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A Comparison of Sediment Metal Concentrations as Potential Stressors to Resident Benthic Communities in an Agricultural Waterbody

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Abstract: This study was designed to (1) determine the relationship between the sediment concentrations of eight total metals (As, Cd, Cr, Cu, Pb, Hg, Ni, and Zn) and five simultaneously extracted metals (SEM) (Ni, Cu, Zn, Cd, and Pb) with 11 benthic metrics based on a three-year data set including two seasons per year for an agricultural water body (Cache Slough, California), and (2) rank the importance of individual metals within a metal mixture as potential stressors to resident benthic communities. The total arsenic, lead, and cadmium showed the highest number of statistically significant and ecologically meaningful relationships with benthic metrics. The total copper, nickel, zinc, chromium, and mercury were not reported to show any statistically significant and ecologically meaningful relationships with any of the benthic metrics. There were also no statistically significant and ecologically meaningful relationships between the benthic metrics and the simultaneously extracted (bioavailable) metals. Both stress tolerant and stress sensitive benthic metrics were reported to have the best discriminatory power for detecting the adverse effects from metals. Mixed agreement results were reported when comparing statistically significant and ecologically meaningful benthic metric relationships with the threshold effect level (TEL) exceedances for the various metals.

Keywords: total metals; bioavailable metals; benthic communities; metals threshold effect levels

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1. Introduction

Heavy metals have been increasing in the global environment since the industrial evolution [1]. Metals in aquatic natural systems are of concern to regulators because they can be toxic at concentrations above ambient conditions, non-biodegradable in the environment, and accumulate in animal and plant tissue [2]. Many metals are essential for living organisms, yet can be toxic at high concentrations [3]. In aquatic ecosystems, sediments are the main sink for metals [4]. Sources for metals in the environment are both anthropogenic and natural and include effluents from wastewater treatment plants, industrial and agricultural wastes, power stations, mining, boating activities, transportation vehicles, urban runoff, and geological processes [2,5,6].

Various approaches are used to determine the potential impacts of metals on benthic communities. These approaches include laboratory toxicity testing with field collected contaminated sediment using single species toxicity tests, microcosms, mescosms, and a comparison of the environmental sediment concentrations with guidelines, standards, criteria or effects thresholds developed for metals. These approaches are considered to be impacted predicted responses, because the condition of resident benthic communities is not being directly assessed with concurrent exposure to metals. The other approach, termed the impacted observed response, that can be used to determine the impact of metals on benthic communities is called bioassessments, where the condition of the resident benthic community with concurrent measurements of various stressors such as metals can be used to determine the condition of the aquatic ecosystem [7,8].

Metals are generally found as mixtures in the aquatic environment [9]. Therefore, in order to assess the impact of combinations of metals and to attempt to rank which metals are most toxic to resident benthic communities, bioassessment field studies are needed that concurrently evaluate the benthic taxa condition and individual metal concentrations from the same sample location. Because of spatial and temporal variability issues, these studies also need to be conducted over multiple years and preferably over multiple seasons. To the best of our knowledge, studies designed to address the above components are rare.

A bioassessment multiple stressor study was conducted over multiple years and multiple seasons in an agricultural water body in California (Cache Slough) in order to determine the relationship between various benthic metrics and sediment characteristics, total metals, simultaneously extracted metals, and pyrethroids [10]. The relationship of 11 benthic metrics representing richness, composition, tolerance/intolerance, and trophic measures to 28 different stressors was evaluated over a three-year period. The results from this study showed that benthic communities were more closely associated with sediment characteristics and some metals, but not pyrethroids. However, a focused extensive univariate and stepwise multiple linear regression analysis was not conducted to determine which of the total metals and simultaneously extracted metals were most important for impacting the benthic communities. The objectives of this study were to (1) determine the relationship between the sediment concentrations of eight total metals (As, Cd, Cr, Cu, Pb, Hg, Ni, and Zn) and five simultaneously extracted metals (SEM) (Ni, Cu, Zn, Cd, and Pb) with 11 benthic metrics based on a three-year data set including two seasons per year for an agricultural waterbody (Cache Slough, California), and (2) rank the importance of individual metals within a metals mixture as potential stressors to resident benthic communities.

2. Materials and Methods

2.1. Site Selection

Field sampling in the Cache Slough was conducted during two seasons (spring and fall) for three consecutive years (2012, 2013, and 2014), as presented in Figure 1 [10]. The 18 km study area included 12 sites with different habitat types, including upstream to the downstream confluence points with the Sacramento River. The study sites were placed after the confluence points of the various water bodies flowing into the Cache Slough.

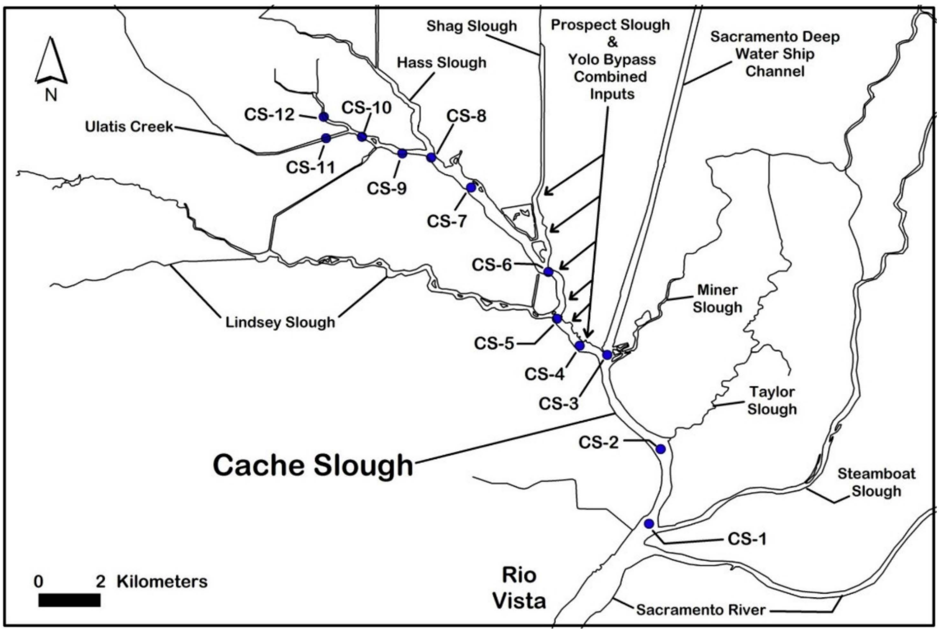

Figure 1. Twelve Cache Slough sites sampled during the spring and fall of 2012, 2013, and 2014.

2.2. Sampling Procedures

The Cache Slough is a non-wadeable water body with a depth ranging from approximately 1 to greater than 9 m, depending on the tidal cycle. An 18 ft. Arimi boat was used for the field sampling. Previously described methods [11] were used to collect the sediment samples for the sediment characteristics, metals, and benthic communities. A petite Ponar sampler was used to collect the surface sediment (approximately the top 2–5 cm). Five locations were randomly selected from a 100 m transect for sampling. One composite sample from the five locations was used to measure the physical, chemistry, and benthic samples described below.

2.3. Water Quality and Sediment Measurements

Previously described methods [12] were used to measure the following water quality parameters: temperature, pH, salinity, specific conductivity, dissolved oxygen, and turbidity. Approximately one liter of sediment was used to measure the grain size [13], TOC [14], and metals, as discussed below. Nitric acid, ethanol, and distilled water were used to clean the sampling equipment between the sampling sites. After collection in the field, the sediment samples were stored in a cooler on ice and then later transferred to a refrigerator. The next step was shipping the samples to the Alpha Analytical Laboratory in Mansfield, Massachusetts, for the metal, grain size, and total organic carbon (TOC) analyses.

2.4. Total Metals and SEM/AVS Analysis

EPA method 6020 m was used to measure the sediment samples for the following total metals: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn). These total metals had method detection limits (MDL) ranging from 0.02 to 1.4 µg/g dry weight. EPA method 245.7 m was used to measure the mercury (Hg), and the MDL for the mercury was 0.02–0.04 µg/g dry weight.

EPA method 200.8 was used to measure simultaneously extracted metals (SEM) for Ni, Cu, Zn, Cd, and Pb. The SEMs had the following MDLs (µmol/dry g): Ni (0.02), Cu (0.009), Zn (0.02), Cd (0.002), and Pb (0.006). Previously described methods [15] were used to measure the acid volatile sulfides (AVS). The bioavailability of these metals in the sediment was then determined by calculating the SEM/AVS ratios. The SEM/AVS model for estimating the toxicity to benthic organisms predicts that when AVS concentrations of a molar basis exceed the SEM concentrations, metals will be bound to sulfides and will be non-toxic [16,17]. When sediments contain an excess of SEM metals they are considered potentially toxic, but not necessarily toxic to benthic organisms.

2.5. Benthic Macroinvertebrate Sampling

Benthic macroinvertebrate samples were collected using a Petite Ponar grab sampler from the 100 m transect consisting of a composite of five samples per site, as described above. The goal was to identify the benthic samples to the species level if possible. However, this was not the case for all samples, and particularly for oligochaetes and chironomids, where the family and genus level, respectively, were often the lowest level of identification possible.

Personnel at the CDFG Laboratory located at the Chico State University were responsible for subsampling and sorting the benthic macroinvertebrate samples. California's Department of Fish and Game (CDFG) conducted the benthic macroinvertebrate subsampling (resulting in a maximum of 300 individuals) and identification. Protocols previously described [18] were used for Level 3 identifications (species level identifications if possible). Protocols developed by the United States Geological Survey National Quality Control Laboratory [19] were used for the slide preparations and mounting for species such as midges and oligochaetes.

The following list of benthic metrics (with the predicted response to stress, such as metals, previously reported [18]) were also developed from the taxonomic data and used in the statistical analysis presented below:

Benthic Metric	Response to Impairment
# Collector/Filterer and Collector/Gatherer	Increase
Number of Individuals (Abundance)	Decrease
% Amphipoda	Variable
% Collector/Filterer and Collector/Gatherer	Increase
% Corbicula	Variable
% Dominant Taxon	Increase
% Oligochaeta	Increase
% Predators	Decrease
% Tolerant Taxa	Increase
Shannon Diversity	Decrease
Taxonomic Richness	Decrease

All benthic metric calculations were conducted using CDFG. Stress sensitive metrics, such as taxonomic richness, were predicted to decrease with impairment, while stress tolerant metrics, such as % dominant taxa, were predicted to increase with impairment.

2.6. Statistical Analysis

The sediment concentrations of the total metals and concentrations of the simultaneously extracted metals (SEM) to acid volatile sulfide (AVS) ratios were merged with benthic metric data sets. Metals in the sediment concentrations from the three-year data set were standardized to their relative toxicities by dividing the dry weight concentrations of each metal by their respective threshold effects level (TEL) values [20].

Indications of significant relationships ($\alpha = 0.01$) between the benthic metrics and specific metals (both the total metals concentration to TEL ratios and SEM to AVS ratios for each metal) were determined using univariate general linear model regressions [21]. In order to determine the potential relationships between the benthic metrics and metal to TEL ratios, a series of stepwise multiple regressions was also used [21]. This statistical approach addresses the effects of combinations of metals to TEL ratios and the relationships of benthic metrics with metals that may be confounded by the effects of the correlated variables.

3. Results

Details about the water quality; sediment measurements; total metal; SEM metal; and benthic community data by site, season, and year are available in various reports [22–24]. The ranges of these data are summarized below.

3.1. Water Quality

The ranges of the water quality parameters for all of the 12 sites for both spring and fall for 2012 were as follows: temperature (C) = 12–19.1; pH = 6.9–8.39; dissolved oxygen (mg/L) = 7.34–10.0; conductivity (uS) = 111–339; salinity (ppt) = 0.1–0.3; and turbidity (NTU) = 10.6–255. For 2013, the ranges of the water quality parameters for all 12 sites for both spring and fall were as follows: temperature (C) = 13.7–18.4; pH = 7.3–8.22; dissolved oxygen (mg/L) = 7.21–10.73; conductivity (uS) = 128–605; salinity (ppt) = 0.1–0.4; and turbidity (NTU) = 4.7–68.9. The ranges of the water quality parameters for all 12 sites for both spring and fall for 2014 were as follows: temperature (C) = 15.1–21.5; pH = 7.34–8.14; dissolved oxygen (mg/L) = 6.4–8.93; conductivity (uS) = 173–689; salinity (ppt) = 0.1–0.4; and turbidity (NTU) = 5.91–32.1.

The ranges for both the pH and salinity measurements were consistent for all three years. The ranges for the other water quality parameters were somewhat variable. For example, the upper range of temperature was slightly higher in 2014 when compared with the other two years. The lower range of dissolved oxygen was lower in 2014 when compared with the other two years. The upper range of turbidity was lower in 2014 when compared with the other years.

3.2. Sediment Measurements

The ranges of the three-year mean % TOC and grain size measurements sampled in the spring and fall for 2012, 2013, and 2014 were as follows: % TOC = 0.75–2.49; % gravel = 0–2.02; % sand = 1.29–49.8; % silt = 34.6–67.29; and % clay = 14.23–37.4. The % gravel range was rather narrow. However, the other sediment measurements showed a variable range of approximately 2 × to 38 × difference between the upper and lower values of the range.

3.3. Total Metals

The ranges of the three-year means for the total metals (ug/g dw) sampled in the spring and fall of 2012, 2013, and 2014 were as follows: arsenic = 4.08–8.15; cadmium = 0.147–0.487; chromium = 38–84.4; copper = 25.1–57.7; lead = 7.52–12.75; mercury = 0.045–0.172; nickel = 58.9–101.9; and zinc = 67.5–114.3. The ranges of the difference in the total metals were 1.7 to 3.8×. The smallest range was reported for lead, nickel, and zinc, while the largest range was reported for mercury.

The NOAA threshold effect levels (TEL) (ug/g dw) for each metal were as follows: arsenic (5.9), cadmium (0.596), chromium (37.3), copper (35.7), lead (35), mercury (0.174), nickel (18), and zinc (123.1) [20]. The chromium and nickel concentrations exceeded the TELs at all of the sites for the three-year period. Copper TEL exceedances occurred for most of the sites over the three-year period. Arsenic TEL exceedances also occurred for approximately half of the sites sampled over the three years. There were no TEL exceedances for the cadmium, lead, mercury and zinc.

3.4. AVS and SEM Metals

The ranges of the three-year means for SEM metals (umoles/g) sampled in the spring and fall of 2012, 2013, and 2014 were as follows: AVS = 0.13–3.13; nickel = 0.20–0.55; copper = 0.20–0.51; zinc = 0.40–0.72; cadmium = 0–0.003; lead = 0.019–0.048; total SEM = 0.83–1.7; and SEM/AVS ratio = 0.41–6.89. For most of the SEM metals (nickel, copper, zinc, cadmium, and lead) and total SEM, there was an approximately 2× to 3× difference in the range of concentrations. For AVS, there was 24× difference between the lowest and highest concentrations. This suggest a high variability for AVS in this study area. The SEM/AVS ratio showed approximately a 17× difference between the low and high values.

3.5. Benthic Communities

The number of different benthic taxa collected during each year ranged from 46 in 2014 to 56 in 2013. The five most dominant taxa collected during the six sampling periods comprised 74 to 85% of the taxa. The two most dominant taxa collected during the six sampling periods were the amphipod *Americorophium sp.* and the polychaete *Manayunka speciosa.* (see [10] for details).

The ranges of the mean benthic metrics across years and seasons by sites were as follows: # collector/filterers and collector/gatherers = 4.8–11.8; abundance = 61–280; % amphipods = 12–73; % collector/filterers and collector/gatherers = 74–97; % Corbicula = 2–18; % dominant taxa = 36.9–69.8; % oligochaete taxa = 9.2–18.3; % predator taxa = 14.5–30.2; % tolerant taxa = 51.5–76.8; Shannon diversity = 1.1–2.25; and taxa richness = 7–22. Most of these metrics showed fairly low variability (less than 3× difference between the low and high values). These metrics were as follows: # collectors/filterers and collectors/gatherers, % collectors/filterers and collectors/gatherers, % dominant taxa, % oligochaetes, % predators, % tolerant taxa, Shannon diversity index, and taxa richness. The abundance, % amphipod, and % Corbicula metrics were more variable, as the difference between the low to high values ranged from approximately 5× to 9×.

3.6. Univariate Regressions of Benthic Metrics vs. Metals to TEL Ratios

There were a total of 12 statistically significant relationships with the benthic metrics and metals to TEL ratios, as presented in Table 1. The number of significant relationships for each metal were as follows: total metals (2), arsenic (2), cadmium (2), chromium (2), copper (1), lead (1), mercury (0), nickel (2), and zinc (0). The relationships of metals to the benthic metrics % amphipods and % Corbicula are considered to be variable (not a clear response to impairment), so the results from these relationships were not used in the following data interpretation. Therefore, there were four significant relationships with benthic metrics and metals as follows: taxonomic richness and cadmium to TEL, % collector/filterers and collector/gatherers and arsenic to TEL, abundance and cadmium to TEL, and abundance and lead to TEL. Of these four significant relationships, the positive relationship of the % collectors/filterers and collectors/gatherers to arsenic (an increase in arsenic caused this stress tolerant metric to increase) was ecologically meaningful, as was the abundance negative relationship with lead (an increase in lead caused abundance to decline). In contrast, two of these four significant relationships that were not ecologically meaningful were the positive relationship of taxonomic richness and cadmium (an increase in cadmium caused this stress sensitive metric to increase) and the positive relationship of abundance and cadmium (an increase in cadmium caused abundance to increase). In summary, the only metals to show significant and ecological relationships with benthic metrics were arsenic and lead.

3.7. Stepwise Multiple Linear Regression Models of Benthic Metrics to Metals TELS

In the stepwise multiple linear regression models in Table 2, there are 13 significant relationships with benthic metrics and metal to TEL ratios. The number of significant relationships by metal were as follows: cadmium (2), lead (2), zinc (2), arsenic (3), chromium (2), nickel (1), and copper (1). The relationships of metals to the benthic metrics % amphipods and % *Corbicula* are considered to be variable (not a clear response to impairment), so the results from these relationships were not used in the following data interpretation. Therefore, there were nine significant relationships of benthic metrics and metals based on the stepwise multiple regression analysis. These nine significant relationships were as follows: taxonomic richness and cadmium, taxonomic richness and lead, taxonomic richness and zinc, percent dominant taxa and arsenic, percent dominant taxa and chromium, percent collector/filterer and collector/gatherer and arsenic, percent collector/filterer and collector/gatherer and copper, percent collector/filterer and collector/gatherer and cadmium, and abundance and lead. The five ecologically meaningful relationships were taxonomic richness and negative relationship with lead (taxonomic richness declined with an increase in lead), percent dominant taxa increased positive response with arsenic (the stress tolerant metric percent dominant taxa increased with an increase in arsenic), percent collector/filterer and collector positive response the arsenic (this stress tolerant metric increased with an increase in arsenic), percent collector/filterer and collector/gatherer positive response with cadmium (this stress tolerant metric increased with an increase in cadmium), and abundance negative response with lead (this stress sensitive metric declined with an increase in lead concentrations). In our view, the four relationships with benthic metrics and metals that were not ecologically meaningful were as follows: taxonomic richness positive relationship with cadmium (richness increases with cadmium), taxonomic richness positive relationship with zinc (richness increases with zinc), percent dominant taxa negative relationship with chromium (this stress tolerant metric decreases with chromium), and percent collector/filterer and collector/gatherer negative relationship with copper (this stress tolerant metric decreases with copper). In summary, the only metals to demonstrate statistically significant and ecologically meaningful relationships were arsenic (two relationships), lead (two relationships), and cadmium (one relationship).

Table 1. Results of univariate linear regression models of benthic metrics versus metals to TEL ratios for sediments from the Cache Slough in the combined 2012, 2013, and 2014 data set, indicating the type of the relationships (+ = direct; - = inverse) and R^2 values for significant relationships (α = 0.01; NS = not significant).

	Total Metals to TELs		As to TEL		Cd to TEL		Cr to TEL		Cu to TEL		Pb to TEL		Hg to TEL		Ni to TEL		Zn to TEL	
BENTHIC METRICS	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**
Taxonomic Richness	NS		NS		+	0.14	NS		NS		NS		NS		NS		NS	
% Dominant Taxon	NS		NS		NS		NS		NS		NS		NS		NS		NS	
Shannon Diversity	NS		NS		NS		NS		NS		NS		NS		NS		NS	
% Amphipods	-	0.10	NS		NS		-	0.17	-	0.11	NS		NS		-	0.13	NS	
% Corbicula	+	0.15	+	0.13	NS		+	0.13	NS		NS		NS		+	0.18	NS	
% Oligochaetes	NS		NS		NS		NS		NS		NS		NS		NS		NS	
% Tolerant Taxa	NS		NS		NS		NS		NS		NS		NS		NS		NS	
% Predators	NS		NS		NS		NS		NS		NS		NS		NS		NS	
# Collectors/Filterers and Collectors/Gatherers	NS		NS		NS		NS		NS		NS		NS		NS		NS	
% Collectors/Filterers and Collectors/Gatherers	NS		+	0.17	NS		NS		NS		NS		NS		NS		NS	
Abundance	NS		NS		+	0.11	NS		NS		-	0.14	NS		NS		NS	

Table 2. Results of the stepwise multiple linear regression models of benthic metrics versus metals in sediments (standardized to toxic units by dividing by TEL values for the metals) for the Cache Slough in the combined 2012, 2013, and 2014 data set. Only variables that were significant at $\alpha = 0.01$ were included in the models (NS = not significant). The direction of the relationship for each significant variable is indicated (+ = direct; - = inverse), as is the contributed R^2 value.

BENTHIC METRICS	Prob.	R^2	Significant Variables (R^2)
Taxonomic Richness	<0.001	0.26	+ Cadmium (0.14), - Lead (0.06), + Zinc (0.06)
% Dominant Taxon	0.004	0.15	+ Arsenic (0.08), - Chromium (0.07)
Shannon Diversity	NS		
% Amphipods	<0.001	0.44	+ Arsenic (0.27), - Chromium (0.17)
% Corbicula	<0.001	0.28	+ Nickel (0.18), - Zinc (0.10)
% Oligochaetes	NS		
% Predators	NS		
% Tolerant Taxa (8–10)	NS		
# Collectors/Filterers and Collectors/Gatherers	NS		
% Collectors/Filterers and Collectors/Gatherers	<0.001	0.26	+ Arsenic (0.17), - Copper (0.09)
Abundance (#/sample)	<0.001	0.27	+ Cadmium (0.16), - Lead (0.11)

3.8. Univariate Linear Regressions of Benthic Metrics Versus SEM/AVS Ratio

The SEM/AVS ratio is an approach used to determine if the bioavailable metal is potentially toxic. The results from Table 3 show that there are five significant relationships with the SEM metals: two for nickel, two for cadmium, and one for lead. The relationships of SEM metals to the benthic metrics % amphipods and % Corbicula are considered to be variable (not a clear response to impairment), so the results from these relationships were not used in the following data interpretation. Therefore, there were only two relationships with benthic metrics and SEM metals. These relationships were taxonomic richness and abundance positive relationships to cadmium to AVS. In our view, this is not an ecologically meaningful relationship, because an increase in cadmium should not cause an increase in taxa richness. The second relationship (abundance and a positive relationship with cadmium) is also not ecologically meaningful either, because an increase in cadmium should not increase abundance. In summary, there were no statistically significant and ecologically meaningful relationships for benthic metrics and bioavailable metals in contrast with the various statistically significant and ecologically meaningful relationships reported above for total metals.

Table 3. Results of the univariate linear regression models of the benthic metrics versus concentrations of simultaneously extracted sediment metals to AVS ratios for sediments from the Cache Slough in the combined 2012, 2013, and 2014 data set, indicating the type of relationships (+ = direct; - = inverse) and R^2 values for significant relationships (α = 0.01; NS = not significant).

	Total Metals SEM to AVS		Ni to AVS		Cu to AVS		Zn to AVS		Cd to AVS		Pb to AVS	
BENTHIC METRICS	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**	**Rel.**	**R^2**
Taxonomic Richness	NS		NS		NS		NS		+	0.12	NS	
% Dominant Taxon	NS		NS		NS		NS		NS		NS	
Shannon Diversity	NS		NS		NS		NS		NS		NS	
% Amphipods	NS		-	0.13	NS		NS		NS		NS	
% Corbicula	NS		+	0.10	NS		NS		NS		+	0.10
% Oligochaetes	NS		NS		NS		NS		NS		NS	
% Tolerant Taxa	NS		NS		NS		NS		NS		NS	
% Predators	NS		NS		NS		NS		NS		NS	
# Collectors/Filterers and Collectors/Gatherers	NS		NS		NS		NS		NS		NS	
% Collectors/Filterers and Collectors/Gatherers	NS		NS		NS		NS		NS		NS	
Abundance	NS		NS		NS		NS		+	0.10	NS	

4. Discussion

Total arsenic, lead, and cadmium were the only total metals to show both statistically significant and ecologically meaningful relationships with benthic metrics in the Cache Slough. In contrast, the total copper, nickel, zinc, chromium, and mercury were not reported to show any statistically significant and ecologically meaningful relationships with any of the benthic metrics. Another key finding is that there were also no statistically significant and ecologically meaningful relationships between the benthic metrics and the bioavailable metals (Ni, Cu, Zn, Cd, and Pb).

The first step in the data interpretation was a comparison of the results from the statistically significant and ecologically meaningful benthic metric relationships of the eight total metals with the TEL exceedances to see if these data were in agreement (Table 4). In other words, we considered whether the compelling significant benthic metric relationships based on field data corresponded to the potentially toxic metal concentrations expressed as TELs based on laboratory toxicity data, as previously reported [25]. There was agreement for the total arsenic, zinc, and mercury. In the case of the total arsenic, three statistically significant and ecologically meaningful benthic metric relationships were reported at approximately the half sites where the arsenic TEL exceedances occurred. Arsenic has also been reported as an important metal impacting benthic metrics in another bioassessment multiple study in five wadeable waterbodies in California [26]. For the total zinc, there were no statistically significant and ecologically meaningful benthic metric relationships reported and there were no TEL exceedances for zinc that would imply stressful conditions. A similar agreement scenario was also reported for mercury, as there were no statistically significant and ecologically meaningful benthic metric relationships and there were no TEL exceedances for mercury.

Table 4. Comparison of the statistically significant and ecologically meaningful benthic metric relationships with the total metals TEL exceedances. ~ means approximately.

Total Metal	# Significant Benthic Metric Relationships	TELs Exceedances for Sites	Agreement with Metric Relationships and TEL Exceedances
Arsenic	3	~Half the sites	Yes
Lead	3	None	No
Cadmium	1	None	No
Copper	0	Most sites	No
Nickel	0	All sites	No
Chromium	0	All sites	No
Zinc	0	None	Yes
Mercury	0	None	Yes

In contrast with the agreement patterns reported above, there was disagreement when comparing the statistically significant and ecologically meaningful benthic metric relationships with the TELs for lead, cadmium, copper, nickel, and chromium (Table 4). In the case for total lead, there were three statistically significant and ecologically meaningful benthic metric relationships reported, but all lead concentrations were below the TEL of 35 ug/g. In fact, the highest total lead concentration of 12.75 ug/g was well below the TEL. Therefore, it appears that the TEL for lead in this aquatic system may have been too high, as various benthic metrics were responding to lower concentrations based on our data set. The total cadmium had a similar interpretation as the total lead. There was one statistically significant and ecologically meaningful benthic metric relationship with total cadmium, but all cadmium concentrations were below the cadmium TEL of 0.596 ug/g. For example, the highest total cadmium concentration of 0.487 ug/g was slightly lower than the TEL. As reported above for lead, it appears that the TEL for cadmium in the Cache Slough may be too high as well, as one benthic metric responded to lower concentrations. The results from other bioassessment multiple stressor studies in California have shown that cadmium is an important stressor impacting benthic community metrics in various wadeable California streams [26]. However, the case for cadmium is not as compelling as the case for lead, as only one benthic metric showed a valid response.

The total copper, nickel, and chromium had similar results when comparing statistically significant and ecologically meaningful benthic metric relationships with TELs. In the case of copper (a micronutrient), there were no statistically significant and ecologically meaningful benthic metric relationships reported, but the TEL of 35.7 ug/g was exceeded at most of the sites. Therefore, for the total copper it appears that the TELs may have been too low as there was no response from 11 benthic metrics. For both the nickel and chromium, there were no statistically significant and ecologically meaningful benthic metric relationships reported, although the TELs for both of these metals were exceeded at all sites during the three-year sampling period. High concentrations of nickel and chromium in the Cache Slough are likely due to the presence of serpentine soils in the area, which are naturally high for these two metals [27]. As it is highly likely that these high concentrations of both nickel and chromium have been present in the Cache Slough for a long period of time, it is certainly plausible that the resident benthic communities have acclimated to these concentrations. Therefore, the benthic metrics used in this study showed no response to these ambient metals concentrations that exceeded the TELs. The other reasonable explanation to explain the above results is that the TELs for both nickel and chromium were too low. There is one other bioassessment multiple stressor study in a wadeable agricultural watershed with a compatible study design, which was conducted in the Santa Maria River watershed California, where nickel TEL exceedances occurred at all of the study sites concurrently with a statistically significant and ecological meaningful relationship with % Predators, but not any of the other benthic metrics [28]. These nickel results from the Santa Maria watershed provide a case for widespread nickel TEL exceedances, with one statistically significant and ecologically meaningful benthic metric response, in contrast with our Cache Slough data.

Another important result from this work is that there were no statistically significant and ecologically meaningful relationships between the benthic metrics and bioavailable metals (nickel, copper, zinc, cadmium, and lead). For bioavailable copper, nickel, and zinc, which showed no statistically significant and ecologically meaningful benthic metric relationships, these results agreed with the total metals results for these three metals, as discussed above. For both bioavailable lead and cadmium, there were no statistically significant and ecologically meaningful benthic metric relationships, but for both the total lead and total cadmium, there were compelling benthic metric relationships. These results suggest that only the total fraction of these metals was responsible for a benthic response, but the bioavailable metal fraction was not.

The benthic community in the Cache Slough is clearly exposed to a suite of metals, as reported in this study. Our analysis of the relationship of various benthic metrics representing richness, tolerance/intolerance, and trophic measures to ambient metal concentrations showed some compelling metal relationships with total arsenic, lead, and cadmium, but not the bioavailable fraction of lead and cadmium. The other metals such as copper, nickel, chromium, zinc, and mercury did not show statistically significant and ecologically meaningful relationships with benthic metrics, and are therefore unlikely stressors for resident benthic communities in this waterbody.

5. Conclusions

Three of the eight total metals measured (arsenic, lead, and cadmium) were reported to have statistically significant and ecologically meaningful relationships with benthic metrics in the Cache Slough, while the other five total metals (copper, nickel, chromium, zinc, and mercury) did not show compelling relationships with the benthic metrics. The benthic metrics that were most important for detecting compelling relationships with the total metals were both a combination of stress tolerant metrics, such as % collector/filterer and collector/gatherer and % dominant taxa, as well as stress sensitive metrics such as abundance and taxa richness. This suite of significant benthic metrics were important ecological measures of the various components of the benthic community, such as richness, tolerance/intolerance, and trophic measures, so the discriminatory power to detect possible adverse impacts from metals was likely present. However, attempting to extrapolate the results from this study based on compelling relationships between the benthic metrics and total metals and the possible impairment or ecological conditions in the Cache Slough is challenging at this time because there is no reference site that can be used for comparison. In addition, the Cache Slough is a tidal freshwater aquatic lotic waterbody that typically has a lower number of benthic species (ranging from 46 to 56 for the three-year period in this study) when compared with either freshwater or saltwater areas. The complex habitat in these types of waterbodies also makes it difficult to understand the effects the on benthic taxa [29]. Based on the results from our study, the conservative interpretation is that total copper, nickel, chromium, zinc, and mercury are not likely to be stressors on the resident benthic communities in the Cache Slough, while the total arsenic, lead, and cadmium are possible stressors.

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