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Seasonal Variations In Mutagenicity of Sediment Samples Collected From Kanjli Wetland (Ramsar Site), Kapurthala Using *Salmonella Typhimurium*

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Abstract

Genotoxicity of sediment samples collected from Kanjli wetland Kapurthala has not been documentedin the literature earlier. This report abridges an investigation of the microbial mutagenicity This report summarizes a study onthe microbial mutagenicity of 18sediment samples collected in dry and wetseasons during 2012-2015. Sediment extracts were analysed for mutagenicity utilizing a short-term bacterial test with pre and post-enzyme activation. Mutagenicity (without and with S9 mix) was detected in most of the samples. Dose dependant increase in mutagenicity was observed, with aqueous extracts showing higher mutagenic activity as compared to acetone extracts in both TA98 and TA100 strain of *Salmonella typhimurium*. Among different season dry period (winter) samples showed higher number of revertants as compared to wet period.Results of present investigation clearly indicate that there is an urgent need for continuous monitoring of freshwater environment, mainly those intended for domesticated usage.

Introduction

Sediments are of significant natural concern since they may act as potential sinks for a large number of contaminants. These residues may accumulate and release contaminants to aquatic medium that can be collected by bottom dwelling organisms. In this way, sediments in water bodies like wetlands, streams and creeks may have a potential risk to the benthic organisms and the human wellbeing through their trophic transfer. In-vitro biological tests are well studied and valuable in evaluating sediments quality [1-4]. These bioassays can be directed in a well-controlled and foreordained condition, they can give fast outcomes,

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Vol-22-Issue-16-August-2019

increasingly reproducible and economic [5]. Mutagenicity has been an expanding worry as of late on the grounds that it is responsible for perpetual changes in the structure and measure of the hereditary material of a living being which results in heritable changes in its structure and capacity [6]. *Salmonella*/microsome assay is a for the most part acknowledged biological assay to distinguish mutagenicity of individual compound and environmental samples [6]. The test utilized the standard analyzer strains TA98 and TA100 to identify frame-shift and base-substitution transformations, separately. In the present investigation, the Ames variance measure was utilized to identify the bacterial toxicity of dregs removes from the Kanjli wetland, Kaputhala, Punjab.

Materials and methods

Sample collection and preparation

Composite sediment samples were collected from Kanjli wetland complex. After collection samples were dried atroom temperature for 3 days, sieved, and further dried in hot air oven (Model: NSW-135; Make: Caltan, India) at 105 ℃ for 24 h followed by grinding to make a fine powder.10 g of finely grounded sample was extracted with 10 mlacetone/water by centrifugation at 7000 rpm for 10 min. Supernatant was collected and dried at 100 º C in hot air oven. Residue was dissolved in 10ml of 0.1% of dimethyl sulphoxide (DMSO) and filtered through 0.22 µm membrane filters. The stock solution was used to prepare various concentrations *viz.,* 1000, 500, 250, 125 and 62.5 mg/ml with 0.1% DMSO and distilled water, for acetone and water extracts respectively. Sediment extract was stored at−80◦C until mutagenicity testing.

Test procedure

Sediment extracts were analysed using Ames fluctuation assay according to the procedure given by Ames and Maron [7] with little modifications.For experimentation 2 ml of top agar and 100 µl fresh overnight bacterial culturewas taken into test tube and 100 µl sample was added and solution was mixed by gentle shaking, mixture was poured to minimal agar plate. For enzymatic activation, 0.5 ml of freshly prepared liver homogenate (S9 mix) was added to test tube containing 2 ml of top agar, 100 µl culture and 100 µl of test sample. Sodium azide (NaN_3) and Nitro-o-phenylendiamine (NPD) was used as positive control in absence of S9 mix for TA100 and TA98, respectively whereas 2-Aminofluorene (AF) was utilized as

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positive control without S9 blend. 0.1 % dimethyl sulphoxide (DMSO) and membrane filtered distilled water weretaken as negative control.Petri plates were incubated at 37° C for 48 h. All the samples were tested in triplicate.

Results and discussion

The mutagenic effects of sediment samples of Kanjli wetland were analyzed using two tester strains *viz.*, TA98 and TA100 of *Salmonella typhimurium* following Ames assays. Two types of extracts *viz.*, aqueous and acetone at different concentrations (62.5, 125, 250, 500 and 1000 mg/ml) per 0.1 ml culture per plate were used during the present study. The results of mutagenic effects of soil and sediments are presented in the following section and are depicted in **Figure 1 - 2**.

At the maximum dose, number of revertants of TA98 strain of *Salmonella typhimurium*, on treatment with aqueous extracts of sediment samples, varied from 299 (KCS6) to 457 (KCS5) in the absence of S9 fraction and 345 (KCS4) to 510 (KCS3) in the presence of S9 fractionwhile for acetone extracts, number of revertants colonies varied from 294.3 (KCS1) to 447(KCS5) in the absence of S9 fraction and 360 (KCS1) to 501(KCS5) in the presence of S9 fraction, respectively (Fig. 1).

TA100

Number of revertants of TA100 strain of *Salmonella typhimurium* in aqueous extracts varied from 387.6 (KCS6) to 489 (KCS2) in the absence of S9 fraction and 447 (KCS6) to 567 (KCS2) in the presence of S9 fraction while for acetone extract as 338 (KCS1) - 479 (KCS2) and 413.6 (KCS4) - 546 (KCS2) in absence and presence of S9 fraction, respectively (Fig. 2).

Among different seasons, samples collected during winter season showed higher mutagenicity than samples collected during summer/monsoon season in both TA98 and TA100 strains. The reason of low mutagenicity during summer/monsoon period may be linked to dilution of contaminants by heavy flow in the water that prevents settling of pollutant in sediments. Hilscherova *et al*. [8] in their study on sediment samples of Morava River, Drevnice River and its tributaries in the Zlı́n region of Czech Republic also reported that sediments samples collected in dry season (autumn) has high toxicity in different bioassays as compared to spring samples that is directly linked with dissemination of pollutants by heavy flow of water in spring season. Similar observations were made by number of earlier studies highlighting the mutagenicity of sediment samples [9-12]

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Vol-22-Issue-16-August-2019

Conclusion

The present study showed that the sediments from the Kanjli wetlandare mutagenic in both the tester strain *i.e* TA98and TA100 with and without metabolic activation. Further, seasonal variations in mutagenicity can reveal the fate of contaminants in a dynamic aquatic ecosystem. Results of the study not only provides the base line information for systematic monitoring of various compartments of Kanjli wetland ecosystem to prevent its further degradation but also highlights toxicological effects of contaminants that may leads to serious health issues in different life forms living in the purlieu of Kanjli wetland.

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ISSN: 0971-1260
Vol-22-Issue-16-August-2019

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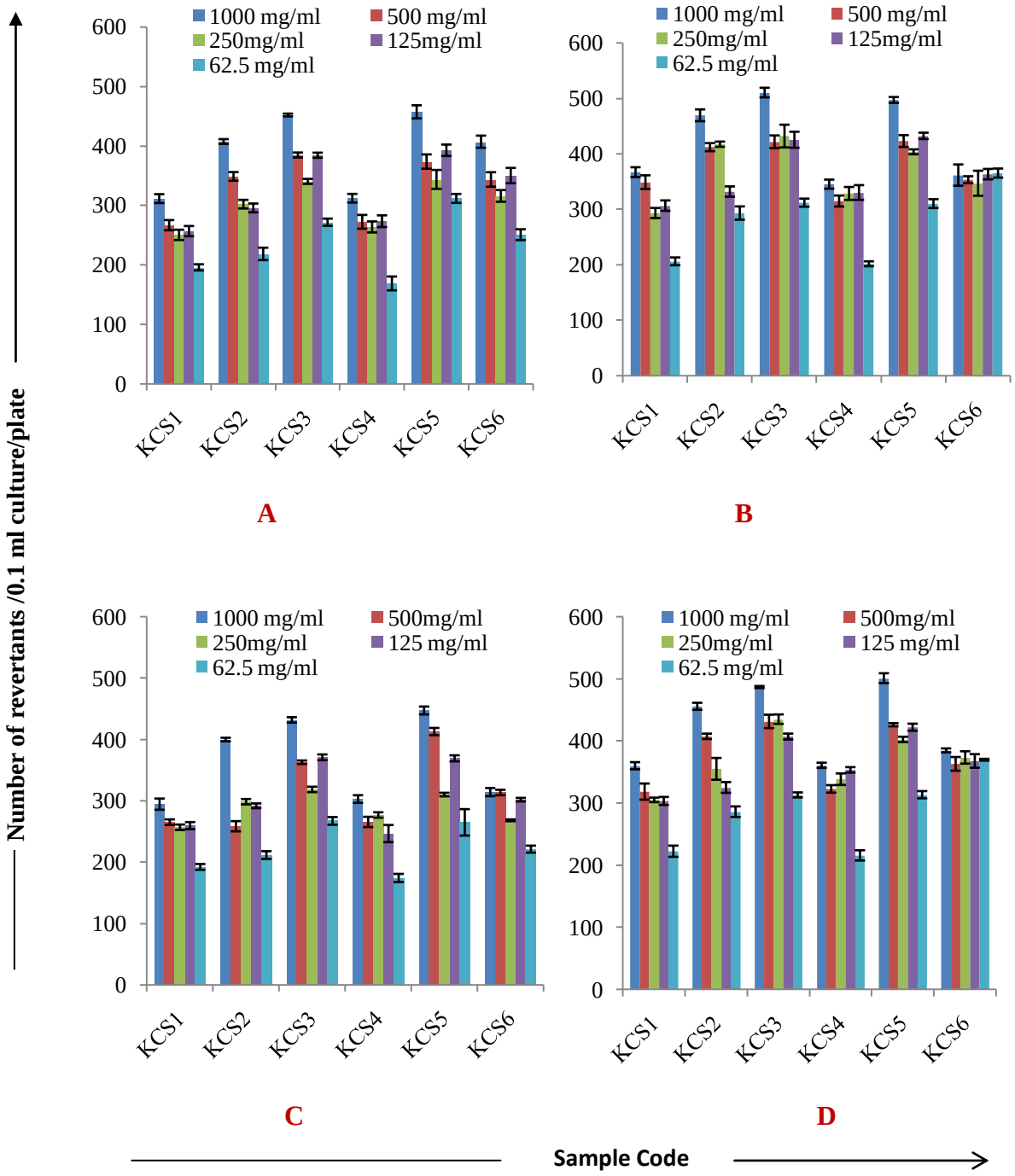

Fig.1.Mutagenic effect of aqueous (A and B) and acetone extracts (C and D) of sediment samples collected from Kanjli wetland, Kapurthala, Punjab (India) employing Ames assay using TA98 strains of *Salmonella typhimurium* in absence (A and C) and presence (B and D) of S9 mix.

ISSN: 0971-1260
Vol-22-Issue-16-August-2019

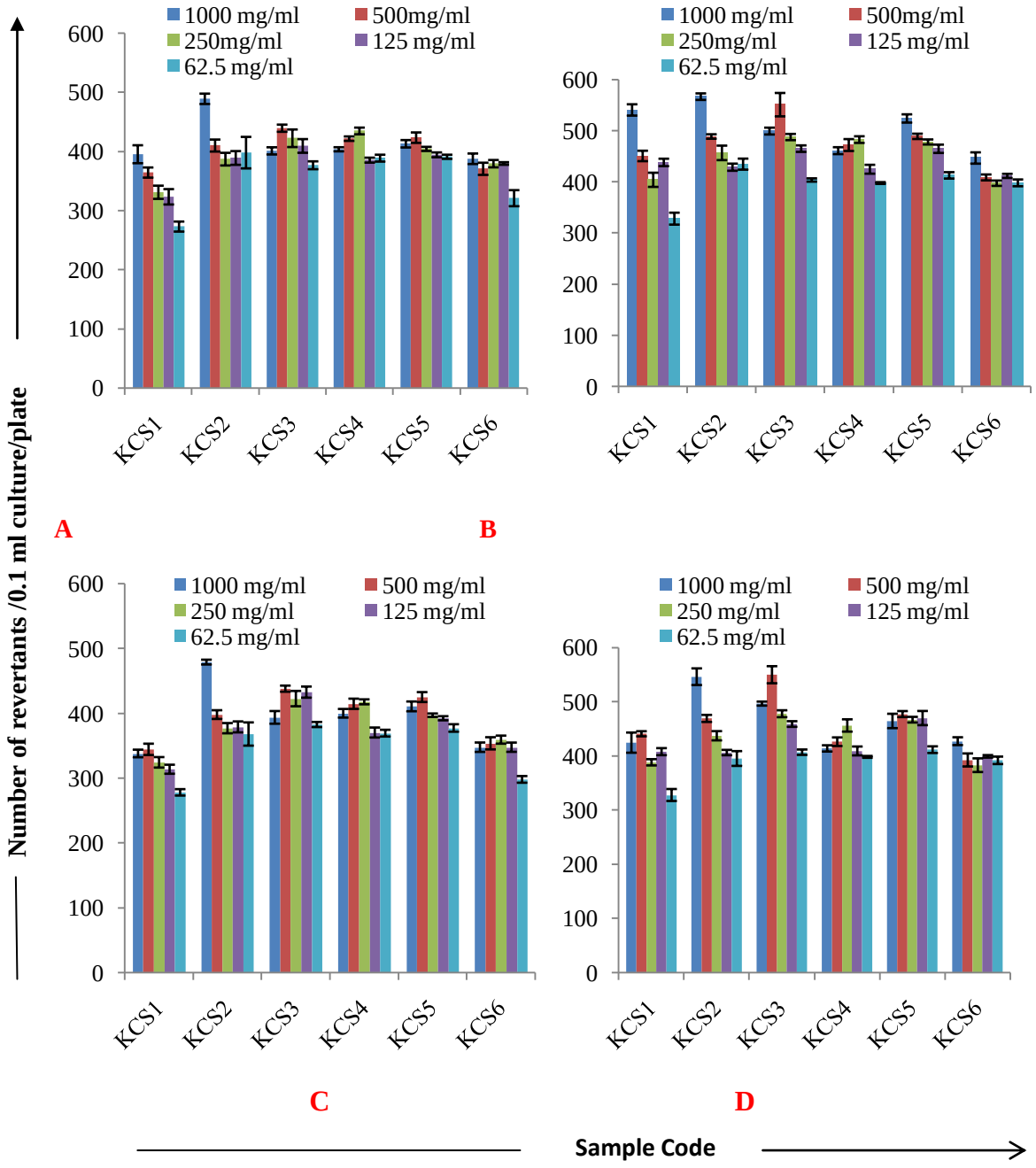

Fig.2. Mutagenic effect of aqueous (A and B) and acetone extracts (C and D) of sediment samples collected from Kanjli wetland, Kapurthala, Punjab (India) employing Ames assay using TA100 strains of *Salmonella typhimurium* in absence (A and C) and presence (B and D) of S9 mix.