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Determination of Endocrine Disrupting Compounds in Water Bodies Around Guwahati City, Assam, India through Gas Chromatography/Mass spectrometry

***Saptadeepa Roy, Jogen Chandra Kalita**

Physiology & Biochemistry Research Laboratory, Department of Zoology, Gauhati University, Guwahati-781014. Assam, India.

****Corres.author: saptadeepa_roy@yahoo.co.in***
Phone Number: (+91) 9435084175

Abstract: Endocrine-disrupting chemicals (EDC) are capable of mimicking or blocking the normal functioning of endogenous hormones in both humans as well as in animals. Effects attributed to endocrine disruptors include the development of breast cancer, testicular and prostate cancer, reduced sperm production in humans, de-masculinization, feminization, altered immune functions and decreased fertility in birds, fish and mammals. The present work dealt with the detection and identification of endocrine disrupting compounds (Pesticides and Phthalates) from three different sites around Guwahati city, India, famous for its polluted water. Water samples were collected and analyzed using Gas chromatography and Mass spectroscopy (GC-MS). The results showed the presence of estrogenic chemicals Aldrin, Dialdrin in Bharalu river (µg/L). The chemicals analyzed were below the detectable limit in Deepor beel, while Borsola beel recorded the presence of Dibutyl Phthalate (µg/L). Some of the other chemicals tested were either not detected or were below the detectable limit.
Keywords: EDC, feminization, phthalates, GC-MS.

Introduction & Experimental

During the past few years there has been a growing concern on possible harmful consequences of exposure to xenobiotic compounds that are capable of modulating or disrupting the endocrine system. This concern for EDCs is directed at both wildlife and humans [1, 2]. Wide varieties of chemicals have been identified as endocrine disruptors or are suspected to be able to affect the endocrine system. As the relationships between the chemicals and the observed effects in many cases are still hypothetical, additional scientific research is required into the nature and severity of the reported phenomena [3]. Some environmental EDCs may be released into the environment intentionally (e.g., pesticides), but for most environmental contaminants release is unintentional. Unintentional release of chemicals can occur throughout part or all of the chemical's life cycle (e.g., manufacturing, use, and disposal). Leakage from landfill areas and distribution via sewage sludge are also sources of exposure [4].

Most developed countries like have established regulatory authorities and requirements for chemical and biological analytical procedures for testing

pesticides, metals, industrial chemicals and PCBs in food and environmental matrices. Endocrine disruptors are usually present in the environment at low concentrations so a pre-concentration technique is usually needed to determine them [5].Because they are so varied, endocrine disruptors are usually determined either by gas chromatography (GC)[6-9] or high performance liquid chromatography. (HPLC)[6].However, methods developed are usually focused on families or groups of chemicals, but not on a large number of compounds with estrogenic activity[5].

water hyacinth, too, are expanding to more and more areas.

Sampling Area:

Guwahati is a major city in eastern India largest cities of eastern India. It is positioned at 26.13° N 91.77° E .The city is situated between the southern bank of the Brahmaputra river and the foothills of the Region. The three sites of water collection are The Bharalu River, the Borsola Beel and the Deepor Beel

The Bharalu River (tributary of Brahmaputra) in Assam once provided potable water to thousands of people living on its banks. It was also a source of variety of fish and other aquatic flora and fauna for the people. The river is now very badly polluted. Scientists have warned for its quick cleaning measures otherwise a disaster is imminent.

Dipor Bil also spelt **Deepor Beel** (*Bil* or *Beel* means "lake" in the local Assamese language is a permanent freshwater lake. It is also called a wetland under the Ramsar Convention. It is considered as one of the largest Beels in the Brahmaputra valley of lower Assam

Deepor Beel has been bearing the brunt of the city's unplanned development. The ills plaguing Deepor Beel are many and have assumed serious proportions. Perhaps foremost among the problems is the accumulation of municipal solid wastes, including toxic disposals, which are increasingly finding their way into the very core of the wetland. The problem gets aggravated during the monsoons, with rainwater sweeping large amounts of garbage from the dumping site to the beel. Growing pollution is indeed a grave threat for long-term survival of the beel that shelters a wide range of avi-fauna including migratory birds.

The **Borsola beel** or the Sola beel, is one of the notable wetlands of Guwahati city. It has been identified as a large water storage lake. The beel once provided a habitat for a large number of flora and fauna and receives rainwater from nearest hills and plains. Continued discharge of the city's untreated sewage and dumping of municipal solid wastes in its close proximity have pushed the wetland's pollution to alarming levels. Municipal garbage, particularly plastic wastes, can be seen floating in the water. A closer look will further reveal a blackish, oily substance coating the water over a large stretch. Invasive weeds such as

Materials and Methods[6-14]

The water samples were collected during the pre-monsoon season in the month of April 2010 from different locations around Guwahati city in glass containers containing sodium thiosulphate. The samples were then shaken thoroughly until dissolved and were kept on ice. They were then refrigerated at 4°C until extraction.

Preparation of stock standard and internal standard solutions:

Stock standard solution:

0.0100 g of pure material (pesticide and phthalate standards) was dissolved in MTBE (Methyl Tert Butyl Ether) and diluted to volume in a 10 mL volumetric flask. The stock standard solutions were transferred into TFE-fluoro-carbon-sealed screw cap amber vials. It was stored at room temperature and protected from light.

Internal Standard Solution:

An internal standard fortifying solution was prepared by accurately weighing approximately 0.0010 g of pure Pentachloronitrobenzene (PCNB). The PCNB was dissolved in MTBE and diluted to volume in a 10 mL volumetric flask. It was then stored into a TFE-fluorocarbon-sealed screw cap bottle and stored at room temperature. Addition of 5 µL of the internal standard fortifying solution to 5 mL of sample extract results in a final internal standard concentration of 0.1 µg/mL.

Liquid-liquid extraction of water sample:

The water meniscus on the side of the sample bottle was marked for later determination of sample volume. The sample was fortified with 50 µL of the surrogate standard fortifying solution and was poured into a 2L separator funnel and was adjusted to pH 7 by adding 50 ml phosphate buffer. The pH 7was checked and H_2SO_4 or NaOH was added as and when necessary. 100 g NaCl was added to the sample sealed and shaken to dissolve the salt. 60 mL methylene chloride was added to the sample bottle, sealed, and shaken for 30

seconds to rinse the inner walls. The solvent was then transferred to the separator funnel and the sample was extracted vigorously shaking the funnel for two minutes with periodic venting to release excess pressure. The organic layer was allowed to separate from the water phase for a minimum of 10 minutes. The methylene chloride extract was collected in a 500 mL Erlenmeyer flask. A second 60 mL volume of methylene chloride was added to the sample bottle and the extraction procedure was repeated for the second and the third time combining the extracts in the Erlenmeyer flask. The original sample volume was determined by refilling the sample bottle to the mark and transferring the water to a 1000mL graduated cylinder.

Extract Concentration:

A Kuderna-Danish (K-D) concentrator was assembled by attaching a 25 mL concentrator tube to a 500 mL evaporative flask. The extract was dried by pouring it through a solvent-rinsed drying column containing about 10 cm of anhydrous sodium sulfate. The extract was collected in the K-D concentrator, and the column was rinsed with 20-30 mL methylene chloride. This rinse was added to the extract. Alternatively, about 5 g of anhydrous sodium sulfate was added to dry the extract in the Erlenmeyer flask. The flask was swirled to dry extract and allowed to sit for 15 minutes. The methylene chloride extract was then decanted into the K-D concentrator and the remaining sodium sulfate was rinsed with two 25 mL portions of methylene chloride and the rinses were decanted into the K-D concentrator.

One to two clean boiling stones were added to the evaporative flask and a macro Snyder column was also attached to it. The Snyder column was pre wet by adding about 1-2 mL of MTBE (Methyl Tert Butyl Ether). Additional 5-10 mL of MTBE and fresh boiling stones were added. A micro-Snyder column was attached to the concentrator tube and the column was pre-wet by adding about 0.5 mL of MTBE to the top. The micro K-D apparatus was placed on the water bath so that the concentrator tube was partially immersed in the hot water. The vertical position of the apparatus was adjusted and the water temperature as required to complete concentration in five to 10 minutes. When the apparent volume of liquid reached 2 mL, the micro K-D is removed from the bath and is allowed to drain and cool. 5-10 mL MTBE was again added to the micro K-D and re-concentrated to 2 mL. It was then removed from the bath and was allowed to drain and cool. The micro Snyder column was removed, the walls of the concentrator tube rinsed while adjusting the volume to 5.0 mL with MTBE. The extract is transferred to an appropriate-sized TFE-fluorocarbon-sealed screw-cap vial and stored, refrigerated at 4°C, until analysis by GC-ECD (Shimadtzu)

Polluted Bharalu River

The deepor beel

The Sola beel

Fig 1: Photomicrographs of water collection sites, around Guwahati city, Assam, India

Table1: Endocrine disrupting chemical concentration in water (µg/L) in three Major water bodies of Guwahati , Assam.

ESTROGENIC CHEMICALS **(PESTICIDES)**	BHARALU RIVER(µg/L)	BORSOLA BEEL(µg/L)	DEEPOR BEEL(µg/L)
Alpha HCH	BDL	BDL	BDL
Lindane	BDL	BDL	BDL
Beta HCH	BDL	BDL	BDL
Delta HCH	ND	ND	ND
Aldrin	0.08	BDL	BDL
Dieldrin	0.76	BDL	BDL
Endrin	BDL	BDL	BDL
OP DDT	BDL	ND	ND
(PTHALATES)			
Benzyl butyl phthalate	BDL	ND	ND
Dibutyl phthalate	BDL	BDL	0.69

Gas Chromatography:
The system was first calibrated. 5 µL of the internal standard fortifying solution was added to the sample extract, sealed, and shook to distribute the internal standard. 2 µL of this sample extract was then injected into the GC packed column. The resulting peak size in area is measured in units.

Identification of Analytes:
A sample component is identified by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive.

Results and Discussion

Endocrine disrupting compound analysis of the water during the study period using GC/MS are presented in Table 1. The results showed the presence of estrogenic chemicals Aldrin, Dialdrin in Bharalu River (µg/L). The chemicals analyzed were below the detectable limit in Deepor beel, while Borsola beel recorded the presence of Dibutyl Phthalate (µg/L). The concentration of some of the other chemicals tested was generally below the limit of detection but was positively identified in three of the different water samples.

The quality of water and sediment in the river system as well as the beels is seriously affected by pollutants which enter through drains that bring domestic as well as industrial effluents. These industrial and domestic waste waters, besides other pollutants also contain high concentration of EDCs. The presence of such compounds is a serious matter of concern because such EDCs enter human body through polluted waters which gets bioaccumulated in body's muscle and tissues. Moreover by eating fishes from such polluted sites is also another route of exposure to such chemicals to human bodies, because EDCs get biomagnified in the food chain.

Conclusion

The GC/MS method was successfully applied for the detection of Endocrine disrupting compounds (Pesticides and Phthalates) in environmental water samples. Further studies of such kind will help in the identification of EDCs.

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