Trace metal speciation in a wastewater wetland and its bioaccumulation in tilapia *Oreochromis niloticus*.

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**ABSTRACT**

Trace metal species in the water column of a canal system forming a wetland filled with wastewater were analyzed to determine their correlation with metal accumulation in the gills of locally fished *Oreochromis niloticus*. The metal concentrations in the suspended particles and water dissolved were analyzed. The metal species were calculated using Windermere Humic Aqueous Model version VII showing that the high organic matter and major cation contents were important parameters. Also, the free ion metal concentration was expected to correlate with the organic matter aromaticity; however, organic compounds other than humic substances seem to be complexing the metals in the system. Additionally, no clear correlation could be found between metal accumulation in gills and any of the dissolved metal species. Nonetheless, certain trends could be seen between the calculated metal species and metal accumulation in the tilapia from the suspended particles.

**1. Introduction**

The toxicity, bioavailability and mobility of heavy metals depend on their speciation rather than on their total concentrations in water [1,2]; therefore trace metal speciation has become an important piece of information in the geochemical and ecotoxicological impacts of metals. Metals have been characterized as highly harmful to aquatic communities and, depending on the magnitude of their concentration and exposure time, can cause numerous alterations at different levels of biological organization [3,4]. Metal toxicity in living beings can occur by the blocking of biological activities, i.e., enzymatic inactivation by the formation of bonds between the metal and the -SH (sulfhydryl) groups of proteins, causing irreversible damage in different organisms. For heavy metals to be toxic to organisms, they must be available to be absorbed, a.k.a. bioavailable. The most toxic metallic chemical species are free ions and small metal complexes in solution [5,6] and these bioavailable forms are proposed as the major forms incorporated by aquatic organisms. However, these metals can also be incorporated via the diet (by the incidental intake of particulate matter), i.e., in benthic and benthopelagic organisms. In this context, digestive processes and chemical conditions prevailing in the organism's digestive tract (pH, digestion time, redox state) determine whether non-bioavailable metals in the external environment are absorbed and assimilated into the gastrointestinal epithelium [7].

The chemical equilibrium of a metal in a solution determines the concentration of the metal species. The toxicity of a metal in aquatic organisms can be mitigated or diminished when the metal exists in a form that is not bioavailable, i.e., either thru complexation with dissolved organic matter (DOM) or inorganic species, or by its association with suspended particles. Chemical species of metals occur in very low concentrations, particularly the free metal ion or small metal complexes. These low concentrations, together with their chemical similarities, pose an analytical challenge. Consequently, two primary approaches have been studied in the determination of metal toxicity: the use of mathematical, chemical equilibrium models and the use of organisms as biomarkers. The use of mathematical models has been of paramount importance to recreate or represent the way in which the chemical species of metals occur in the water [8].

Green areas within cities provide a range of environmental services of vital importance. One of these green areas is Xochimilco in the southern part of Mexico City. Xochimilco is a wetland site whose clear spring waters now its 180 km of canals are filled with wastewater, making this space a repository for a variety of pollutants. Nevertheless, Xochimilco is an agricultural and...
recreational area with essential environmental services such as being a place for local fauna and migration birds, a lung for the metropolis and an important periurban agricultural site. Metal pollution is one of the area’s primary concerns, and several studies have analyzed the metal concentrations in the area [9,10], including in the canal waters, which are the habitat for endemic species such as *Ambystoma mexicanum, Cambarellus montezumae* or *Nymphaea mexicana*. However, no studies have been conducted on the possible metal species present or their bioavailability. Since the waters of the canals are rich in organic matter and soluble salts, the toxicity of the metals could be lower than previously thought [11]. This study aims to determine the metal concentrations in the water column, including in the suspended particles, and to identify the different metal species present using mathematical equilibrium models so as to relate these concentrations with metal accumulated in the gills of the locally fished tilapia *Oreochromis niloticus*.

2. Materials and methods

2.1. Study area

The studied canal system is fed with waste water from water treatment plants and also receives contributions of surface runoff from agricultural parcels and clandestine sewages, making it a very polluted system. Four sampling sites were selected based on the following criteria:

![Figure 1. Sketch showing the geographical location of the Natural Protected Area of Xochimilco and sampling points. Source: http://www.conabio.gob.mx/informacion/gis/](image)

To determine the total metal concentrations in the water including the suspended particles, the water samples were digested (modified EPA SW-3015a and EPA SW-3052 methods) by placing 10 mL of water in Teflon tubes and adding 5 mL of HNO₃ (70%, Aldrich® purified by redistillation, ≥99.999% trace metals basis), 1 mL H₂O₂ and 2 mL HF and microwaving in a Microwave Oven (MARSX, CEM, Matthews, NC, USA). At the end of the digestion, a post-treatment was performed in which 2 mL of H₃BO₄ and deionized water were added to a final volume of 15 mL.

The organic carbon content in the suspended particles was determined in a carbon analyzer (TOC Perkin Elmer 2400 Weltham MA, USA) after filtration through a 0.45 μm filter; while the dissolved organic carbon (DOC) content in the water was determined in a TOC analyzer (Apollo 9000 from Tekmar Dohrmann, Mason, OH, USA).

For the analysis of the dissolved metals, upon reaching the laboratory, the obtained water samples were
filtered using a system with 0.45 μm pore membranes (HT Tuffryn® Polysulfone Membrane Disc Filters, Pall Corporation, USA) and placed in thoroughly washed, high-pressure polyethylene bottles (Nalgene® bottles). The determination of the metals concentration in the water was conducted using ICP-MS (Thermo Scientific, Model: iCap Q, USA). For the quality control of the analyses, two replicates of the certified reference standard (SLRS-5 Riverine Water, NRC - CNRC, Canada) were used, and three aliquots of MilliQ grade deionized water were used as blanks (Barnstead®, 18 MΩcm⁻¹).

The cations and anions analysis of the water was conducted using a 10 mL sample of water that was field filtered using 0.45 μm nitrocellulose membranes (MFTM-Millipore Membrane Filters, 0.45 μm HAWPO2500, Ireland), and then injected into a 10 mL Vacutainer® Serum, Plus Blood Collection Tubes, 367820, USA). This sample was stored on ice at 4 °C for liquid chromatography analysis consisting of a binary pump (Waters model 1525, MA, USA), a self-sampler (Waters model 717 plus, MA, USA) and a conductivity detector (Waters model 432, MA, USA).

Since the chemical equilibrium models of metals require data on the concentrations of humic substances, the concentration of humic acids in the water was determined utilizing the technique described by Van Zomeren et al. (2007) [12]. Briefly, humic substances are polar substances of polymeric organic acids derived from humus, soils, and terrestrial and aquatic plants; they comprise approximately one-third to one-half of the total dissolved carbon (COD). Because of their polar characteristics, humic substances can be adsorbed and/or exchanged in certain resins. Thus, this method is based on the use of a macroporous resin to extract the aquatic humic substances by solid phase extraction. The substances are then selectively extracted and quantified in solution in a TOC analyzer (Apollo 9000 Tekmar Dohrmann, Mason OH, USA). According to Van Zomeren [12], the recovery of humic acids (HA) decreases markedly when the concentrations are below 50 mgL⁻¹, which coincides with the recovery values of 30% found in this study. Recoveries were determined with a laboratory-made solution containing humic acid standard, the Suwannee River Humic Acid Standard II (2S101H) from the International Humic Substances Society matching the concentrations found in the studied site. In spite of the low HA recovery, these values were not corrected to a 100% recovery in the parameters fed to the chemical equilibrium models of the metals. The metal species calculated with the chemical equilibrium models did not change substantially since, for a difference to be observed, the concentrations of HA should be orders of magnitude higher.

Fulvic acids were calculated as 65% of the DOM, where the ratio DOC-DOM is assumed to be two [13].

### 2.3. Freshwater fish sampling

With the help of a professional fisherman, Oreochromis niloticus (O. niloticus) at its commercial size were collected from the same areas as the water samples. The fish were visually examined for the presence of any morphological body abnormalities, and any abnormal fish were excluded. From the remaining fish, ten organism were selected randomly and their gills lyophilized and analyzed for metals after a microwave nitric acid digestion (Sigma Aldrich Nitric acid 70%, purified by redistillation, ≥99.999% trace metals basis) with ICP-MS.

### 2.4. Mathematical models of metal chemical equilibrium

The mathematical models considered in this study were selected because they consider the chemical equilibrium with non-polar substances. The Windermere Humic Aqueous Model (WHAM), Biotic Ligand Model (BLM) and VisualMINTEQ were compared. The WHAM model determines the concentration of the metal ion or ‘free metal’ (bioavailable) and the other metallic species thereof. Visual MINTEQ is a chemical equilibrium model for the calculation of metal speciation, solubility equilibria, sorption, etc. For natural waters, the WHAM model combines the state-of-the-art description of sorption and complexation reactions used for chemical balancing modeling. The BLM is a tool for predicting toxicity in aquatic systems using information on the chemistry of the local water sources. The BLM was initially designed to determine copper and silver toxicity in freshwater fish and invertebrates and is therefore considered to be a more classic model for evaluating toxicity. Because the BLM model can only be used for four metals (Cd, Cu, Pb and Zn), these metals were used to make comparisons between the chemical equilibrium models. For the comparison, the same parameters analyzed in the water were used: dissolved metal, anions and cations concentrations, HA, FA and dissolved organic carbon (DOC). The models were run to obtain free/bioavailable ion concentration, and the model results were compared.

### 3. Results and discussion

#### 3.1. Background water chemistry

The studied canals varied from the widest and longest in the Agricultural site (AI); the medium sized Urban canal (UI), which ends in a lagoon surrounded by houses with clandestine domestic waters; the wide Recreational canal (RI), almost a minor lagoon with a small island used for occasional theatrical representations; and the Wastewater canal (WW) near an important dock with a high volume outlet from the wastewater treatment plant, which imparts the canal with the highest flow of all the canals and has almost no sediment. The Recreational and
Agricultural sites are more isolated and have additional local fauna relative to the Urban and Wastewater sites. The water quality parameters can be seen in Table 1. Based on the results obtained from the physicochemical parameters of the different sites (canals), a comparative boxplot analysis (not shown) was made. This plot revealed that the AI sites presented the highest pH, temperature and dissolved oxygen relative to the other sites. Conductivity was different for all the canals, although the AI site showed the highest standard deviation. The highest concentrations of DOC were found at the AI site, and the average lowest in the RI site; while the WW site had similar values to the RI site but a higher standard deviation.

In aquatic environments, DOC is primarily composed of recalcitrant high molecular weight aromatic humic substances (HS) that can interact with metal ions and organic compounds to form soluble or water-insoluble complexes with various chemical and biological stabilities. Such interactions can affect the behavior of the contaminants, including the rate and degree of decomposition, photolysis, volatilization, migration and biological absorption. The Specific UV Absorbance (SUVA) is used to determine the aromaticity of DOC, where the measured aromaticity is a general characteristic of the pool of molecules that compose the DOC [14]. SUVA254, determined at 254 nm, is strongly correlated with percent aromaticity determined by 13C NMR; therefore, it is a useful parameter for estimating the content of aromatic systems in water. Table 1 shows the data for the SUVA254, where it can be seen that most sites had a high aromatic carbon content (25%–30% of total carbon). This elevated content indicates the HS was derived from plant litter and soils and reflects the contribution of lignin degradation to their formation [15]. The AI site showed the highest values; values that are typical for SUVA254 from materials extracted from peat, which has been found to be around 35 [16]. The high aromaticity of the DOC in the AI site could be explained by its strong agricultural influence, i.e., the established greenhouses dedicated to the cultivation of ornamental plants. The lowest SUVA values were found in the UI and WW sites, indicating low aromaticity of the DOC, which was possibly microbially derived and explained by the DOC originating from the wastewater and drain with a higher content of aliphatic compounds such as fatty acids, carbohydrates, etc.

A hierarchical exploratory cluster analysis was used to differentiate the sites according to their physicochemical properties. The hierarchical tree can be seen in Figure 2. There is a definite group formation with the RI samples and with the AI samples, except for AI5, which is in the cross section with two other canals, thus indicating common environments in the formed groups. The WW sites furthest away from wastewater outlet (WW3, 4 and 5) were grouped in a common environment, as were the UI sites (with small difference between them), while WW1 WW2 and AI5 showed very particular environments. The parameters that had the highest influence in the grouping of the sites were pH and depth, followed by temperature.

For the humic substance concentrations, the HA values were not significantly different between the sites, while the FA had a wider range of values, with the UI and WW sites showing the highest concentrations.

### 3.2. Chemical metal equilibrium models

A simple calculation was made to determine the correlation between the different chemical equilibrium models for our water system. On the basis that the important

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**Table 1. Water quality parameters for the studied sites.**

| Site Zone | Depth [cm] | Width [m] | Conductivity [µS] | Temperature [°C] | Dissolved Oxygen [mgL⁻¹] | Suspended particles [mgL⁻¹] | COD [mgCL⁻¹] | SUVA254 [LgC⁻¹ cm⁻¹] | AH [mgCL⁻¹] | AF [mgCL⁻¹] |
|-----------|------------|-----------|-------------------|------------------|--------------------------|-----------------------------|---------------|-----------------------|--------------|--------------|
| RI 1      | 110        | 24.0      | 7.15              | 680              | 19.52                    | 2.91                        | 12.6          | 13.3                  | 29           | 1.4          |
| 2         | 100        | 24.0      | 7.11              | 684              | 19.63                    | 3.5                         | 22.1          | 12.6                  | 27           | 2.0          |
| 3         | 70         | 24.3      | 7.09              | 681              | 19.61                    | 3.2                         | 25.7          | 12.3                  | 28           | 1.4          |
| 4         | 100        | 24.7      | 7.18              | 680              | 19.62                    | 4.02                        | 41.3          | 11.9                  | 29           | 1.4          |
| 5         | 120        | 50.2      | 7.14              | 937              | 19.97                    | 4.02                        | 4.3           | 23.1                  | 40           | 1.2          |
| AI 1      | 200        | 25.3      | 6.93              | 743              | 18.86                    | ND                          | 8.45          | 17.9                  | 26           | 2.3          |
| 2         | 200        | 25.3      | 6.93              | 743              | 18.86                    | ND                          | 13.8          | 16.9                  | 25           | 1.3          |
| 3         | 100        | 18.5      | 6.68              | 741              | 19.99                    | ND                          | 69.12         | 15.5                  | 26           | 3.0          |
| 4         | 110        | 18.5      | 6.9                | 740              | 19.81                    | ND                          | 30.4          | 16.2                  | 29           | 2.4          |
| 5         | 150        | 25.3      | 6.93              | 691              | 18.66                    | ND                          | 30.4          | 16.2                  | 29           | 2.4          |
| UI 1      | 170        | 32.5      | 7.72              | 773              | 19.69                    | 3.98                        | 8.0           | 12.2                  | 21           | 1.3          |
| 2         | 70         | 32.5      | 7.27              | 1150             | 19.08                    | 1.28                        | 7.5           | 21.0                  | 36           | 1.1          |
| 3         | 50         | 32.5      | 7.22              | 777              | 19.67                    | 4.21                        | 24            | 12.0                  | 21           | 1.2          |
| 4         | 100        | 32.5      | 7.15              | 790              | 19.36                    | 4.11                        | 15.6          | 11.5                  | 23           | 1.2          |
| 5         | 130        | 32.5      | 7.14              | 793              | 18.98                    | 3.84                        | 14.2          | 11.6                  | 23           | 1.3          |

Note: AI: Agricultural Impact, UI: Urban Impact, RI: Recreational Impact, WW: Wastewater Impact, ND: Not Detected.
metal species to study were the free dissolved ions, the statistical correlation was obtained between the concentrations of the free metal ions calculated from the models of all the sampled sites. Significant correlation (0.9722) was obtained between the BLM and WHAM VII models, followed by the VisualMINTEQ and BLM models (0.7037). No significant correlation was found between the WHAM VII and VisualMINTEQ models. These results were expected since Visual MINTEQ was developed primarily to calculate equilibria for inorganic ions and trace metals in water, and MINTEQ is a robust model of chemical equilibrium that is actively supplied with databases of thermodynamic constants. On the other hand, the BLM and WHAM VII models incorporate equilibrium reactions with dissolved organic carbon, with WHAM specializing in interactions between metals and Natural Organic Matter (MON) in aqueous environments. The WHAM model is not as complete as that of MINTEQ. However, WHAM is the most appropriate model to apply where interactions between metals and natural organic matter are important or considerable [17,18], as in our studied site.

The WHAM VII mode was chosen for the metal speciation study of the studied sites since it can accommodate several metals and yields information on other metal species apart from their free ions. In this way, metal species were calculated using the WHAM VII program after measuring humic substances, anions and cations, and the total dissolved (0.45 μm filter) metal concentrations in the water column from the twenty different sites (Table 2). The Fe III concentration was estimated by assuming the presence of equilibrium colloidal hydrous ferric oxide after passing through the 0.45 μm filter.

### 3.3. Suspended particles’ metal concentration

Many studies have shown the importance of sorption of trace metals by suspended particles (SP) that ultimately affects the concentration, bioavailability and residence times of metals in surface waters. Furthermore, transfer of metals from suspended particles to the aqueous phase facilitates their entry into neighboring fauna and flora organisms and thus into the food chain [19,20]. Additionally, dietary intake is an important route for metal toxicity in aquatic invertebrates [21]. Therefore, metal concentrations in suspended particles, and more precisely, extractable metal concentrations, are of high importance. The extractable metal concentration is the metal concentration that participates in the particulate-aqueous equilibrium and is not embedded in crystal lattices. The suspended particles’ metal concentration was more precisely obtained by subtracting the total metal concentration of the water column from the dissolved metal concentration shown in Table 2. This table shows that the metal with the lowest percent associated with suspended particles was Cd (93.7% average for all sites) followed by Ni (98.9% average for all sites). The rest of the metals were all above 99%. For the difference between sites, the RI had the lowest metal concentration in the SP, followed by the UI site; while the AI and
WW sites had similar metal concentrations in the SP. The metal concentrations in the SPs from the different sites were expected to correlate with the organic matter (OM) contents of the SPs from the different sites. The RI site had the highest average OM content (31%), followed by the AI site (26%) and the UI and WW sites with an average OM content of 24%. However, that was not the case, as the lowest metal content associated with the SPs was in the RI site. A possible explanation could be that this site exhibited a significantly lower conductivity and as a result, a lower ionic strength that might have enhanced the formation of soluble ionic complexes and thus shifted the equilibrium of the particulate/aqueous phase toward the aqueous. Either way, these results show that more complex interactions are taking place in the particulate/aqueous interphase than association to OM and needs further consideration.

### 3.4. Dissolved metal species’ concentration

The dissolved metal concentration refers to the sum of metal species that can pass through a 0.45 μm filter. These concentrations are shown in Table 2. None of these metal concentrations was above the NOAA SQUIRT for Inorganics in Water [22] metal concentrations for chronic exposure and acute thresholds for freshwater organisms.

| Site | Suspended particles metal concentrations (μgKg⁻¹) | Dissolved metal concentrations (μgL⁻¹) |
|------|-------------------------------------------------|--------------------------------------|
| RI   | Cd 4.9  Co 829.7  Cu 276.3  Cr 1289.8  Fe 182251.6  Mn 4715.4  Ni 11795  Pb 207.0  | Cd 0.5  Co 0.2  Cu 0.3  Cr 0.9  Fe 22.5  Mn 3.7  Ni 0.1  Pb 0.1 |
| RI2  | Cd 2.4  Co 784.9  Cu 140.0  Cr 501.7  Fe 44453.5  Mn 3059.7  Ni 8136.8  Pb 197.6  | Cd 0.1  Co 0.2  Cu 0.4  Cr 0.8  Fe 17.5  Mn 1.4  Ni 0.1  Pb 0.1 |
| RI3  | Cd 0.5  Co 789.2  Cu 89.5  Cr 1044.4  Fe 25714.5  Mn 2793.8  Ni 1999.2  Pb 51.2  | Cd 0.1  Co 0.2  Cu 0.4  Cr 1.2  Fe 27.2  Mn 1.9  Ni 0.1  Pb 0.1 |
| RI4  | Cd 2.9  Co 671.2  Cu 93.9  Cr 1051.6  Fe 35098.7  Mn 2897.8  Ni 738.2  Pb 223.2  | Cd 0.7  Co 0.2  Cu 0.4  Cr 1.0  Fe 27.0  Mn 4.5  Ni 0.1  Pb 0.1 |
| RI5  | Cd 1.4  Co 458.3  Cu 104.8  Cr 985.7  Fe 29147.2  Mn 3100.4  Ni 332.7  Pb 56.6  | Cd 0.2  Co 0.3  Cu 0.6  Cr 1.6  Fe 16.3  Mn 1.6  Ni 0.1  Pb 0.1 |
| Al1  | Cd 24.5  Co 515.5  Cu 137.7  Cr 1144.6  Fe 19988.8  Mn 5601.9  Ni 4114  Pb 431.2  | Cd 0.2  Co 0.3  Cu 0.5  Cr 1.2  Fe 27.0  Mn 4.2  Ni 0.1  Pb 0.1 |
| Al2  | Cd 1.3  Co 328.5  Cu 120.3  Cr 2152.4  Fe 25398.5  Mn 5222.7  Ni 375.4  Pb 60.8  | Cd 0.1  Co 0.3  Cu 0.7  Cr 1.2  Fe 40.7  Mn 0.6  Ni 0.1  Pb 0.1 |
| Al3  | Cd 2.7  Co 384.9  Cu 114.3  Cr 731.2  Fe 19061.8  Mn 5601.9  Ni 3756  Pb 90.1  | Cd 0.1  Co 0.3  Cu 0.9  Cr 1.9  Fe 36.2  Mn 1.4  Ni 0.1  Pb 0.1 |
| Al4  | Cd 3.1  Co 408.9  Cu 331.9  Cr 2198.5  Fe 57327.3  Mn 9946.6  Ni 2897.8  Pb 8136.8  | Cd 0.7  Co 0.3  Cu 1.3  Cr 2.3  Fe 197.6  Mn 4.1  Ni 0.1  Pb 0.1 |
| Al5  | Cd 4.1  Co 555.4  Cu 112.2  Cr 829.3  Fe 30670.3  Mn 4833.0  Ni 35098.7  Pb 197.6  | Cd 0.2  Co 0.6  Cu 0.8  Cr 1.4  Fe 52.0  Mn 1.4  Ni 0.1  Pb 0.1 |
| UI1  | Cd 0.6  Co 488.7  Cu 73.7  Cr 21183  Fe 14648.0  Mn 2852.3  Ni 240.6  Pb 88.2  | Cd 0.5  Co 0.3  Cu 0.6  Cr 2.6  Fe 57.9  Mn 1.4  Ni 0.1  Pb 0.1 |
| UI2  | Cd 3.2  Co 195.9  Cu 58.9  Cr 858.3  Fe 26756.8  Mn 3790.0  Ni 89.4  Pb 50.7  | Cd 0.3  Co 0.6  Cu 2.3  Cr 91.8  Fe 62.3  Mn 2.0  Ni 0.1  Pb 0.1 |
| UI3  | Cd 3.8  Co 202.9  Cu 54.1  Cr 15123.5  Fe 32668  Mn 233.5  Ni 375.4  Pb 78.1  | Cd 0.3  Co 0.8  Cu 0.6  Cr 5.2  Fe 56.6  Mn 3.8  Ni 0.1  Pb 0.1 |
| UI4  | Cd 3.4  Co 123.9  Cu 63.6  Cr 842.1  Fe 19461.6  Mn 3682.9  Ni 182.8  Pb 73.1  | Cd 0.1  Co 0.6  Cu 1.2  Cr 48.5  Fe 30.3  Mn 2.6  Ni 0.1  Pb 0.1 |
| UI5  | Cd 1.1  Co 72.7  Cu 105.0  Cr 888.7  Fe 90967.7  Mn 3410.2  Ni 784.8  Pb 154.0  | Cd 0.2  Co 0.5  Cu 0.9  Cr 43.5  Mn 33.9  Ni 0.1  Pb 0.1 |
| WW1  | Cd 5.5  Co 102.7  Cu 71.9  Cr 14800  Fe 31495.1  Mn 2994.0  Ni 340.3  Pb 212.4  | Cd 0.0  Co 0.2  Cu 0.5  Cr 3.3  Mn 16.5  Ni 1.4  Pb 0.1 |
| WW2  | Cd 1.3  Co 111.9  Cu 156.1  Cr 704.7  Fe 44655.5  Mn 31078  Ni 348.8  Pb 70.3  | Cd 0.3  Co 0.8  Cu 0.9  Cr 79.2  Mn 47.7  Ni 0.2  Pb 0.1 |
| WW3  | Cd 2.3  Co 89.4  Cu 128.9  Cr 458.2  Fe 30346.5  Mn 2462.7  Ni 180.9  Pb 5.6  | Cd 0.1  Co 0.2  Cu 0.5  Cr 2.1  Fe 28.2  Mn 2.0  Ni 0.1  Pb 0.1 |
| WW4  | Cd 2.5  Co 120.8  Cu 81.5  Cr 779.0  Fe 32721.0  Mn 1950.1  Ni 225.9  Pb 92.8  | Cd 0.4  Co 0.3  Cu 0.5  Cr 3.0  Fe 30.5  Mn 19.3  Ni 0.1  Pb 0.1 |
| WW5  | Cd 2.1  Co 52.2  Cu 89.4  Cr 351.3  Fe 32573.3  Mn 18570  Ni 323.8  Pb 96.5  | Cd 0.1  Co 0.2  Cu 0.4  Cr 1.1  Fe 25.2  Mn 9.0  Ni 0.1  Pb 0.1 |

As expected, the free ion metals, the most important metal species from the ecotoxicological point of view, showed the lowest concentration of the dissolved metal species, with Mn being the most abundant and Cr the least. For all the metals, the UI site had the highest concentration of free metal ions, followed by the RI site. The dissolved metal concentrations from this study showed...
three orders of magnitude) of free metal ions determined in our study compared to the concentrations reported by Mueller.

Comparisons of the theoretical and empirical free metal concentration in the literature, have found discrepancies between the two [26,27]. Nevertheless, a

Table 3. Correlation matrix between physicochemical parameters and free metal ions.

|     | pH   | Cond | Temp | DOC | SUVA | HA    | FA    | Free Cd | Free Cr | Free Fe | Free Mn | Free Ni |
|-----|------|------|------|-----|------|-------|-------|---------|---------|---------|---------|---------|
| pH  | 1.00000 |      |      |     |      |       |       |         |         |         |         |         |
| Cond| 0.31011 | 1.00000 |      |     |      |       |       |         |         |         |         |         |
| Temp| 0.53638 | 0.30867 | 1.00000 |     |      |       |       |         |         |         |         |         |
| DOC | -0.11703 | 0.72395 | 0.28672 | 1.00000 |      |       |       |         |         |         |         |         |
| SUVA| -0.04476 | 0.59598 | 0.49594 | 0.81921 | 1.00000 |      |       |         |         |         |         |         |
| HA  | -0.33339 | -0.42928 | -0.43358 | -0.13946 | -0.21573 | 1.00000 |       |         |         |         |         |         |
| FA  | 0.02412 | -0.05582 | -0.66198 | -0.26063 | -0.64153 | 0.19908 | 1.00000 |         |         |         |         |         |
| Free Cd| -0.41171 | -0.32633 | -0.24054 | -0.20008 | -0.26392 | -0.10904 | -0.01710 | 1.00000 |         |         |         |         |
| Free Cr| -0.76137 | -0.09403 | -0.45308 | 0.26792 | 0.05740 | 0.04820 | 0.07464 |         | 1.00000 |         |         |         |
| Free Fe| -0.58860 | -0.29072 | -0.52325 | -0.12454 | -0.43310 | 0.16345 | 0.36241 |         |         | 1.00000 |         |         |
| Free Mn| -0.53946 | -0.121353 | -0.711693 | 0.083102 | -0.298734 | 0.471689 | 0.532341 |         |         |         | 1.00000 |         |
| Free Ni| -0.54994 | -0.434873 | -0.437990 | -0.223163 | -0.345437 | 0.318420 | 0.045198 |         |         |         |         | 1.00000 |

Note: Significant correlation are shown in bold.
comprehensive study by Fortin and coworkers in lake waters with physicochemical parameters similar to those in this study, showed at least good agreement for Cd and Zn, although Cu was underestimated by WHAM [28].

To elucidate which physicochemical variables were most influential in the occurrence of free metal ion, a correlation matrix was constructed as shown in Table 3.

A significant negative correlation with temperature was found for most of the free metals, which is unanticipated since it would be expected that a more important correlation would be found with DOC, HS or SUVA254. As expected, the DOC and SUVA had a significant positive correlation since SUVA254 derives from the DOC concentration. Nevertheless, the likely correlation between SUVA and HA was not found, and a significant negative correlation with FAs was identified; Mueller and coworkers found similar results [25]. No correlation was found with the SUVA254 or DOC and the free metal ions; the only metal that correlated with the HS was Mn, and it was Pb that correlated with FA. This result suggests that organic ligands other than HS were involved in metal complexation [25,29].

3.5. Metal concentration in tilapia gills

Metals originating from both natural and human-made sources are present in all environments, and water is no exception. Fish can encounter metals in their water environments. As fish take up metals from the water and from their food, metal levels in their tissues increase, and after certain thresholds, whether essential or not, all metals can become toxic, causing dysfunction or alteration in certain vital systems [30]. Nevertheless, the uptake of metals by aquatic organisms depends on water chemistry parameters such as salinity, hardness, pH, etc. [31]. Through respiration, gills are the first organ of contact with metals and, in sufficiently low concentrations, metals can be regulated in the gill membrane. However, higher metal concentrations can cause metal accumulation that can produce gill damage and mucus accumulation, triggering respiratory effects [32]. For this reason, fish have been used as biomarkers, and metal bioaccumulated in gills also provides a tool for biomonitoring [33]. Additionally, the metal accumulation in tilapia in the area is of concern due to its use as forage, prohibited human consumption and bioaccumulation into the food chain by ways of migratory birds.

Metal concentration in *O. niloticus* s gills can be observed in Figure 4 for the different studied sites. The WW site shows the highest concentration of most metals, and Mn was present at the highest concentration on the gills, followed by Cu, Ni, Pb and Cr. Cadmium was not detected in the gills. None of the tilapia’s metal concentrations were above the FAO guidelines [34].

A comparison of bioaccumulation factors (BAF) (not shown) for Mn, Pb, Cd and Cu in *O. niloticus* gills from wastewater-impacted sites in Egypt [35] showed lower BAFs compared to this study: Pb was 5–7 times lower, Cu up to 2 times lower, and Mn up to 10 times lower, while Cd was present in the gills unlike this study. Another wastewater study in India [36], showed BAFs in fish to be similar or lower: Ni, Cu and Cr were similar, while Pb and Mn were up to 1 and 2 orders of magnitude lower, respectively.

To try to explain metal bioaccumulation in fish, correlation matrices were made with free metal (not shown) and soluble metal concentrations (Table 4). No correlation was found “with free metal ion concentrations and gill metal concentrations”. The only significant correlation ($p < 0.05000$) was found with soluble and gill Cr concentrations. Other, not so obvious, correlations were found between soluble Cr and Mn and Ni in the gills. Even when no significant correlation was found between free metal ions and metal accumulation in the gills, WHAM VII calculations found that Mn was the most abundant free metal ion, and it was also the most bioaccumulated metal. Unfortunately, no further trends could be identified.

The lack of correlations between accumulated metal in tilapia gills and metal species could be due to having insufficiently accurate parameters fed into the WHAM VII program [24]. Additionally, WHAM VII metal species calculations have proven to correlate to organisms metal accumulation in specific cases [24,37,38]. This correlation trend is particularly true for macroinvertebrates [27], and further software fine-tuning is necessary to obtain a good correlation between predicted metal species and organisms metal accumulation.

Nevertheless, in this study, WHAM VII has proven to be a valuable tool for assessing the potential metal toxicity of the metals since WHAM VII calculations suggested a very low bioavailability of the metals. The comparison of tilapia metal concentrations from this study and other *O. niloticus* metal accumulation studies [35,39] shows lower

| Pb sol   | Cd sol   | Cr sol   | Mn sol   | Ni sol   | Cu sol   |
|----------|----------|----------|----------|----------|----------|
| Pb gill  | 0.251206 | 0.223658 | 0.236589 | 0.12945  | 0.031373 | 0.016510 |
| Cd gill  | 0.258953 | 0.196233 | 0.198524 | 0.403853 | 0.314387 | 0.142283 |
| Cr gill  | 0.114461 | 0.122850 | 0.560001 | 0.013590 | 0.030152 | -0.339557 |
| Mn gill  | -0.362176| -0.213838| 0.502050 | -0.270162| 0.057900 | 0.030152 |
| Ni gill  | -0.208761| -0.183742| 0.548870 | -0.111021| 0.013157 | -0.083200 |
| Cu gill  | 0.245465 | 0.271741 | 0.307087 | 0.224896 | 0.142283 | 0.020945 |

Note: Marked correlations are significant at $p < .05000$. Significant correlation are shown in bold.
results show that other chemical equilibria in the water column and additional accumulation routes into the fish have to be taken into account in order to correctly predict metal bioaccumulation. Nonetheless, the WHAM calculated metal species gave us important information as to the metal dynamics in the wastewater filled canals impacted with different socioeconomic activities in their vicinity.

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metal accumulation in our studies, possibly due to the predicted low bioavailability of the metals.

4. Conclusions

Urban water bodies give a variety of environmental services, even those supplied with wastewater. Nevertheless they are a source of pollutants including metals. The studied site, a network of water canals, has different socioeconomic activities that impact or modulate the pollutants in the nearby canals. When studying metals as pollutants, their bioavailability is of great importance to infer their toxicity to organisms. Organic matter and more specifically humic substances (with high aromaticity) have been identified as an important parameter regulating metal bioavailability both in water and suspended particles. However, our study did not show a clear relationship between organic matter and metal content in suspended particles, although most of the metal content in the water column was associated to suspended particles (>90%). As for the metals in water, the WHAM VII model calculated that a very low percentage (less than 1%) is present as the bioavailable free metal ions and the metals were mostly as soluble species (sum of all soluble inorganic species and simple ligand complexes) followed by fulvic acids; also no correlation was found between metals associated to HS and SUVA254 (aromaticity measurement). These results suggest that the bioavailability of the metals in the water column are not mainly driven by the organic matter content; the high salts content and small organic molecules might play a significant role, particularly in wastewater bodies [40,41]. On the other hand, when the WHAM calculated metal species was compared with the metal concentration in O. niloticus’ gill from the different site, no clear correlation was found with any of the calculated metal species because either there was insufficient accuracy in the parameters fed to the WHAM VII model or there were tilapia’s gill bioaccumulation routes other than free metal ion association. However higher bioaccumulation of Mn was found, which was the metal with a higher percentage of the free ion. Our

Figure 4. Metal concentration in gills of Oreochromis niloticus at the different sites. ND: Not detected.
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