DISTRIBUTION OF ARSENIC AND OTHER HEAVY METALS IN SEDIMENTS AND THEIR EFFECTS ON BENTHIC MACROINVERTEBRATES IN THE GALLINAS RIVER, SAN MIGUEL COUNTY, NEW MEXICO

A THESIS

Presented to the Graduate Division

College of Science and Mathematics

New Mexico Highlands University

In Partial Fulfilment

Of the Requirements for the Degree

Master of Science

By

Bildad Eta Eyong

July 2008

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ABSTRACT

Sediments and benthic macroinvertebrates (BMIs) were used to investigate the distribution of heavy metals and their impact on the aquatic biota in the Gallinas River basin, an 84 square mile watershed in San Miguel County, North Central New Mexico. Sampling was carried out during Spring and Fall 2006 at two locations upstream of the City of Las Vegas, and two downstream. Chemical analysis of the BMIs and streambed sediments reveal that in the Spring, the Shredders had higher concentrations of metals than any other feeding guild at the two upstream sites while the Collectors and Predators bioaccumulated higher concentrations at the two downstream sites. In the Fall, the bioaccumulation trend shifted toward the Collectors and Grazers. Of the eight metals investigated, Ni is the only metal whose levels in sediments correlated strongly ($p < 0.05$) with those in all four BMI feeding guilds. Cd levels in sediments correlated strongly with those in the Collectors ($p = 0.01$). Cr in sediments correlated strongly with those in Shredders ($p = 0.04$) and Predators ($p = 0.01$), while sediment Zn levels correlated strongly with concentrations in Collectors ($p = 0.04$) and Predators ($p = 0.01$). Correlation between these metals and biotic metrics revealed that As, Cd, Ni, and Zn have the highest negative effect on the biological community in the River. The metals index had strong correlations ($p < 0.05$) with four of the five biotic metrics used. Although other physical parameters assessed during the sampling periods reveal that organic pollution is important at the downstream sites, the strong relationship between the biotic condition of the River and the heavy metal contamination of the sediments gave enough reason to conclude that the heavy metals are influencing water quality adversely and impeding BMI communities in the Gallinas River, especially downstream.

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ACKNOWLEDGEMENTS

I would like to offer my sincere gratitude to my advisor, Dr. Edward Martinez, who has supported me throughout my thesis with his patience and knowledge, whilst allowing me to explore my own ideas. I could not wish for a better advisor.

My heartfelt thanks to Dr. Mike Meyer and Mr. Bob Mishler, who served on my thesis committee. I deeply appreciate their criticisms and scrutiny. A big ‘Thank you’ also to faculty members of Biology and Natural Resources at New Mexico Highlands University for the knowledge they imparted in me during my Master’s Degree studies. Dr. Brian Miller was of tremendous help to me during the writing of this thesis. I would also like to thank Dr. Ian Williamson of Behavioral Sciences, who taught me the fundamentals I needed to know in statistical analysis using SPSS.

Furthermore, I wish to express my profound gratitude to the following students who supported me in one way or the other during my studies here in Las Vegas: Adel Enoru, Mbiatem Gordon, Mirabel Nongni, Che Shu-Nyamboli, Adelphe Mfuh, Carlos Gonzales, Julie Trujillo, Jason Martinez, Roger Griego, Daniel Wimber, Amin Tazifor, and the entire Cameroonian student body in Las Vegas, NM.

The Department of Life Science provided me with financial support through graduate assistantships during my studies.

This study was supported in part by funds from Sigma Xi, WAESO, and WRRI.

Finally, I thank my sisters, Francisca and Nerradine, my brothers, Ernest and Simon, and my step mom, Pauline Eyong, for the unconditional support they have always given me. Without them I would not have come to Las Vegas in the first place.

DEDICATION

To my parents, John and Cecilia Eyong, who instilled the quest for knowledge in me from a very tender age, but did not live long enough to reap the fruits of their labor.

INTRODUCTION

Water is unquestionably the most precious natural resource on earth and constitutes over 70% of the earth's surface. Without it life on earth would be nonexistent. However, morbidity and mortality throughout the world resulting from water related hazards are significant and increasing. "Diseases related to contamination of drinking-water constitute a major burden on human health" (WHO, 2006). Due to their exposure, surface waters are subject to direct deposits from the atmosphere, runoffs, erosion of the geology, and anthropogenic activities. Rivers and streams play a major role in the transportation and distribution of materials of all kinds originated, eroded and/or deposited at different levels along their course. These deposits and exchanges greatly affect the quality of the water and may render it unsafe for aquatic life and human use. In the USA, 66% of people are served by water systems that use surface water as their major water source (USEPA, 2007). Continuous critical assessment is necessary to ensure the protection and restoration of the quality of surface waters (fresh/marine/estuarine) and groundwater as the two have been shown to be directly related and entertain physical and chemical exchanges (Winter et al., 1998). Pollutants in the surrounding and/or underlying environments enter into water bodies and have been shown to affect aquatic life depending on their chemical speciation, toxicity, bioavailability and rate of uptake and metabolic regulation by specific organisms (Mason and Jenkins, 1995; Rainbow, 2002).

Many methods have been developed to analyze impairments to water quality. Amongst these, physicochemical analyses have been widely used (Alabaster and Llyod, 1980; Ingersoll et al., 2000). Physical characteristics usually involve evaluating stream bank erosion, turbidity, sedimentation, siltation, flow patterns, water temperature, riparian cover, plant debris, etc., on survival and reproduction of aquatic species. Chemical analyses take into consideration parameters such as dissolved oxygen, biochemical oxygen demand, pH, alkalinity, hardness, nutrients, metals, and organic compounds (Hunt and Wilson, 1985). Physicochemical evaluations are usually done using direct observations as well as laboratory analyses of water and waterbed sediments for the pollutant(s) impeding water quality (Soares et al., 1999; Upadhyay et al., 2006; Szalinska et al., 2006; Gaboury et al., 1999; Jain and Sharma, 2001). However, physicochemical criteria do not always take into consideration the protection of aquatic life.

The integration of biological parameters in addition to physicochemical assessments has proven to be a more complete method to fully assess pollutant effects in aquatic ecosystems in general and lotic systems in particular (USEPA, 1988; Karr, 1991; Makepeace et al., 1995). Bioassessment gives more reliability in evaluating the presence and impact of pollutants because lotic systems are subject to flushing during storm events and contaminants may be swept away without any apparent effect (Lynch et al., 1988). The most important biota used for bioassessment studies are aquatic plants and macroinvertebrates. Benthic macroinvertebrates (BMIs) have been used in numerous bioassessment studies because of their importance as bioindicators and the uncountable advantages they offer in evaluating the presence and extent of environmental pollutants (Nehring et al., 1979; Colborn, 1982; Namminga and Whilm, 1977; Mathis and Cummings, 1973; Burrows and Whitton, 1983).

Investigations on the Gallinas River, San Miguel County, New Mexico have shown that the surrounding and underlying geology contribute substantial amounts of

Arsenic in the water (Duran et al., 2005). The biological distribution and the impact of this heavy metal on the lotic ecosystem of this River have, however, not been determined because physicochemical analytical measurements of soil, rock, and water samples used by the above studies only provide the sources and loads of Arsenic in the water and sediments. Therefore, traditional techniques do not provide a full assessment of the level and distribution of contaminants nor an assessment of their impact on the overall health of the ecosystem.

The purpose of the present study was to use BMIs, in addition to sediment metal concentrations, to evaluate the contamination and distribution of Arsenic and other heavy metals in the Gallinas River.

The use of aquatic organisms to assess water quality is not a new technique; although algae and BMIs are the most recommended (Hellawell, 1986), BMIs have been more commonly used than any other group of organisms (Hawkes, 1979; Wiederholm, 1980; Hellawell, 1986; Abel, 1989). BMIs are excellent indicators of the distribution of heavy metals in aquatic systems due to their intimate contact with the sediments, their relative immobile state, their feeding habits, and their extended residence time in aquatic systems (Hare, 1992). BMIs are therefore a reliable and cost-effective mechanism for monitoring water sources such as the Gallinas River. These insect larvae are a primary food source for many organisms and, therefore, play a large role in transmission of contaminants from their environment to higher levels of the food chain. Some species of aquatic larvae are very tolerant of various types of water conditions (Krantzberg and Stokes 1989), their distribution is worldwide, and they are sensitive to aquatic pollutants. Furthermore, heavy metals bioaccumulate in the fatty tissues of these invertebrates through the consumption of algae, other invertebrates, phytoplankton, macrophyte tissues, and through ingestion and absorption of the pollutants in the surrounding water and sediment.

Streambed sediments were selected as the possible media for metal uptake because of their high sensitivity compared to water as indicators of contamination in hydrologic systems (Solomons and Forstner, 1984; Duzzin et al., 1988; Lietz and Galling, 1989; Luoma, 1990; Jha et al., 1990; Pardo et al., 1990; Gonçalves and Boaventura, 1991; Huang et al., 1994; Lapaquellerie et al., 1995; Borovec, 1996; Wardas et al., 1996; Soares et al., 1999). They are considered to be the ultimate sink for a variety of toxicants because pollutants may persist in sediments long after the original sources of contamination are eliminated (Reynoldson, 1987). In fact sediments have the capacity to accumulate and integrate low concentrations of trace elements in water over time and, therefore, allow for metal determination even when levels in overlying waters are extremely low and undetectable (Soares et al., 1999). Although the bioavailability of sediment-bound metals most often depends on variations of physical and chemical parameters of the overlying water, geochemical (speciation, adsorption to oxides, sulfides and organic materials etc.), and biological (digestion, extracellular secretions, physiological transport, etc.) processes have been shown to alter metal bioavailability and bioaccumulation (Luoma, 1983).

The present study investigates the following hypotheses:

- Hypothesis 1: There is no significant difference in metal concentrations in streambed sediments between sampling seasons;

- Hypothesis 2: There is no significant difference in metal concentration between sampling sites;
- Hypothesis 3: There is no significant correlation between concentrations of heavy metals in aquatic insects and those in sediments;
- Hypothesis 4: There is no biomagnification of heavy metals in the BMI food chain;
- Hypothesis 5: There is no significant correlation between sediment metal contamination and biotic metrics.

Metals Investigated

Extensive analysis of heavy metals in aquatic ecosystems have been undertaken worldwide (e.g., Turekian, 1969; Lenvik et al., 1978, Borg and Johansson, 1989; Runnels et al., 1992, Gadh et al., 1993, Farag et al., 1997; Moore, 1992; Van der Verde and Leuven, 1999), but very few studies on their concentrations and biological effects have been undertaken in river systems in New Mexico (Lynch et al., 1988; Roy et al., 1992). Such information is critical when considering the natural geochemistry of this region and the proximity of many of the water systems to the Los Alamos laboratory.

Weathering of minerals, industrial effluents, atmospheric precipitation and non-point discharges are important sources of high heavy metal concentrations in river systems (Solomons and Förstner, 1984; Förstner and Wittman, 1979). Also, geologic formations usually contain many different heavy metals in nature and more than one metal may be entering into nearby water systems through erosion. Furthermore, heavy metals have been reported to entertain synergistic and antagonistic interactions whenever in a mixture (Sprague, 1985). Therefore, Arsenic may not be the only metal causing adverse effects on the biota of the Gallinas River.

The metals selected for investigation were assumed to be from nonpoint sources because no known point source has been identified in the Gallinas Watershed. In addition to Arsenic (As), seven other metals, namely Nickel (Ni), Cadmium (Cd), Silver (Ag), Copper (Cu), Zinc (Zn), Chromium (Cr) and Lead (Pb) are investigated by the present study. However, As, was selected because previous studies indicated it represents a potential impairment to water quality of the Gallinas River (Duran et al., 2005).

Zn, Cu, Pb and Cd are the most frequently researched heavy metals in bioaccumulation studies (Goodyear and McNeill, 1999). Ni and Cr were considered because they have been found to be in high concentrations in urban runoff due to corrosion-induced release from metal alloys (Wallinder et al., 2006; Novotny, 1995). Ag on the other hand has no apparent source in the Gallinas watershed but many studies have stated that Ag is characteristic of sewage sludge (Chapman et al., 1988; Luoma and Phillips, 1988) and is mostly associated with oxidized sediments characterized by high organic content (Luoma and Bryan, 1981). Ag in solution is considered one of the most toxic metals (Bryan and Langston, 1992).

Description of the Gallinas Watershed

The Gallinas River

The Gallinas River originates in a forested mountainous area in the Pecos Wilderness in the Southern Rocky Mountains physiographic province, from the eastern flank of Elk Mountain, at an elevation of more than 2600m above sea level. It flows southeast draining a watershed of about 84 square miles before entering into the Pecos River (NM State Engineer, 1991). An average annual discharge of 19.8ft^3/s is reported for the period of 1927 to 2005 (USGS, 2007). Like most streams originating in mountainous watersheds in New Mexico, the Gallinas River and its tributaries provide a wealth of freshwater and sustain abundant and diverse populations of plants and animals. These significant water resources are exploited at various sites along the course of the river. The majority of people living in Las Vegas, New Mexico rely mainly on the Gallinas River to serve their agricultural, private, recreational and potable, water needs (Garn and Jacobi, 1996). This diverse and multipurpose utilization makes the overall water quality and impact to water quality, important to over 15,000 people living in Las Vegas and its environs. Monitoring water quality is therefore important in order for the River to support vital socioeconomic strength for the region.

Land Use

According to a report by the Surface Water Quality Bureau of the New Mexico Environment Department (NMED/SWQB (2005), land use in the Gallinas watershed comprises of about 92% forest land, 6% rangeland, 2% desert, and less than 1% farming and tundra, and includes activities such as grazing, residential and commercial properties, small-scale forestry, agriculture and recreational activities such as fishing, hiking, and camping. The same report indicates that 52% of this land belongs to the U.S. Forest Service, and 48% belongs to private owners including the City of Las Vegas. It contains the City's two canyon water storage reservoirs plus Storrie Lake on the high plains. The city's drinking water supply intake is located about seven miles downstream from the Santa Fe National Forest boundary and about half mile downstream from the USGS gauging station number 08380500, at Montezuma. Garn and Jacobi (1996) reported that the Gallinas watershed presents a wide variety of vegetation species such as pinion, juniper, ponderosa pine, mountain mahogany, oak, douglas fir, blue spruce, white fir, and aspen.

Climate

Because of its location between the southern extension of the Rocky Mountains and the eastern Plains of New Mexico, Las Vegas has a temperate climate that is typical of these two regions. Low relative humidity, abundant sunshine, and a wide variation in diurnal and annual temperature are some of the climatic features that make up the area (Houghton, 1972). Precipitation varies widely both seasonally and annually. Average annual precipitation varied from 15 inches at the City of Las Vegas to more than 30 inches at the higher elevations during the record period, 1951-1980 (Garn and Jacobi, 1996). Rains are usually brief and intense thunderstorms which occur mostly during the summer. Snowfall occurs during the winter months. Temperatures in the Gallinas watershed vary according to seasons and locations. July is the warmest month of the year and January the coldest. The average maximum temperature ranges from 82^{o}C at the lower altitudes to about 70^{o}C at the higher altitudes. In general, the Las Vegas area shows an average annual temperature of about 50ºC while that of the higher altitude is a little below 40ºC. Temperatures as low as –17ºC have been recorded in the Las Vegas area (Houghton, 1972).

Hydrology

The Gallinas River is a perennial stream which runs about 85 miles from its headwaters to its confluence with the Pecos River (NM State Engineer, 1991; Hopkins, 2001). It is the main tributary to the Pecos River and constitutes the principal source of drinking water for the City of Las Vegas. In addition to providing 95% of the water for Storrie Lake, the River also supplies water to the Peterson and Bradner reservoirs, which belong to the local Drinking Water Treatment Plant. The stream shows high levels of turbidity and TSS during storm events (Evans and Lindline, 2003). Stream water is relatively clear during base flow and snowmelt runoffs, and it is during these periods that water is diverted into the city's reservoirs for treatment by the Drinking Water Treatment Plant. The Gallinas River gets its water from four main sources.

Snowmelt

Snowmelt constitutes the principal source of runoff in the Gallinas River, and this is a typical feature of rivers flowing in mountainous areas. The greatest snowmelt usually occurs during the months of April and May. Average snowfall ranges annually between 30 inches in the lower altitudes to more than 100 inches at the higher altitudes. According to a study by Tuan et al. (1973), winter precipitation shows more inconsistency year after year than summer precipitation.

Rainfall

Rainfall occurs as a result of general southeasterly air circulation from the Gulf of Mexico and the Western Pacific Ocean. This air undergoes surface heating and orographic lifting producing convective air currents and condensation (Houghton, 1972). Intense summer thunderstorms produce annual peak discharges in the Gallinas River (USGS, 2007). The greatest rainfall usually occurs between July and September. July and August are the wettest months with about 40 % of the total average annual precipitation. Unlike snowmelt, which usually causes infiltration and slow moving runoff, intense rainfalls erode the topsoil and results in high turbidity and high total suspended solids.

Groundwater

Groundwater is an important source of drinking water serving a large number of households in the Gallinas watershed. The hyporheic discharges of groundwater into the Gallinas River come from the Greenhorn limestone, which is the main aquifer that underlies the lower Gallinas canyon (Evans and Lindline 2004). Effects of natural and anthropogenic impairments of water have been shown to be transmitted from surface water to groundwater and vice-versa (Duran et al., 2005; Evans and Lindline, 2003, Evans et al., 2004, Johns-Kaysing and Lindline, 2006).

Perennial Springs

There is a wide array of perennial springs at Montezuma commonly known as "Hot Springs" that discharge their waters into the Gallinas River (Lessard and Bejnar, 1976). These are naturally occurring water sources which originate from infiltration of snow and rain at the Sangre de Cristo Mountains. This water, which travels east to the mouth of the Gallinas canyon, percolates to great depths until it meets the Peterson reservoir fault Breccia zone. This zone contains clay-sized sediments and extends about three miles to the north and five miles to the south of Montezuma, forming a large dam. The water moves to the surface with a temperature of about $56^{o}C$ at the lowest site of the dam located at Montezuma. The geothermal gradient caused by the difference in elevation of the hot springs compared to the nearby mountains, the heat associated with relatively recently uplifted Precambrian rocks, as well as magma that extend under the hot springs, are possible sources of heat (Lessard and Bejnar, 1976). The Springs water has a chemical composition of Ca = 7.9 ppm, Mg = 2.2 ppm, Na = 168 ppm, K = 168 ppm, HCO_3 = 79 ppm, CO_3 = 15 ppm, SO_4 = 50 ppm, Cl = 157 ppm, Fe = 20 ppm, NO_3 = 0.1 ppm, and 534 ppm total solids. This ionic composition may influence water chemistry in the Gallinas River downstream of the hot springs.

Geology

The geology of the Gallinas watershed is characterized by a rugged, mountainous topography with broad ridges, deep canyons and steep side slopes. Outcroppings of different rock types ranging from fractured igneous, metamorphic and sedimentary rocks make the watershed very heterogeneous. The main geologic formations range from a few feet to over 1,000 feet thick and include in chronological order the Precambrian, the Mississippian and Devonian, Pennsylvanian, the Cretaceous, and the Quaternary (Baltz, 1972) (Figure 1).

A detail description of the geologic formations and their outcroppings in the Gallinas watershed can be found in Baltz (1972), Baltz and Bachman (1956), Callender et al. (1976), Garn and Jacobi (1996), Lessard and Bejnar (1976), and Skotnicki (2003). Studies of the effects of weathering on rocks in the watershed suggest that the Pennsylvanian Madera formations found outcropping in the upper and mid sections of the watershed around Johnson Mesa, and the Cretaceous Carlisle and Niobrara formations found outcropping northeast of the mouth of the Gallinas canyon, are primary sources of As in the Gallinas River (Duran et al., 2005; Evans and Lindline, 2003; Evans et al., 2004; Johns-Kaysing and Lindline, 2006). Because of the diverse geologic formations of the watershed it is possible that the aforementioned rock formations may be releasing more than just As.

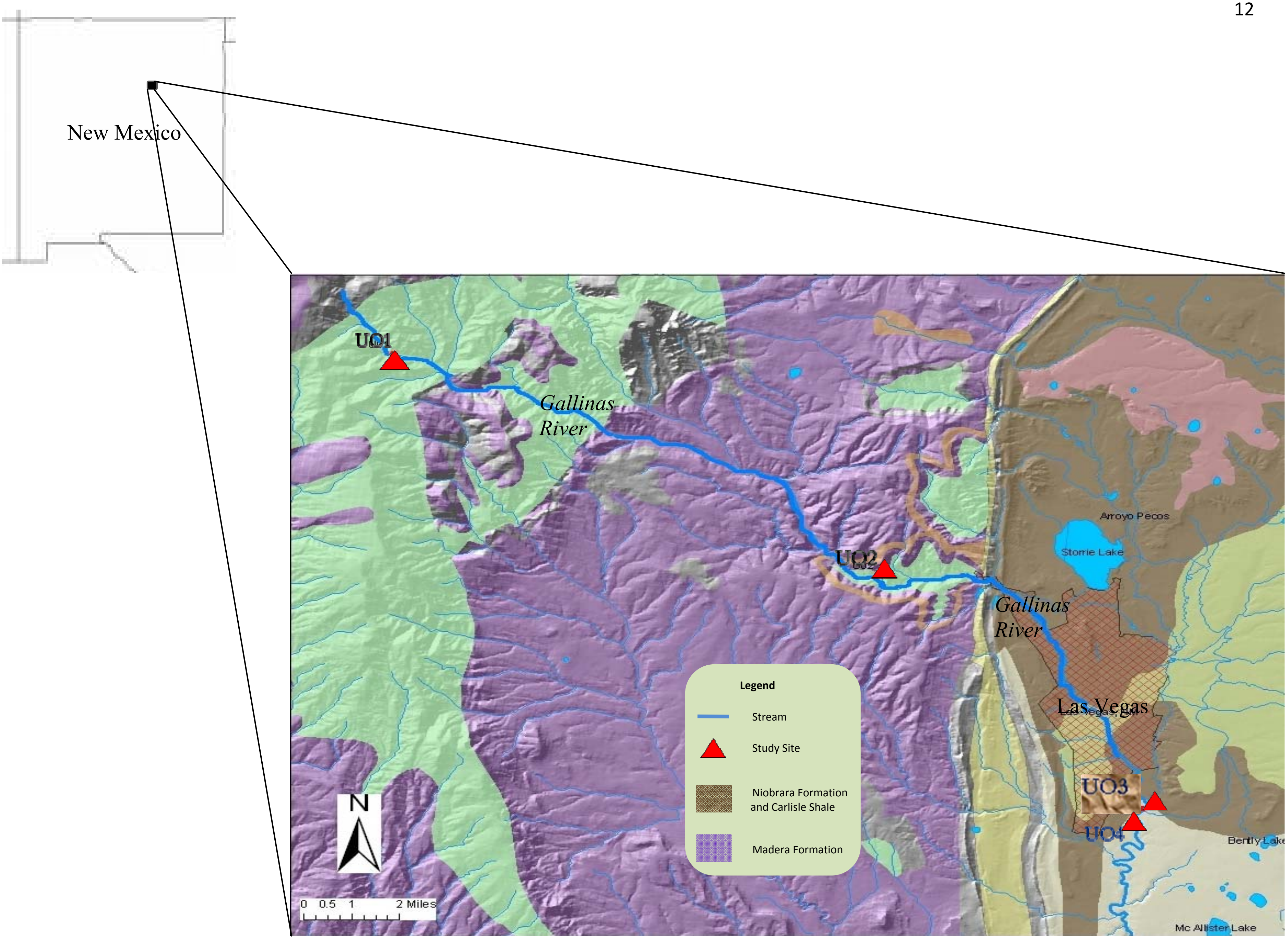


Figure 1. Map showing study sites and main rock outcrop formations in the Gallinas Watershed

LITERATURE REVIEW

Förstner and Wittman (1981) have reported that metals from terrestrial and atmospheric origins have increased enormously in recent years. Biomonitoring of environmental toxicants using benthic macroinvertebrates (BMIs) has served as a reliable means of assessing ecological transfer and potential ecotoxicological effects of these pollutants. Many studies have demonstrated that heavy metal contamination of aquatic environments triggers functional and/or structural ecosystem responses. Luoma and Carter (1991) reported that the most frequently used measures of ecosystem responses are structural changes in species composition, species richness, and population sizes. However, Schindler (1987) has indicated that ecosystems usually respond to metal exposures by ecophysiological compensations, and therefore functional changes are not easy to interpret. A list of some of the most significant studies of bioaccumulation of heavy metals in/with lotic waters carried out in the past fourteen years is given in Appendix A.

Johnson et al. (1993) reported that BMIs have the potential to bioaccumulate organic and heavy metal contaminants from their surrounding environment. They provide numerous advantages over direct analyses of water and sediments (Phillips, 1980; Metcalfe et al., 1989). One of these advantages is the fact that the presence of a pollutant in an organism means that the pollutant is bioavailable and may pose a threat to the organism and other members of the aquatic trophic chain (Van-Balogh, 1988; Lower and Kendall, 1990). Additionally, Nehring et al. (1979) reported that an ideal biomonitor provides long term measure of pollutant bioavailability unlike the punctual levels in water and/or sediment. As a matter of fact, analyzing the biomonitor may be the ideal procedure for contaminant detection when levels in the surrounding environment are too low for direct analysis (Nehring, 1976; Nehring et al., 1979, Graney et al., 1983). Although there is still limited information about the biology of freshwater organisms (Hare, 1992; Rainbow and Dallinger, 1992; Goodyear and McNeil, 1999), investigations on trace and heavy metal bioaccumulation by lotic BMIs started in the 1970s and has been undertaken at an increasing rate since then (Goodyear and McNeill, 1999) (See Appendix A). The degree of success of this method is highly dependent on habitat type, food source, source of the pollutant, physical and chemical state of the pollutant, metabolic differences among taxa, as well as whether the study was experimental or a survey.

Mathis and Cummings (1973) reported that although the tubificid worm in the Illinois River accumulated all metals present in sediments with a bioaccumulation factor (BAF) < 1, its BAF for Cu was greater than 1. Chapman et al. (1979) working on the Fraser River, British Columbia, found BAFs of less than 1 for all metals except Hg (BAF of 4 and 1.7 at two separate stations respectively) between tubificid worms and sediments. Bindra and Hall (1977) had noted earlier that tubificid worms had a BAF greater than 1 for Cu, Zn, Pb, and Mn, from sediments at the lower reach of the same river. These variations in reports may indicate differences in feeding habits, as well as in local bioavailability of metals and metal excretion mechanisms.

Enk and Mathis (1977) evaluating baseline concentrations of Cd and Pb in Jubilee Creek, Peoria County, Illinois, found that concentrations of Cd in aquatic insects were about five times those of the sediments and over 10 times those found in water. Pb concentrations, on the other hand, were similar between sediments and aquatic insects and highest in snails. Farag et al. (1998) found that body burdens of the metals As, Cd, Pb, Zn, and Cu correlated positively with concentrations in sediments and biofilm in the Coeur d'Alene River, which has a history of mining waste deposits. No biomagnification was occurring except for Cu, the highest concentration of which was found in the engulfers. Zauke (1982) showed that elevated levels of Cd in five *Gammarus* species occurred in most contaminated localities of different rivers in north-western Germany.

Eyres and Pugh-Thomas (1978), comparing bioaccumulation of Pb, Cu, and Zn between a scud (*Asellus sp*.) and a leech (*Erpobdella sp*.) in River Irwell, Lancashire, observed that the leech had lower CFs for Pb and Cu and a higher CF for Zn whereas the opposite was found for the scud. However, they also observed spatial differences in accumulation efficiency based on bioavailability and sediment concentration. In a comprehensive study on the same River, Dixit and Witcomb (1983) found that levels of Cu, Pb, and Zn were much higher in animals collected from areas with higher sediment concentrations of these metals for all animal species, and invertebrates that fed directly on sediments showed even greater bioaccumulation rates.

Positive correlations between metals in sediments and those in macroinvertebrates were also found by Burrows and Whitton (1983) for Pb, Cd, and Zn in Mayflies, and Namminga and Wilhm (1977) for Cu, Zn, and Pb in Chironomids. Although metal uptake has been shown to be variable depending on organisms (Prosi, 1979; Burrows and Whitton, 1983), many authors have demonstrated that the major pathway for uptake and bioaccumulation is through food (Patrick and Loutit 1976; Brown, 1977). Pip (1992) found that although concentrations of Cd, Cu, and Pb were not significantly correlated with levels in sediments of the Lower Nelson River, Manitoba, Canada, food was the main source of metals for the gastropods.

To provide further evidence of bioaccumulation of metals by aquatic macroinvertebrates, Tochimoto et al. (2003) transferred instar V larvae of *Stenopsyche marmorata* from a non-contaminated site to a metal contaminated site in the Tamagawa River, near Tokyo. Metal concentrations in the organisms increased rapidly and the organisms synchronized their Ni, Cu, Zn, and Pb body burdens to approach those of native larvae within 5 to 30 days. Fialkowski et al. (2003) used bioaccumulated concentrations of Cd, Pb, and Zn to provide evidence of general contamination of the River Biala Przemsza system by these three metals. Gerhardt et al. (2005) found that metal body burdens of the Mayfly *Choroterpes picteti* exposed to acid mine drainage (AMD) in the Monteirão Stream were significantly higher than those of individuals of the reference water from River Vascão (SE Portugal). In a holistic study on mercury pollution in the Ganga River system, Varanasi, India, Sinha et al. (2007) found bioaccumulation factors of 3×10^2 in sediment and 5×10^2 in benthic macroinvertebrates.

Patrick and Loutit (1976) reported bioacumulation and trophic transfer of Cr, Cu, Mn, Fe, Pb, and Zn from bacteria to tubificid worms. Also, the metals were transmitted and biomagnified up to the fish that fed on tubificid, and Pb had the highest transfer efficiency. Nikanorov et al. (1988) found that aquatic macroinvertebrates bioaccumulated Hg irrespective of their food type. Food may be just one of numerous means of bioaccumulation of heavy metals. However, many studies have shown that for many benthic taxa and for many metal species, body burdens are more often than not, correlated with levels in their food. Also, biomagnification has been shown to occur for many metals from benthic macroinverbrates to their predators. In a study of bioamplification in the marine environment, Bryan (1976) reported that the crab *Carcinus maenas* absorbed 31% of the Cu, 58% of the Zn, and 35% of the Fe from its prey *Nereis diversicolor* (a marine polychaete). In another study, *N. diversicolor* was shown to transfer up to 25% of its Co, 18-36% of its Zn, 32% of its Fe, and 37-40% of its Mn contents to its fish predator (Pentreath, 1973a, b, 1976). A study by Young and Mearns (1979) on food webs impacted by sewage discharges from Los Angeles County, CA, reported that biomagnification occurred for Hg but not for Ag, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn.

Some authors have, however, refuted the possibility of biomagnification of metal contaminants in aquatic food chains. This is the case of Burrows and Whitton (1983) who examined tissue concentrations of metals in a number of taxa from the River Derwent, England, and found that concentrations of Pb, Cd, and Zn from sites close to mining activities were highest in the mayflies and lowest in carnivorous stoneflies. Clements and Kiffney (1994) studied the impact of heavy-metals at the Arkansas River, Colorado, and found that although concentrations of Cd, Cu, and Zn in the organisms were relatively higher at contaminated sites, levels were generally much higher in periphyton than in benthic macroinvertebrates. Also, levels in the mayfly *Baetis spp*. (Grazer) were usually higher than in other benthos. Farag et al. (1998) found that although metals in sediments of the Coeur d'Alene River are bioavailable and do biotransfer, they do not biomagnify.

Nonetheless, the absence of proof of bioaccumulation or biomagnification has been most often associated with chemical composition of the surrounding environment, metal speciation, difference in metabolism, and life stages. Bindra and Hall (1977) showed that tubificids for instance have the capacity to bioregulate their tissue levels of Cu, Fe, Pb, and Zn and thus may not transfer all the metals initially bioaccumulated to their predator. Jop and Wojtan (1982) associated the non-consistence of Cd and Pb contaminant levels in streams with levels in aquatic invertebrates to the high Ca concentrations in streams of Southern Poland. Basic biological differences among species have been shown to be more important than body size in determining rates of uptake of Cu and Cd (Buchwalter and Luoma, 2005).

Moreover, whereas bioaccumulation may be an indication of metal pollution, negative effects on the individual organism and the BMI community structure most often give a better idea as to the extent the perturbation caused to the aquatic biota. Many workers have shown that aquatic insect taxa and individuals tend to reduce in number in rivers polluted from metal mining (Dills and Rogers, 1974; Armitage, 1980; Chadwick et al., 1986, Roline, 1988). A number of investigations have reported that mine effluents influence reduction of macroinvertebrate communities (Clements et al., 1988; Roline, 1988; Moore et al., 1991). In a toxicology assessment study of two rivers in the Northern Pennine Orefield, Northern England, Abel and Green (1981) reported significant qualitative and quantitative restrictions of invertebrate fauna in River West Allen, Cumbria (UK) compared to unpolluted rivers. Moreso, Winner et al. (1980), Clements (1991), and Clements and Kiffney (1994) have indicated that exposure of stream benthic communities to heavy metals usually results in effects such as reduced abundance, reduced species richness, and a shift in community composition.

The results of the studies cited herein were obtained either in field lotic settings, micro- or mesocosms, or in laboratory bioassays. These studies provide substantial evidence that macroinvertebrates are the most reliable biomonitors of metal pollution in lotic systems. Although some of these studies suggest that sediments may not always be the main source of metals and that these metals do not always biomagnify, a close examination of their results reveals that food is always the most important metal transfer pathway and particulate food in natural lotic systems is always associated with sediment components. Furthermore, almost all of the studies used individual species or feeding guilds to examine bioaccumulation rates and toxicity levels. However, a survey of metal body burdens of different taxa pooled into functional feeding guilds gives a better inference into accumulation risks that face members of the different members of the aquatic trophic chain. Most of the studies also reveal that biotic metrics, more often than not, provide a positive correlation with metal contamination.

MATERIALS AND METHOD

Benthic macroinvertebrates (BMIs) and stream bed sediments were collected from four different locations in the Gallinas River, Northern New Mexico. Sampling took place on two days during low flow seasons on April 14 and October 10, 2006, in order to minimize the influence of high runoff pulses of heavy metals (Figure 2). Periods of high flow alter stream habitat characteristics, wash organisms downstream and may influence temporary hikes in metal body burdens. Consequently, BMIs collected during high flow episodes may not be representative of the community that normally inhabits an area. At each location, sediments were collected at random while BMIs were collected in riffle sections within an approximately 100m long reach. Samples collected during April 2006 were intended to represent spatial variability with respect to snow runoff that occurred during spring snowmelt. October 2006 samples were intended to represent the effects of runoff and erosion from summer 2006 rainfalls. The two sampling periods were intended to detect seasonal changes in metal concentrations. Physical conditions of the sampling location were collected during each sampling period to determine how these parameters influence the distribution of BMIs or metal bioavailability.

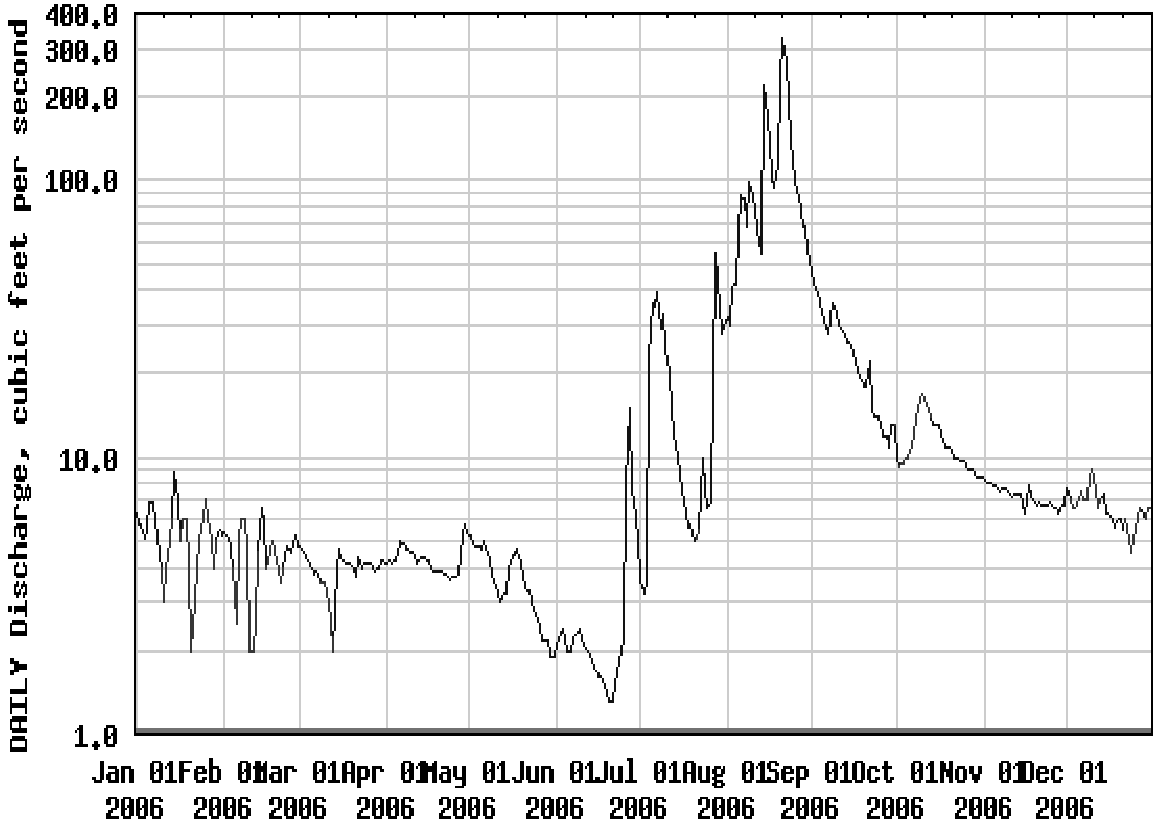


*Figure 2***.** Daily mean discharge in the Gallinas River near Montezuma for the Year 2006 (USGS, 2007)

Site Selection

Samples for the present study were collected at four locations in the Gallinas River. These sampling sites are the same ones that previous researchers in the Gallinas River studied. They represent different stream orders and reflect the influence of the geology and the different land uses along the Gallinas River on the concentrations of heavy metals in the sediments and in the BMIs. Stream order was determined by the physiographic configuration of tributary creeks above a sampling site and by using a USGS 7.5-minute map. Table 1 provides geographical coordinates of sampling locations.

Table 1. *Geographical coordinates of sampling locations*

Study site	Longitude	Latitude	Elevation (m)
UO1	N 35^{o} 43.427’	W 105^{o} 30.606’	2628
UO2	N 35^{o} 39.119’	W 105^{o} 19.133’	2110
UO3	N 35^{o} 34.133’	W 105^{o} 12.550’	1939
UO4	N 35^{o} 33.871’	W 105^{o} 12.753’	1929

The first selected sampling site, UO1, is northwest of Las Vegas, NM, on the eastern flank of the Sangre de Cristo Mountains, at the end of Highway 65 (FR 263), and it is designated as first order. UO1 is located at an elevation of 2628 m in the heavily forested area of the Elk Mountain (Figure 1). Here, there is little or no anthropogenic activity as it is reserved for hiking, sightseeing, and fishing; activities that, of course, depend on weather conditions and permission from the US Forest Service. This site was expected to represent background concentrations of metals and was therefore designated the reference site for this study. Its waters derive mainly from runoff. It is dominated by spruce-fir, ponderosa pine, and aspen trees. The soil is covered by litter and could be described texturally as silty-clay.

The second site, UO2, located northwest of Las Vegas, at the site of the USGS gauging station (No. 08380500) is some thirty kilometers downstream from UO1 and roughly one kilometer above the diversion to the City’s Drinking Water Treatment Plant (DWTP). This site is situated at an elevation of 2110m. The surrounding vegetation consists of ponderosa pine and juniper. The riparian area, which is mostly rocky, has some scanty hedges of grass and willows. This site has limited human activities, except prescribed thinning by the US Forest Service. Based on the number of tributaries (Porvenir Creek is the only perennial tributary) and the limited extent of anthropogenic activities in the watershed of this location, UO2 was designated as second order and was selected to distinguish background metal concentrations associated with the heavily erodible canyon above UO2.

The third sampling site, UO3, is located approximately two kilometers southeast of Las Vegas, and about 100 m above the Wastewater Treatment Plant (WWTP). At an elevation of 1939 m, this site has unstable banks. The streambed is made up of about 90% compact bedrock with silt, sand, and gravel comprising the remaining 10%. Thick grass, about 0.1m high, and willows dominate the riparian area. Cattle grazing is the main activity in the area, causing the stream bed to be covered by algae. Site UO3 was selected to detect metal inputs from erosion and the extensive anthropogenic activities of the City, as well as a number of tributaries that flow into the Gallinas River before reaching this site.

UO4 is the last sampling reach and is located downstream and southeast of UO3, some 100m below the WWTP at an elevation of 1929 m. UO4 was selected to represent the influence of inputs from the WWTP. Water quality at this site is affected by the integrated impairments deposited at different levels along the course of the River and discharges from the WWTP. The stream bed is composed of 20% embedded rocks, 30% cobble, about 20% gravel, and 30% silt and sand. The right bank is unstable while the left bank is made up of compact steep rock limiting the floodplain. The riparian area is covered by grass and a few juniper trees. UO3 and UO4 were designated as second order because all the tributaries entering the River downstream of site UO2 are seasonal streams (Figure 1).

Sample Collection, Preparation, and Analysis

During sampling, a number of environmental parameters were collected. Environmental parameters are important in determining metal toxicity because they affect the bioavailability of toxicants and more often than not, they play an important role in determining the availability of the BMIs in general. The parameters measured during sampling were water temperature, specific conductance, dissolved oxygen (DO), pH, hardness, oxygen saturation, and alkalinity. Dissolved oxygen, oxygen saturation, and water temperature were measured using the YSI portable DO meter model EcoSense DO200. pH was measured using the YSI handheld pH and Temperature System model 60. Specific conductance was measured with a YSI handheld model 30 conductivity instrument. Alkalinity and hardness were analyzed in the laboratory following the Hach buret titration method 8221 and 8222 respectively (Hach Company, 2003).

Benthic macroinvertebrates (BMIs) were collected using a modified hand-held circular Hess sampler with a 1.0mm diameter mesh (Jacobi, 1978). The samples were collected with the minimum amount of water possible and put in sterilized polyethylene bags. A sufficient amount of 95% Ethanol was added before each sample was put in a cooler containing ice blocks to maintain the samples at $0^{o}C$. The samples were then taken to the laboratory at New Mexico Highlands University where the BMIs were sorted, separated according to taxonomic groups, counted, and stored in 5ml glass sample vials filled with 95% ethanol. Visual identifications were verified by microscopic examination using standard identification keys provided by several publications (Merrit and Cummins, 1996; Voshell, 2002; McCafferty and Provonsha, 1998; and Ward and Kondratieff, 1992). After the BMIs were separated into families, they were then sorted into four functional feeding guilds, namely grazers, collectors, shredders, and predators; the categories are based on classification provided by Farag et al. (1998), Vanote et al. (1980) Rohasliney and Jackson (2008), and Cain et al. (1992).

A revised procedure by Lynch et al. (1988) was followed for sample preparation and chemical analysis. The samples were oven-dried for 24hrs. Because of the small number of individuals and the small dry-weight found for some feeding guilds at certain sites, entire samples were used in the digestion process. All glassware, beakers, forceps, sample bags, and vials were pre-cleaned with 10% nitric acid (HNO_3), then rinsed with high-purity double de-ionized water (DDI water) purified with a UV/UF (ultraviolet and ultrafilter) analytical-reagent grade water-purification system (Barnstread EASYpure II). After cooling in the desiccator for 24 hours, the BMIs were acid digested with redistilled HNO_3 and hydrogen peroxide (H_2O_2). The dried samples were placed in 50ml Teflon beakers with 10ml of concentrated HNO_3 for samples less than 1.0g; for concentrations of 1.0g and more, additional HNO_3 was used. Each mixture was gently heated for 1hr, allowed to cool, and 5ml of 30% H_2O_2 was added. Then the solution was heated gradually to a boil (approximately 10min) and 5ml of HNO_3 was added. The solution was then reduced to 10ml by boiling, followed by cooling. The cooled solutions were passed through a 0.2 micrometer (μm) membrane filter into polyethylene bottles and diluted with reagent water to various volumes within the linear range of the inductively coupled plasma mass spectrophotometer (ICP-MS) for analysis. The entire digestion process was done in a fume hood. All filters, beakers, and bottles were rinsed with 10% redistilled HNO_3 and DDI water before and after each digestion process to avoid cross contamination of samples and biasing of the results. Samples were stored at room temperature (about 25°C) until analysis.

Waterbed sediments were collected from the same sites as the BMIs, where possible, or at the nearest possible spots in the River, where enough silt-sized particles could be obtained within the 100m sampling reach. Acid-washed watchglass was used for the grab collection and the samples were stored at 0°C in acid-washed (10% v/v HCl) glass jars. The samples were later thawed and wet sieved through a 63μm pore-size stainless steel mesh to segregate the clay-size sediment fraction. Reagent water was used during sieving to help in the separation. The samples were left to settle and water was later decanted. Silt and clay-size sediments usually contain the highest concentrations of metals due to their negative electronic state and the high surface area to volume ratios associated with these materials. The clay-fraction sediments were oven-dried to a constant weight at 60°C for 24hrs in order to prevent the loss of possible volatile metallic compounds, and to facilitate sample grinding and sieving. The samples were later homogenized by grinding in a mortar and pestle, and dry-sieved through a 5μm pore-size polypropylene mesh. The mortar, pestle, and sieve were cleaned before and after every sample with 10% redistilled HNO_3 and rinsed with reagent water.

Digestion and analytes extraction in preparation for ICP-MS analysis were performed using an acid mixture procedure (Creed et al., 1994). One gram of each sediment sample was measured and transferred into a 250ml Phillips beaker to which 4ml of HNO_3 (1+1) and 10 ml of HCl (1+4) were added and the solution was covered with a watch glass. The beaker was then placed on a hot plate for extraction of the analytes at an adjusted reflux temperature of 95^{o}C. The sample was heated for two hours while avoiding vigorous boiling of the solution (though very slight boiling could be tolerated). Heating was done in a fume hood. The solution was left to cool and the extract was transferred into a 100ml volumetric flask. The extract was then diluted with reagent water, covered with a stopper and mixed. The sample extract was later left to stand overnight to separate the insoluble material. Filtration was carried out to remove suspended solids that could clog the nebulizer. Twenty ml of the filtered solution was pipetted into a 100ml volumetric flask, diluted to volume with reagent water to bring it within the linear range of the ICP-MS. Samples were analyzed as soon as possible to minimize the effect of the various matrices on the stability of the diluted samples.

A total of twelve sediment samples were digested, that is, three per sampling site. The sediment samples were fortified after digestion with various quantities of metals in an external fortification process. The amounts of metals added to various samples were not known to the analyst. This series of sediment analyses were used to determine the validity of the analytical procedure, including metal species interferences, and instrument detection limits.

Analysis of the sediments and macroinvertebrate samples for metals was done by Activation Laboratories Inc., Ontario Canada, using an inductively coupled plasma mass spectrophotometer (Perkin Elmer SCIEX ELAN 6100). Table 2 presents the detection limits for all investigated metals.

Table 2. *Instrumental detection limits of all metals investigated*

	Ag	As	Cd	Cr	Cu	Ni	Pb	Zn
Unit	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$
Detection Limit	0.002	0.1	0.01	0.5	0.01	0.1	0.01	0.1

Quality Assurance and Quality Control

To insure quality assurance and quality control, samples that had sufficient dry weight were divided and duplicate analysis was performed. All duplicate BMI samples were digested and internally fortified before analysis. Sediment samples were internally and externally fortified before analysis. Fortification gave the opportunity to improve validity of results because metal differences between fortified and unfortified solutions should equal the quantity of metal added to fortified BMI or sediment solutions.

International certified materials (USGS standards GXR-1, GXR-2, GXR-4 and GXR-6) were used as quality control reference materials and were digested and analyzed at the beginning and end of each set of sediment samples. Reference tissues for quality control of BMI analysis were not available. Internal control standards were analyzed every 10 samples and a duplicate was run for every 10 samples. Sample data are reported in units of $\mu g.g^{-1}$ dry weight.

For each type of material analyzed a method blank consisting of all reagents and procedures used in a particular digestion, with the exception of the sample material, was prepared and analyzed to determine internal contagion associated with sample manipulation and digestion procedure. Results of the external fortification process for the sediments and quality control measures are shown in Appendix B.

A rinse blank was used to flush the system between solution changes for blanks, standards, and samples. Sufficient flushing time was allowed and each sample was aspirated for 30sec before the collection of the data to establish equilibrium.

Data Treatment and Analysis

It is not always easy to analyze water quality data because most often they are characterized by non-normal distributions, seasonality, missing values, values below the limit of detection, just to name a few (Hirsch et al., 1982). For these reasons, a mixture of methods is usually employed to give the most adequate explanations of variability in data points (Hirsch et al., 1982; Farag et al., 1998).

The biological relevance of aquatic contaminants are usually evaluated by comparing their concentrations to aquatic sediment quality standards. Several guidelines have been established by Long et al. (1995) and Smith et al. (1996). These are relevant, based on the type of contaminant, its concentration and the time of exposure (acute or chronic). For the purposes of this study, the Threshold Effects Levels (TEL), the Probable Effects Levels (PEL), the Effects Range-Median (ERM) and the Upper Effects Threshold (UET), were considered as sediment screening criteria because they have been used in similar aquatic sediments assessment studies (Buchman, 1999; MacDonald et al., 2000; Ingersoll et al., 2000). Sediment concentrations of each metal were compared to these criteria to determine if these metals are major stressors to the aquatic biota.

Ecological effects of individual environmental contaminants are often difficult to determine. Any successful prediction and inference of long-term trends require understanding temporal and spatial variations (Mcguire, 1990). Statistical analyses were done with the SPSS software for Windows version 13.0.1 (SPSS, 2004). Spatial and temporal differences in metal concentrations in streambed sediments were determined using the nonparametric Kruskal-Wallis method. Post-Hoc tests were used to follow-up the findings in cases where significant differences existed and the Bonferroni correction was applied to adjust the level of significance of all effects to 0.5/6 = 0.0083.

Biological metrics were derived from the BMI community distribution at each site. Data analysis of the BMI assessment was based on the seven-criteria metrics of Plafkin et al. (1989). The metrics or indices of comparison are the following:

- Standing crop: It is simply the BMI density or number of individuals per square meter. This metric is used because aquatic biota generally responds to organic enrichment by an increase in standing crop while low standing crops usually indicate the presence of toxins or habitat degradation.
- The EPT/(EPT plus Chironomidae): This is the total number of organisms belonging to the Ephemroptera (E), Plecoptera (P), and Trichoptera (T) divided by the number of EPT plus Chironomidae. This index gives a measure of community balance. A value close to 1 indicates an even distribution whereas a lower ratio indicates high numbers of Chironomids denoting a poor biotic condition.
- Taxa richness: It represents the number of taxa per site. A good water quality is usually accompanied by a high number of taxa while degradation in water quality usually leads to reduction in number of taxa present.

- EPT index: This represents the number of taxa in the perturbation-sensitive Ephemroptera (E), Plecoptera (P), and Trichoptera (T) orders. The higher the number of individuals of these taxa present, the better the water quality.
- The CTQd value: This is the community tolerance dominance quotient, which was used as the basic tolerance metric for nonorganic disturbances in the bioassessment protocol by Winget and Mangum (1979). Based on individual taxa tolerances, which ranged from 2 to 108, the CTQd of a site is estimated as the summation of the product of each taxa's tolerance value by the number of individuals in that taxa divided by the total number of individuals of that site. Values close to 100 indicate a population dominated by more tolerant taxa while values less than 60 indicate sensitive organisms.
- The percent dominant taxon: This is the number of organisms in the taxon that has the highest number of individuals in the total community. A dominance by one or few taxa is usually indicative of a stressed environment.
- Community loss: This is a measure of the similarity between a reference site and a comparison site. Similarity amongst the two sites is expressed by low values whereas the opposite indicates disparity.

Scores were assigned to the different metrics to characterize the BMI community at each site. Except for percent dominant taxon and community loss, scores were assigned to the metrics according to their comparability following the criteria in Table 3. Scores were then totaled and each total compared with that of the reference site (site UO1) and to represent the biotic condition for a given site (Table 4).

Table 3. *Criteria followed in giving scores to biotic metrics according to Plafkin et al. (1989)*

[%, percent; <, less than; >, greater than; ≥, equal to or greater than]

Metric	Scoring criteria for score of 6	4	2	0
Standing crop[1]	50-149%	35-49% or 150-199%	20-34% or 200-249%	<20% or ≥250%
Taxa richness[1]	≥80%	60-79%	40-59%	<40%
CTQ_d[2]	≥85%	70-84%	50-69%	<50%
EPT/(EPT+Chironomidae)[1]	≥75%	50-74%	25-49%	<25%
Percent dominant taxon[3]	<20%	20-29%	30-39%	≥40%
EPT index[1]	≥90%	80-89%	70-79%	<70%
Community loss[4]	<0.5	0.5-1.4	1.5-3.9	≥4.0

Note. [1]Score is a ratio of study site to reference site x 100
[2] Score is a ratio of reference site to study site x 100
[3]Actual percent composition for study and reference sites, not percent comparability to the reference site
[4]Range of values obtained incorporates a comparison with the reference; therefore actual index values are used

Table 4. *Bioassessment attributes followed while rating water quality condition categories (following Plafkin et al., 1989)*

[%, percent; <, less than; >, greater than]

Percent comparability to reference score	Biological condition category	Attributes
>83%	Nonimpaired	Comparable to the best situation to be expected within an ecoregion. Balanced trophic structure. Optimum community structure (composition and dominance) for stream size and habitat quality.
[1]54-79	Slightly impaired	Community structure less than expected. Composition (species richness) lower than expected due to loss of some intolerant forms. Percent contribution of tolerant forms increases.
[1]21-49	Moderately impaired	Fewer species due to loss of most intolerant forms. Reduction in EPT index.
[1]<17	Severely impaired	Fewer species present. If high densities of organisms, then dominated by one or two taxa.

Note. [1]Percentage values obtained that are intermediate to the indicated ranges require subjective judgment as to the correct placement. Use of the habitat assessment and physicochemical data may be necessary to aid in the decision process.

In addition to the seven-criteria metrics, a Shannon Index (Shannon and Wiener, 1949) was also calculated for each sampling site to evaluate evenness of taxa distribution across the sites. A fairly evenly distributed community amongst all taxa indicates good water quality whereas a community dominated by a few taxa indicates stress.

The sediment bioaccumulation factor (BAF) of trace metals in aquatic invertebrates, according to Harraby and Clements (1997), was used to determine the extent to which metals were concentrated in tissues of BMIs. This factor is the same as the concentration factor of Reynoldson (1987). The bioaccumulation factor is calculated using the following formula:

$$\mathrm{BAF} = C_{\mathrm{org}}/C_{\mathrm{sediment}}$$

Where C_{org} is the element mass fraction in the organism (μg.g^{-1} dry weight) and C_{sediment} is the element concentration of the sediment (μg.g^{-1} dry weight).

Metal concentrations in streambed sediments were correlated with those in the different macroinvertebrate functional feeding guilds using the nonparametric Spearman's Rho correlation factor (r_s) to evaluate the response of the biota to metals in sediments. Selected biotic metrics were also correlated with sediment metals to determine the effect of the latter on the macroinvertebrate community structure and their influence on the River's aquatic biota as a whole.

To assess the influence of the heavy metals collectively, as they occur in the environment, a metals index (Giddings et al., 2001) was calculated for each site. Concentrations (μg.g^{-1}) of individual metals in sediments were standardized for each sampling site on a scale of 0 to 10 based on the following formula:

$$\text{Metals index} = \sum_{i=1}^{N} \left(\frac{X_i}{X_{imax}}\right)10$$

Where:

N is the number of metals in the index

X_i is the concentration of one of the N metals at a site

X_{imax} is the maximum concentration of the metal observed at all sites

A correlation (r_s) between the metals index and the selected biotic metrics was performed to assess the relationship of the cumulative effect of all metals present and the response of the biological community.

The biotic metrics selected for comparison with metal contamination were the Shannon Index, standing crop, CTQd, EPT/ (EPT plus Chironomidae), taxa richness, and percent dominant taxon.

RESULTS

A total of 24 sediment and 24 BMI samples were collected from all four sites during the two sampling seasons. Sediment metal concentration guidelines found in the literature were used to establish the possibility of an environmental risk at the study sites. In order to determine the potential cause and effect relationship between metals in sediments and the response of macroinvertebrates, metal concentrations in sediments were considered first, followed by those in BMIs, then community structure responses. The BMI samples were analyzed as composite samples for each sampling site and date because in some replicate samples the number of organisms of some feeding guilds was very small and their dry weight was below the minimum requirement for the ICP-MS analysis.

Environmental Field Parameters

Results of the environmental parameters measured at the time of sampling were primarily below New Mexico water quality standards (Table 5). Temperature ranged between 1.1^{o}C and 16^{o}C. Specific conductance ranged from 62 at site UO1 to 1000 µS/cm at site UO3 during the Spring sampling. pH values were between 7.8 and 8.6. Alkalinity was lowest at site UO1 during both sampling, and relatively high at sites UO2, UO3 and UO4. DO values ranged between 7.4 and 8.9mg/l during the Spring sampling and between 10 and 13.12mg/l during the Fall sampling. Oxygen saturation ranged from 84.3% to 107.6%. Hardness ranged from 40 at site UO1 to 305mg$CaCO_3$/l during the Spring sampling and between 65 and 275mg$CaCO_3$/l during the Fall sampling.

Table 5. *Environmental parameters at the four sampling sites at time of sampling during the two sampling dates.*

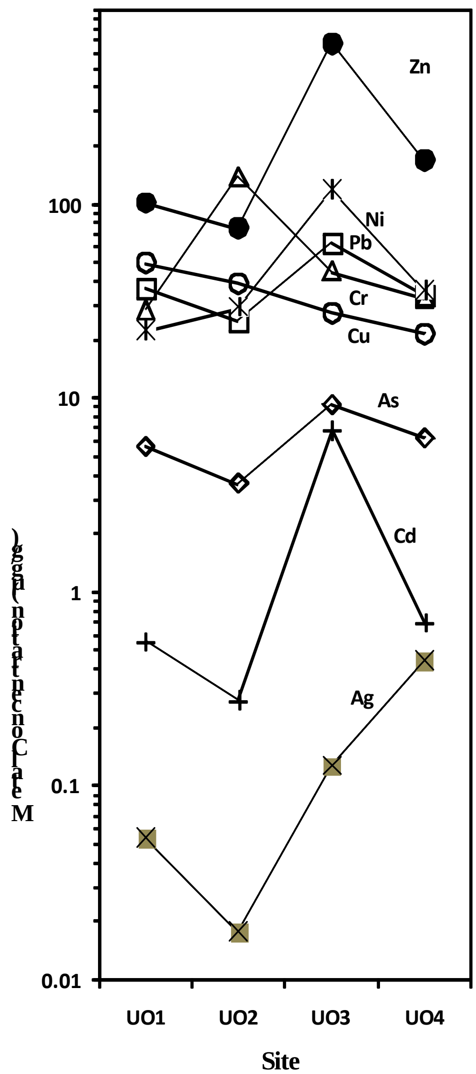
Sampling Season	Site	Temp. (^{o}C)	Conduct. (µS.cm^{-1})	pH	Alkalinity ($mgCaCO_3/l$)	DO (mg/l)	Oxygen saturation %	Hardness ($mgCaCO_3/l$)
		Parameter						
Spring 2006	UO1	3.0	62	8.1	48	8.9	93.0	40
	UO2	16	210	8.6	144	7.4	97.2	65
	UO3	9.5	1000	7.8	170	7.7	85.4	305
	UO4	12.4	800	8.0	110	8.8	104.7	225
Fall 2006	UO1	1.1	130	8.2	67	13.12	92.4	65
	UO2	5.4	248	8.6	200	11.98	84.3	90
	UO3	12.0	943	7.98	165	11.67	107.6	275
	UO4	13.2	921	8.0	145	10.0	95.00	250
	NMWQCC[1]	< 20	< 300	6.6-8.8	--	>6.0	> 85.0	--

Note. NMWQCC – New Mexico Water Quality Control Criteria

Sediment Metal Distribution

Results of the ICP/MS analyses of the sediments from the different study sites are found in Appendix C. Mean sediment concentrations indicate that during both sampling dates and at all sites, Zn concentrations were generally the highest, ranging from 74.9 to 683μg.g^{-1} in the Spring and from 71.63 to 235.33μg.g^{-1} in the Fall (Appendix D). However, the mean concentration of Cr (138.67μg.g^{-1}) was the highest at site UO2 (Figure 3 and Appendix D). Mean concentrations of Ag were consistently the lowest at all sites during both sampling dates and ranged from 0.02 to 0.44μg.g^{-1} in the Spring and from 0.02 to 0.15μg.g^{-1} in the Fall. Dispersions were concordant with mean concentrations for all metals and at all sites except at site UO1 where Pb had the highest standard deviation (±13.5) whereas its concentration of 37.2μg.g^{-1} was lower than those of Cu (49.97 $\mu g.g^{-1}$) and Zn (101.5 $\mu g.g^{-1}$) which had standard deviations of ±11.12 and ±12.9 respectively (Appendix D).

(a)


Metal Concentration (ug/g)
Zn
Ni
Pb
Cr
Cu
As
Cd
Ag
100
10
1
0.1
0.01
UO1
UO2
UO3
UO4
Site

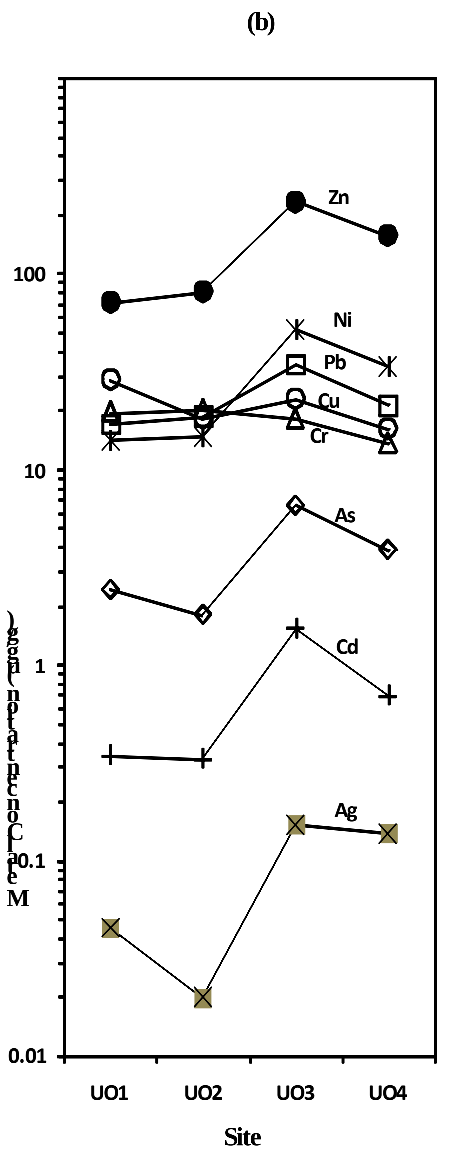


Figure 3. Average distribution of sediment metal concentration at each of the four sites along the Gallinas River for Spring 2006 (a) and Fall 2006 (b).

Temporal Distribution

Overall, streambed sediment metal concentrations in Fall 2006 showed a decrease compared to those of Spring 2006 (Figure 3). The reverse was observed for a few cases such as Zn at site UO2 where mean concentration during the Fall (81.47 $\mu g.g^{-1}$) was higher than during the Spring (74.9 $\mu g.g^{-1}$), Cd at UO2, where the mean concentration in the Spring (0.28 $\mu g.g^{-1}$) was lower than in the Fall (0.33 $\mu g.g^{-1}$), and Ag at site UO3, where the mean concentration in the Fall (0.15 $\mu g.g^{-1}$) was higher than in the Spring (0.13 $\mu g.g^{-1}$). Statistical assessment reveals that there was no significant difference in metal concentrations between the two sampling dates at all sites, and for all metals, as indicated by significance level using the Mann-Whitney *U-test* given in Table 6 (all *p*-values > 0.05).

Table 6. *Significance level (p-values) of Mann-Whitney tests comparing temporal metal concentrations at the four study sites along the Gallinas River*

	Metal							
Site	As	Cd	Cr	Cu	Pb	Ni	Ag	Zn
UO1	0.05	0.15	0.1	0.1	0.1	0.1	0.35	0.1
UO2	0.05	0.5	0.5	0.25	0.2	0.05	0.5	0.5
UO3	0.1	0.05	0.05	0.1	0.1	0.05	0.35	0.05
UO4	0.5	0.15	0.05	0.1	0.1	0.2	0.1	0.5

Spatial Distribution and Aquatic Sediment Quality Criteria

Metal concentrations in sediments were generally highest at site UO3 during both sampling dates for all selected metals except for Cr and Cu. The comparison of sediment metal concentrations with the screening criteria indicate that at least one metal's concentration in sediments exceeded the metal screening criteria for seven of the eight metals at all sites and for both sampling dates (Figure 4).

The highest mean concentration of As in sediments was recorded at site UO3 during the Spring 2006 sampling ($9.3\mu g.g^{-1}$) and was about 1.5 fold higher than TEL ($5.9\mu g.g^{-1}$), but lower than the PEL ($17\mu g.g^{-1}$) for this metal. Site UO2 of Fall sampling had the lowest mean concentration for this metal (Figure 4 and Appendix D). Statistical analysis reveals that these differences in As concentrations were significant between sites ($H(3) = 12.79$, $p < 0.05$) (Table 7). Post-hoc comparisons revealed that the difference was found only between site UO2 and site UO3 ($z = -2.56$) (Table 8).

With a mean concentration of $6.79\mu g.g^{-1}$, site UO3 had the highest level of Cd while site UO2 had the lowest ($0.3\mu g.g^{-1}$) and both these concentrations were observed during the Spring 2006 sampling. The above-mentioned Cd concentration at site UO3 was as much as tenfold higher than the TEL ($0.596\mu g.g^{-1}$) and about 1.9 fold higher than PEL ($3.53\mu g.g^{-1}$) (Figure 4 and Appendix D). Statistical analysis (Table 8) indicates that significant differences existed in Cd concentrations in sediments between sites ($H(3) = 14.47$, $p < 0.05$). The significant differences were between site UO3 and both sites UO1 ($z = -2.89$) and UO2 ($z = -2.88$) for this metal (Table 8).

With the highest mean concentration of sediment Cr, site UO2 ($138.7\mu g.g^{-1}$), during Spring 2006 sampling period, was enriched 1.5 fold compared to PEL ($90\mu g.g^{-1}$) and 3.7 fold compared to TEL ($37.3\mu g.g^{-1}$). The lowest concentration was found at site UO4 ($13.7\mu g.g^{-1}$) during the Fall sampling (Figure 4 and Appendix D). However, statistical comparisons reveal that there was no significant difference in sediment Cr concentrations between sites ($H(3) = 2.85$, $p = 0.42$) (Table 7).

Although Cu concentrations all fell below the PEL criterion value of 197μg.g^{-1}, the highest concentrations of this metal found at site UO1 (49.97μg.g^{-1}) and the second highest found at site UO2 (39.33μg.g^{-1}) during the Spring 2006 sampling both exceeded the TEL value of 35.5μg.g^{-1} (Figure 4 and Appendix D). Site UO4 had the lowest concentration with 21.57μg.g^{-1} of Cu. However, these site differences were not statistically significant ($H(3) = 2.61$, $p = 0.48$) (Table 7).

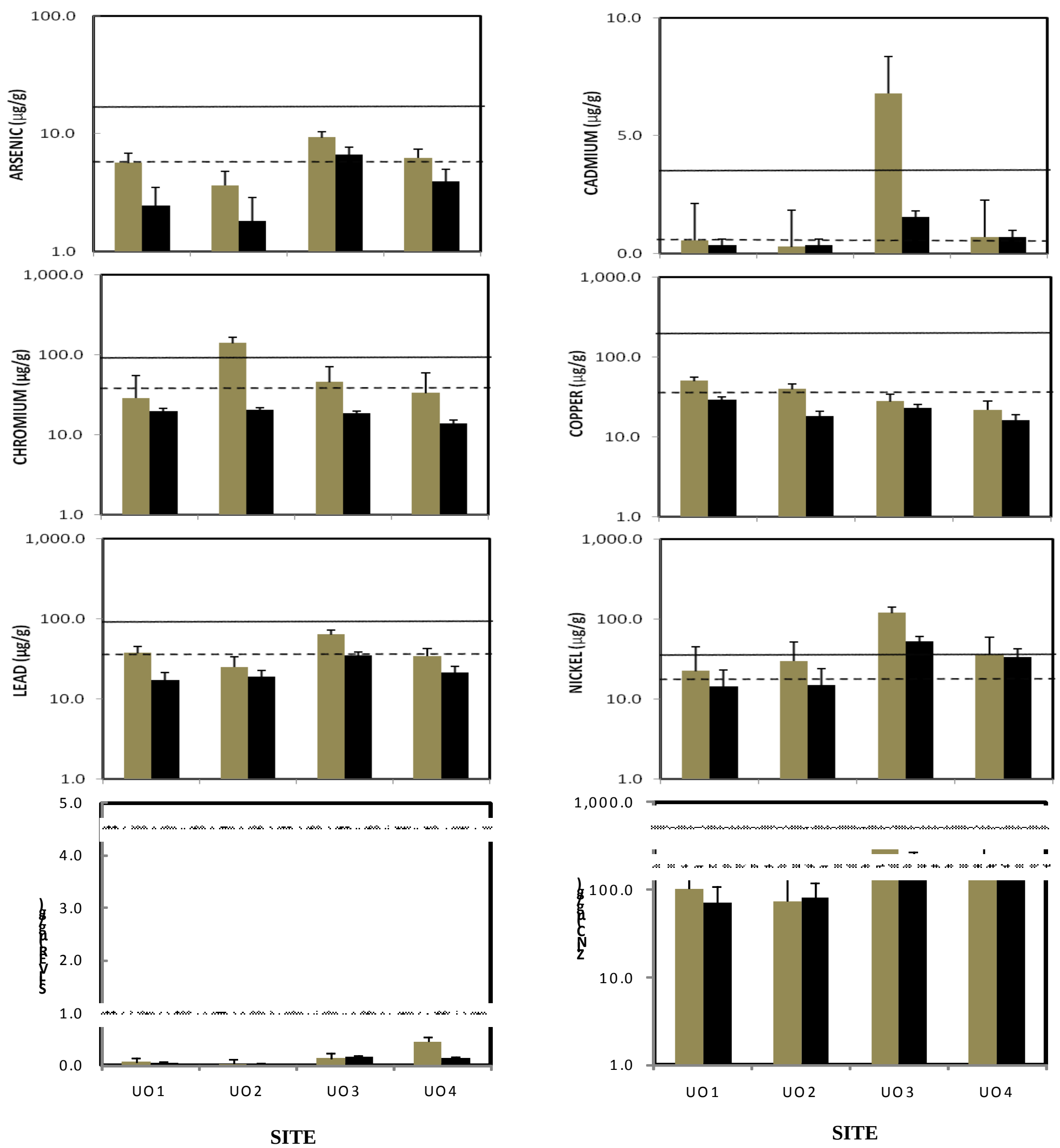
Pb concentrations were below the PEL (91.3 μg.g^{-1}) at all sites during both sampling dates and exceeded the TEL (35μg.g^{-1}) at site UO1 and UO3 during the Spring only. Site UO3 (63.47μg.g^{-1}), during the Spring, had the highest mean concentration which was found to be 1.8 fold higher than the TEL. Site UO1 during the Fall (14.13μg.g^{-1}) had the lowest mean sediment Pb level (Figure 4 and Appendix D). Table 7 indicates that site differences were also non-significant for this metal ($H(3) = 6.04$, $p = 0.11$).

Ni concentrations during the Spring sampling were higher than the TEL value of 18μg.g^{-1} at all sites. This was not the case during the Fall. The highest mean Ni concentration was found at site UO3 (119.1μg.g^{-1}) during the Spring, and was more than 3 fold higher than the PEL value of 36μg.g^{-1}. The lowest concentration of Ni was found at site UO1 (14.13μg.g^{-1}) during the Fall (Figure 4 and Appendix D). Site differences did affect Ni concentrations significantly ($H(3) = 17.0$, $p < 0.05$) (Table 7). Post-hoc tests reveal that the differences were between site UO1 and both sites UO3 ($z = -2.88$) and UO4 ($z = -2.88$), as well as between sites UO2 and UO3 ($z = -2.88$) (Table 8).

Mean concentrations of Ag in streambed sediments were all below both screening criteria, that is, the UET value of 4.5μg.g^{-1} and the ERM value of 1.0μg.g^{-1}, respectively

(Figure 4 and Appendix D). The highest concentrations were found at site UO4 ($0.44\mu g.g^{-1}$) during the Spring and the lowest was found at site UO2 ($0.02\mu g.g^{-1}$) during both sampling dates. Statistical analysis indicates that site differences did affect Ni concentrations significantly ($H(3) = 14.51$, $p < 0.05$) (Table 7). These differences were between site UO1 and site UO4 ($z = -2.56$), and also between site UO2 and both sites UO3 ($z = -2.56$) and UO4 ($z = -2.88$) (Table 8).

With the highest mean concentrations of Zn, site UO3 ($683\mu g.g^{-1}$) during the Spring, was the only site whose level exceeded the PEL value of $315\mu g.g^{-1}$, by more than 2 fold, and the TEL value of $123\mu g.g^{-1}$, by about 5.6 fold. The concentration at site UO1 during the Fall ($71.6\mu g.g^{-1}$) was the lowest of all Zn concentrations (Figure 4 and Appendix D). Statistical analysis indicates significant differences between sampling sites ($H(3) = 18.49$, $p < 0.05$) (Table 7). These differences were between site UO1 and both sites UO3 ($z = -2.88$) and UO4 ($z = -2.72$), and also between site UO2 and both sites UO3 ($z = -2.88$) and UO4 ($z = -2.88$) (Table 8).



EXPLANATION

(spring bar)	Metal concentration (Spring sampling)	- · - · ·	Upper effects threshold
(fall bar)	Metal concentration (Fall sampling)	— · · —	Effects range median
———	Probable effects level	μg/g	Micrograms per gram
- - - -	Threshold effects level	⊤	Standard Error

Figure 4. Concentration of selected metals in benthic sediments of the Gallinas River, Las Vegas, New Mexico, collected during two sampling dates in April and October 2006, in comparison with sediment quality guidelines.

Table 7. *Kruskal-Wallis tests to distinguish if heavy metal concentrations are equal in sediments at all four sampling sites in the Gallinas River (significance level p = 0.05)*

Metal	*H*-value	*df*	*p*-value
As	12.79	3	**0.001**
Cd	14.47	3	**< 0.0001**
Cr	2.85	3	0.434
Cu	2.61	3	0.477
Pb	6.04	3	0.110
Ni	17.1	3	**< 0.0001**
Ag	14.51	3	**< 0.0001**
Zn	18.49	3	**< 0.0001**

Note. Bold – significant *p*-value

Table 8. *Significance level of post-hoc tests (with the Bonferroni correction, significance level p = 0.0083) to determine site differences in sediment metal concentrations*

	Contrasted sites					
Metal	UO1 vs. UO2	UO1 vs. UO3	UO1 vs. UO4	UO2 vs. UO3	UO2 vs. UO4	UO3 vs. UO4
As	0.21	0.013	0.129	**0.001**	0.013	0.013
Cd	0.09	**0.001**	0.279	**0.001**	0.013	0.013
Ni	0.19	**0.001**	**0.001**	**0.001**	0.036	0.0084
Ag	0.04	0.07	**0.004**	**0.005**	**0.002**	0.15
Zn	0.19	**0.001**	**0.002**	**0.001**	**0.001**	0.013

Note. Bold – significant *p*-value

Metals Index

When the metals were considered together, values obtained for the Metals Index indicated that in the Spring, the cumulative effect was highest at site UO3 (61.7) below Las Vegas (Table 9). Sites UO1, UO2, and UO4 had fairly similar index numbers of 29.39, 30.10, and 35.32, respectively. However, site UO4 had the second highest cumulative concentration followed by site UO2 and site UO1 as third and fourth. The

Metals Index numbers in the Fall increased at all sites compared to those found in the Spring. With a Metals Index of 76.85, site UO3 still clearly had the highest index of all sites. Site UO4 maintained the second highest contamination with an index number of 50.99, whereas site UO1 became the third site in the contamination hierarchy with an index of 39.27. Site UO2 had the lowest index of 34.17.

Table 9. *Metals indices at four sampling sites along the Gallinas River during two sampling dates*

Site	Sampling period	
	Spring	Fall
UO1	32.09	39.27
UO2	32.23	34.17
UO3	63.20	76.85
UO4	36.49	50.99

Benthic Macroinvertebrates

Aquatic macroinvertebrates collected from the four sites combined and during the two sampling dates represented a total of 43 taxa. The Spring sampling had a total of 39 taxa (Appendix E) while the Fall sampling had a total of 40 taxa (Appendix F). Taxa representatives by order for both sampling dates are as follows: Ephemeroptera (Mayflies) – 5 taxa; Plecoptera (Stoneflies) – 6 taxa; Trichoptera (Caddisflies) – 8 taxa; Diptera (True Flies) – 9 taxa; Odonata (Dragonflies and Damselflies) – 1 taxon; Coleoptera (Water Beetles) – 3 taxa; Gastropoda (Snails and Limpets) – 1 taxon; Pelecypoda (Clams and Mussels) – 1 taxon; Hemiptera (Water Bugs) – 1 taxon; Lepidoptera (Water Moths) – 1 taxon; Annelida (Segmented Worms) – 2 taxa;

Nematoda (Round Worms) – 1 taxon; Nematomorpha (Horsehair Worms) – 1 taxon; Turbellaria (Flat Worms) – 1 taxon; Amphipoda (Scuds) – 1 taxon; Decapoda (Crayfish and Shrimps) – 1 taxon. Complete taxonomic and functional feeding guilds lists for the two sampling dates are given in Appendixes E and F.

Biotic Metrics

Based on the seven-criteria bioassessment (Plafkin et al., 1989), scores were very similar between the two sampling dates. Summary comparisons indicate that slight differences exist between sampling dates based on individual indices. Site UO1 was rated as non-impaired, site UO2 as slightly impaired, while sites UO3 and UO4 where both moderately impaired for both sampling dates (Appendices G and H).

Spring Sampling: Standing crops showed a positive trend ranging from a low of 1754.25 organisms per square meter (organisms / m^2) at site UO1 to a high of 9483.89 organisms / m^2 at site UO4 (Appendix G). Number of taxa per site decreased from 20 at sites UO1 and UO2 to 15 at site UO4. The Shannon Index (Shannon and Weaver, 1963) also decreased in an upstream-downstream direction and ranged from a high of 2.34 at site UO1 to a low of 0.59 at site UO4. This is concordant with the low percent dominant taxon found at site UO1 (26.82% for the Mayfly Baetidae), the high percent dominant taxon found at site UO4 (86.99% for the Chironomidae), and the drop in EPT index and the EPT/EPT + Chironomidae ratio from site UO1 to site UO4 (Appendix G). Although none of the CTQd values were below 60, those of UO3 and UO4 were above 100 (106.94 and 107.91).

Fall Sampling: Standing crops showed an increase at sites UO1, UO2 and UO3 compared to the Spring sampling but dropped drastically at site UO4 (Appendix H). Site

UO2 had the highest standing crop of 4353.64 organisms / m^2 followed by site UO3 (4180.04 organisms / m^2). Site UO1 had the third highest macroinvertebrate density with 2425.79 organisms / m^2 whereas site UO4 had the lowest (2046.62 organisms / m^2). Number of taxa per site were high at site UO1 (27) and site UO2 (23) but decreased at sites UO3 (13) and site UO4 (14). Diversity indices showed a similar trend with a high of 2.74 at site UO1 and a low of 1.43 at sites UO3 and UO4. The percent dominant taxon was still low at site UO1 (24.29% for the Caddisfly Limnephilidae), but decreased for site UO3 (21.42% for the worm Nematoda) while site UO2 saw its dominant taxon increase to 40.61% of the total community. Site UO4 still had the highest percentage of one dominant taxon (58.04% for the worm Nematoda). Nonetheless, the EPT index was still high for site UO1 (12) and site UO2 (10) compared to site UO3 (4) and site UO4 (3). The EPT/EPT+Chironomidae ratio had a similar pattern with site UO1 and site UO2 having high values (0.98 and 0.99, respectively) while site UO3 and site UO4 had lower values (0.26 for both). The CTQd values ranged from 80.68 at site UO1 to 100.18 at site UO4 (Appendix H).

Metal Bioassessment

Comparing the metal levels found in the macroinvertebrates with those of the streambed sediments revealed that bioaccumulation occurred for all metals studied in the Gallinas River. This was not the case for bioamplification. Bioaccumulation was considered efficient for cases where bioaccumulation factors were greater than 1. The total number of individual organisms composing each functional feeding guild and the concentration of selected metals in the feeding guilds are presented in Appendix I. No metal was bioaccumulated in a steady manner across all sites or for any feeding guild. Bioaccumulation factors (BAFs) for this study are found in Appendix J.

Spring Sampling

The Shredders had the highest BAFs for As (BAF = 12.78), Cd (BAF = 5.42), Cr (BAF = 0.28), Cu (BAF = 2.10), Pb (BAF = 2.02) and Zn (BAF = 2.84) at site UO1 (Appendix J). The Predators had the highest BAF for Zn (BAF = 3.69).

At site UO2, the Shredders again seem to be the highest bioaccumulators with BAFs being highest for As (BAF = 9.69), Cd (BAF = 15.9), Cu (BAF = 8.92), Pb (BAF = 5.7), and Zn (BAF = 11.39). The group with the second highest bioaccumulation was the Grazers, which accumulated Cr (BAF = 0.22), Pb (BAF = 6.22) and Ni (BAF = 3.05) more than the other groups.

At site UO3, the Collectors were so reduced that their pooled sample of all three replicates did not meet the minimum dry weight required for analysis using the ICP-MS. The Predators had the highest BAFs for all metals (0.76 for As, 0.96 for Cd, 0.66 for Cr, 99.82 for Cu, 1.26 for Ni and 1.12 for Zn). The Grazers did not bioaccumulate any metal above the level in their surrounding sediments. This was the only site where BAFs for Predators were higher than those of all other feeding groups. An abnormally high metal body burden was observed for Cu in Predators at site UO3 (1820μg/g). However, this observation was most probably due to a manipulation error of the ICP-MS (Appendix I). In spite of the high metal contamination observed in the sediments at this site (Appendix D), the bioaccumulation exceeded sediment concentration only rarely.

The Collectors group found at site UO4 bioaccumulated As (BAF = 1.72), Cd (BAF = 3.84), Cu (BAF = 2.46), and Zn (BAF = 2.76) at levels exceeding sediment concentrations, and more than the other groups. The Shredders bioaccumulated Pb (BAF = 0.63) at higher levels compared to the other groups. The Grazers bioaccumulated Cr (BAF = 0.29), while the Predators bioaccumulated Ni (BAF = 1.14) more than the other groups.

Fall Sampling

The first thing to notice here is that although concentration of all metals increased in the Grazers at site UO1 and site UO3, compared to the Spring, most groups showed a rather inconsistent pattern between the two sampling dates; some metals increased at some sites and for some feeding guilds and decreased at others. Instrument manipulation error was likely responsible for the unusually high BAFs observed for Cd in the collectors at site UO3 (BAF=140.52) (Appendix J).

The Grazers bioaccumulated Cd (BAF = 8.16), Cr (BAF = 1.93), Cu (BAF = 2.91), Ni (BAF = 1), and Zn (BAF = 5.28), more than the other guilds at site UO1. They were also the highest bioaccumualtors of As (BAF = 1.21), Cr (BAF = 0.44), Cu (BAF = 5.89), and Pb (BAF = 1) at site UO3. The Collectors were the highest bioaccumulators of As (BAF = 6.16) and Pb (BAF = 1.02) at site UO1, as well as of Cr (BAF= 0.26), Pb (BAF= 1.19), and Zn (BAF = 3.24) at site UO2. They also bioaccumulated Cd (BAF = 140.52), Ni (BAF = 10.25), and Zn (BAF = 23.37) at site UO3, and Cd ((BAF = 2.8), Cu (BAF = 1.87), and Pb (BAF = 0.33) at site UO4, at higher levels compared to the other groups. The Shredders were completely absent at sites UO3 and UO4. Their levels of Cu (BAF = 5.25) and Ni (BAF = 0.54) at site UO2 were higher than those of the other guilds. The Predators had the highest BAFs for As (BAF = 17.11) and Cd (BAF = 7.27) at site UO2 and for As (BAF = 0.44), Ni (BAF = 1.11), and Zn (BAF = 9.96) at site UO4.

Relationships between Metal Contamination in Streambed Sediments and Concentrations in BMIs

The relationship of metal concentrations in streambed sediments and BMIs from the four sampling sites was examined. All seven metals were detected in almost all BMI functional feeding groups, except for a few cases.

Nonparametric Spearman's correlations were used to determine the relationships between metals in BMIs and streambed sediments. Individual functional feeding group's metal accumulations were assessed using specific relationships between metals in Collectors, Grazers, Shredders, and Predators and corresponding sediment samples. Only correlation factors greater than 0.5 with a *p*-value less than 0.05 were considered significant.

Although most sediment metal concentrations increased at the downstream sites compared to upstream from the City, concentrations in BMIs did not follow this pattern for all feeding guilds. There was a limited number of cases where metal concentrations in Grazers and Shredders significantly related to corresponding sediment metal concentrations (Appendix J). Apparently As concentrations in sediment did not affect levels in corresponding BMIs in a positive way (r_s ranging from -0.52 to 0, $p > 0.05$). Concentrations of Cu in BMIs were only marginally to moderately related to those in corresponding sediments (r_s ranging from 0.18 to 0.6, $p > 0.05$). Pb concentrations in Collectors and Grazers were marginally related with those in corresponding sediments (r_s = - 0.07 and 0.17 respectively, $p > 0.05$) whereas those in Shredders and Predators were moderately related to sediment levels (r_s = 0.6 and 0.52 respectively, $p > 0.05$). Concentrations of Cd in Collectors increased (r_s = 0.85) with increases in sediment Cd concentrations (Table 10). Cd concentrations in the other three BMI feeding guilds seems to have only marginal to moderate relationships (r_s ranging from 0.14 to 0.56, $p > 0.05$) with corresponding sediment Cd concentrations. A significant correlation for Shredders and Predators (r_s = 0.82, and 0.77, respectively) exists with respect to Cr, whereas Cr concentrations in Collectors and Grazers were only moderately related to corresponding sediment concentrations (r_s = 0.54 and 0.36 respectively, $p > 0.05$). Zn concentrations in Grazers were not affected by those in sediments (r_s = -0.12, $p > 0.05$). Zn accumulation by Shredders only moderately correlated to levels in streambed sediments. Ni was the only metal whose concentrations in all four feeding guilds (r_s = 0.86, 0.71, 0.89, and 1 for Collectors, Grazers, Shredders and Predators respectively, $p < 0.05$) were strongly related to those in streambed sediments.

Table 10. *Correlation between sediment contamination and BMI concentration of metals (p-values in bracket, significance level at p = 0.05)*

	Metal						
Feeding Guild	As	Cd	Cr	Cu	Pb	Ni	Zn
Collectors	-0.31	**0.85**	0.54	0.18	-0.07	**0.86**	**0.71**
	(0.27)	**(0.01)**	(0.11)	(0.35)	(0.44)	**(0.01)**	**(0.04)**
Grazers	0	0.56	0.36	0.6	0.17	**0.71**	-0.12
	(0.51)	(0.07)	(0.19)	(0.06)	(0.35)	**(0.02)**	(0.39)
Shredders	-0.429	0.14	**0.82**	0.37	0.6	**0.89**	0.37
	(0.2)	(0.39)	**(0.04)**	(0.23)	(0.1)	**(0.01)**	(0.23)
Predators	-0.52	0.3	**0.77**	0.38	0.52	**1**	**0.76**
	(0.1)	(0.24)	**(0.01)**	(0.18)	(0.09)	**(< 0.001)**	**(0.01)**

Note. Bold – significant correlation between sediment and BMI samples metals concentrations

Relationship between Metal Contamination and Biotic Metrics

The relationships between metal contamination in streambed sediments and selected biotic metrics from respective study locations in the Gallinas River were examined. Relationships between Diversity Index, ratio of EPT/EPT+Chironomidae, CTQd, Standing Crop, and Percentage of Dominant taxon were correlated using Spearman's Rho factor against the Metals Index (Figure 5). Results reveal that the Metals Index was moderately negatively correlated with the Diversity Index ($r_s = -0.50$, $p > 0.05$) and EPT/EPT+ Chironomidae ($r_s = -0.48$, $p > 0.05$), and moderately positively correlated with CTQd ($r_s = 0.57$, $p > 0.05$) and Standing Crop ($r_s = 0.36$, $p > 0.05$). Percent dominant taxon on the other hand had a small negative correlation with the metals ($r_s = -0.14$). This indicates that, although moderately, diversity decreased as metal concentrations increased and the number of Chironomidae also increased as metal concentration increased. CTQd increased moderately with increase in sediment metal concentration. Standing crop also did increase as sediment metal concentrations increased, although very moderately. Percent dominant taxon on the other hand did not increase proportionately with an increase in streambed metals concentration. Site UO3 had the highest Metals Index during both sampling dates but the greatest standing crop was at site UO4 during the Spring and at site UO2 during the Fall (Appendices G and H).

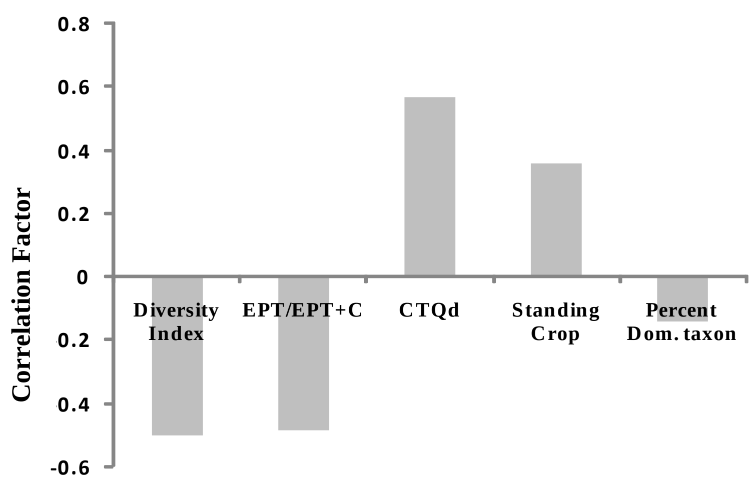


Figure 5. Correlation between metals index and selected biotic metrics (significance level at $p = 0.05$)

Results of relationships between individual metals and biotic metrics indicate that significant relationships existed between metals and biotic metrics 17 out of 48 times (Table 11). Of these, only nine were strong correlations ($0.5 \leq r_s \leq 1$) and the others were moderate ($0.3 \leq r_s < 5$). Diversity Index was significantly negatively related to sediment concentrations of Ni and Zn (r_s = -0.67 and -0.62 respectively, $p < 0.05$). Cd, As, Ni, and Zn strongly influenced EPT/EPT+Chironomidae (r_s = -0.69, -0.54, -0.75, and -0.73 respectively, $p < 0.05$). CTQd was positively related to Ni and Zn concentrations in streambed sediments (r_s = -0.67 and -0.55 respectively, $p < 0.05$), while percentage of dominant taxon was strongly correlated only with Zn concentrations (r_s = -0.58, $p <$ 0.05). There was no relationship between standing crop and any metal.

Table 11. *Correlation between individual metal concentrations and selected biotic metrics (p-values in bracket)*

Biotic metric	Metal						
	As	Cd	Cr	Cu	Pb	Ni	Zn
Diversity index	**-0.43 (0.02)**	**-0.39 (0.03)**	0.02 (0.47)	-0.05 (0.40)	-0.251 (0.12)	**-0.67* (0.0002)**	**-0.62* (0.001)**
EPT/EPT+C	**-0.69* (0.00)**	**-0.54* (0.003)**	-0.12 (0.3)	-0.004 (0.49)	**-0.440 (0.02)**	**-0.75* (0.00)**	**-0.73* (0.00)**
CTQd	**0.43 (0.02)**	**0.35 (0.05)**	0.21 (0.17)	-0.23 (0.15)	0.253 (0.12)	**0.67* (0.0002)**	**0.55* (0.003)**
Standing Crop	-0.09 (0.33)	-0.15 (0.25)	0.02 (0.47)	-0.02 (0.47)	-0.021 (0.46)	-0.061 (0.39)	-0.064 (0.38)
Percent Dom. Taxon	**0.40 (0.04)**	**0.40 (0.03)**	-0.24 (0.13)	0.32 (0.06)	0.213 (0.16)	**0.45 (0.01)**	**0.57* (0.002)**

Note. Bold – significant correlation, *strong correlation

DISCUSSION

Standards set forth by the New Mexico Water Quality Control Commission (NMWCC, 1991), indicate that all measurements of temperature, pH, dissolved oxygen concentration, and saturation recorded during this study were within the range of 'good quality' (Table 5). Conductivity exceeded its New Mexico standard of < 300µS/cm at sites UO3 and UO4 during both sampling dates. This is concomitant with the fact that these sites are characterized by erosion of rocks in the steep canyons before Montezuma and runoff from the City of Las Vegas. There were no standards available for alkalinity and hardness scores. For these reasons, the environmental parameters were not considered as potential factors influencing metal toxicity in the Gallinas River.

Hypothesis 1

There is no significant difference in metal concentrations in streambed sediments between sampling seasons.

A previous study by Garn and Jacobi (1996) in the Gallinas River found that most metals were below the instrumental detection limits in collected water samples. Except for Al and Fe, which showed elevated concentrations, the other trace metals that exceeded their detection limits, namely Cd, Cr, Mn, and Zn, did so only slightly. However, all samples for the aforementioned study were collected in the upper region of the River, above USGS gauging station No. 08380500. Sites UO1 and UO2 of the present study are both located in the same area as the sites studied by Garn and Jacobi (1996). Metal concentrations in the streambed sediments at these two sites were high enough to be detected and some of them were even above standard sediment guideline criteria

(Figure 4 and Appendix D). This finding confirms that there are inputs of heavy metals in the Gallinas River in this part of the watershed. It also further supports the idea that sediments can serve as a sink and a better medium of contaminant assessment in aquatic environments (Soares et al., 1999; Duzzin et al., 1988; Jha et al., 1990, Borovec, 1996; Luoma, 1989). However, during this study, the distribution of metals did not differ significantly between the two sampling dates. This indicates that time is not a factor affecting metal distribution in the Gallinas River. The data of the present study supports Hypothesis 1.

Hypothesis 2

There is no significant difference in metal concentration between sampling sites.

The discrepancy in distribution trends signifies that the spatial distribution of metals in streambed sediments is metal-specific and mostly influenced by physical and/or geochemical processes.

Analysis of the heavy metals investigated in this study showed that with respect to sediment quality guidelines (Smith et al., 1996; Buchman, 1999; MacDonald et al., 2000; Ingersoll et al., 2000), Ni is the metal that should be of highest concern in the Gallinas River. Although six other metals did exceed these criteria as well, they did not do so consistently. Concentrations of Ag were compared to the Effects Range-Median (ERM) (Long et al., 1995) and the Upper Effects Threshold (UET) (Buchman, 1999) because there are no standard Threshold Effects Level (TEL) and Probable Effects Level (PEL) values for this metal. The UET value for this metal is considered a similar criterion to the

PEL for other metals (Giddings et al., 2001). Only metals that exceeded at least one of these guidelines at least at one sampling site were further investigated. For this reason Ag was not included in subsequent metal analyses because its concentration in all sediment samples was below both the ERM and the UET guidelines (Figure 4).

Site UO3 should be monitored more frequently because metal concentrations in most samples collected here were higher than the other sites. Whereas TEL values were frequently exceeded at many sites, there were only four instances during which the PEL values were exceeded, three of which occurred at site UO3 (Zn in the Spring, and Ni in the Spring and Fall). The fourth exceedence was observed with Cr concentrations at site UO2 during the Spring sampling. Upadhyay et al. (2006), and Lynch et al. (1988) suggested that although the highest metal concentrations in aquatic systems are recorded during runoff events, most of the metals during these events are found in dissolved or suspended form. The greatest amounts of contaminants from runoff that concentrate in the sediments do so during snowmelt in the Spring. This may be the reason why most of the concentrations that exceeded streambed sediment guidelines occurred during the Spring whereas concentrations in the Fall were mostly below these criteria (Figure 4).

Although high concentrations of As exist in the local geology and have been reported in the Gallinas River waters during stormflow events (Duran et al., 2005), As concentrations in streambed sediments during this study exceeded TEL values only three times, two of which were by a very slight margin (Figure 4). This is probably due in part to the high desorption capacity of As from Al and Fe oxides in water with pH > 6 (Table 5) (Dzombak and Morel, 1990; Waychunas et al., 1993). The other five metals assessed in this study do not have any known point source in the Gallinas Watershed but at least two of them were detected in trace quantities by a previous study (Garn and Jacobi, 1996) in the upper reaches of the Gallinas River. In addition, these metals have been shown to increase in water systems due to urban anthropogenic activities (Beasley and Kneale, 2002).

In addition to levels supplied as by-products of transportation and energy production, Cu compounds, such as cuprous oxide, cupric sulfate, and cupric acetate are used as fungicides and pesticides, as well as in paint and in wood preservative materials (Beasley and Kneale, 2002). This may explain the higher concentrations of this metal observed during this study upstream of Las Vegas where habitations are mostly constructed with wood. However, these concentrations were not significantly different from those observed downstream.

Cr was also found to be highest at upstream sites compared to downstream sites (Figure 4). Although Cr could be leached as a result of corrosion-induced metal release, its distribution in sediments suggests that its principal source in the Gallinas River could be leachates from chromate copper arsenate treated residential wood structures (Shibata et al, 2007). Pb, Ni and Zn were all higher in streambed sediments downstream from the City than upstream. Emissions from gasoline powered vehicles, wear of moving parts of engines, wear from tires, and corrosion of building materials and metal objects, which characterize urban settings (Novotny, 1995; Beasley and Kneale, 2002) like the City of Las Vegas, are the possible major contributors of these metals into the Gallinas River. In the case of Cr, Cu, and Pb the spatial distributions did not show any significant difference between sites.

Comparison between the study sites indicates that no difference exists between sites UO1 and UO2 in one hand and between sites UO3 and UO4 on the other hand (Table 8). The five metals that had significant differences did so more often between the upstream and the downstream sites. Hypothesis 2 was therefore supported for Cr, Cu, and Pb, but not for As, Cd, Ni, Ag, and Zn (Tables 7 and 8).

Hypothesis 3

There is no significant correlation between concentrations of heavy metals in aquatic insects and those in sediments.

Metals persist in various sections of the Gallinas River and are biologically available. Composite samples of BMI functional feeding groups symbolize a more adequate representation of the BMI community as it is available to predatory fish (Farag et al., 1998).

Ni is being taken up by all BMIs in the Gallinas River, in direct proportions to levels in sediments as indicated by the strong correlations that it exhibits with all the four functional feeding guilds. Tochimoto et al. (2003) found that Ni was one of the metals whose level increased in BMIs when the latter were transferred from a non contaminated site to a metal contaminated site. In a review paper, Goodyear and McNeill (1999) stated that most bioaccumulation studies have noted differences in uptake between heavy metals, based on functional feeding groups. But whenever bioaccumulation did occur, concentrations in BMIs were in direct proportions to those found in sediments. Except for Ni, information provided by this study does not support results of the literature reviewed by the aforementioned study. The analysis of the relationships of metal contamination and concentrations in BMIs in the present study showed very few strong patterns. In most cases, metal levels in BMIs are not correlated to corresponding metal contamination. Only a few cases existed where metals in sediments were significantly related to those in BMIs (Table 11). Marquenie (1985) reported that because organisms accumulate only the biologically available species of the polluting metals, there is not always a correlation between metal concentrations in sediments with those in BMIs from the same sampling reach. However, Goodyear and McNeill (1999) observed that a concentration gradient between metals in organisms and sediments (Cd < Pb < Cu < Zn) is most often the same for all feeding guilds in rivers around the world. A very similar gradient (Cd < As < Cr < Pb < Ni < Cu < Zn) was observed in about 50% of BMI samples collected from the Gallinas River during this study. Ni was the only metal whose correlation data from the present study support Hypothesis 3. The relationships between Cd, Cr and Zn in BMIs and in sediments supported Hypothesis 3 for some feeding guilds but not for others. However, Hypothesis 3 was supported for Cu, Pb and As in all feeding guilds (Table 10).

Hypothesis 4

There is no biomagnification of heavy metals in the macroinvertebrate food chain.

Eyres and Pugh-Thomas (1978), Timmermans et al. (1989), Nehring (1976), Besser and Rabeni (1987), and Burrows and Whitton (1983) suggested that biomagnification of metal contaminants does not occur in BMI trophic chain except for Pb. Kiffney and Clements (1993) and Farag et al. (1998) have opined that because they feed on biofilm, which may accumulate great concentrations of metals, Grazers (herbivores) most often have a greater concentration of heavy metals compared to other feeding guilds. During this study, metal enrichment in BMIs at levels greater than those found in sediments were observed in about half of the total samples (Appendix J). In most cases, metal concentrations were higher in other feeding guilds compared to Predators, making it impossible to conclude that biomagnification is occurring.

Although there is enough evidence to show that some of these metals can increase with trophic level (Young and Mearns, 1979; Goodyear and McNeill, 1999; Reynoldson, 1987), several reasons can explain the lack of biomagnification observed during this study. One of these reasons is the complexity of aquatic food chains. BMIs usually feed on different species of food based on seasonal availability. Also, the predatory BMIs investigated may either prey on other BMIs or on smaller organisms like zooplankton and bacteria. Differences in diet definitely bring about differences in the amount of metals biotransferred from the prey to the predator (Bindra and Hall, 1978). Another reason for the lack of biomagnification is seasonal migrations and drifting. When there is habitat disturbance, BMIs get dislodged and are carried away by water currents to new favorable locations (Wolfe et al., 1976). Excretion rates, biodegradation/biotransformation, chemical changes of metals in sediments, are yet other reasons that can influence how much metal is transferred from one aquatic organism to the next in the food chain (Boddington et al., 1979). The present study supports Hypothesis 4 (Appendix J)

Hypothesis 5

There is no significant correlation between sediment metal contamination and biotic metrics.

The biological health of the Gallinas River indicates that the BMI communities ranged from nonimpaired to moderately impaired, from UO1 to UO4 respectively, with each site maintaining its rating during both sampling periods (Appendices G and H). The seven-criteria bioassessment and the diversity index all indicated that the downstream sites are under perturbation. The decrease in taxa richness, EPT Index, and the increase in community loss and standing crop between upstream and downstream sites indicate the possibility of organic enrichment at the downstream sites (Keup and Zarba, 1987; Garn and Jacobi, 1996; Courtemanch and Davis, 1987). Nonetheless, the higher CTQd indicate that in addition to organic pollution, other non-organic toxins may be affecting BMIs at these same downstream sites (Winget and Mangum, 1979). Moreover, a decrease in the EPT/EPT+Chironomidae ratio observed at these same sites indicate that, unlike the upstream sites, the downstream sites have a higher density of individuals belonging to the family Chironomidae compared to individuals belonging to the orders Ephemeroptera, Plecoptera, and Trichoptera combined. Winner et al. (1980) have positively correlated richness in Chironomidae genera (number/site) with increasing metal concentrations in sediments. Kiffney and Clements (1994), while carrying out an experiment to evaluate the effects of the addition of a mixture of metals on macroinvertebrate assemblages from a Rocky Mountain stream, found that the number of Plecoptera and Ephemeroptera genera declined rapidly while the Chironomidae became dominant. In the present study, the EPT/EPT+Chironomidae metric showed that with the high ratios (greater than 0.8 for both sampling dates) found at sites UO1 and UO2, aquatic conditions at these two sites do not pose any threat to BMI community. On the contrary, the heavy metal concentrations are affecting the BMI community at sites UO3 and UO4 where EPT/EPT+Chironomidae ratios are as low as 0.19 and 0.002 respectively, during the Spring, and 0.26 at both sites during the Fall (Tables 11 and 12). As stated by Smolders et al. (2003), ecological risks usually vary considerably in different reaches of a river because toxicants are gradually broken down and/or immobilized after release into the river. The correlations between the biotic metrics and the Metals Index show that heavy metal contamination in the Gallinas River is a possible factor influencing water quality and should be considered in management plans of the River.

As, Cd, Pb, and Zn are the metals that are most related to biological health of BMI communities in the Gallinas River. However, most of these metals fall below accepted sediment quality guidelines and do not seem to show any consistency in bioaccumulation pattern by the various feeding guilds. Moreover, apart from As, whose source has been determined in the Gallinas Watershed (Evans et al., 2004), no individualized source has been identified for the other metals (Garn and Jacobi, 1996).

The virtual absence of Shredders from the two downstream sites during the Fall and the low number of Collectors at site UO3 during the Spring could be attributed to the poor water quality observed at these sites (Appendices G and H). Additionally, Rohasliney and Jackson (2008) suggested that the absence of Shredders could indicate the virtual lack or insufficiency of coarse particulate organic matter. Site assessment of the present study showed that sites UO3 and UO4 are dominated by compact bedrock covered by clay and silt materials and the riparian areas lack trees that could provide allochthonous material to the River. Nonetheless, there is sufficient evidence demonstrating that the lack of Shredder taxa in a rocky mountain stream can be attributed to high sensitivities of many members of this feeding guild to trace metals (Clements et al., 2000; Clements and Kiffney, 1995; Carlisle and Clements, 2005). Six of the eight metals investigated during this study showed relatively high concentrations in sediments at the two downstream sites compared to the upstream sites (Figure 4). Although the present study does not provide sufficient data to assert all the causes of perturbation in the lower reaches of the Gallinas River, it most certainly confirms that heavy metals may be a contributing factor in this equation and does not support Hypothesis 5.

CONCLUSION AND RECOMMENDATIONS

The findings of the present study verify the null hypothesis that there was no significant difference in streambed sediment metal concentrations between sampling seasons, implying that metal concentrations do not change with time. However, significant differences do exist between stream reaches, with the downstream sites having higher concentrations compared to upstream. This is concordant with poorer bioassessment scores at the downstream sites.

Levels of Ni in all BMIs appear to be strongly related with sediments levels. Levels of a few other metals like Cr, Zn and Cd in some BMIs are also strongly related with sediment levels. The high levels of metals in Shredders at the upstream sites during the Spring sampling are a possible indication that the riparian vegetation is taking up considerable amounts of heavy metals from the geology. Metal speciation, feeding habits and varying uptake rates may be responsible for the differences in bioaccumulation found in the BMIs. Overall, biomagnification is not occurring in the Gallinas River.

Site assessment indicates that sites UO3 and UO4 have undercut banks and lots of silt and algae in the streambed sediments, suggesting that the habitats at these sites are degraded and unstable for BMI colonization. Anthropogenic activities at these sites, like cattle grazing, which reduces riparian vegetation and increases fecal matter in the River, farming, which increases fertilizer and pesticides, and urban activities, which promote increased erosion, may be responsible for this observation. Nonetheless, the significant relationships observed between most biotic metrics and metals like As, Cd, Ni, and Zn, as well as the Metals Index, indicate that heavy metal contamination is a strong contributing factor to the poor biotic condition of these two sites.

Although this study did not find any adverse contribution from discharges from the City's Wastewater Treatment Plant, the biotic condition at this site suggest that the flushing from the Plant's discharges is not sufficient to improve the quality of the water. Also, Cd and Ag, two metals that are known to be linked with high sewage sludge inputs in river systems did not show any marked increase downstream of the WWTP (Figure 4 and Appendix D).

In a nutshell, the Gallinas River water quality is 'Fairly Good' to 'Good' upstream of the City of Las Vegas and 'Poor' downstream. However, there is no indication that the heavy metal contamination could be biomagnifying in the aquatic food chain. It can be assumed that the heavy metals in the Gallinas River are not hazardous to the surrounding human population. Nonetheless, the present levels appeared to exceed BMI sediment quality criteria at many sites. Therefore, these concentrations should be watched carefully and control measures such as stormwater treatment be implemented because small, insignificant increases may be biotransferred and become hazardous to humans over time.

Methods used in this study were adequate to indicate that in addition to As, other metals are present in the Gallinas River and are bioavailable. However, the data provided in this study are not definitive in asserting the level of heavy metal toxicity in the Gallinas River. Consequently, additional investigations are warranted. These studies should, first, identify specific bioindicator BMI taxa for the different contaminant species available in the River and investigate those species. Also, future research should consider depurating the BMIs to eliminate adsorbed contaminants and gut contents. Furthermore, analysis of BMI body parts separately is an effective way of determining where exactly these organisms bioaccumulate heavy metals the most.

The next step would be to conduct comparative studies of controlled laboratory bioassays and stream mesocosms in order to control factors that may not be accounted for in the field. These controlled experiments should test the BMIs for physical deformities using the metals (As, Cd, Ni, and Zn), which were found by the present study to strongly correlate with most biotic measures. Such investigations would provide information on sublethal concentrations of these metals individually and collectively. Significant information on whether these metals have synergistic or antagonistic behaviors would be provided and would help in management, cleanup and protecting of the aquatic biota of the Gallinas River.

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APPENDICES

Appendix A

Summary of reported bioaccumulation studies in the past fourteen years.

Location	***Taxa/Feeding Guild***	***Metals investigated***	***Comments***	***Reference***
Upper Arkansas River, Colorado	*Baetis spp.* (G), *Arctopsyche grandis* (C), and *Rhyacophyla spp.* (P).	Cd, Cu, and Zn	In spite of spatial and temporal variations in metal concentrations among taxa, the mayfly *Baetis spp.* bioaccumulated more than other taxa.	Clements and Kiffney, 1994
Cottonwood River, Kansas	*Orconectes*. Feeding guild not indicated.	Pb, Cd, Al, Zn and Cu	Although metal concentrations in sediments were higher than those of water and all organism, the crayfish *Orconects nais* accumulated more metals than the sunfish (*Lepmis humilis*)	Morrissey and Edds, 1994
River Gaula, Norway	*Baetis*, *Diura*, *Rhyacophila*. Feeding guild not indicated.	Cu, Cd, and Zn	Foraging species concentrated metals two to threefold compared to Carnivores. Levels of Cu and Zn related to water levels	Arnekleiv and Størset, 1995

Location	***Taxa/Feeding Guild***	***Metals investigated***	***Comments***	***Reference***
Upper Mississippi River	*Hexagenia* (C)	Cd, Pb, Cu, Zn, Hg	Cd was the most pronounced metal with levels in mayflies from sites closest to the Twin Cities (Minneapolis-St. Paul, Minnesota, orders of magnitude higher than those of BMIs found at sites further downstream.	Beauvais et al., 1995
Upper Sacramento River, California	Baetidae (C), Chironomidae (C).	Cd, Pb, Cu, Zn	Chironomids and mayflies from sites downstream from inputs had higher concentrations of metals compared to uncontaminated sites.	Saiki et al., 1995
River Kyronjoki, Finland	*Hydropsyche pellucidula* (C)	Al, Cd, Cu, Fe, Pb, and Zn	Metal concentrations in the larvae strongly depend on the life stage with Cd and Cu significantly higher in newly moulted larvae.	Vuori and Kukkonen, 1996
Four rivers in Flanders, Belgium	*Chironomus gr. thummi* (C), *Tubifex tubifex* (C)	Cu, Cd, Zn, and Pb	Metal levels in tubificid worms were related to total metal concentration in sediments whereas levels in chironomid were most often related only to the reducible fractions in sediments except for Pb.	Bervoets et al., 1997

Location	***Taxa/Feeding Guild***	***Metals investigated***	***Comments***	***Reference***
River Hayle, Cornwall	Ephemeridae (C), Ecdyonuridae (C), Perlodidae (P), Hydropsychidae (C)	Cd, Pb, Cu, Zn, Fe, Mn	Significant correlations were observed between Zn and Cu levels in sediments and BMIs for predators, grazers and collectors.	Goodyear and McNeill, 1998
River Caine, Bolivia	Chironomidae (C)	Cd, Pb, Ni, Cr, Cu, and Zn	Levels of Cd, Pb, Zn, and Cu were higher in larvae downstream after the confluence with the polluted River Molinero, although there was no difference in sediment levels.	Bervoets et al., 1998
Coeur d'Alene River, Idaho	Pterenarcella (G), Pterenarcys (G), Tipula (S), Arctopsyche (P)	Cd, As, Cu, Hg, Pb, and Zn	Shredders-scrapers had significantly higher concentrations of As, Cd, Hg and Zn than other feeding groups. No biomagnification was found to be occurring.	Farag et al., 1998
Blackbird Creek, Big Deer Creek, Panther Creek, Idaho.	Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Chironomids and few other Diptera. Feeding guild not indicated.	As, Co, and Cu	Metal concentrations in invertebrates were highest at the closest site downstream from mine inputs.	Beltman et al., 1999

Location	***Taxa/Feeding Guild***	***Metals investigated***	***Comments***	***Reference***
Runoff discharge at road crosings in Eastern England, UK	*Gammarus*, *Asellus* and *Sialis*. Feeding guild not indicated.	Pb, Cd, and Zn	Concentrations of Pb in the BMIs were related significantly with those in sediments. However, Cd did not show the same trend and Zn levels were higher than in sediments.	Perdikaki and Mason, 1999
Slippery Rock Creek and Wolf Creek, Pennsylvania	Hydropsych caddisfly. Feeding guild not indicated.	Fe, Al, Mn, Zn, Cd, and Pb	Body burdens of most metals were higher in the AMD site than in the reference stream.	DeNicola and Stapleton, 2002
Tamagawa River, near Tokyo (Japan)	*Stenopsyche marmorata* (C)	Ni, Cu, Zn, and Pb	Cu and Ni uptake was predominantly through food. Zn levels decreased in larvae over time.	Tochimoto et al., 2003
Many sites on the Pilcomayo River, South America	Chironomidae (C) and semi-aquatic Pisauridae (P)	Cd, Cu, Pb, S and Zn	Metal concentrations is positively correlated with levels in sediments and water and the metals are biotransferred to predators via chironomid larvae	Smolders et al, 2003
The Clark Fork, the Blackfoot River, and Rock Creek (MT, USA)	*Hydropsyche spp*., *Baetis spp*., *Arctopsyche grandis*, *Epeorus albertae* and *Serratella tibialis.* Feeding guild not indicated.	Cd, Cu, and Zn	In general, spatial and temporal variation in metal body burdens corresponded to sediment concentrations. Metal concentrations were relatively high in sensitive species compared to tolerant species.	Cain et al., 2004

Location	Taxa/Feeding Guild	Metals investigated	Comments	Reference
Several streams in California	Ephemerellidae (G), Heptageniidae (G), Ameletidae (C), Siphlonuridae (C), Hydropsychidae (S), Perlidae (P)	Cd and Zn	Although the Ephemerellidae accumulated metals more rapidly than the other taxa, uptake rates were generally very variable and were not related to body size except for the mayfly *Drunella gandis*.	Buchwalter and Luoma, 2005
River Vascão and Mosteirão stream, SE Portugal	*Choroterpes* - Leptophlebiidae (S)	Mn, As, Pb, Zn, Cu, Co, and Cd	Metal body burdens higher at site impacted with AMD compared to reference unimpacted sites.	Gerhardt et al., 2005
River Guadiamar, Spain	*Procambarus*. Feeding guild not indicated.	Cd, Cu, Zn, Pb and As	Metal concentrations in crayfish at the sites having the polluted sediments were higher than sites with no polluted sediment.	Alcorlo et al., 2006
River Tisza, River Szamos and River Maros, Romania.	*Chironomus* (C)	Mn, Fe, Ni, Cu, Zn, As, Sr, Pb	Calculated BAFs were always higher in heavily impacted compared to other rivers	Woefl et al., 2006
Biala Przemsza river system, southern Poland	*Gammarus fossarum, Baetis rhodani* and *Baetis vernus.* Feeding guild not indicated.	Cd, Cu, Fe, Pb, and Zn	Mayfly larvae were more sensitive to high metal influxes than the amphipod and metal concentrations in the former dropped more slowly than the later in less contaminated reaches.	Fialkowski and Rainbow, 2006

Location	***Taxa/Feeding Guild***	***Metals investigated***	***Comments***	***Reference***
Boulder River, Montana	Taxa and feeding guild not indicated.	As, Cd, Cu, Pb, and Zn	Although BMIs had lower concentrations compared to biofilm, metal levels in BMIs from metal impacted sites were generally higher than in those from reference sites.	Farag et al., 2007
Ganga River, India	Gastropoda (S), and Pelecypoda (C)	Hg	Higher concentrations of Hg were found in shells of the gastropods compared to the pelecypods and were attributed to feeding habits	Sinha et al., (2007)

Appendix B

Quality assurance and quality control for external fortifications of sediments and USGS materials.

Material	**Element**							
	Ag	As	Cd	Cr	Cu	Ni	Pb	Zn
Method Blank	< 0.002	< 0.1	< 0.01	< 0.5	< 0.01	< 0.1	< 0.01	< 0.1
GXR-6 Meas	0.131	210	0.11	76.3	70.7	24.4	109	121
GXR-6 Cert	1.3	330	1	96	66	27	101	118
GXR-2 Meas	12.5	3.3	3.31	20.5	78	17	510	526
GXR-2 Cert	17	25	4.1	36	76	21	690	530
GXR-1 Meas	29.7	459	2.45	6.7	1000	40.7	635	801
GXR-1 Cert	31	427	3.3	12	1110	41	730	760
GXR-4 Meas	3.3	113	< 0.01	55	5640	40.6	42.7	69.5
GXR-4 Cert	4	98	0.9	64	6520	42	52	73

Appendix C

ICP-MS concentrations of metals (µg/g) in streambed sediments from the Gallinas River during two sampling dates in 2006.

Sampling period	Site	Metal							
		As	Cd	Cr	Cu	Pb	Ni	Ag	Zn
Spring 2006	UO1	8.8	0.58	27.6	54.5	50.5	22.4	0.068	104.0
		4.1	0.41	22.2	37.3	23.5	17.6	0.029	87.5
		4.0	0.67	35.8	58.1	37.6	27.1	0.064	113.0
	UO2	4.7	0.21	100.0	34.3	22.4	32.9	< 0.002	62.5
		3.7	0.34	202.0	55.3	37.4	29.3	0.019	87.3
		2.5	0.28	114.0	28.4	15.0	26.4	0.032	74.9
	UO3	7.4	3.49	33.6	21.6	32.0	85.3	0.024	401.0
		10.3	7.79	55.7	31.8	79.5	114.0	0.208	719.0
		10.1	9.09	46.5	30.0	78.9	158.0	0.149	929.0
	UO4	5.9	0.47	26.6	18.4	26.1	29.5	0.203	128.0
		7.0	0.36	31.2	20.4	29.2	29.3	0.294	111.0
		5.8	1.25	41.1	25.9	46.8	49.8	0.83	269.0
Fall 2006	UO1	1.6	0.19	15.9	20.7	11.3	11.4	0.02	55.1
		3.5	0.58	26.2	40.8	26.2	18.7	0.088	96.8
		2.2	0.26	17.0	25.0	13.9	12.3	0.027	63.0
	UO2	1.6	0.23	17.5	12.9	14.6	12.0	0.010	81.4
		1.4	0.19	19.1	13.0	14.7	13.2	0.008	62.0
		2.4	0.57	24.5	28.4	26.9	19.2	0.041	101.0
	UO3	5.4	1.03	12.0	14.8	21.6	46.8	0.051	198.0
		8.4	2.47	24.4	30.0	51.5	66.8	0.216	307.0
		6.0	1.09	18.3	23.5	30.9	42.1	0.189	201.0
	UO4	4.4	1.14	17.3	19.8	26.2	43.2	0.215	227.0
		3.8	0.39	11.5	13.8	16.5	28.0	0.066	117.0
		3.5	0.55	12.2	14.5	21.2	29.2	0.129	129.0

Appendix D

Mean concentrations of metals in streambed sediments (Mean ± Standard Deviation) at four sampling stations along the Gallinas River collected in 2006

Date	Site		Ag (μg/g)	As (μg/g)	Cd (μg/g)	Cr (μg/g)	Cu (μg/g)	Pb (μg/g)	Ni (μg/g)	Zn (μg/g)
Spring 2006	UO1	Mn	0.05	5.63	0.55[b]	28.53	49.97[b]	37.2[b]	22.37[b]	101.5
		Std	±0.02	±0.3	±0.1	±6.9	±11.12	±13.5	±4.8	±12.9
	UO2	Mn	0.02	3.63	0.28	138.67[a]	39.33[b]	24.93	29.53[b]	74.9
		Std	±0.02	±1.1	±0.1	±55.3	±14.14	±11.4	±3.3	±12.4
	UO3	Mn	0.13	9.27[b]	6.79[a]	45.27[b]	27.80	63.47[b]	119.1[d]	683.0[d]
		Std	±0.1	±1.6	±2.9	±11.1	±5.4	±27.3	±36.6	±265.8
	UO4	Mn	0.44	6.23[b]	0.69[b]	32.97	21.57	34.03	36.2[a]	169.33[b]
		Std	±0.3	±0.7	±0.5	±7.4	±3.88	±11.2	±11.8	±86.7
Fall 2006	UO1	Mn	0.05	2.43	0.34	19.7	28.83	17.13	14.13	71.63
		Std	±0.04	±0.9	±0.2	±5.7	±10.6	±7.9	±3.9	±22.2
	UO2	Mn	0.02	1.8	0.33	20.37	18.1	18.73	14.8	81.47
		Std	±0.02	±0.5	±0.2	±3.7	±8.9	±7.1	±3.9	±19.5
	UO3	Mn	0.15	6.6[b]	1.53[b]	18.23	22.77	34.67	51.9[d]	235.33[b]
		Std	±0.1	±1.6	±0.8	±6.2	±7.6	±15.3	±13.1	±62.1
	UO4	Mn	0.14	3.9	0.69[b]	13. 67	16.03	21.3	33.47[b]	157. 67[b]
		Std	±0.1	±0.5	±0.4	±3.2	±3.3	±4.9	±8.5	±60.3
Guideline (PEL)			--	17	3.53	90	197	91.3	36	315
Guideline (TEL)			--	5.9	0.596	37.3	35.7	35	18	123
Guideline (UET)			4.5	--	--	--	--	--	--	--
Guideline (ERM)			1.0	85	9	145	390	110	50	270

Note. [a]value above PEL; [b]value above TEL; [c]value above UET; [d]value above ERM;
-- value omitted or not available.

Appendix E

Quantitative BMI collections from four sites along the Gallinas River, April 2006 sampling.

Taxa	FG	Site Name											
		UO1			UO2			UO3			UO4		
EPHEMEROPTERA – mayflies													
Baetidae	C	61	28	14	8	1	7			1			
Ephemerellidae	G	13	4	9	1								
Heptageniidae	G	12	19	28	2					2			
Tricorhythidae	C					8	32						
PLECOPTERA – stoneflies													
Leuctridae	S			1									
Chloroperlidae	S	1											
Perlidae	P	1	2	3						1			
Nemouridae	S	10	14	5									
Perlodidae	P	6	3	2									
Taenipterygidae	S	19	1	1	1								
TRICHOPTERA – caddisflies													
Hydropsychidae	C	2	1	2	9	4	17	9	3	4	1		1
Hydroptilidae	-							4	4	1			
Limnephilidae	S		2	9					64				
Psychomyiidae	C	1											
Rhyacophilidae	P	13	7	3	9	6	1					1	
Glossomatidae	G				6	2						3	
Polycentropodidae	C											1	
DIPTERA – true flies													
Ceratopogonidae	P		2			1			3	40	2	1	1
Chironomidae	C	11	18	17	11	9	6	5	4	1	527	536	732
Ephydridae	C				1								
Stratiomyidae	C					1	1						
Empididae	P				1	6	4	2	194	5	1		2
Simulidae	C	11	2		1					1	35		20
Culicidae	-										2		
Psychodidae	C			1									
Tipulidae	S		1		2	25	17		1		12	11	
ODONATA – damsel/dragonflies													
Coenagrionidae	P											1	1
HEMIPTERA – true bugs													
Naucoridae	P				3	2							
COLEOPTERA – beetles													
Elmidae	C	7	5	9	52	47	33		1				
LEPIDOPTERA – moths													
Pyralidae	G				6	1	6		14				
DECAPODA – crayfish and shrimps													
Astacidae	C								1				
AMPHIPODA – scuds													
Talitridae	C							1					
GASTROPODA – snails and limpets													
Planorbidae	G				1				144		5	15	1
PELECYPODA – clams and mussels													
Sphaeriidae	C								2				
ANNELIDA – segmented worms													
Hirudinea	P										1		
Oligochaeta	C		3		35	35	10		7		6		
ASCHELMINTHES – round worms													
Nematoda	C										24	50	19
NEMATOMORPHA – gordian worms													
Gordioidae	-								2		6	47	21
PLATYHELMINTHES – flatworms													
Turbellaria	P				2		2						
Standing Crop			1754			1937			2371			9484	
Total taxa			20			20			18			15	
CTQd			72.50			92.76			106.94			107.91	
Diversity Index			2.34			2.16			1.66			0.59	

Note. FG – functional feeding guild: C – collectors; G – grazers; S – shredders; P – predators

Appendix F

Quantitative BMI collections from four sites along the Gallinas River, October 2006 sampling

Taxa	FG	Site Name											
		UO1			UO2			UO3			UO4		
EPHEMEROPTERA – mayflies													
Baetidae	C	6						1			6		1
Caenidae	C								2				
Ephemerellidae	G	38			33	12	16			4	1		
Heptageniidae	G	64	12	4					7		30	10	10
Tricorhythidae	C										9		
PLECOPTERA – stoneflies													
Leuctridae	S		6	2	1	14	23						
Chloroperlidae	S	6					2						
Perlidae	P	3	17	5	50	3	16	1	25	69	2	7	2
Nemouridae	S	1											
Perlodidae	P	10	5	5	3	1		2	31	6		1	1
Taenipterygidae	S	15		1									
TRICHOPTERA – caddisflies													
Hydropsychidae	G	10											
Limnephilidae	S	16	49	29	1								
Psychomyiidae	C		28	37									
Hydroptilidae	-		4	7	35		19	4	4	20	54		23
Lepidostomatidae	S				3								
Rhyacophilidae	P	5											
Glossomatidae	G		24	13	7	1							
Polycentropodidae	C		2	5									
DIPTERA – true flies													
Ceratopogonidae	P		1		3			2	63	23			
Chironomidae	C	9			1						3	9	
Ephydridae	C							3	12	17			
Empididae	P	2											1
Simulidae	C									2	4		
Psychodidae	C	5	2	5									
Tipulidae	S	1											
ODONATA – damsel/dragonflies													
Coenagrionidae	P					1			25	9		8	
HEMIPTERA – true bugs													
Naucoridae	P				21								
COLEOPTERA – beetles													
Elmidae	C	14	14	10	35	69	38		19	3		2	
Dysticidae	P				4	12	19				4		
Hydrophilidae	P				3		2						
LEPIDOPTERA – moths													
Pyralidae	G			2	1								
DECAPODA – crayfish and shrimps													
Astacidae	C											2	
AMPHIPODA – scuds													
Talitridae	C											2	
GASTROPODA – snails and limpets													
Planorbidae	G				1			3	12	17			
PELECYPODA – clams and mussels													
Sphaeriidae	C		1	5	66		13						
ANNELIDA – segmented worms													
Hirudinea	P											2	
Oligochaeta	C	7			1								
ASCHELMINTHES – round worms													
Nematoda	C	5		13	1	5		109	162	290	196	15	49
PLATYHELMINTHES – flatworms													
Turbellaria	P				342	14	31						
Standing Crop			2426			4354			4180			2047	
Total taxa			27			23			13			14	
CTQd			80.68			94.45			95.15			100.18	
Diversity Index			2.74			1.94			1.43			1.43	

Note. FG – functional feeding guild: C – collectors; G – grazers; S – shredders; P – predators.

Appendix G

Bioassessment of the Gallinas River, April 2006 following Plafkin et al. (1989)

	Site			
Biological metric	UO1 (Reference)	UO2	UO3	UO4
Calculated value				
Standing crop (number of organisms per square meter)	1754.25	1936.98	2370.97	9483.89
Taxa Richness	20	20	18	15
CTQd	72.50	92.76	106.94	107.91
EPT/(EPT + Chironomidae)	0.87	0.81	0.19	0.002
Percent dominant taxon	26.82	31.13	37.38	86.99
EPT index	13	8	5	3
Community Loss	0.00	0.40	0.50	0.87
Percentage of reference				
Standing crop (number/m2)	100	110.42	135.16	540.62
Taxa Richness	100	100	90	75.00
CTQd	100	78.16	67.79	67.19
EPT/(EPT + Chironomidae)	100	93.10	21.84	0.23
Percent dominant taxon	26.82	31.13	37.38	86.99
EPT index	100	61.54	38.46	23.08
Community Loss[1]	0	0.4	0.50	0.87
Score				
Taxa Richness	6	6	6	4
EPT index	6	0	0	4
EPT/(EPT+Chironomidae)	6	6	0	0
Percent dominant taxon	4	2	2	0
Standing crop (number/m2)	6	6	6	0
CTQd	6	4	2	2
Community Loss	6	6	4	4
Biological condition				
Total of metric score	40	30	20	14
Percentage of reference	100	75	50	35
Stream condition	Nonimpaired	Slightly impaired	Moderately impaired	Moderately impaired

Note. [1]Actual values, not a percent comparability to reference site

Appendix H

Bioassessment of the Gallinas River, October 2006 following Plafkin et al. (1989)

Biological metric	UO1 (Reference)	UO2	UO3	UO4
	Site			
Calculated value				
Standing crop (number of organisms per square meter)	2425.79	4353.64	4180.04	2046.62
Taxa Richness	27	23	13	14
CTQd	80.68	94.45	95.15	100.18
EPT/(EPT+Chironomidae)	0.98	0.99	0.26	0.26
Percent dominant taxon	24.29	40.61	21.42	58.04
EPT index	12	10	4	3
Community Loss	0.00	0.30	1.00	1.00
Percentage of reference				
Standing crop (number/m2)	100.00	179.47	172.32	84.37
Taxa Richness	100.00	85.19	48.15	51.85
CTQd	100.00	85.42	84.79	80.54
EPT/(EPT+Chironomidae)	100.00	101.02	26.53	26.53
Percent dominant taxon	24.29	40.61	21.42	58.04
EPT index	100.00	83.33	33.33	25.00
Community Loss[1]	0.00	0.30	1.00	1.00
Score				
Taxa Richness	6	6	2	2
EPT index	6	4	0	0
EPT/(EPT+Chironomidae)	6	6	2	2
Percent dominant taxon	4	0	4	0
Standing crop (number/m2)	6	4	4	6
CTQd	6	6	2	4
Community Loss	6	6	4	4
Biological condition				
Total of metric score	40	32	18	18
Percentage of reference	100	80	45	45
Stream condition	Nonimpaired	Slightly impaired	Modertely impaired	Modertely impaired

Note. [1]Actual values, not a percent comparability to reference site

Appendix I

ICP-MS metal concentrations in benthic macroinvertebrates

Sampling date	Site/Guild	Organisms per sample	Metal						
			As	Cd	Cr	Cu	Pb	Ni	Zn
Spring 2006	UO1								
	Shredders	64	72.0	3.0	8.0	105.0	75.0	6.0	288.0
	Grazers	85	4.1	2.9	3.1	41.5	11.5	3.6	228.0
	Collectors	193	2.3	0.8	5.3	43.6	12.6	5.7	167.0
	Predators	42	23.7	1.2	3.0	66.9	47.4	4.2	375.0
	UO2								
	Shredders	45	35.2	4.4	11.0	351.0	142.0	8.8	853.0
	Grazers	25	10.0	0.5	30.0	335.0	155.0	90.0	355.0
	Collectors	328	2.0	0.7	8.9	30.1	30.2	6.3	203.0
	Predators	37	9.0	3.0	8.0	97.5	27.0	5.0	227.0
	UO3								
	Shredders	65	3.0	5.0	10.0	230.0	45.0	10.0	575.0
	Grazers	146	-	3.3	3.8	19.2	14.6	82.6	268.0
	Collectors	40	---	---	---	---	---	---	---
	Predators	145	7.0	6.5	30.0	1820.0	45.5	150.0	767.0
	UO4								
	Shredders	23	0.6	0.6	3.0	28.8	21.6	22.8	200.0
	Grazers	24	1.5	0.6	9.4	17.7	16.2	19.1	75.4
	Collectors	1942	10.7	2.7	6.0	53.0	20.2	24.2	468.0
	Predators	11	0.2	0.8	2.0	27.6	8.6	41.4	286.0
Fall 2006	UO1								
	Shredders	126	8.4	0.04	-	6.6	3.5	0.1	13.7
	Grazers	167	4.6	2.8	38.0	83.8	13.4	14.2	378.0
	Collectors	168	15.0	0.9	6.1	32.4	17.4	5.0	206.0
	Predators	53	4.3	0.3	4.8	25.4	3.1	3.9	75.4
	UO2								
	Shredders	44	11.0	1.0	3.0	95.0	18.5	8.0	177.0
	Grazers	71	2.0	0.5	3.6	16.5	14.6	4.0	104.0
	Collectors	229	2.1	1.6	5.2	39.8	22.2	7.0	264.0
	Predators	525	30.8	2.4	2.0	45.2	16.4	4.0	191.0
	UO3								
	Shredders	--	--	--	--	--	--	--	--
	Grazers	43	8.0	16.5	8.0	134.0	34.5	153.0	897.0
	Collectors	620	0.3	215.0	6.0	73.5	20.4	532.0	5500.0
	Predators	256	0.2	11.8	1.0	37.0	2.2	68.2	995.0
	UO4								
	Shredders	--	--	--	--	--	--	--	--
	Grazers	51	0.2	0.4	2.4	25.5	3.8	18.1	73.5
	Collectors	298	-	1.9	2.0	30.0	7.1	35.5	494.0
	Predators	28	1.7	0.7	1.3	28.7	1.5	37.3	1570.0

Note. - BMI metal concentration below instrument detection limit
-- No BMI organism available
--- BMI sample below ICP-MS minimum weight

Appendix J

Bioaccumulation factors (BAFs) of metals in benthic macroinvertebrates

Sampling date	Site/Guild		Metal						
			As	Cd	Cr	Cu	Pb	Ni	Zn
Spring 2006	UO1	**Shredders**	12.78	5.42	0.28	5.33	2.02	0.27	2.84
		Grazers	0.73	5.17	0.11	2.11	0.31	0.16	2.25
		Collectors	0.41	1.43	0.19	2.21	0.34	0.25	1.65
		Predators	4.21	2.17	0.11	3.40	1.27	0.19	3.69
	UO2	**Shredders**	9.69	15.90	0.08	17.23	5.70	0.30	11.39
		Grazers	2.75	1.81	0.22	16.45	6.22	3.05	4.74
		Collectors	0.55	2.35	0.06	1.48	1.21	0.21	2.71
		Predators	2.48	10.84	0.06	4.79	1.08	0.17	3.03
	UO3	Shredders	0.32	0.74	0.22	12.61	0.71	0.08	0.84
		Grazers	-	0.48	0.08	1.05	0.23	0.69	0.39
		Collectors	---	---	---	---	---	---	---
		Predators	0.76	0.96	0.66	99.82	0.72	1.26	1.12
	UO4	Shredders	0.10	0.87	0.09	2.11	0.63	0.63	1.18
		Grazers	0.24	0.87	0.29	1.30	0.48	0.53	0.45
		Collectors	1.72	3.84	0.18	3.88	0.59	0.67	2.76
		Predators	0.03	1.15	0.06	2.02	0.25	1.14	1.69
Fall 2006	UO1	Shredders	3.45	0.12	-	0.23	0.20	0.01	0.19
		Grazers	1.89	8.16	1.93	2.91	0.78	1.00	5.28
		Collectors	6.16	2.56	0.31	1.12	1.02	0.35	2.88
		Predators	1.77	0.93	0.24	0.88	0.18	0.28	1.05
	UO2	Shredders	6.11	3.03	0.15	5.25	0.99	0.54	2.17
		Grazers	1.11	1.42	0.18	0.91	0.78	0.27	1.28
		Collectors	1.17	4.85	0.26	2.20	1.19	0.47	3.24
		Predators	17.11	7.27	0.10	2.50	0.88	0.27	2.34
	UO3	Shredders	--	--	--	--	--	--	--
		Grazers	1.21	10.78	0.44	5.89	1.00	2.95	3.81
		Collectors	0.05	140.52	0.33	3.23	0.59	10.25	23.37
		Predators	0.03	7.71	0.05	1.63	0.06	1.31	4.23
	UO4	Shredders	--	--	--	--	--	--	--
		Grazers	0.05	0.50	0.18	1.59	0.18	0.54	0.47
		Collectors	-	2.80	0.15	1.87	0.33	1.06	3.13
		Predators	0.44	0.94	0.10	1.79	0.07	1.11	9.96

Note. - BMI metal concentration below instrument detection limit
-- No BMI organism available
--- BMI sample weight too small to measure