East Calcutta Wetlands: A Comprehensive Study on its Contribution in Wastewater Remediation and Reuse

Conference: 13th AICLST, Kolkata, 6–8 March 2026

Paper reference: TC-31/O

Printed pages: 128–133

Source: *Leather 2050: Challenges & Opportunities*, ILTA (2026)

East Calcutta Wetlands: A Comprehensive Study on its

Contribution in Wastewater Remediation and Reuse

Soumya Chatterjee 1,2,*,#, Buddhadeb Chattopadhyay[1], Siddhartha Datta[2],

1Government College of Engineering & Leather Technology, LB/III, Salt Lake City, Kolkata 700098, India

2Department of Chemical Engineering, Jadavpur University, Kolkata 700032, India

Ref. No. TC-31/O

Subhra Kumar Mukhopadhyay[1]

*Present address: Defence Research Laboratory, DRDO, Solmara Military Station, Tezpur 784501, Assam, India

Abstract

Wetlands are cardinal ecosystems yet utterly neglected in most parts of the globe. They are one of the important sources of environmental oxygen and replenish subsoil water and nutrients through retention & reuse. Kolkata, India, has a vast wetland area, the East Calcutta Wetlands (ECW 22°25’ N – 22°40’ N and 88°20’ E – 88°35’ E) located at the eastern fringe of the city, that cleans the city’s wastewater and uses waste nutrients productively in producing fish and vegetables, besides other ecological benefits. The comprehensive years-long examinations on ECW were carried out by using instruments like AAS, PIXE, XRD, EDAX, CHN, SEM, TEM, IEM, AFM, flow cytometry, fluorescence microscopy, etc. to study i) the sequestration of different elements (viz., Ca, Cr, Mn, Fe, Cu, Zn, and Pb) in water and soil of wastewatercarrying canals, pisciculture ponds, & agricultural lands; ii) metal uptake by plants grown for floriculture and agriculture, naturally occurring wetland plants, and fish species; iii) the process of metal amelioration at the tissue level (including toxic responses of fish tissue, if any). The study indicated the cumulative accumulation of metals in sediments from water and thus comparatively lower uptake by the organisms, where wetland plants in ECW play a crucial role in bioremediation of metals, and fish tissues resorted to metal accumulation by methylation of the metals using metalloproteins (metallothionein). The study reveals

Corresponding author email : schatterjee.drl@gov.in / drlsoumya@gmail.com

that ECW, a Ramsar Site (no. 1208), though decimated by >37% due to anthropogenic interventions, naturally converts composite wastewater, generates wealth, and provides many ecological benefits that our city of Kolkata has been enjoying for over the last hundred years.

Key words: phytoremediation, metal sequestration, metalloproteins, water hyacinth, tilapia

1. Introduction

Kolkata (previously Calcutta) has grown along the Ganga levee. A typical water scheme with a natural inclination toward east and southeast supports this heavily populated eastern Indian city. The Ganga flows west of the city. Kultigong, another river 40 km east of the metropolis, joins Ganga. Located east of the city, the East Calcutta Wetlands (ECW, lat. 220 27’ – 220 40’ N; long. 880 27’ – 880 35’ E) span 12,500 ha. The many uses of this Ramsar site (Ramsar site number. 1208; Wetland International site reference no. 2IN013) make it of international importance. ECW is a model wise-use wetland ecosystem, with local farmers using mixed city sewage for agriculture and pisciculture to maintain the environment and manage development (www.wwfindia.org). ECW receives ~600 MLD of untreated wastewater from the city, which is pumped into the SWF canal and discharged into the Kultigong river after roughly 40 km of travel (Chattopadhyay et al. 1999, 2004). Water from about 6,000

enterprises, including tanneries, battery, electroplating, rubber, pottery, and pigment producing units, and the city’s urban sewage, carries composite effluents year-round. The original municipal drainage plan was established in 1803, but an upgraded one was implemented in the early 1890s. Today, sustainable development concerns about discharge and dumping untreated industrial effluents polluted with heavy metals have grown (Chatterjee et al. 2006). This comprehensive investigation monitored the concentration profile of S, Ca, Cr, Mn,

Fe, Ni, Cu Zn, Rb, Sr, and Pb in water and sediments at chosen SWF canal sites, as well as common fish and plant species.

2. Materials and methods

a. Sampling sites and sample collection

Sampling was done at four selected sites along the major SWF canal passing through the ECW area. Site 1 is located around 1 km away from Tangra tannery agglomerate (Chinatown) of Kolkata on a canal carrying raw composite tannery effluent. Site 2 is located at Chowbaga, beyond 8 km from the source point, where the composite tannery effluent is siphoned into the SWF canal, being mixed with municipal sewage. Site 3 is on the SWF canal near the Calcutta Leather Complex at Bantala, around 15 km away from the source point. Site 4 is on the same canal near Kultigong lock gate, around 40 km away from the source point. Samples (water and bottom sediment) were collected from the selected sites of the SWF canal throughout the year, during the first week of each month and at three selected times of the day between January and December for five consecutive years starting from 2002.

Water was collected in clean stopper glass bottles (1 L) by immersing them completely into the water (about 10–12 cm below the surface of the water to avoid floating debris). Bottom sediments were collected from the upper 10-cm layer of the canal sites using Ekman dredge. Similar abiotic samples were also collected from the wastewaterfed fishponds along with three economically important fish species (Rohu: Labeo rohita, Mrigala: Cirrhinus mrigala, and Nile Tilapia: Oreochomis niloticus) cultivated in the

region and predominant aquatic macrophytes, water hyacinth (Eichhornia crassipes) and common arum (Colocasia) and sunflower, marigold, and cock’s comb were also collected as samples for the present study. Following the established protocol, samples taken from control and contaminated sites were cleaned carefully by Millipore water. All of these samples were processed as per the requirements to carry out different experiments.

b. Analysis of samples

Detection of elements in the samples was done by protoninduced X-ray emission (PIXE) technique after preparation of targets. Thin targets (water) were prepared on 25-mm-diameter Nucleopore membranes with pore size of 0.4 ìm following the technique used by Aprilesi et al. (1984). Thick targets (bottom sediment) were prepared by making pellets of 10-mm diameter from powdered samples, and standards (NIST SRM for water: 1643d, estuarine sediments 1646a, and bovine liver: SRM 1577b). Targets were analyzed using 2-mm collimated 3-MeV proton beam, generated from a 3-MV tandem pelletron accelerator (at the Institute of Physics, Bhubaneswar, India). The data were analyzed by using GUPIX software as described in earlier reports (Chatterjee et al. 2007). Furthermore, samples were subjected to analysis by flame absorption mode of atomic absorption spectrophotometer (AAS, Perkin-Elmer AAnalyst-100 with interfacing AAWinlab Software), using element-specific hollow cathode lamps in default condition (Chatterjee et al. 2010).

c. Studies on physicochemical parameters of wastewater

Physicochemical parameters of wastewater of canal were also examined along with the samples collected each time. Potentiometric determination of pH, total dissolved solids (TDS), and dissolved oxygen (DO; using Multiline P4 (WTW, Germany) and Mettler Checkmate 90 (Toledo)), calorimetric and titrimetric determination of NO_3, Cl^-, total hardness, carbonate hardness, alkalinity, and acidity (using E. Merck, Germany, Field Testing Aquamerck reagent

kits), and gravimetric analyses of total suspended solids (TSS) were done following standard methods. pH and other reagent factors were determined in the laboratory (using a Mettler Autotitrator DL25).

d. Optical microscopy of liver tissue

Following dissection, fish liver tissue was immediately cleaned (in cold 0.67% normal saline water). After fixation, 8 µm thick sections were cut using a microtome, de-paraffinized, double stained (Hematoxylin and Eosin), mounted in DPX (Qualigens Fine Chemicals) and visualized under optical microscope (Leica, DMR) following the methods described elsewhere (Pearse, 1980; Ray, 1986).

e. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) of liver and gill tissue

After stabilizing in modified Karnovsky’s fixative (4% paraformaldehyde and 1% glutaraldehyde in 0.1 M sodium phosphate buffer) tissues were processed for Critical Point Drying (CPD). Dried samples were mounted on Al-stubs using adherent material. Specimens were observed in SEM (LEO 435VP with Robinson detector for secondary electron). Similarly, modified Karnovsky’s Fixative were used to stabilize the samples followed by fixative 1% osmium tetroxide (OsO_4) at 40C for 2 h (Kaul et al., 2012). Samples were embedded (TAAB, UK) and

the blocks were used to prepare ultrathin (70- 90 nm) sections (ultramicrotome of Reichert-Jung-ultracut E). The sections were then double stained using Uranyl acetate and Lead citrate (20% alkaline aqueous solution) and kept in grid box for observation under TEM (Philips Morgagni 268).

f. Atomic Force Microscopy (AFM) of fish blood

To examine metal-induced morphological changes of red blood cells by AFM, blood samples of the fish from experimental and control ponds (n = 9, each group) were

examined. One drop of blood from each sample was taken separately on grease free slide, smeared and stained with Wright's stains and observed under optical microscope (Leica, DMR). Subsequently, blood sample was stabilized using modified Karnovsky's Fixative, pellets were obtained by centrifugation followed by repeated washing and resuspension in phosphate buffer. One drop of resuspended cells was viewed through AFM in tapping mode with Nanoscope-IIIa (VEECO Instru ments) with a tip made up of Si3N4 (silicon nitride).

g. Localization of MT

Immunological localization of metallothionein was examined using three techniques, namely, Flow Cytometry (using primary antibody against MT (Rabbit polyclonal IgG: FL-61: sc-11377; & Fluorescein Isothiocyanate (FITC) conjugated secondary antibody (Goat anti rabbit IgG FITC, sc2012; Santa Cruz Inc., Santa Cruz, CA, USA and analysed by FACS Calibur (Becton-Dickinson, San Jose, CA, USA) using CellQuest software), Immuno Histochemistry (using fluorescence microscope (Leica DRM) and Immuno-Electron Microscopy (Rabbit polyclonal IgG: FL-61: sc-11377; Santa Cruz Inc., Santa Cruz, CA, USA, followed by Goat anti-rabbit IgG immunogold-conjugated (TAAB, GEMO24) secondary antibody and viewed under electron microscope (FEI Philips Morgagni 268D).

h. Statistical analysis

Analytical results were evaluated using SPSS (version 14.0). The standard deviation values were calculated, and the representative graphs were made using Origin (version 6.1). ANOVA (Ftest) was performed to compare the different sites studied. Other statistical analyses like principal component analysis and determination of correlation matrix and clusters were performed using SPSS (version 14.0) to evaluate further the different components and parameters of the dataset.

3. Results

a. Metal concentrations in different samples

Physicochemical factors and the concentration levels of different elements (six elements viz., Cr, Mn, Fe, Cu, Zn and Pb) in SWF canal wastewater and bottom sediment of four selected sites (sites 1–4) were examined. Cr, a major constituent of tannery effluent, was recorded highest in site1 (3.2±0.21 mg.L-1). Fe and Ni concentrations were recorded highest in site1 (3.3±0.33 and 0.45±0.06 mg.L-1). Interestingly, Cu and Zn concentrations were recorded highest in site2 at 0.39±0.03 and 3.2 ±0.21mg.L-1, respectively. Concentration of Pb was 1.5±0.11 mg.L-1 in site 1; however, a gradual decline in the concentration level from site2 (0.85±0.03 mg.L-1) to site4 (0.17± 0.05 mg.L-1) was evident from the study. The concentrations of Ca and Cr in BS1 were recorded highest (110,078.9 ± 5,442.51 and15,561.1± 1,824.11 mg.kg-1dw, respectively). Fe (23478.2 ± 567.51 mg.kg-1dw) was found as the most major component of the bottom sediment of wastewater fed fishponds, followed by Cr (882.2 ± 78.28 mg.kg-1dw), while in the water the concentration of Fe (4.4 ± 0.14 mg.L-1) was found highest and Pb lowest (0.46 ± 0.1 mg.L-1).

Higher concentrations of metals was found in the fish tissues collected from the wastewater fed fishpond in comparison with the fish collected from the uncontaminated ponds. Liver was found to be the main site for metal accumulation with a metal profile of Fe > Zn > Cu > Mn > Cr > Pb. The concentration of nonessential toxic elements like Pb was found much higher in the liver of fish from contaminated site (17.9 ± 3.37) than from the uncontaminated site (0.2 ± .04). While, in fish gill of contaminated site, highest accumulated metal was Fe (1351.1 ± 134.27) and lowest was Pb (6.9 ± 1.02).

b. Histopathology of fish liver

Optical microscopic studies: The results of H/Estained liver tissue histology were depicted in Fig. 3a"h for both fish collected from uncontaminated and contaminated sites. The liver of fish from uncontaminated

site showed hepatocytes arrayed in closely arranged cords; the intra-hepatic exocrine pancreatic tissue, as a result of its acinar arrangement and its diffused distribution in the hepatic parenchyma, was also evident in the observations. The tissue of the fish collected from contaminated ponds showed alteration of the parenchymatous tissue density along with the modification especially in the acinar region at the hepatopancreatic region was seen in liver from contaminated sites

Electron microscopic studies of liver: The scanning images of liver tissues collected from uncontaminated and contaminated ponds apparently showed negligible changes. The picture showed even surface of the uncontaminated liver tissue forming uniform cellular masses with a few scattered blood cells. However, few aggregates were clearly visible in the contaminated liver tissue samples.

Ultrastructure studies using TEM of the hepatocytes of the uncontaminated site showed nucleus with vesicular rounded shape, a bi-layered nuclear membrane, granular peripheral heterochromatin scattered within the nucleus, and cytoplasm with numerous mitochondria of variable shapes and sizes, Golgi system (with saccules and cisternae), smooth endoplasmic reticulum (sER), and scanty rough endoplasmic reticulum (rER). The liver cells of the contaminated site showed a vast array of rough endoplasmic reticulum (rER) with polysome formation was found in the cell cytoplasm. The main structural unit of the Golgi system was a flattened membranous vesicle, the Golgi saccule. Prosecretory granules were found in the system with secretory granules nearby, which was a part of the GERL (Golgi-endoplasmic reticulum-lysosome) system.

The AFM study of fish blood revealed interesting results. The red blood cells collected from the fish of the uncontaminated site showed round or elliptical structures with smooth outer surface. However, the blood cells from fish of contaminated pond demonstrated small dot-like structures (‘basophilic stippling’) on the membranes of the erythrocytes. Within a scan size of 30 m, the dot-like

protrusions on the membrane surface of erythrocytes were clearly visible.

Higher expression of metallothionein was found in contaminated fish hepatocytes when compared with uncontaminated site, as evident by studies using flow cytometry, immunohistochemistry and immuno-gold labelling. FACS analysis of intracellular MT in fish hepatocytes was observed. Mean fluorescent intensity (MFI) for FITC labelled antibody against MT in hepatocytes collected from contaminated fishpond (MFI: 249.67) was much higher than in hepatocytes of fish from uncontaminated pond (MFI: 110.09). Again, immunohistochemical staining using FITC demonstrated higher expression of MT fluorescence in the hepatocytes of contaminated fish. The results of immuno-gold labeling of MT through immunoelectron microscopy also found to be in concordance with flow cytometric and immunohistochemical studies. Very few, scattered gold particles indicated the cytoplasmic presence of MT in the cytoplasm of hepatocytes of fish of uncontaminated site. On the contrary, higher cytoplasmic distribution (mainly as aggregation attached to membranes in different organelle of the cytoplasm, especially with the endoplasmic reticulum and mitochondrial membranes) of gold particles in liver parenchyma cells of fish from contaminated pond was noticed.

4. Discussion:

As the SWF canal naturally sediments year-round, bottom sediment concentrations of all elements were higher than overlying water, according to the study. The study found element-specific sedimentation from water into canal bottom sediment, showing the relevance of metal–metal interaction in this environment (Chatterjee et al., 2011). The principal component model showed that metal ions like Cr, Fe, Pb, Cu, and Mn affect bio-concentration, absorption, accumulation, and toxicity of both fish and plants. Bioactive metals including Fe, Mn, Cu, and Zn were actively controlled by the organism; however, ambient quantities affected non-essential harmful elements like Pb (Chatterjee et al., 2016). Heavy metal exposure causes

prevalent hepatocyte peribiliary destruction in fish. TEM shows that heavy metal buildup in fish hepatocytes causes toxicopathic response, with alterations in the rER and GERL systems indicating differentiation in cell secretion. Gill hyperplasia and lamellar dilatation are prevalent with aquatic pollution (Chatterjee et al., 2016), as shown by SEM in the fish gills of contaminated ponds. Pb-rich effluent caused punctate basophilia (basophilic stippling) on fish erythrocytes (Pattee and Pain, 2003). Basophilic stippling in fish erythrocytes in this experiment was caused by aberrant RNA aggregation. Chronic exposure to metal complexes and organic compounds causes tissue-specific alterations. The fish reared at the contaminated study site may have been able to adapt to their stressed environment by producing more metal-binding proteins like metallothioneien (MT). Through its amino acid composition, metal binding ability, and induction by a wide variety of metals, MT quenches free radicals created by tissue metal ions (Chatterjee et al., 2016). Numerous compounds, including transition and d10 metal ions, significantly improved MT biosynthesis in vivo and in cultured cells (Martín-Díaz et al., 2007; Chen and Chan, 2011). The use of wastewater for years in the agriculture fields heavily contaminated the farmlands, and accumulation in the vegetables grown in the region was apparent. The present study indicated that sunflower, marigold, and cock’s comb, usual cultivable species of the areas, were able to take up metals from the contaminated sites. It could, therefore, be suggested to culture these common economically important flowering plants for a season or two, making use of the deeply contaminated agro-soil, to ameliorate the hazardous metal load of the sites.

5. Conclusion

East Calcutta Wetlands' components could handle a wide range of industrial contaminants, especially waste metals. As the "kidney of nature," ECW trap and modify metals year-round. In this study, the metal content of both water and sediment decreased significantly along the sites of the wastewater-carrying canal of ECW, demonstrating the role of wetlands in wastewater remediation. Fish

cultured in wastewater-fed fishponds developed adaptability through methylation of the metal to reduce its toxicity with the help of metalloproteins, like metallothionein, to counter the high metal concentrations. This study may help us to understand the natural and biological processes at this Ramsar site on Kolkata's eastern edge. Motivated conservation efforts and better understanding of the ecosystem under a wide range of contaminants can only resist further pressure and systematic use of this exceptionally productive wetland in Calcutta's backyard.

Acknowledgements: SC sincerely acknowledges the Director, DRL DRDO Tezpur.

References

1. Chatterjee, S., Chattopadhyay, B., Mukhopadhyay, S. K., Mohanta, B., Sudarshan, M., & Chakraborty, A. (2007). East Calcutta Wetlands as a sink of industrial heavy metals: A PIXE study. Intl. J. PIXE, 17(3–4), 129–142.

2. Chatterjee, S., Chattopadhyay, B., Mukhopadhyay, S.K. (2006). Trace metal distribution in tissues of Cichlids (Oreochromis niloticus and O. mossambicus) collected from wastewater-fed fishponds in East Calcutta Wetlands, A Ramsar Site. Acta Ichthyol. Et. Piscat. 36, 119–125.

3. Chatterjee, S., Chattopadhyay, B., Mukhopadhyay, S.K. (2010). Monitoring waste metal pollution at Ganga estuary via the East Calcutta Wetland areas. Environ. Monit. Assess. 170, 23–31.

4. Chatterjee, S., Chattopadhyay, B., Mukhopadhyay, S.K., Mohanta, B., Sudarshan, M., Chakraborty, A. (2007). East Calcutta Wetlands as a sink of industrial heavy metals: a PIXE Study. Int. J. PIXE 17, 129–142.

5. Chatterjee, S., Chetia, M., Singh, L., Chattopadhyay, B., Datta, S., Mukhopadhyay, S.K. (2011). A study on the phytoaccumulation of waste elements in wetland plants of a Ramsar site in India. Environ. Monit. Assess. 178, 361–371.

6. Chatterjee, S., Datta, S., Das, T.K., Veer, V., Mishra, D., Chakraborty, A., Chattopadhyay, B., Datta, S., Mukhopadhyay, S. K., Gupta D.K. (2016) Metal accumulation and metallothionein induction in Oreochromis niloticus grown in wastewater fed fishponds . Ecol Eng. 90 405–416

7. Chattopadhyay, B., Chatterjee, S., & Mukhopadhyay, S. K. (2004). Seasonality in physico-chemical parameters of tannery wastewater passing through East Calcutta Wetland ecosystem. Journal of Society for Leather Technologists & Chemists, 88, 27–36.

8. Chattopadhyay, B., Gupta, R., Chatterjee, A., & Mukhopadhyay, S. K. (1999). Characterization of tannery effluents envisaging environmental impact assessment. Journal of American Leather Chemists' Association, 94(9), 338–347.

9. Martín-Díaz, M.L., Kalman, J., Riba, I., Fernández de la Reguera, D., Blasco, J., DelValls, Á. (2007). The use of a metallothionein-like-proteins (MTLP) kinetic approach for metal bioavailability monitoring in dredged material. Environ. Int. 33, 463–468.

10. Pearse, A.G.E. (1980). Histochemistry –

Theoretical and Applied, vols. 1 and 2., fourth ed. Churchill Livingstone, Edinburgh, London and New York.

11. Ray, A.K. (1986). Manual of Practical Zoology,

Part-I and II. Medical Allied Agency, Calcutta, India.