THINK INDIA JOURNAL

ISSN:0971-1260
Vol-22-Issue-37-December -2019

Assessment of the mutagenicity of soil samples in the vicinity of Kanjli wetland (Ramsar site), Kapurthala Using *Salmonella Typhimurium*

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Abstract

Soil samples are of significant environmental concern because they may act as sinks for different contaminants and can be accumulated by organisms at different trophic levels. In the present study soil extracts from Kanjli wetland Kapurthala, Punjab (India) was investigated for mutagenic potential using Ames fluctuation assay. Most of the soil samples were mutagenic to the strain TA98, irrespective of the presence or absence of S9 mix. The mutagenicity of the samples increased in dose dependent mannerwith maximum effect at 1000 mg/ml of soil extract.

Introduction

Soil isn't just a medium for plant to raise but also act as source of contamination for surface water and food crops. Soil near aquatic bodies tends to accumulate various pollutants during floods and release them back to water through leaching and surface runoff. Increasing food demands and extensive use of agro-chemicals to meet the demand, further intensify the burden on soil ecosystem. Concern has been raised over the long term sustainability and environmental consequences due to overuse of agro-chemicals in agriculture. Many of these chemicals used have already been proved to showgenotoxic, carcinogenic and mutagenic effects in different living organisms. Therefore, use of bioassays has been suggested as reliable and useful tools to correlate biological responses with environmental contaminants [1-3]. Among different bioassays, Ames test has been widely used to monitor the mutagenicity of various environmental segments such as soil [4-6]. In the present study, soil samples from agricultural fields adjoining Kanjli wetland were collected and analyzed for mutagenicity.

Materials and Methods

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Soil samples were collected from agricultural fields located in the vicinity of Kanjli wetland by digging the soil to the depth of approximately 10 - 15 cm.Soil sampleswere collected from 3 different sites *viz.*,upstream (KUA), Kanjli wetland complex (KCA) and downstream (KDA)under agricultural cultivationsin four different seasons. All samples were collected in triplicates. Soil samples were collected in polyethylene bags, labeled and brought to the research facility. Samples were dried at room temperature for 72 hours followed by drying in hot air oven (Model: NSW-135; Make: Caltan, India) at 105 °C for 24 h. The samples were then grounded to fine powder.

Sample Preparation

Two types of extracts (acetone and aqueous extracts) were prepared. For acetone extracts, 10 g of soil sample was mixed with 10 ml of acetone and centrifuged at 7,000 rpm to make organic extracts of soil. Supernatant was collected and evaporated to dryness on water bath and reconstituted to 10 ml with 0.1% Dimethyl sulphoxide (DMSO) to make 2000 mg/ml soil extract. For aqueous extract, 10 g of sediment/soil sample was centrifuged using 10 ml of distilled water. After centrifugation supernatant was collected, evaporated to dryness and reconstituted to 10 ml with distilled water. Further, both the extracts were diluted to prepare the concentrations of 62.5, 125, 250, 500 and 1000 mg/ml equivalent to mg/g of soil samples with 0.1 % DMSO and distilled water.

Procedure

Salmonella typhimurium mutagenicity assay i.e. Ames assay was used to estimate the mutagenicity of the aqueous and acetone soil samples. It is a transient bacterial examine designed to identify substances that can cause genetic impairment which results in mutations. The procedure used for Ames assay was same as given by [7] with little modifications. Samples were passed through 0.2 micron membrane filter before experimentation and test was performed using TA98 strains to detect frame-shift in presence and absence of metabolic activation. *Salmonella typhimurium* strains were obtained Institute of Microbial Technology (IMTECH), Chandigarh. To a test tube containing 2 ml of top agar and 100 µl fresh overnight bacterial culture100 µl sample was added and mixture was poured to minimal agar plate. For metabolic 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. Nitro-o-phenylendiamine (NPD) was used as positive control in absence of S9 mix for TA98 whereas 2-Aminofluorene (AF) was used as positive control in presence of S9 mix. Membrane filtered

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distilled water was used as negative control. The plates were incubated at 37° C for 48 h. All the samples were tested in triplicate. Two fold surge in the number of revertant colonies when compared to negative control was considered to be mutagenic [8].

Result and discussion

The mutagenic effects ofsoil samples of Kanjli wetland were analyzed using TA98strain of *Salmonella typhimurium* following Ames assay. 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 samples are presented in **Tables 1 - 3**.

Number of revertants of TA98 strain of *Salmonella typhimurium* varied from 236.6 (KUA3) to 441.6 (KUA4) and 362.3 (KUA1) to 526.6 (KUA4) in absence and presence of S9 mix, respectively for aqueous extracts while from 289.6 (KUA2) to 420 (KUA5) in the absence of S9 mix and 357.6 (KUA6) to 437.6 (KUA5) in presence of S9 for acetone extract of soil sample collected from upstream site (Table 1).

The aqueous extracts of soil samples collected from agricultural field adjacent to Kanjli wetland showed the mutagenic response using TA98 strain of *Salmonella typhimurium*as varying from 324 (KCA3) - 475.6 (KCA2) in absence of S9 mix and 408.3 (KCA3) to 544.6 (KCA4) in the presence of S9 mix for aqueous extracts. The acetone extracts of soil samples collected from agricultural field adjacent to Kanjli wetland showed the mutagenic response using TA98 strain of *Salmonella typhimurium*varying from 307.3 (KCA1) to 407.3 (KCA4) in absence of S9 mix and 338.3 (KCA1) to 485.3 (KCA4) in the presence of S9 mix at highest dose (Table 2).

Mutagenic response of aqueous extracts of soil samples collected from an agricultuiral field downstream of Kanjli wetland using TA98 strain of *Salmonella typhimurium* at highest dose induced number of revertants in the range of 306.3 (KDA1) to 445 (KDA6) in absence of S9 mix and from 428 (KDA1) to 537.3 (KDA2) in presence of S9 mix. For acetone extracts of KDA, number of revertants varied as 316.3 (KDA1) to 438.3 (KDA6) in absence of S9 mix and from 388 (KDA1) to 516.3 (KDA5) in presence of S9 mix (Table 3). Mutagenicity of soil samples has been well documented in earlier studies [1, 9, 10, 11, 12, 13].

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Analysis of mutagenic effects of soil samples revealed that both types of extracts *viz.*, aqueous and acetone of soil samples were low to moderately mutagenic at different concentrations used.Similarly,investigation on soil tests from industrial territory of Ghaziabad City, UP (India) demonstrated that fluid soil separates showed positive outcomes and organic (methanol) extricate was the most mutagenic with TA98 strain in thepresence as well as absence of S9 mix. nearness just as nonattendance of S9 part. They reasoned that TA98 variant of *Salmonella Typhimurium* was the most responsive strain among every one of the strains in term of mutagenicity and initiation factor [18].Different investigations likewise detailed TA98 variant recognized the mutagenicity of more soil tests (either agrarian, woodland or car traffic defiled soils) than some other strain [15-17].

Conclusion

Mutagenicity assessment of aqueous and acetone extract of soil samples from Kanjli wetland, Kapurthala, Punjab(India) showed that it has been contaminated by a blend of toxins responsible for mutagenic effects. Results of the present study points that the samples are capable of causing base pairinsertions or deletions of a number of nucleotides in a DNA sequence that leads toframe shift type of mutations. In perspective on the normal act of utilization of untreated wastewater and water from contaminated water bodies to farming area in the neighboring region ought to be carefully restricted as the contaminant may enter into the natural way of life and cause wellbeing perils to people.

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Table 1. Mutagenic response acetone extracts of soil sample collected from agricultural field (KUA) at upstream of Kanjli wetland, Kapurthala, Punjab (India).

Sample Code	Aqueous extracts									
	TA98 without S9					TA98 with S9				
KUA1	322.6 ± 8.412	262 ± 11.718	241.6 ± 12.01	267.6 ± 6.960	216.3 ± 9.171	362.3 ± 19.95	376 ± 12.42	303 ±11.789	352.6 ± 23.94	277 ± 7.0
KUA2	289.6 ± 15.16	247.3 ± 5.364	237.3 ± 16.49	311.3 ± 7.531	232 ± 9.165	393 ± 14.22	392.6 ± 11.85	343 ± 12.76	371.6 ± 11.28	346 ± 14.46
KUA3	236.6 ± 15.89	326.3 ± 15.34	337.6 ± 14.76	350.3 ± 15.34	328 ± 6.110	423 ± 7.234	427 ± 4.04	463.6 ± 4.484	443.3 ± 11.56	411 ± 8.386
KUA4	441.6 ± 10.13	374.3 ± 12.03	348.3 ± 9.279	364 ± 13.57	278 ± 7.234	526.6 ± 10.13	456.6 ± 7.264	423.3 ± 15.89	414.6 ± 8.373	368.3 ± 10.13
KUA5	431 ± 12.28	372.3 ± 11.34	369.6 ± 12.97	441 ± 23.89	326.6 ± 8.81	497 ± 4.932	422.6 ± 10.68	428.3 ± 11.66	540 ± 17.32	438.6 ± 16.17
KUA6	318 ± 11.93	276.6 ± 16.63	276.3 ± 6.839	326.6± 10.13	238.6 ± 24.91	385.3 ± 7.859	330.6 ± 11.56	374.3 ± 14.67	376.3 ± 12.12	375 ± 4.041
F ratio (5, 12)	41.20*	19.01*	21.12*	11.05*	14.98*	31.10*	19.39*	23.35*	21.01*	26.46*
HSD	56.47	57.82	56.32	63.74	56.93	53.41	45.54	56.04	67.69	49.04
Acetone extracts										
KUA1	316 ± 3.605	271± 3.214	232.6 ± 3.929	292.6 ± 6.839	222.3 ± 3.179	385.6 ± 12.83	376 ± 6.658	311.3 ± 4.096	376 ± 18.23	238.3 ± 18.65
KUA2	289.6± 15.16	247.3 ± 5.364	237.3 ± 3.929	311.3 ± 7.535	232 ± 9.165	393 ± 14.22	392.6 ± 11.85	349.6 ± 9.701	377.3 ± 6.044	356 ± 7.023
KUA3	313.3 ± 11.66	340.3 ± 9.134	356 ± 6.082	343.6 ± 9.769	321.3 ± 4.666	383 ± 2.886	424.3 ± 5.783	427.3 ± 8.192	418.6 ± 9.333	406 ± 4.358
KUA4	400.6 ± 3.179	373 ± 3.511	346.6 ± 11.66	364 ± 4.932	275.6 ± 3.333	419 ± 2.645	404 ± 4.582	399.3 ± 2.962	404.6 ± 3.711	345 ± 10.40
KUA5	420 ± 8.736	369 ± 9.609	353 ± 8.0	405.6 ± 4.977	321.6 ± 6.009	437.6 ± 14.94	406.3 ± 5.607	407.6 ± 5.044	479.6 ± 11.25	404.6 ± 3.527
KUA6	297.6 ± 5.811	272.6 ± 4.255	273.3 ± 6.009	285.6 ± 8.253	245.3 ± 6.641	357.6 ± 6.641	317.6 ± 3.844	344.3 ± 8.089	389.6 ± 3.711	249.3 ± 26.20
F ratio (5, 12)	37.70*	74.26*	37.0*	40.55*	55.948	7.42*	29.49*	43.28*	14.86*	26.56*
HSD	41.14	28.83	43.60	32.74	26.50	46.94	31.40	30.68	45.49	64.41

Data shown are Mean ± S.E. of three replicates
KUA: Soil samples from agricultural field upstream of Kanjli wetland collected during different seasons.
KUA1: July, 2013 (Summer/Monsoon)**; KUA2:** November, 2013 (Winter)**; KUA3**: February, 2014 (Winter)**; KUA4:** June, 2014 (Summer/Premonsoon)**; KUA5:** November, 2014 (Winter)**: KUA6:** April, 2015 (Summer/Premonsoon)
HSD: Honestly significant value as calculated from one way ANOVA between parameter and seasons

ISSN:0971-1260
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*Significant at $p \leq 0.05$ level of significance.

Table 2. Mutagenic response of aqueous and acetone extracts of soil sample collected from agricultural field (KCA) adjacent to Kanjli wetland, Kapurthala, Punjab (India).

Sample Code	Aqueous extracts									
	TA98 without S9					TA98 with S9				
	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
KCA1	326.6 ± 10.13	281 ± 8.621	224 ± 9.539	201.6 ± 6.009	193.3 ± 2.333	445 ± 12.70	347.3 12.19	264 ± 7.371	244 ± 12.288	226.6 ± 10.13
KCA2	475.6 ± 24.36	360 ± 8.660	332.6 ± 5.783	337.6 ± 19.40	318 ± 13.86	540.6 ± 10.47	404 ± 3.214	399 ± 10.96	395 ± 9.643	369.3 ± 15.05
KCA3	324 ± 15.94	302.3 ± 9.061	264.6 ± 7.535	284.6 ± 16.02	208.3 ± 10.13	408.3 ± 10.13	399.6 ± 4.841	393.3 ± 3.480	369.6± 3.711	378 ± 6.350
KCA4	409.6 ± 6.641	366.6 ± 12.41	323.3 ± 10.13	293 ± 11.93	234.3 ± 16.18	544.6 ± 22.76	425 ± 8.660	405.3 ± 3.711	381.6 ± 9.333	329.6 ± 11.83
KCA5	415 ± 7.637	375.3 ± 8.666	345 ± 10.40	357.3 ± 10.74	281 ± 9.865	520 ± 10.40	456.6 ± 6.00	415.3 ± 15.49	428 ± 7.234	361.6 ± 6.009
KCA6	338.3± 7.264	285.3 ± 4.176	283.3 ± 7.264	299 ± 6.082	175 ± 7.637	443 ± 8.504	341.3 ± 5.60	345 ± 10.40	346.6 ± 10.92	220 ± 15.27
F ratio (5, 12)	20.57*	23.70*	29.16*	18.09*	25.14*	39.52*	36.74*	36.17*	46.54*	40.20*
HSD	61.22	42.24	38.84	57.16	49.34	59.14	33.19	43.14	41.85	51.40
Acetone extracts										
KCA1	307.3 ± 6.173	266.6 ± 11.34	216.6 ± 3.382	208.6 ± 4.096	194.3 ± 2.185	338.3 ± 9.955	327.3 ± 5.044	267.3 ± 8.685	241.3 ± 3.480	223.3 ± 10.92
KCA2	372 ± 15.30	350 ± 2.886	346.6 ± 10.10	330.3 ± 16.90	298 ± 4.358	408.3 ± 2.962	405 ± 3.214	370.6 ± 3.282	405 ± 3.605	365.6 ± 11.46
KCA3	323 ± 4.163	290.6 ± 9.134	248 ± 9.609	274.6 ± 7.310	202.6 ± 8.969	392.3 ± 2.848	327.3 ± 10.15	342.6 ± 7.310	329.6 ± 8.969	272 ± 11.532
KCA4	407.3 ± 3.844	352 ± 8.736	320 ± 2.886	279.3 ± 5.487	214.3 ± 2.333	485.3 ± 8.192	413.3 ± 6.009	400 ± 3.055	319.6 ± 15.38	330.3 ± 3.929
KCA5	405.3 ± 3.756	371.3 ± 7.688	361 ± 6.658	353.6 ± 8.110	280 ± 8.962	435 ± 5.507	397.3 ± 4.055	418.6 ± 3.756	408 ± 4.358	353.3 ± 11.66
KCA6	328 ± 5.686	288.6 ± 0.881	295.3 ± 3.756	293.3 ± 3.382	201.6 ± 1.763	404 ± 7.211	348 ± 2.645	340 ± 8.660	333.3 ± 6.009	239.6 ± 5.783
F ratio (5, 12)	32.67*	31.09*	70.16*	32.77*	63.20*	53.27*	48.46*	72.42*	57.81*	38.68*
HSD	34.49	34.77	30.40	39.63	25.58	29.99	25.87	28.41	36.70	43.91

Data shown are Mean ± S.E. of three replicates
KCA: Soil samples from agricultural field adjacent to Kanjli wetland complex collected during different seasons.

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KCA1: July, 2013 (Summer/Monsoon)**; KCA2:** November, 2013 (Winter)**; KCA3**: February, 2014 (Winter)**; KCA4:** June, 2014 (Summer/Premonsoon)**; KCA5:** November, 2014 (Winter)**: KCA6:** April, 2015 (Summer/Premonsoon)
HSD: Honestly significant value as calculated from one way ANOVA between parameter and seasons
*Significant at $p \leq 0.05$ level of significance.

Table 3. Mutagenic response of aqueous extracts of soil sample collected from agricultural field (KDA) at downstream of Kanjli wetland, Kapurthala, Punjab (India).

Sample Code	Aqueous extracts									
	TA98 without S9					TA98 with S9				
	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
KDA1	306.3 ± 6.839	291.3 ± 4.096	270 ± 9.073	258 ± 10.69	218 ± 7	428 ± 15.13	386 ± 6.658	343.6 ± 9.837	381.6 ± 4.255	283.6 ± 5.840
KDA2	436 ± 6.658	405.6 ± 3.844	377.6 ± 10.26	365 ± 7.937	335 ± 7.637	537.3 ± 9.024	461 ± 8.621	427.6 ± 18.67	426.3 ± 16.17	413.3 ± 6.009
KDA3	363.3 ± 6.658	327.3 ± 3.844	309 ± 10.26	324 ± 7.937	221.6 ± 7.637	428.3 ± 9.024	426 ± 8.621	393.3 ± 18.67	400.6 ± 16.17	378 ± 6.009
KDA4	340.3 ± 4.409	318.3 ± 8.969	302.6 ± 6.082	322.3 ± 2.645	290 ± 6.009	428.6 ± 11.66	313.3 ±11.59	348.3 ± 3.480	407 ± 6.984	392 ± 6.350
KDA5	436.3 ± 10.98	370 ± 5.773	370 ± 12.66	461 ± 18.73	341.6 ± 7.264	513 ± 5.686	442.6 ± 11.86	436.6 ± 7.264	561.6 ± 10.92	445.3 ± 10.72
KDA6	445 ± 7.637	364.3 ± 6.437	355 ± 2.889	374 ± 6.658	271.3 ± 4.096	526.6 ± 10.13	456.6 ± 7.264	418.3 ± 16.41	411.3 ± 5.783	365 ± 7.637
F ratio (5, 12)	52.34*	35.37*	25.30*	46.47*	54.56*	26.09*	39.568	11.14*	50.78*	40.05*
HSD	36.81	31.40	38.63	44.89	32.59	47.55	40.45	54.58	41.41	39.97
Acetone extracts										
KDA1	316.3 ± 7.838	299.3 ± 0.333	263 ± 9.451	267 ± 5.773	216.6 ± 3.929	388 ± 5.686	310.6 ± 9.386	340.3 ± 8.373	327.6 ± 10.39	231.3 ± 7.535
KDA2	405 ± 5.131	392 ± 3.214	361.6 ± 6.009	357 ± 6.429	314.3 ± 7.859	441 ± 8.504	458.3 ± 9.683	424.6 ± 10.49	385.3 ± 8.412	363 ± 9.451
KDA3	368.3 ± 8.819	330.3 ± 0.881	332.3 ± 14.31	337.6 ± 6.960	231.6 ± 4.409	425 ± 8.660	406 ± 4.358	393.3 ± 4.702	393.6 ± 5.696	371.3 ± 3.382
KDA4	335.3 ± 6.064	319.3 ± 11.05	289.6 ± 11.55	321 ± 5.507	295.6 ± 4.371	421.6 ± 8.171	318.3 ± 4.409	329.3 ± 10.83	405 ± 3.785	338.3 ± 9.279
KDA5	436.3 ± 5.238	356 ± 14.57	368.6 ± 3.282	381.6 ± 4.176	328.3 ± 8.333	516.3 ± 4.096	415 ± 6.244	446.6 ± 6.009	456 ± 12.42	426.3 ± 0.881
KDA6	438.3 ± 8.819	400 ± 1.154	347.6 ± 8.685	370.6 ± 2.603	248.6 ± 13.29	512.3 ± 7.838	483 ± 6.506	383.3 ± 8.41	409 ± 5.507	355 ± 5.0
F ratio (5, 12)	51.97*	28.33*	19.38*	58.47*	35.65*	50.39*	100.2*	29.62*	25.26*	92.0*
HSD	32.28	34.29	43.17	24.54	35.03	33.70	31.97	38.03	37.29	30.22

ISSN:0971-1260
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Data shown are Mean ± S.E. of three replicates
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HSD: Honestly significant value as calculated from one way ANOVA between parameter and seasons