	<0.195
10/00	CANAL	D230CS	44	17.3	14260	5618	<0.0214	<0.00843	5079	2001	294	116	<5.34	<2.1
07/01	CANAL	D230CSa	23.9	12.4	16784	8696	<0.2	<0.104	7841	4063	394	204	<5.0	<2.59
07/01	CANAL	D230CSb	17.7	9.1	15206	7833	<0.2	<0.103	4585	2362	304	157	<5.0	<2.58
03/00	CW1	320CW11S	15.6	6.47	12040	4985	<0.0202	<0.00836	4333	1794	354	146	<5.06	<2.1
06/00	CW1	607CW12S	16.8	7.91	16148	7622	<0.0676	<0.0319	4583	2163	322	152	<0.5	<0.236
10/00	CW1	CW1S	13	7.76	13360	7976	<0.021	<0.0125	3571	2132	262	156	<5.25	<3.14
07/01	CW1	CW1Sa	25.2	24.2	20442	19594	<0.2	<0.192	7470	7160	403	387	<5.0	<4.79
07/01	CW1	CW1Sb	26.2	24.3	19310	17904	<0.2	<0.185	7280	6750	523	485	<5.0	<4.64
07/01	CW2	CW2Sa	12	4.47	16727	6226	<0.2	<0.0744	5293	1970	231	86.1	<5.0	<1.86
07/01	CW2	CW2Sb	13.3	4.99	19302	7259	<0.2	<0.0752	6392	2404	220	82.8	<5.0	<1.88
07/01	CW3	CW3Sa	20.2	8.24	19987	8163	<0.2	<0.0817	6895	2816	638	261	<5.0	<2.04
07/01	CW3	CW3Sb	20.8	8.49	18094	7369	<0.2	<0.0815	7066	2878	716	291	<5.0	<2.04
07/01	CW4	CW4Sa	12.8	4.83	17410	6571	<0.2	<0.0755	6198	2339	456	172	<5.0	<1.89
07/01	CW4	CW4Sb	19.5	7.39	21986	8337	<0.2	<0.0758	6699	2540	791	300	<5.0	<1.9
03/00	CW5	320CW51S	14.8	6.43	12270	5350	0.0249	0.0109	4271	1862	481	210	<5.04	<2.2
06/00	CW5	607CW52S	10.6	5	10841	5106	<0.0619	<0.0291	3475	1637	302	142	<0.455	<0.214
10/00	CW5	CW5S	10.4	5.99	10420	5992	<0.021	<0.0121	3566	2050	230	132	<5.25	<3.02
07/01	CW5	CW5Sa	20	7.11	21806	7741	<0.2	<0.071	6889	2445	643	228	<5.0	<1.78
07/01	CW5	CW5Sb	20.1	8.18	21314	8690	<0.2	<0.0815	6659	2715	1035	422	<5.0	<2.04
07/01	CW6	CW6Sa	17.8	6.41	21190	7635	<0.2	<0.0721	7404	2668	404	146	<5.0	<1.8
07/01	CW6	CW6Sb	19.2	6.4	21742	7242	<0.2	<0.0666	7379	2458	394	131	<5.0	<1.67
07/01	CW7	CW7Sa	8.76	4.92	13353	7499	<0.2	<0.112	4728	2655	307	172	<5.0	<2.81
07/01	CW7	CW7Sb	9.41	4.73	14287	7182	<0.2	<0.101	5298	2663	291	146	<5.0	<2.51
03/00	McMurtrey	320ME1S	16.2	6.38	9543	3750	0.0475	0.0187	2428	954	209	82.1	<5.09	<2.0
10/00	McMurtrey	MS	14.8	7.7	10850	5642	0.0406	0.0211	2278	1185	133	69.4	<5.42	<2.82

Table A.9. Continued.

			Ni		Pb		Se		Sr		V		Zn	
Date Col.	Site	Sample ID	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.
07/01	LAGOON	A127LSa	30.7	6.43	7.19	1.51	6.78	1.42	396	82.9	28.8	6.04	1712	359
07/01	LAGOON	A127LSb	32.9	6.39	8.77	1.7	7.86	1.52	240	46.6	32.5	6.29	2059	399
07/01	LAGOON	A63LSa	22.9	10.2	11.7	5.22	2.58	1.15	226	100	29.7	13.2	614	273
07/01	LAGOON	A63LSb	15.3	7.18	7.51	3.52	1.51	0.706	499	233	33.9	15.9	394	184
07/01	LAGOON	C124LSa	26.3	4.29	5.58	0.911	6.81	1.11	206	33.6	29.9	4.89	1969	322
07/01	LAGOON	C124LSb	27.2	3.58	<5.0	<0.658	7.66	1.01	208	27.3	28.3	3.73	2364	311
07/01	LAGOON	D327LSa	23.9	8.32	16.6	5.78	3.81	1.32	81.3	28.3	40.6	14.1	1390	483
07/01	LAGOON	D327LSb	28.9	6.8	9.72	2.29	7.06	1.66	132	31.1	40.1	9.42	3101	729
07/01	LAGOON	D415LSa	32.3	5.44	6.37	1.07	10	1.69	174	29.3	37.2	6.26	4820	811
07/01	LAGOON	D415LSb	27.2	5.35	5.52	1.08	7.08	1.39	196	38.5	33.6	6.61	2916	573
03/00	CANAL	320CC1S	17.9	9.23	13.8	7.16	<0.495	<0.256	87.2	45.1	22.1	11.4	110	56.6
07/01	CANAL	C118CSa	22.9	10.5	11.8	5.4	2.76	1.26	290	133	32.4	14.8	386	177
07/01	CANAL	C118CSb	23.2	12.7	11.4	6.26	2.96	1.62	430	236	36.4	20	318	175
06/00	CANAL	607DC2S	15	6.25	12.8	5.33	0.428	0.179	38.5	16.1	21.9	9.13	91.3	38.2
10/00	CANAL	D230CS	17.7	6.99	10.6	4.18	1.48	0.583	103	40.6	20.9	8.25	206	81.3
07/01	CANAL	D230CSa	18.1	9.38	15.9	8.23	1.23	0.637	110	56.9	25.6	13.2	120	61.9
07/01	CANAL	D230CSb	15.7	8.07	11.6	5.97	*1	<0.515	46.6	24	25.5	13.1	91.4	47.1
03/00	CW1	320CW11S	16.2	6.69	12.6	5.2	<0.506	<0.21	45.3	18.7	22.9	9.46	59.6	24.7
06/00	CW1	607CW12S	17	8.03	14.9	7.03	0.31	0.146	42.6	20.1	26	12.3	64.4	30.4
10/00	CW1	CW1S	14.3	8.54	16.1	9.6	<0.42	<0.251	30.3	18.1	20.6	12.3	49.2	29.4
07/01	CW1	CW1Sa	20.6	19.7	15.4	14.8	<1.0	<0.958	97.7	93.6	37.5	35.9	119	114
07/01	CW1	CW1Sb	21.2	19.7	15.6	14.5	1.06	0.985	131	121	36.2	33.6	125	115
07/01	CW2	CW2Sa	14.9	5.54	13	4.83	<1.0	<0.372	42.2	15.7	28.1	10.5	56.6	21.1
07/01	CW2	CW2Sb	15.7	5.92	14.4	5.4	<1.0	<0.376	41.6	15.7	39.6	14.9	58	21.8
07/01	CW3	CW3Sa	20.3	8.28	15.9	6.5	<1.0	<0.408	99.6	40.7	44.3	18.1	85.7	35
07/01	CW3	CW3Sb	21.7	8.85	15.1	6.16	<1.0	<0.407	115	46.8	41.2	16.8	94.1	38.3
07/01	CW4	CW4Sa	14.5	5.47	11.4	4.32	<1.0	<0.377	57.7	21.8	36.9	13.9	56.6	21.4
07/01	CW4	CW4Sb	23.2	8.81	16.4	6.22	<1.0	<0.379	73.7	27.9	45.3	17.2	85.2	32.3
03/00	CW5	320CW51S	16.4	7.17	17.1	7.47	<0.504	<0.22	47.3	20.6	24.1	10.5	58.2	25.4
06/00	CW5	607CW52S	11.5	5.42	12.3	5.78	0.255	0.12	37	17.4	18	8.45	42.8	20.2
10/00	CW5	CW5S	11.5	6.6	13	7.46	0.57	0.328	31.6	18.1	17.4	10	36.6	21.1
07/01	CW5	CW5Sa	20.7	7.33	18.8	6.67	<1.0	<0.355	81.8	29	44.2	15.7	94	33.4
07/01	CW5	CW5Sb	20.8	8.49	18.6	7.6	<1.0	<0.408	112	45.6	43.7	17.8	93.3	38
07/01	CW6	CW6Sa	20.1	7.25	15.9	5.72	<1.0	<0.36	66.1	23.8	41.8	15.1	86.3	31.1
07/01	CW6	CW6Sb	21	7	17	5.65	<1.0	<0.333	64.1	21.4	42.1	14	91.4	30.4
07/01	CW7	CW7Sa	12.7	7.12	12	6.71	<1.0	<0.562	37.6	21.1	27.7	15.6	42.3	23.8
07/01	CW7	CW7Sb	12.4	6.25	10.6	5.34	<1.0	<0.503	45	22.6	30.8	15.5	42.6	21.4
03/00	McMurtrey	320ME1S	11.8	4.62	22.4	8.82	1.22	0.479	47.1	18.5	25.2	9.92	63	24.8
10/00	McMurtrey	MS	10.9	5.66	20.3	10.5	1.33	0.692	32.9	17.1	19.9	10.4	57.5	29.9

< Indicates the sample was below the detection limit (value = detection limit). Date Col. = approximate date of sample collection, CW = created wetland, D.W. = dry weigh, W.W. = wet weight.

Table A.10. Concentrations (mg/L) of trace elements in water samples collected from MM Marsh and Hastings Pork created wetlands and canals, Clay County, Nebraska, 2000.

Date Col.	Site	Sample ID	Al	As	B	Ba	Be	Cd	Cr	Cu	Fe	Hg
03/00	CANAL	320CC1	0.321	<0.0056	0.373	0.0514	<0.0011	0.0013	<0.0056	0.106	1.18	>0.0002
06/00	CANAL	607DC2	0.879	0.00499	0.549	0.033	<0.0003	<0.0004	0.00266	0.0227	0.796	>0.0002
10/00	CANAL	D230CW	0.154	0.0077	0.407	0.0326	<0.0006	<0.0006	<0.0056	0.0334	0.261	>0.0002
03/00	CW1	320CW11	3.61	0.0082	0.248	0.122	<0.0011	<0.0011	<0.0056	0.02	3.36	>0.0002
06/00	CW1	607CW12	2.64	0.017	0.548	0.109	<0.0003	<0.0004	0.0034	0.016	1.91	>0.0002
10/00	CW1	CW1W	0.173	0.0101	0.427	0.0438	<0.0006	<0.0006	<0.0056	0.0205	0.198	>0.0002
03/00	CW5	320CW51	14.7	0.0166	0.204	0.335	<0.0011	0.0014	0.0141	0.0293	13.7	>0.0002
06/00	CW5	607CW52	8.71	0.029	0.707	0.278	0.00069	<0.0004	0.00827	0.0277	5.15	>0.0002
10/00	CW5	CW5W	<0.111	0.0192	0.375	0.0726	<0.0006	<0.0006	<0.0056	0.0172	0.171	>0.0002
03/00	McMurtrey	320ME1	0.657	<0.0056	0.047	0.0827	<0.0011	<0.0011	<0.0056	0.0062	0.398	>0.0002
10/00	McMurtrey	MW	11.9	0.0119	<0.111	0.59	0.0019	0.0014	0.0113	0.0302	12.2	>0.0002

Date Col.	Site	Sample ID	Mg	Mn	Mo	Ni	Pb	Se	Sr	V	Zn
03/00	CANAL	320CC1	31.6	0.307	0.0167	0.034	0.0086	<0.0056	0.442	0.006	0.566
06/00	CANAL	607DC2	51.4	0.11	0.00533	0.0437	0.00102	0.0035	0.261	0.0166	0.101
10/00	CANAL	D230CW	50.8	0.0951	<0.0556	0.0358	<0.0111	<0.0044	0.22	0.006	0.103
03/00	CW1	320CW11	25.2	0.287	0.0073	0.0233	0.0099	<0.0056	0.244	0.0165	0.0663
06/00	CW1	607CW12	50.9	0.244	<0.004	0.0506	0.00295	0.0027	0.256	0.019	0.0584
10/00	CW1	CW1W	52.3	0.0856	<0.0556	0.0289	<0.0111	<0.0044	0.188	0.0086	0.0399
03/00	CW5	320CW51	31.4	0.874	<0.0556	0.0323	0.0245	<0.0056	0.261	0.0458	0.0944
06/00	CW5	607CW52	55.8	0.556	0.0144	0.0739	0.00861	0.0069	0.366	0.0399	0.0667
10/00	CW5	CW5W	35	0.131	<0.0556	0.0271	<0.0111	<0.0044	0.182	0.0119	0.013
03/00	McMurtrey	320ME1	9.46	0.01	<0.0556	<0.0556	<0.0556	<0.0556	0.19	<0.0556	<0.0111
10/00	McMurtrey	MW	11.5	0.85	<0.0556	0.017	0.0448	<0.0044	0.226	0.026	0.0881

< Indicates the sample was below the detection limit (value = detection limit). Date Col. = approximate date of sample collection, CW = created wetland.