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Toxicity and Trace Metal Concentrations of Sediments from Lake Maryut, Alexandria, Egypt

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Lake Maryut is a shallow, brackish-water lake situated to the south of Alexandria, Egypt. The lake is 90 km^2 and is divided into sub-basins comprising the main basin (MB), south-east basin (SEB), south-west basin (SWB), and Mallahet Maryut (Figure 1). The main basin is the largest, measuring 25 km^2 in area. Since 1950 the total area of the lake has been reduced to a third of its original size, and current depths range from 40 to 220 cm. Lake Maryut is heavily polluted, largely due to the discharge of raw sewage from Alexandria, untreated industrial waste and agricultural run-off (El-Rayis and El-Sabrouti, 1997). The lake has no natural outlet to the Mediterranean Sea, and the main water inlets are the El Qalaa and El Umum drains, which traverse the lakes to the El Max pumping station where water is then pumped into the sea. Analysis of lake sediment reveals a 40% clay fraction together with silt and sand and a large proportion of Mollusca, Polychaeta, Ostracoda, Foraminifera, and plant debris from previous eras (Bernasconi and Stanley, 1994).

The aim of the present study is to use *Chironomus riparius* bioassays and chemical analyses to identify areas of high and low sediment pollution. Specific objectives included, (1) Trace metals analysis of sediment samples from different sites in Lake Maryut, (2) the use of a *Chironomus riparius* bioassay to identify pollution hot spots, and (3) a combination of analytical chemical data with the *C. riparius* bioassays to aid the identification of polluted areas.

MATERIALS AND METHODS

In February 2001 samples were taken from several stations in Lake Maryut ((latitude 31.204° longitude 29.914°); Figure 1) and at various points along the main drains (e.g. Qalaa Drain). In the main basin (MB) samples were taken along two transects: one along the north shore (MB/N) and one parallel to the south shore (MB/S). Point samples were taken from a basin separated from the main basin by the Nubia Canal (MB/W), which is directly connected to the MB via small openings in the bank. Samples were also taken from the south-west basin (SWB), which is separated from the MB by a dam. The only exchange of water between the SWB and the MB is via an interstitial flow through the dam and through small breaks in the canal banks. At each sampling point an Ekman grab sample, of 300cm^2 surface area and approximately 5 cm deep, was transferred to a

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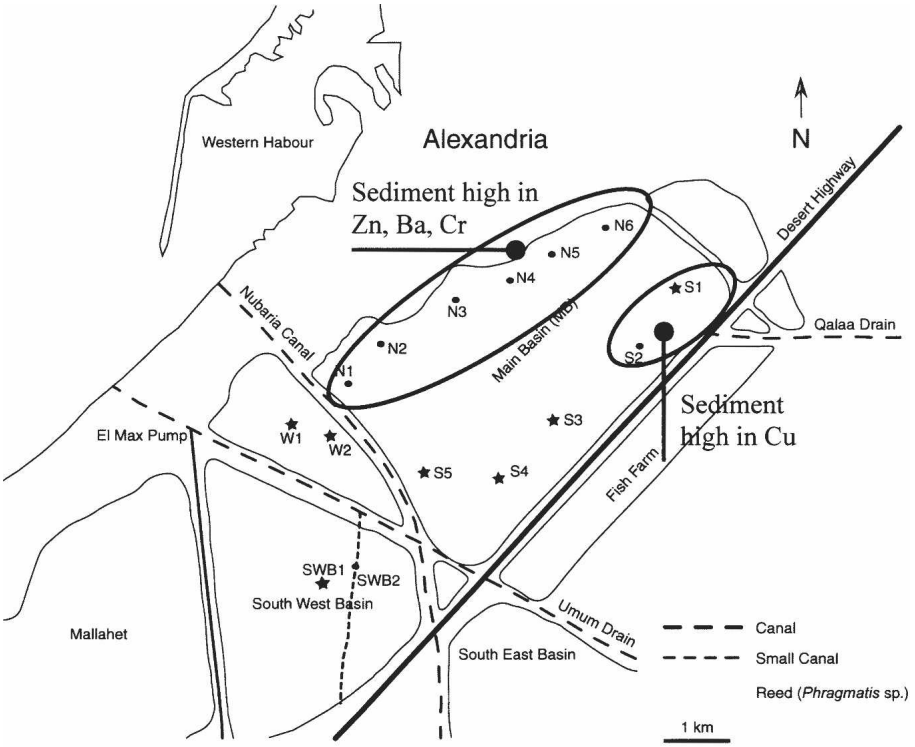

Figure 1. Schematic map of Lake Maryut (N, S, W, SWB = sediment sampling points); (•) sampling point where no larvae; (✶) where larvae were recovered from *C. riparius* bioassays. Including indication of high concentration for certain trace metals.

labelled polyethylene bag, returned immediately to the laboratory and stored for 12 hours at 4°C before being bioassayed.

Sediment bioassays were performed according to ASTM guidelines (ASTM, 1998). In the laboratory, sediment samples from each station were sieved through two meshes (1180 μm and 250μm). Three replicate test containers (300 ml polyethylene beakers, Woolworth, UK) were filled with 50g of sieved sediment from each station and weighed into the test containers using a Sartorius 1405 balance (Sartorius AG, Goettingen, Germany). All equipment was rinsed with 10% nitric acid and washed with tap water between samples from different stations to avoid cross-contamination.

Beakers were filled with 200 ml of reconstituted water (ASTM, 1998; temp. 17.5°C, conductivity 228μS, pH 8.8), with care being taken not to disturb the sediment in the beakers. Each beaker was then covered with polythene cling film. Silica sand controls (Tilcon (South) Ltd, Harrogate, UK) were set up in an additional three beakers. The beakers were placed in a constant temperature room (20±2°C) with a fixed light regime of 16:8 hrs (light/dark). All test containers

were randomly placed on a bench and an air supply system was installed using short glass pipettes and an air pump. After 10 hours the air supply was suspended and 10 individual early instar larvae (instars I-II) of *Chironomus riparius* (originally cultured at Royal Holloway, University of London, UK) were counted into each test beaker (control and lake sediment) using a low power microscope. Beakers were then aerated once more and sealed with cling-film. Chironomids in control beakers were fed every other day with 1ml of 0.9g/100ml finely ground TetraMin© (TetraMin Werke, Melle, Germany) in water. Chironomids from Lake Maryut sediments remained unfed. After 10 days the overlying water from each beaker was passed through a 250 μm sieve to avoid loss of free-swimming larvae, and the sediment was emptied onto a clean tray. Surviving *Chironomus* larvae were removed using soft paint brushes, counted, and the size of their head capsules was measured in μm under a low power microscope. Larvae were then blotted dry on tissue paper and weighed (OHAUS Analytical Standard AS 120, Ohaus Corporation, Pine Brook, NJ, USA).

All surviving larvae were snap frozen in liquid nitrogen and stored at -80° and because of their small size, larvae were pooled prior to measurement. Sample actelycholinesterase (AChE) was measured using a spectrophotometric method (Ellman et al. 1961). Chironomids recovered from each beaker were homogenized in a hand-held homogenizer for 30 seconds in a solution consisting of 0.25 M sucrose and Triton-X100 in phosphate buffer pH 7.4. The homogenate was centrifuged for 10 min at 10,000 rpm to provide a clear supernatant for AChE and protein content assays. The AChE bioassay was performed at a controlled temperature of 20°C and all reagents were kept on ice. Reagents were added to a 1-ml cuvette in the following order: 0.83 ml phosphate buffer, 0.03 ml 10mM 5,5' –dithio bis-2-nitrobenzoic acid (DTNB, Sigma D-8130) in pH 7.0 phosphate buffer, 0.01 ml 10mM acetylthiocholine iodide (ATCI, Sigma A-5751), and 0.01 ml *C. riparius* supernatant. The cuvette was gently shaken to ensure thorough mixing. Absorbance was read at 412 nm on the spectrophotometer (Novaspec II, visible spectrophotometer, Pharmacia LKB) every 30 s for a total of 7 min. The protein content was determined according to Lowry et al. (1951) using a series of protein standards.

For the trace metal analysis 60 g of wet sediment from each station was dried in an oven at 60°C for 12 h. Dried sediment samples (approximately 20g of dry mud) were used for trace metal analysis at the Royal Holloway NERC ICP-AES facility. All sediment samples were crushed with a laboratory 'swing mill' (Gry-Ro Mill; Glen Creston Ltd, Stanmore; Middlesex, UK). 0.2g of each powdered sample was placed into 25ml PTFE (Teflon® PTFE resin) crucibles and eight procedural blanks were added. A 1:2 mixture of $HClO_4$ and HF acids was added to each crucible (6ml per crucible) which was placed onto a hotplate in a fume cupboard for 4 hours until all the liquid had evaporated. After cooling, 2 ml HCl was added to each crucible using a dispensing pipette and each crucible was then filled with distilled water and placed on a hotplate for 10 min. To ensure that all of the HCl mixed with the water, a small amount of distilled water was added to each crucible, which was left on the hotplate for another 10 min, and then allowed

to cool. White tubes (23ml, polypropylene (PP)) were rinsed three times with distilled water, placed on a balance, and filled with the contents of the crucibles to a weight of 20.4g (=20ml). All samples were analysed using inductively coupled plasma-atomic emission spectrometry (ICP-AES, Perkin Elmer) for the following trace metals: Al_2O_3, Fe_2O_3, MgO, CaO, Na_2O, K_2O, TiO_2, P_2O_5, MnO, Ba, Co, Cr, Cu, Li, Ni, Sc, Sr, V, Y, Zn, and Zr*.

Data were tested for normality and variance homogeneity before analysis of variance (ANOVA), followed by Tukey's honestly significantly different (HSD) or Dunnett's multiple comparison test. Variance heterogeneity in some data sets required log transformation before ANOVA. All statistical analyses were performed using Unistat 4.53 (Unistat Ltd. 1999, London, UK.).

RESULTS AND DISCUSSION

Over the past 50 years Lake Maryut has changed more than any other Nile Delta lake. The lake is highly eutrophic as a result of discharges of untreated wastewater. For example, sewage effluent containing trace metals such as zinc is still being discharged into Lake Maryut, with concentrations of zinc ranging between 0.03-8.31mg/L in untreated wastewater (Clapham et al. 1999). Existing sewage treatment plants further upstream of the Qalaa Drain and north-west of the city have only primary treatment facilities. The high concentrations of toxic heavy metals and increasing amounts of organic pollutants also pose a risk for the health of people living in the vicinity of the lake and their livelihoods.

The *C. riparius* sediment bioassays showed that no larvae survived in samples MB/N1 to MB/N6, MB/S2, and SWB2 (dredged canal in SW basin)(Figure 1). All larvae were recovered alive from control sediments. There was a significant difference in the size of the head capsule of chironomids recovered alive from the different sites (Kruskal-Wallis H =149.3, $p<0.001$; Dunn's test $p<0.01$; Figure 2), again due to a significant difference between controls (mean=99.41μm; SD=17.87) when compared with samples MB/S1 (mean=70.4μm; SD=12.41), MB/S4 (mean=70.63μm; SD=11.9), MB/S5 (mean=51.95μm; SD =8.31), MB/W2 (mean=63.04μm; SD=10.63), SWB1 (mean=44.62μm; SD=5.08) (Dunn's test $p<0.001$) and MB/S3 (mean=69.5μm; SD=15.6) (Dunn's test $p<0.01$). There was no significant difference between controls and MB/W1 (mean=93.79μm; SD=14.49). Larvae recovered from SWB1 and MB/S5 were significantly smaller than any other larvae recovered alive from the sediments of Lake Maryut.

There was a significant reduction in AChE activity in larvae which survived in lake sediments when compared with controls ($F_{(10,22)}$=38.18, $p<0.0001$; Figure 3). Enzyme activity in larvae recovered from sediment sample MB/S3 was also significantly lower than in larvae from site MB/S1, MB/W1, SWB1 (Tukey HSD $p<0.05$). AChE activity in larvae recovered from sampling site SWB1 was significantly higher than in those from sites MB/S1, MB/S3, MB/S4, MB/S5, and MB/W1 (Tukey HSD $p<0.05$). The highest inhibition of AChE (only 8.2% of the activity measured in larvae from the control group) was recorded from larvae

recovered alive from the sediment of MB/S5. Larvae recovered from MB/S4 and MB/W1 had the highest AChE activities of 37% and 39.7% respectively, compared with controls.

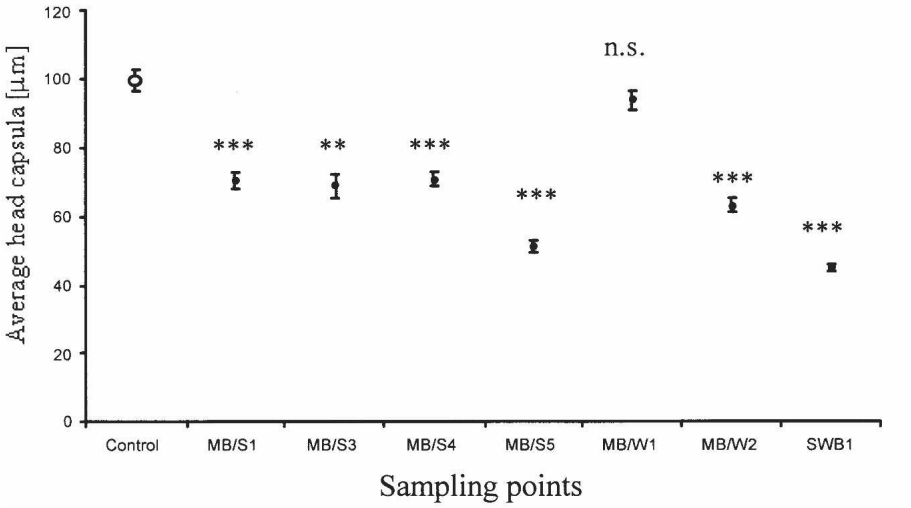

Figure 2. *C. riparius* head capsule length (test animals recovered alive from sediment after 10d exposure). Asterisks (*** $p<0.001$; ** $p< 0.01$; ns. not significant): indicate a significant difference in the size of the larval head capsule when compared to controls. Error bars indicate the standard error of the mean.

Looking at trace metal concentration in the sediment of Lake Maryut it can be said that the highest values for most trace metals were found in samples from MB/N1 (Table 1; Figure 1). Values for Zn and Ba in the sediment were particular high in MB/N1. MB/S1 had high amounts of trace metals compared to other sampling points along the south transect, with values often as high as on the north side. A comparison of sampling point MB/S5, where larvae were recovered alive after the 10 day exposure, to sampling points where no larvae survived showed that some trace elements (Ba, Cr, Cu,and Zn) were present at substantially higher concentrations at the sampling points with no larval survival (Table 1). A comparison of values for some metals with toxicological benchmarks established by Jones and Suter (1997) showed that Cu and Ni substantially exceed No Effect Concentration (NEC) values (NEC value for Cu= 54.8µg/g and for Ni = 37.9µg/g) at all sampling points. The concentration of Zn measured at MB/N1 was substantially higher than its NEC value (541µg/g), but Zn levels at MB/N6, and MB/S1 did not exceed the NEC value. Furthermore, this data could also be assessed using a set of sediment quality guidelines that identify concentration ranges of contaminants associated with biological effects (Long et al. 1995), though these have been developed for marine sediments. According to Long at al. (1995) adverse effects would occasionally occur at sediment concentrations

between the Effects Range-Low (ERL) and Effects Range-Median (ERM), and adverse effects were frequently associated with concentrations above the ERM (for example, Cu EPL= 34μg/g, ERM=270μg/g and Zinc EPL= 150μg/g, ERM=410μg/g). The concentration of Cu and Zn measured at north shore of lake Maryut (MB/N) were substantially higher than their respective ERM values (except at MB/N2). There appears to be two sources of copper contamination as high values were recorded in sediment from sampling points between MB/N1 – N4 and at sampling points MB/S1 and S2. Nickel occurs at higher concentrations on the east side of the main basin, where high levels of barium and chromium were also detected (Table 1).

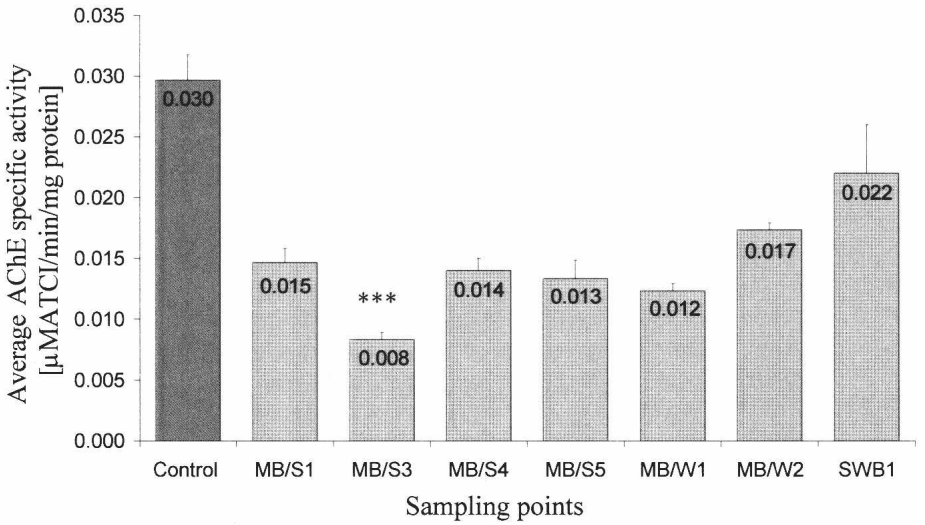

Figure 3. Acetylcholinesterase (AChE) activity (±sd; means are indicated) measured in *C.riparius* larvae recovered from different sediment samples after a 10 d exposure (*** $p<0.0001$ compared to control).

Table 1. Mean value (± se) of selected trace elements (mg g-1) from certain sampling points in Lake Maryut (For position of sampling see Figure 1).

	Ba	Cr	Cu	Zn
MB/N1	1048,0 (±) 82,0	142,7 (±) 3,0	440,7 (±) 13,4	1184,0 (±) 31,1
MB/N2	280,7 (±) 3,2	80,7 (±) 2,2	220,0 (±) 5,9	373,3 (±) 5,4
MB/N4	431,7 (±) 3,8	89,3 (±) 1,5	283,3 (±) 5,2	499,7 (±) 9,6
MB/N5	425,7 (±) 8,3	100,0 (±) 2,0	276,0 (±) 3,5	507,3 (±) 9,2
MB/N6	558,3 (±) 53,4	147,3 (±) 1,2	347,7 (±) 4,7	676,0 (±) 9,5
MB/S1	587,0 (±) 11,0	119,5 (±) 1,5	423,5 (±) 1,5	689,0 (±) 1,0
MB/S2	414,3 (±) 7,2	110,3 (±) 1,9	335,7 (±) 9,0	526,3 (±) 15,1
MB/S4	159,0 (±) 2,2	70,5 (±) 1,2	297,0 (±) 2,7	385,0 (±) 8,1

The use of *C. riparius* sediment bioassays proved a useful and inexpensive tool for identifying areas of poor biological quality in Lake Maryut. Bioassays are important in identifying potential hazards, for example the bioavailable fraction of

metals, that might not be detected using chemical analytical methods alone (Hill et al. 1993). This biologically-based method helps to highlight areas which would require remedial action in the future (Traunspurger and Drews, 1996). Several endpoints proved useful, i.e., survival, growth, development, and AChE activity. Larvae did not survive in sediments taken from the north shore of Lake Maryut, or in sediments from the south basin into which effluents are pumped from petrochemical plants. The toxic effects of high metal concentrations could explain larval mortality in some of these samples. However, a decrease in growth and significant mortality were also observed in samples with relatively low metal concentrations. This suggests that factors, such as unmeasured toxic organic chemicals may be responsible for the poor sediment quality in parts of the lake. For example, pesticides detected in earlier studies include lindane, p,p'-DDE, o,p'-DDT, and p,p'-DDT (Saad et al. 1982). Concentrations of trace metals detected in SWB1 and SWB2 were lower than in other sample sites. However, only 86.67% larval survival was recorded in SWB1 with no survival being recorded from SWB2, indicating that other pollutants were responsible for such a high mortality. A reduced AChE activity was measured in larvae recovered from SWB1 compared with controls. However, AChE activity was still significantly higher than in larvae from the main basin (MB). As metals do not seem to affect AChE activity (Kheir et al. 2001) it seems that pollutants such as organophosphate or carbamate insecticides might be present at sufficiently high concentrations to influence enzyme activity (Kheir et al. 2001). Potentially toxic levels of more volatile compounds, such as ammonia in the sediment would not have been detected due to the 10 hour pre-aeration step of the bioassay.

Results from analyses of sediment samples collected along the north side of the main basin of Lake Maryut showed high concentrations of common polluting trace metals, e.g. copper and zinc. Radioactive dating has shown that sediment, especially on the north side of Lake Maryut, was deposited after 1950 (El-Masry and Friedman, 2000), so contamination has occurred in recent times. However, despite high concentrations of trace metals in sediments from the sampling point MB/S1, which were very similar to concentrations measured in the samples from MB/N, *C. riparius* larvae survived for 10 days in the MB/S1 sediment sample. This again might be due to the presence of additional and unmeasured toxicants in samples from MB/N.

The use of chironomid assays in the present study has highlighted 'hotspots' of contamination in the lake, and this tool could be used, for example, in the future to direct and focus any remediation efforts. As remediation effort could be focused on *toxic* areas, as identified by bioassays, rather than *contaminated* areas, as identified by analytical chemistry alone, a chironomid assay is likely to provide a more biologically relevant and more cost-effective solution to the problems of environmental quality in Lake Maryut.

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