Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
e-ISSN: 2395-3454

Vol. 18 | No. 2 | pp. 369-378 | 2019

Original Research Paper | Open Access

Biomonitoring Effluents from Perungudi MSW/STP Facility and its Impact on Surface Waters of Pallikaranai Wetland, Chennai, Tamil Nadu, India

Juniet Maria Jose*†, Akhilesh Vats, Sharmila Rajendran** and M.C. John Milton***

*Environmental Monitoring Laboratory, PG and Research Department of Advanced Zoology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India

**Centre for Toxicology and Development Research, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India

†Corresponding author: Juniet Maria Jose

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 25-11-2018
Accepted: 04-02-2019

Key Words:
Pallikaranai wetland
Biomonitoring
Aquatic pollution
Genotoxicity

ABSTRACT

The presence of a major MSW landfill and sewage treatment plant operated in the ecologically sensitive areas of Pallikaranai marshland poses a great threat to the biodiversity of the wetland. The mutagenic potential of Perungudi MSW leachate and STP effluents on the receiving waters into which they are discharged was monitored using bacterial reverse mutation assay. *Oreochromis mossambicus* inhabiting the surface waters was also sampled and subjected to single cell gel electrophoresis. At the vicinity of the discharge point (i.e. from Perungudi dumping ground and sewage treatment facility) the ecology of the receiving waters showed marked increase in the mean revertant colony counts in both the tested strains, TA98 and TA100, with and without metabolic activation system. On the basis of the study results obtained, Pallikaranai surface waters pose ecological hazards, and the olive tail moments of *O. mossambicus* have further validated the presence of genotoxic compounds.

INTRODUCTION

Landfill leachate and sewage may be characterized as a water-based solution of four groups of pollutants - dissolved organic matter, inorganic macro components, heavy metals, and xenobiotic organic compounds (Christensen et al. 1994). Surface waters around the world are contaminated with direct and indirect acting frame shift and base substitution mutagens (Ohe et al. 2004). Matured refuse in the landfill over the years has improved organic matter water holding, soil buffering, and cation exchange capacity; the calorific value for the waste comes from materials such as plastics, aromatics, chlorinated aliphatics, phenols, organic compounds, pesticides, and metals (Zhang et al. 2016, Ghosh et al. 2015). Chlorinated plastics such as polyvinyl chloride (PVC) when burned/combusted to recover copper wires give rise to carcinogenic chemicals like persistent organic pollutants such as dioxins and furans (Wong et al. 2007). Perungudi STP not only receives 70% of sewage to be treated from domestic households, but also receives a mixture of toxic effluents coming from industries such as electrical equipment assembly factories; metal smelting units; paper, printing, publishing and allied product industries; textile mills; stone, concrete, glass, lumber and construction products; photographic; medical; food industry; tanneries; chemical sewage from educational and research institutes; variegated manufacturing units operating in and around the vicinity (White & Ramussen 1998). Researchers, Rappaport et al. (1979) and Meier et al. (1985), reported that chemical contaminants are not effectively removed by the conventional wastewater purification process, which is currently being followed at the Perungudi STP, inadvertently leading to increase in toxicity of treated waters.

Bacterial reverse mutation assay: Thus, taking the complex character of the cocktail of pollutants at the vicinity of the discharge point, mutagenicity assessment in surface water by bacterial reverse mutation assay in *Salmonella typhimurium* TA 98, TA 100 strains with and without metabolic activation system was used to demonstrate whether the environmental mixtures contain any unidentified and unregulated toxicants, that may have potential cytotoxicity (Masood & Malik 2013, Tejs 2008).

Bacterial reverse mutation assay is used as a sensitive indirect indicator of DNA damage that is capable of detecting a wide range of chemical substances leading to gene mutations (Hamel et al. 2016). It recognizes pertinent point mutations (base-pair and frameshift mutagens) that inactivate a gene involved in the biochemical synthesis of an essential amino acid, which constitutes the cause of many gene associated diseases and assumes an essential part in tumour commencement and advancement (Mortelmans & Zeiger 2000).

The assay involves the usage of *S. typhimurium* that already carries defined mutations in the histidine operon

(TA100-hisG46 mutation, DgalchID bio *uvr*Brfa genotype, base pair substitution mutation at G-C site; TA 98-hisD3052 mutation, rfaDgalchID bio *uvr*B genotype, and frame shift mutation at G-C site). When treated with a range of concentration of test chemicals, a second mutation can be induced that directly reverses or suppresses the original mutation. *S. typhimurium* has increased sensitivity and permeability to carcinogen molecules (e.g. benzopyrene does not penetrate the normal bacterial cell wall) due to an induced *rfa* mutation that results in a partial defective lipopolysaccharide cell wall (Maron & Ames 1983, Baskaran et al. 2018). When auxotrophic bacterial strains are grown on agar plates containing amino acid histidine, only those bacteria that revert to amino acid independence (His^+) will grow to form visible colonies. Bacteria do not have a compatible metabolic system (cytochrome P450) to metabolize chemicals like vertebrates, hence, exogenous mammalian microsomal enzyme (S9 liver fraction) metabolic activation system is required to detect carcinogenic chemicals (James & Elizabeth 2012). The number of spontaneously induced revertant colonies per plate is relatively constant, but when a mutagen is introduced into the plate, the number of revertant colonies per plate is increased reflecting the potency of the tester compound (Chen & White 2004).

Comet assay: In aquatic ecosystems, cell components such as DNA and nuclear proteins have been found to interact with xenobiotic compounds forming adducts with toxic molecules inducing conformational changes and damages to DNA due to their toxicity by causing base oxidation induced ROS forming single and double-strand breakages leading to changes in DNA repair mechanism and cross-linking (Moller 2006, Lee & Steinert 2003, Fatima et al. 2014). In the final stage, cells show altered function leading to cell proliferation and cancer (Frenzilli et al. 2009). Many studies (Tice et al. 2000, Fahim et al. 2017) report that Comet assay is more reliable when compared to sister chromatid exchanges or micronucleus test due to its high sensitivity (Singh et al. 1988) for detecting low levels of DNA damage (0.1 DNA break/ 10^9Da) in any eukaryotic population and also due to the requirement of small numbers of cells per sample (Cotelle et al. 1999). Reviews (Fatima et al. 2014, Fairbairn et al. 1995, Russo et al. 2004) show individual indicator field collected organisms' sensitivity being tested by many workers to various genotoxicants using SCGE (Kosmehl et al. 2004, Scalon et al. 2010, Steinert et al. 1998, Wirzinger et al. 2007, Hartmann et al. 2003).

In vivo alkaline single cell gel electrophoresis (SCGE), also referred to as comet assay is a quantitative micro electropheretic technique by which multiple classes of DNA damage (single-strand breaks (SSBs), alkali labile sites, DNA-DNA/DNA-protein cross-linking, and SSB associated with incomplete excision repair sites) (Wirzinger et al. 2007) can be detected at the level of the single cell with relative application to any tissue (Hartmann et al. 2003) by measuring the migration of DNA from immobilized nuclear DNA. The resultant image that is obtained with this technique looks like a "comet" with a distinct head consisting of intact DNA, and a tail that contains damaged or broken pieces of DNA when the negatively super coiled DNA migrates in the electric field towards the anode (Collins et al. 2004). The appearance of comets is linearly related to a wide range of DNA break frequency reflecting the extensive DNA damage. The bigger the DNA tail area (%) or the longer the DNA tail length, the more significant the damage (Liao et al. 2009).

MATERIALS AND METHODS

Study Area

Perungudi landfill produces 5,000 metric tonnes of urban solid waste daily. Decomposition of organic matter and the matured compost in the landfill produces a dark melanized alkaline extract oozing from the heaps to produce leachates.

Perungudi sewage treatment plant (STP) operated by CMWSSB, the second largest of the five macrosystems in Chennai treats around 1500 to 2000 MLD of sewage wherein the plant is designed for a treating capacity of 727 MLD, while the remaining sewage is let off into the water bodies (APHA 1998). The landfill and STP are situated near a residential area and located inside the Pallikaranai marshland which is a wetland freshwater body harbouring a variety of migratory avian species. The area is low lying close to the sea level and is poorly drained because of occupying extensive areas of marshlands that are permanently wet and seasonally inundated.

The study area (Fig. 1) is divided into two halves by 200 ft wide Thoraipakkam-Pallavaram radial road. Parts of the marsh in the northern part under the Greater Corporation of Chennai have sampling points 1 and 2, while the southern part of the marsh under the forest department has sampling points 3 and 4. Two of the main channels in the drainage network under the 200 ft Thoraippakam-Pallavaram radial road direct water from the northern part of the marshland (Point 2) to the low-lying protected wetlands (Point 3).

Their distance from Perungudi landfill is as follows:

Point 1 (80°13'37.5"E; 12°57'20"N): Point source pollution site - Perungudi landfill, northern part of the marsh.

Point 2 (80°13'14"E; 12°57'2"N): 457.6 m from the landfill. Site from northern part of the 200-feet road. Point 2 receives excess sewage from the pumping station that cannot be treated by Perungudi STP through sewage outlet directed

Fig. 1: Study area sampling points.

into the Pallikaranai marsh.

Point 3 (80°13'17.9"E; 12°56'51.6"N): 912.86 m from the landfill. Site on the southern part of the 200-feet road, which is under the forest department.

Point 4 (80°12'46.2"E; 12°56'39"N): Marshland area located about 1.73 km away from the contaminated site behind a research institute and opposite to a dental college.

Methodology

Bacterial Reverse Mutation Assay on Leachate and Surface Water Samples

Surface water samples were evaluated to determine its ability to induce reverse mutation at selected histidine loci in two strains of *S. typhimurium* in the absence and presence of exogenous metabolic activation system.

Preparation of test samples: Leachate run offs were collected from different sites at PDG (Point 1) and mixed in equal proportion to get one composite sample. Surface water samples were collected from the fixed sampling points 2, 3 and 4. According to APHA (1998), samples were collected 20 cm below the surface of water in sterile polypropylene 50 mL tubes and transported to the laboratory in ice box. Crude surface water samples without any dilution were filtered using 0.2 µm membrane and tested for their genotoxic potential.

Bacterial reverse mutation assay was conducted as described by OECD 471 (1997) guidelines. All reagents used were of analytical grade supplied by Moltox (Molecular Toxicology Inc) Hi Media, MERCK, Sigma Aldrich and SRL.

Metabolic activation system (S9 Liver Fraction): Freeze dried pre-certified S9 liver fractions of Aroclor 1254 induced Sprague Dawley male rat vials of 1.0 mL were purchased from Moltox. The aliquots were maintained by cryo preserving at -70°C. The aliquot concentrations were thawed at -70°C, and 0.5 mL of 10% v/v S9 mix was used as the working solution for metabolic activation.

***Positive controls*:** Appropriate positive control in both the absence and presence of S9 listed by OECD was used to affirm the sensitivity of the test framework and the metabolic activity of the S9 mix. In the absence of S9, working concentrations 0.1 mL per plate of sodium azide and 2-nitroflurene were prepared from stock solution concentration of 10 mg/10mL DMSO as positive control for TA 100 and TA 98 strains respectively. In the presence of S9, Benzo(a) pyrene (0.1mL per plate prepared from 10 mg/10 mL stock solution) was used as the positive control for both the tester strains.

Tester strains: Lyophilized disc of TA98 (hisD3052, uvrB, rfa, pKM101) and TA 100 (hisG46, uvrB, rfa, pKM101) tester strains were purchased from Moltox. The analyser strains contained within the STDiscs were removed and introduced into 25 mL nutrient broth. The inoculated nutrient broth was allowed to grow at 37°C for 48 h. Cell density of over-

night bacterial culture growth was read using a spectrophotometer at 600 nm using non-inoculated nutrient broth as the control. The viable bacterial cells with a density of approximately 1.5×10^9 cells/mL were used in the plate incorporation assay.

***Salmonella* histidine mutation plate incorporation assay:** Minimal glucose agar plates (basal agar) were prepared by mixing 15 g of Bacto agar, 20 mL of 50 × Vogel Bonner solution (magnesium sulphate-10 g, citric acid monohydrate- 100 g, dipotassium hydrogen phosphate-500 g, sodium ammonium phosphate -75 g were sequentially dissolved in warm water at 45°C adjusted to 1000 mL and autoclaved), 50 mL of 40% glucose, and 0.5 mM of histidine/biotin solution (D-Biotin -30.9 mg, L-Histidine-24 mg for 250 mL of sterilized water) in 930 mL of sterilized water. The homogenous contents were poured from the Erlenmeyer flask onto sterilized standard polystyrene Petri dishes. To 2 mL of molten top agar (0.6 g of bacto agar, 0.5 g of sodium chloride melted in 100 mL of distilled water), 0.1 mL of various test water samples, 0.1 mL bacterial culture and 0.5 mL of S9 mix (metabolic activation required plates) were added in succession, apparently repeating the same for negative (sterilized water/ DMSO solvent) and positive control plates. The contents were mixed and incubated at 37°C for 30 min before pouring and evenly spreaded on top of the basal agar. After solidification of the soft agar the plates were inverted followed by incubation at 37°C for 48-72 h.

The plates were observed and recorded for the presence and absence of the background lawn using a colony counter. The number of colonies (CFUs) in test item, positive and negative controls was compared. The scores for mean revertant colonies per plate with standard deviation are given in Table 1.

Statistical analysis: Experimental data were expressed as mean± S.D. of three replicates. Statistical comparisons between the control and test plates were made by Dunnett's method using $p<0.05$ to determine the significance. Mutagenic index value was calculated by comparing the number of his^+ revertants in the sample to the negative control.

$$\text{Mutagenic index} = \frac{\text{No. of } his^+ \text{ revertants induced in the sample}}{\text{No. of } his^+ \text{ revertants induced in the negative control}}$$

Comet Assay in Oreochromis mossambicus

Indigenous aquatic organisms have been used as sentinels to monitor the potential contamination of surface water ecosystems, since they can metabolize, concentrate and store waterborne pollutants. The genotoxic effects were evaluated at molecular level by the alkaline micro-electrophoretic comet assay standardized by Singh et al. (1988).

Negative controls were liver tissues obtained from organisms inhabiting the less polluted environment. The positive control hydrogen peroxide (H_2O_2) is an established genotoxic substance (Prise et al. 1989) known to induce DNA single strand breaks at low doses.

Preparation of slides for the SCGE/Comet assay: 1% Low Melting Point Agarose (LMPA) (Sigma A9414) (500 mg per 50 mL PBS) for the bottom layer, 0.5% LMPA (250 mg per 50 mL PBS) for the cell containing layer and 1% NMA (HiMedia RM273) (500 mg per 50 mL in Milli Q water) for the third layer were prepared and microwave heated till the agarose was dissolved. The slides were dipped in methanol and burnt over a blue flame to remove the oil and dust.

Processing and preparation of *O. mossambicus* cell nuclei: 500 g of liver tissue (major organ for the metabolism of absorbed compounds) was placed in 1 mL cold HBSS containing 10% DMSO (to prevent oxidant induced DNA damage). The tissue was minced into fine pieces, homogenized, made to settle, removed and mixed with 5-10 µL with 75 µL LMPA.

The slides were dipped sequentially into the hot 1% LMPA, 0.5% LMPA with liver cells, 1% NMA up to one-third of the frosted area and gently removed. The underside of the slide was wiped to remove agarose and laid on a flat tray surface to solidify.

Electrophoresis under pH>13 alkaline conditions and neutralization of microgel slides: The solidified slides were placed in lysing solution (to prevent DNA damage associated with the iron released during lysis from erythrocytes present in the tissue) for 2 hours at 4°C and removed. The slides were placed side by side in a horizontal gel box and the buffer reservoirs were filled with freshly made electrophoretic buffer solution of pH>13 until the solution completely covered the slides. The slides were immersed for 20 minutes for the DNA to unwind and express the alkali-labile damage.

The slides were electrophoresed for 30 minutes by connecting horizontal electrophoretic unit to 24 V and current to -300 mA. Depending on the extent of migration in the control sample, the power was turned off, and the slides were gently lifted and placed on a draining tray. The slides were dropwise coated with neutralization buffer thrice to neutralize the alkali in the gels.

Staining: Slides were stained for 5 minutes with 80 µL 1X ethidium bromide (Sigma E-8751) and then dipped in chilled distilled water to remove excess stain. The coverslips are placed over it and immediately scored.

Measurement of DNA damage: DNA damage was visualized by observing ethidium bromide stained DNA using 40x objective on fluorescent microscope. The qualitative

Table 1: Ames mutagenicity of surface water samples collected from different points at Pallikaranai wetland.

Treatment	Strain	S9	Revertant colonies (Mean ± SD)	Mutagenic Index
NC (DMSO)	TA 98	+	25.50 ± 2.12	NA
Point 1			215.00 ± 24.04	8.3-8.6
Point 2			169.00 ± 14.14	5.9-7.5
Point 3			198.50 ± 12.02	7.7-7.9
Point 4			174.50 ± 19.09	6.0-7.8
PC (2 Nitroflourene)			145.00 ± 7.07	NA
NC (DMSO)		-	26.00 ± 4.24	NA
Point 1			180.50 ± 10.61	6.0-8.1
Point 2			174.00 ± 9.90	5.8-7.8
Point 3			163.00 ± 14.14	6.0-6.7
Point 4			141.00 ± 22.63	4.3-6.8
PC (Benzo(a)pyrene)			128.50 ± 12.02	NA
NC (Water)	TA100	+	68.00 ± 2.83	NA
Point 1			226.50 ± 14.85	3.1-3.6
Point 2			253.00 ± 5.66	3.6-3.9
Point 3			263.50 ± 7.78	3.8-4.0
Point 4			295.50 ± 10.61	4.3-4.4
PC (Sodium azide)			222.00 ± 18.38	NA
NC (Water)		-	62.00 ± 4.24	NA
Point 1			243.00 ± 11.31	3.6
Point 2			274.00 ± 8.49	4.1
Point 3			240.50 ± 14.85	3.3-3.9
Point 4			273.00 ± 2.83	3.9-4.2
PC (Benzo(a)pyrene)			202.50 ± 14.85	NA

'+' - with; '-' - without; DMSO- Dimethyl sulfoxide; NC- Negative control; PC- Positive control; NA - Not applicable

and quantitative extent of DNA damage in the cells were assessed by measuring the length of the DNA migration and the percentage of the migrated DNA (tail moment) in computerized image analysis system using Image J software with open Comet plug-in.

Comet scoring and analysis: Three slides per tissue with 50 cells per slide were scored. DNA damage was calculated according to Olive tail moments (OTM) (μM) by multiplying the percentage of DNA in the tail by the mean distance between the intensity centroids of the head and the tail along the x-axis of the comet (comet tail length) (Olive et al. 1990). Statistical analysis using Dunnett's multiple comparison test was performed on the data. Differences were considered significant at $p< 0.05$.

RESULTS

Experimentation by Mortelmans & Zeiger (2000) and Maron & Ames (1983) states that the revertant standard values of negative and positive control when using TA98 and TA100 (with/without metabolic activation) ranges from 20 to 50 and 75 to 200 respectively. In the present study, mean revertant colony counts of negative controls ranged from 25.50 ± 2.12 in TA98(S9+), 26.00 ± 4.24 in TA98(S9-), 68.00 ± 2.83 in TA100(S9+) and 62.00 ± 4.24 in TA 100(S9-) while positive controls ranged from 145.00 ± 7.07 in TA 98(S9+), 128.50 ± 12.02 in TA 98(S9-), 222.00 ± 18.38 in TA 100(S9+) and 202.50 ± 14.85 in TA 100(S9-). The positive control agents affirmed the sensitivity of tester strains to mutagens TA98 and TA100 with increased colonies. Based on the results obtained in Ames test, i.e. 169-215 induced TA98 revertants with S9, 141-180 induced TA98 revertants without S9, 226-295 induced TA100 revertants with S9, 240- 274 induced TA100 without S9, the water samples from different sites showed a marked increase in the mean revertant colony counts in both the tester strains when compared to negative and positive controls (Fig. 7).

Mean tail length in test tissues of *O. mossambicus* from Pallikaranai ranged between 1.88 ± 0.7 and 2.203 ± 0.71 μM while control specimen from the reference site was of 1.149 ± 0.502 μM, positive control, i.e. 100 μg/mL of H_2O_2 for validating the experiment measured up to 5.16 ± 0.62 μM. Average comet length was shorter in the liver cells of the control specimens than those from Pallikaranai wetland, depicting increased levels of DNA damage in Pallikaranai specimens. Dunnett's multiple comparison between the control and H_2O_2 (positive control); as well as control and test specimens yielded significantly positive results at $p< 0.05$, hence demonstrating significant difference between the liver cells of the test organisms from Pallikaranai waters and the control organisms.

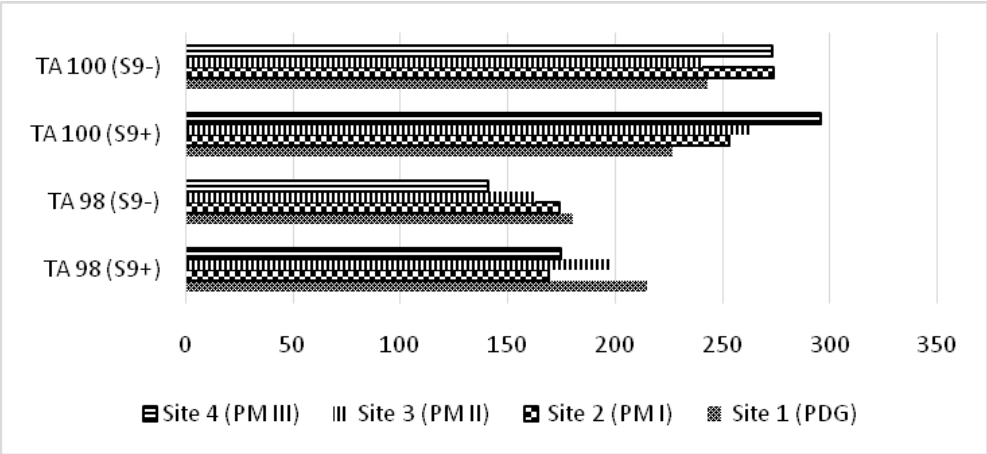


Fig. 2: Mutagenic potencies of surface water samples analysed by the *Salmonella* assay.

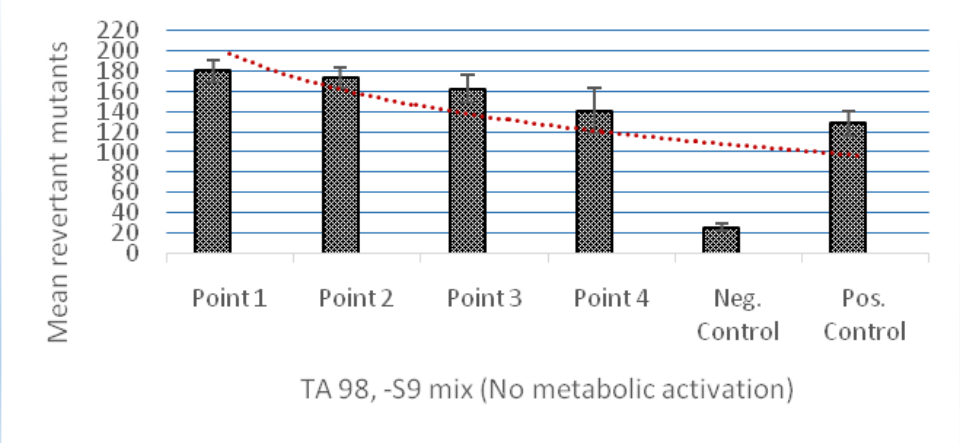


Fig. 3: Mutagenic potency of water samples tested with no metabolic activation system using TA98 strain.

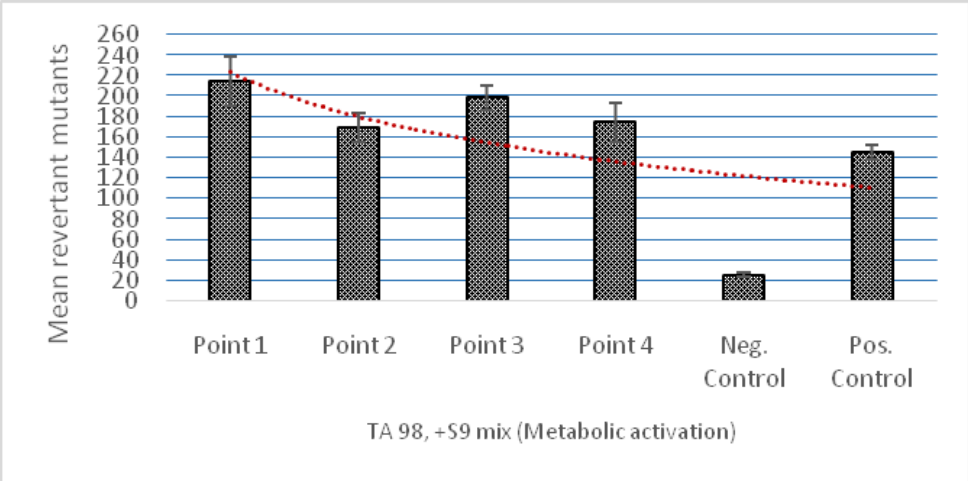


Fig. 4: Mutagenic potency of water samples tested with metabolic activation system using TA98 strain.

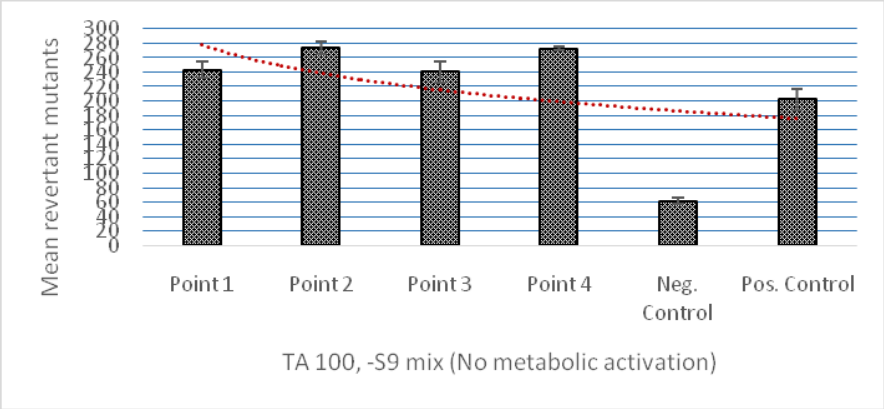


Fig. 5: Mutagenic potency of water samples tested without metabolic activation system using TA100 strain.

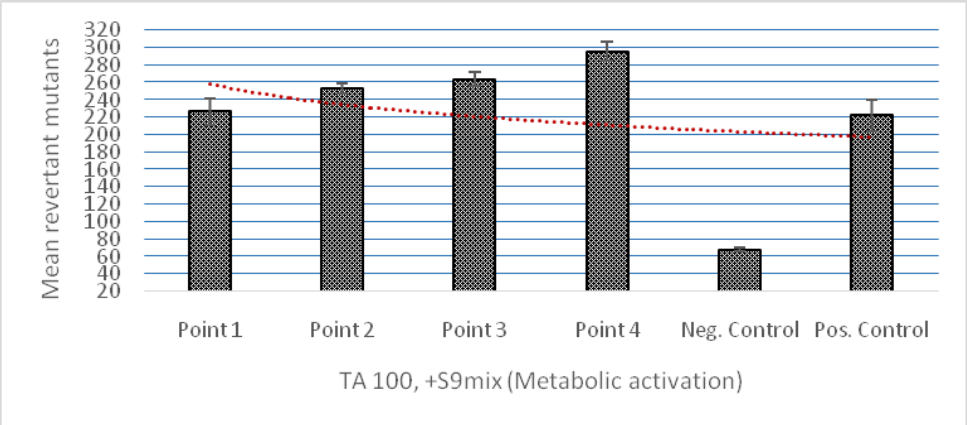


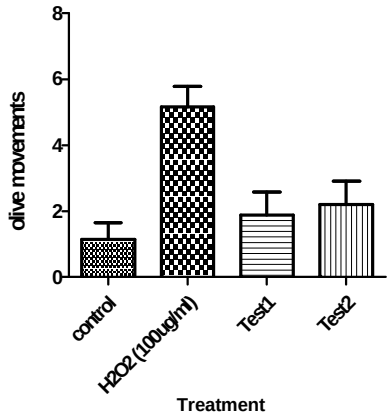
Fig. 6: Mutagenic potency of water samples tested with metabolic activation system using TA100 strain.

DISCUSSION

The water samples were found to be cytotoxic and mutagenic under the tested conditions. At the vicinity of the discharge point (Point 1 from PDG MSW and STP facility) the ecology of the receiving waters showed increased microbial activity in TA98 strains indicating the presence of direct and indirect frame shift mutagens while Point 4 showed the highest microbial activity in TA100 strains indicating that Point 4 has increased the presence of base pair substitution mutations (Fig. 2). The maximum degree of mutagenicity was observed with TA100 tester strains. TA98 was found to be the most responsive strain indicating the dominance of frame shift mutagens.

Mathur et al. (2016) stated that mutagenicity proportion of 2.0 is viewed as a significant indication of mutagenicity. The samples collected from Point 4 showed 4.3-4.4 folds higher mutagenic potential as compared to negative control in TA100 (S9+) strain, while strains of TA98 (S9+) samples collected from Point 1 had higher mutagenic potential of 8.3-8.6 than the control. Mutagenicity level of samples increased when the samples were tested in the presence of TA100 strains with and without metabolic activation. Results of water samples collected from different sites showed mutagenic response of 3.1-8.6 increased response than the control plates. The order of mutagenicity in the test samples (Figs. 3 to 6) for TA98 with metabolic activation was Point 1> Point 3> Point4> Point2; TA98 without metabolic activation Point 1>Point 2>Point 3>Point 4, while TA100 with metabolic activation is in the order Point 4> Point 3>Point 2>Point 1, TA 100 without metabolic activation Point 4>Point 2>Point 3>Point 1. According to the results of Dunnett's method ($p<0.05$), the test revertant colonies showed a significant difference with respect to the negative control confirming genotoxic prevalence.

In the work carried out at Harike wetland (Kaur et al. 2014) water samples collected from Satluj reported maxi-



	Control	H_2O_2 (100µg/mL)	Test 1	Test 2
Mean	1.149	5.16	1.88	2.203
Std. Deviation	0.5023	0.6202	0.6965	0.7143

Fig. 7: DNA damage expressed in olive moments in control and test samples of *O. mossambicus* in µg/mL. The values are the means of three slides per tissue with 50 cells per slide. The bars represent the SE values.

mum mutagenicity with frame shift mutagens than the other 4 sites which is in concordance with the highest heavy metal concentration observed among the 5 sites (Table 2). A similar work carried out by Malik & Ahmad (1995) showed a revertant rate of 27-52 for TA98, 97-210 for TA100 revertants per plate in Aligarh wastewaters (domestic and industrial).

Average comet length was shorter in the liver cells of the control specimens than those from Pallikaranai wetland, depicting increased levels of DNA damage in Pallikaranai specimens. Higher DNA damage in liver tissues found in the present study is in agreement with the findings of Akter et al. (2009) in *A. testudineus* and Ahmed et al. (2011) in *O. mossambicus* due to arsenic/mercury and arsenic toxicity respectively. In a similar work conducted by Bamini et al. (2014) in *O. mossambicus* from Chrompet lake receiving domestic and industrial discharge, the mean tail lengths were observed to range from 8.21 ± 0.87 to 16.54 ± 1.02 µM indicating the results of the current study at lower levels when compared to a severely polluted lake in Chennai.

Hence, the olive tail moments of *O. mossambicus* validate the presence of genotoxic compounds in Pallikaranai, already confirmed in the surface water samples via the Ames assay.

CONCLUSIONS

Genotoxicity tests on water quality and aquatic organisms depict that, regardless of discharge of various reckless dangerous substances into the surface waters, can cause unspecified consequences on different aquatic life forms. The results of the current study show that Pallikaranai water resources pose ecological hazards. These toxic chemicals present in the leachate and sewage channels may act synergistically in Pallikaranai water body causing toxic effects to biological organisms by potentially altering the genotype and phenotype of organisms through binding of xenobiotics to the phosphate, deoxyribose and heterocyclic base residues (Wong 1988) of DNA, thus inducing tumours, malformation, endocrine disruption and immune deficiency (Shen et al. 2001). This should be prevented by a better control of the industrial and communal wastewater before it is allowed to flow into the wetland.

ACKNOWLEDGEMENTS

The study was financially supported by University Grants Commission, New Delhi to the first author, Juniet Maria Jose.

REFERENCES

Ahmed, K., Hossain, M.A., Arif, M., Parvin, E., Salma, M., Shahneawz, M. and Islam, M. 2011. Assessing the genotoxic potentials of

Table 2: Review on mutagenic effect of water samples.

Location	TA 98 (S9+)	TA 98(S9-)	TA 100 (S9+)	TA 100(S9-)	Reference
Point 1 (PDG)	215.00±24.04	180.50±10.61	226.50±14.85	243.00±11.31	Present study
Point 2 (PM I)	169.00±14.14	174.00±9.90	253.00±5.66	274.00±8.49	Present study
Point 3 (PM II)	198.50±12.02	163.00±14.14	263.50±7.78	240.50±14.85	Present study
Point 4 (PM III)	174.50±19.09	141.00±22.63	295.50±10.61	273.00±2.83	Present study
Taihu Lake, surface water, China	NA	NA	151-160	313-351	Wu et al. 2004
Rudnany, waste water,Slovakia	27±2.9	33±2.0	157±11.5	270±23.4	Miadokova et al. 1999
Beas, Harika wetland, India	3093.33	1744.33	1815.66	1058.33	Kaur et al. 2014
Satluj, Harika wetland, India	7944	5271.67	3688.66	1793.33	Kaur et al. 2014
Riyasad, Harika wetland, India	4062	2764.66	1532.66	1054.66	Kaur et al. 2014
Khatan, Harika wetland, India	2064	888.66	1308.66	774.33	Kaur et al. 2014
Sangam, Harika wetland, India	530	380.33	630.66	441	Kaur et al. 2014
Guelma, waste water, Algeria	94.0-4316.33	94.0-3236.66	94.0-3348.33	89.6 - 3250.33	Tabetet al. 2015
Jajmau(Kanpur), Industrial water, India	152-181	143-162	210-253	209 -238	Masood & Malik 2015

arsenic in tilapia (*Oreochromis mossambicus*) using alkaline comet assay and micronucleus test. Chemosphere, 84(1): 143-149.

Akter, M.S., Ahmed, M.K., Akhand, A.A., Ahsan, N., Islam, M.M. and Khan, M.S. 2009. Arsenic and mercury induce death of *Anabas testudineus* (Bloch) involving fragmentation of chromosomal DNA. TerrAquat Environ Toxicol., 3: 42-47.

APHA 1998. Standard Methods for the Examination of Water and Wastewater, 20thedn. American Public Health Association, Washington, DC.

Bamini, B., Ganesan, S. and Kavitha, P. 2014. DNA damage responses to oxidative stress in tilapia (*Oreochromis mo-ssambicus*) exposed to polluted water. Der Pharma Chemica, 6(2): 83-87.

Baskaran, J., Gnanamani, A., Stanley, V.A. and Stanley, S.A. 2018. Genotoxic evaluation of Rasagenthi Mezhugu, a Siddha formulation, using *Salmonella typhimurium* strains. Int. J. of Res. In Biosciences, 7(1): 70-76.

Chen, G. and White, P.A. 2004. The mutagenic hazards of aquatic sediments- A review. Mutation Research, 567: 151-225.

Christensen, T.H., Kjeldsen, P., Hans-Jørgen, A., Albrechtsen, A. Heron, G., Nielsen, P.H., Bjerg, P.L. and Holm, P.E. 1994. Attenuation of landfill leachate pollutants in aquifers. Critical Reviews in Environmental Science and Technology, 24(2): 119-202.

Collins, A.R. 2004. The comet assay for DNA damage and repair. Molecular Biotechnology, 26: 249-261.

Cotelle, S. and Ferard, J.F. 1999. Comet assay in genetic ecotoxicology- A review. Environmental and Molecular Mutagenesis, 34: 246-255.

Fahim, M. and Ahmed, A. 2017. Single cell gel electrophoresis and its applications in different fields. MOJ Bioequivalence & Bioavail ability, 4(2): 256-259.

Fairbairn, D.W., Olive, P.L. and Neill, K.L.O. 1995. The comet assay: A comprehensive review. Mutation Research, 339: 37-59.

Fatima, M., Usmani, N., Mobarak, Hossain, M., Siddiqui, M.F., Zafeer, M.F., Firdaus. F. and Ahmad, S. 2014. Assessment of genotoxic induction and deterioration of fish quality in commercial species due to heavy-metal exposure in an urban reservoir. Archives of Environmental Contamination and Toxicology, 67(2): 203-213.

Frenzilli, G., Nigro, M. and Lyons, B.P. 2009. The Comet assay for the evaluation of genotoxic impact in aquatic environments. Mutat Res., 681: 80-92.

Ghosh, P., Gupta, A. and Thakur, I.S. 2015. Combined chemical and toxicological evaluation of leachate from municipal solid waste landfill sites of Delhi, India. Environmental Science and Pollution Research, 22(12): 9148-9158.

Hamel, A., Roy, M. and Proudlock, R. 2016. The bacterial reverse mutation test. Genetic Toxicology Testing, Elsevier Inc., pp. 79-138.

Hartmann, A., Agurell, E., Beevers, C., Smith, A., Speit, G., Thybaud, V. and Tice, R.R. 2003. Recommendations for conducting the *in vivo* alkaline comet assay. Mutagenesis, 18(1): 45-51.

James, M.P. and Elizabeth, M.P. (eds.). 2012. Genetic toxicology: Principles and methods. Methods in Molecular Biology, 817.

Kaur, J., Chaudhary, A., Kaur, R. and Arora, S. 2014. Assessment of mutagenic, genotoxic and cytotoxic potential of water samples of Harike wetland: A Ramsar site in India using different ex vivo biological systems. Ecotoxicology, 23(6): 967-977.

Kosmehl, T., Krebs, F., Manz, W., Erdinger, L., Braunbeck, T. and Hollert, H. 2004. Comparative genotoxicity testing of Rhine river sediment extracts using the comet assay with permanent fish cell lines (RTG-2 and RTL-W1) and the Ames test. Journal of Soils and Sediments, 4(2): 84-94.

Lee, R.F. and Steinert, S. 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutation Research, 544: 43-64.

Liao, W., Mcnutt, M.A. and Zhu, W. 2009. The comet assay- A sensitive method for detecting DNA damage in individual cells. Methods, 48(1): 46-53.

Malik, A. and Ahmad, M. 1995. Genotoxicity of some wastewaters in India. Environmental Toxicology and Water Quality, 10(4): 287-293.

Maron, D.M. and Ames, B.N. 1983. Revised methods for the *Salmonella* mutagenicity test. Mutation Research/Environmental Mutagenesis, 113(3-4): 173-215.

Masood, F. and Malik, A. 2013. Mutagenicity and genotoxicity assessment of industrial wastewaters. Environmental Science and Pollution Research, 20(10): 7386-7397.

Mathur, N., Kaur, K., Pareek, S. and Nepalia, A. 2016. Genotoxicity hazard assessment of industrial effluent discharge and domestic waste discharge on surface river water. Austin Journal of Environmental Toxicology, 2(2): 1014.

Meier, J.R. and Bishop, D.F. 1985. Evaluation of conventional treatment processes for removal of mutagenic activity from municipal wastewaters. Journal of the Water Pollution Control Federation, 57(10): 999-1005.

Miadoková, E., Dúhová, V., Vlcková, V., Sládková, L., Sucha, V. and Vlcek, D. 1999. Genetic risk assessment of acid waste water containing heavy metals. General Physiology and Biophysics, 18: 92-98.

Moller, P. 2006. The alkaline comet assay- towards validation in biomonitoring of DNA damaging exposures. Pharmacol. Toxicology, 98: 336-345.

Mortelmans, K. and Zeiger, E. 2000. The Ames Salmonella/microsome mutagenicity assay. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis, 455(1-2): 29-60.

OECD 1997. Test No. 471: Bacterial Reverse Mutation Test, OECD Publishing, Paris.

Ohe, T., Watanabe, T. and Wakabayashi, K. 2004. Mutagens in surface waters: A review. Mutation Research-Reviews in Mutation Research, 567: 109-149.

Olive, P.L., Ban, J.P. and Durand, R.E. 1990. DNA damage and repair in tumor heterogeneity in radiation-induced and normal cells measured using the "Comet" assay. Radiation Research, 122: 86-94.

Prise, K.M., Davies, S. and Michael, B.C. 1989. Cell killing and DNA damage in Chinese hamster V79 cells treated with hydrogen peroxide. Int. J. Radiat. Biol., 55: 583-592.

Rappaport, S.S.M., Richard, M.G., Hollstein, M.C. and Talcott, R.E. 1979. Mutagenic activity in organic wastewater concentrates. Environ. Sci. Technol., 13: 957-961.

Russo, C., Rocco, Ã.L., Morescalchi, M.A., Stingo, V. and Universita, S. 2004. Assessment of environmental stress by the micronucleus test and the Comet assay on the genome of teleost populations from two natural environments. Ecotoxicology and Environment Safety, 57: 168-174.

Scalon, M.C.S., Rechenmacher, C., Siebel, A.M., Kayser, M.L., Rodrigues, M.T., Maluf, S.W., Rodrigues, M.A.S. and Silva, L.B. 2010. Evaluation of Sinos river water genotoxicity using the comet assay in fish. Brazilian Journal of Biology, 70(4): 1217-1222.

Shen, J.H., Gutendorf, B., Vahl, H.H., Shen, L. and Westendorf, J. 2001. Toxicological profile of pollutants in surface water from an area in Taihu Lake, Yangtze Delta. Toxicology, 166(1-2): 71-78.

Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res., 175(1): 184-191.

Steinert, S.A., Streib-Montee, R., Leather, J.M. and Chadwick, D.B. 1998. DNA damage in mussels at sites in San Diego Bay. Mutat. Res., 399: 65-85.

Tabet, M., Abda, A., Benouareth, D.E., Liman, R., Konuk, M. Khallef, M. and Taher, A. 2015. Mutagenic and genotoxic effects of Guelma's urban wastewater, Algeria. Environmental Monitoring and Assessment, 187(2): 26.

Tejs, S. 2008. The Ames test: a methodological short review. Environmental Biotechnology, 4(1): 7-14.

Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J. and Sasaki, Y.F. 2000. Single cell gel/comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environmental and Molecular Mutagenesis, 35: 206-221.

White, P.A. and Rasmussen, J.B. 1998. The genotoxic hazards of domestic wastes in surface waters. Mutation Research-Reviews in Mutation Research, 410(3): 223-236.

Wirzinger, G., Weltje, L., Gercken, J. and Sordyl, H. 2007. Genotoxic damage in field-collected three-spined sticklebacks (*Gasterosteusa culeatus* L.): A suitable biomonitoring tool. Mutation Research, 628: 19-30.

Wong, M.H., Wu, S.C., Deng, W.J., Yu, X.Z., Luo, Q., Leung, A.O.W., Wong, C.S.C., Luksemburg, W.J. and Wong, A.S. 2007. Export of toxic chemicals- A review of the case of uncontrolled electronic-waste recycling. Environmental Pollution, 149(2): 131-140.

Wong, P.K. 1988. Mutagenicity of heavy metals. Bulletin of Environmental Contamination and Toxicology, 40(4): 597-603.

Wu, J.Y., Shen, L., Gao, G., Lin, G.F., Ke, X. and Shen, J.H. 2004. A season-dependent variation of genotoxicity of surface water samples from Taihu Lake, Yangzte delta. Environ. Monit. Assess., 98: 225-234.

Zhang, H., Yang, B., Zhang, G. and Zhang, X. 2016. Sewage sludge as barrier material for heavy metals in waste landfill. Archives of Environmental Protection, 42(2): 52-58.