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"A study on mutagenic potency of industrial effluents in and around Bhilai city of Chhattisgarh, India"

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ABSTRACT: Mutagens are the chemical or physical agents which are capable of altering the genetic makeup of living cells, thus causing mutations. These mutations result into various diseases including cancers in human and other live stocks and confer antibiotic resistance to the pathogenic microorganisms. Such mutagens also cause cytogenotoxicity in plants and animals. With the increasing pollution problem, people are much more exposed to such mutagens and even encounter them routinely in their day to day life, hence risking their health unknowingly. This study was carried out with the aim of assessing the mutagenic potency of the effluent wastes discharged by five industries located at Bhilai and Raipur cities of Chhattisgarh, India. The effluent samples were subjected to Ames Mutagenicity assay using bacterial tester strains of *Salmonella typhimurium (TA-100)* and *Escherichia coli (JW1254-2)*. The number of revertant colonies increased significantly on addition of effluent samples (in contrast to controls) emphasizing the mutagenic potential of the respective samples which was recorded in the decreasing order as of Shri ShyamJi Chemicals, Ultratech Cement Pvt. Ltd., Bhilai Engineesring Coorporation, Simplex and Bhilai Steel Plant, respectively for *E. coli* JW1254-2, while mixed results were obtained for *S. typhimurium.*
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Key Words: Ame's Test, mutagenic potency, industrial effluewnts.

1. Introduction

Mutagens are the chemical or physical agents which are capable of altering the genetic makeup of living cells, thus causing mutations. Effluents from various industries contain number of chemicals which may be harmful to both mankind and other livestock in many ways, one of which is the mutagenic or carcinogenic nature of these chemical wastes. Most of the compounds which show mutagenic nature are found to induce cancer in living organisms[1,2]. Generally, industries drain their wastes directly into water bodies or into sewer drains which ultimately end up in the water bodies thus polluting the water. People directly or indirectly get exposed to these chemicals and are at risk of getting cancers. Some studies have also reported cytogenotoxic effects of industrial effluents on certain food crops[3]. Thus, it becomes necessary to monitor the quality of the effluents released by the industries and ensure the minimum possible discharge of such chemicals into the environment. The Environment (Protection) Rules, 1986 (Section 3A)[4] provides an insight and guidelines for the acceptable amount of such chemicals, which could be considered tolerable or non toxic for the livestock. Bruce N. Ames and his colleagues published a protocol to assess the mutagenicity of various compounds[5,6] and enlisted as much as 300 compounds[7]. Of these compounds, many were reported as common industrial wastes. Thus, this study was undertaken to assess the mutagenicity of the effluents discharged off by the various industries in and around the Bhilai city of Chhattisgarh state in India, through Ames Mutagenicity Assay[2,5,6].

2. Materials and method

The effluent samples were collected from different areas of Bhilai and Raipur. Four samples were collected from Bhilai Steel Plant(metallurgy), Sector-5,Bhilai, Chhattisgarh; Bhilai Engineering Corporation (metallurgy), Hathkhoj Village, Industrial Area, Bhilai, Chhattisgarh - 490026, Simplex Castings Ltd. (metallurgy), Light Industrial Area, Bhilai, Chhattisgarh - 490011; Shri ShyamJi Chemicals (chemicals), Heavy Industrial Area Hathkhoj, Bhilai, Chhattisgarh - 490024 and one from Raipur Ultratech Cement Ltd. (mineral), Taluka Simga, Hirmi, Raipur, Chhattisgarh – 493195, respectively. All the water samples were collected in BOD bottles with precautions (gloves, mask and goggles) and were marked separately.

The standard Ames test protocol[2] was followed with few modifications. The tester strain cultures were initiated on Glucose minimal media, supplemented with histidine - biotin and tryptophan – biotin, for *Salmonella typhimurium* TA-100 and *Escherichia coli* JW1254-2, respectively. Table 1 gives the detailed account of culture initiation from lyophilized bacteria. Mutagenicity of the samples was assessed by growing

the tester strains in presence of the test samples (crude and 10^{-5}dilution) on glucose minimal medium supplemented with 0.5mM histidine and biotin respectively, with and without metabolic activator (S9).

3. Results

Table 1, shows the various compositions of GM agar medium for establishment of tester strain cultures. The best growth was observed in GM supplemented with 0.5mM histidine and 0.5mM biotin for *S.typhimurium* TA-100 and 0.5mM tryptophan for *E. coli* JW1254-2.The five industrial samples under study were subjected to Ames Mutagenicity assay in the presence and absence of S9 rat liver extract. The number of revertant colonies obtained by spontaneous mutations was low and remained within 0-7 in every control plate without S9 and between 0-53 on addition of S9 extract. Tests were performed using the master sample and its one dilution (10^{-5} times) and the results were recorded as represented in graph 1 and 2. The number of revertant colonies increased significantly on addition of effluent samples (in contrast to controls) emphasizing the mutagenic potential of the respective samples which was recorded in the decreasing order as of Shri ShyamJi Chemicals, Ultratech Cement Pvt. Ltd., Bhilai Engineesring Coorporation, Simplex and Bhilai Steel Plant, respectively for *E. coli* JW1254-2, while mixed results were obtained for *S. typhimurium.* The number of revertant colonies dropped significantly for Bhilai Engineering Corporation and Bhilai Steel Plant in Salmonella after addition of S9, however an increase in revertant colonies was observed for Simplex Castings Ltd.

4. Discussion

Ames mutagenicity assay given by Bruce N. Ames (1973) was designed specifically to detect mutagens using the enteric bacteria[5,6] like *Salmonella typhimurium* and Escherichia coli. The strains used in the Ames test are highly sensitive to mutagens and are developed by imparting point mutations in the plasmids carrying special genes for metabolism of known bio-active compounds. Such characters are induced by genetic engineering.

The *Salmonella typhimurium* TA-100 strain was derived from TA-1535 strain and contains resistance transfer plasmid "R factor pKM 101. This plasmid confers the bacteria, resistance against Tetracycline and Ampicillin, hence designated as "TA" while the number 100 depicts the serial number of the strain developed in lieu to obtain the desired characters. The plasmid also increased the sensitivity against certain mutagens possibly through error prone repair and makes the bacteria susceptible to point mutations[8].

Similarly, *Escherichia coli* JW1254-*2* strain is derived from Keio collection of the Coli Genetic Stock Centre (CGSC), USA, which is a derivative of K-12 strain of *E. coli*[9]. This strain consists of an F-chromosome marker with mutations at lac-Z gene promoter region and it also makes the bacteria knockout for tryptophan production. The plasmid also imparts resistance against Kanaymycin, which acts as a selectable marker[10].

In the present study, effluents from five large and small industries were assessed for mutagens using Ames Test.

Bhilai is surrounded by a number of metallurgy industries, principal among which are Bhilai Steel Plant (BSP) and Bhilai Engineering Corporation (BEC). Also, these two industries are the biggest sources of industrial effluents in and around Bhilai city. In this study, it was observed that effluents from all the industries were potentially mutagenic. The study thus highlights the fact that the not only the metallurgy but also the other industries are the primary cause of mutagenic contaminants in water. According to the report of Fraser River Estuary Study of Water Quality, Canada, the effects of municipal pollutants in Fraser River downstream from Kanaka Creek to Roberts Band and Sturgeon Bank, had acute toxicity for aquatic life, however, accumulations of heavy metals and other toxicants in benthic region was having detrimental effects upon the benthic fauna. It was estimated that by 2020 the levels would reach to unsafe and lethal levels for fishes in the region[11].

The effluents, when drained into water bodies do not show their effects instantly, as they get diluted. Also the solubility of different chemicals varies with the water quality. However, continuous discharge increases both the concentration and solubility of these chemicals, which ultimately causes genotoxicity and bioaccumulation of heavy metals[12] and xenobiotic compounds.

Industrial effluents not only cause water pollution but also result in soil contamination when drained on open lands. Serious concerns had been reported for heavy metal contamination of agricultural land in Dhaka[13]. The contaminants include heavy metals and persistent organic compounds including dyes[14] and petrochemicals, both of the classes are highly toxic and carcinogenic. Mutagenic and genotoxic effects had been reported not only in micro flora but also in many cultivated plants. Samuel et.al, (2010)[3] and Abu Ngozi E. (2012)[15] reported that heavy metals and compounds like benzene derivatives[16] cause clastogenic and aneugenic effects, including mitosis and cytokinesis disturbances in *Allium cepa*. Such genotoxicity poses threat not only to the flora but for the fauna as well.

Mutagenesis is the base of carcinogenesis as most of the cancers arise either due to mutagenic activation or deactivation of certain genes which are responsible for controlling the cell cycles. All carcinogens are mutagens[1] and thus, the present study provides a base

to evaluate and assess the upcoming effects of these industrial effluents upon environment and on the public health as well.

Conclusion:

The present research reveals that the industries in Bhilai region have been discharging potential mutagens along with their effluents. Most of the mutagens are being discharged by the chemical and mineral industries; still the potential of the metallurgy industries could not be diluted. Since they had been draining these mutagens since a long period, their accumulation in the environment is likely. Still, none of the industries could be blamed completely for the increasing incidents of cancer in Bhilai city, as this requires further investigation. At last, this study provides a base to evaluate and assess the upcoming effects of these industrial effluents upon environment and on the public health as well.

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Table 1: Establishment of Cultures

S.no.	Bacteria	Culture media	Histidine (0.5mM)	Biotine (0.5mM)	Tryptophan (0.5mM)	Incubation time	Culture status
	TA-100	Nutrient agar	Absent	Absent	Absent	48 hours	Nil
		Nutrient agar + Glucose	Absent	Absent	Absent	48 hours	Nil
		Glucose minimal agar	Absent	Absent	Absent	48 hours	Nil
		Glucose minimal agar	Present	Absent	Absent	48 hours	Nil
		Glucose minimal agar	Present	Present	Absent	48 hours	Growth observed
	***E.coli* JW 1254-2**	Nutrient agar	Absent	Absent	Absent	48 hours	Growth observed
		Glucose minimal agar	Absent	Absent	Absent	48 hours	Nil
		Glucose minimal agar	Absent	Absent	Present	48 hours	Growth observed

Figures:

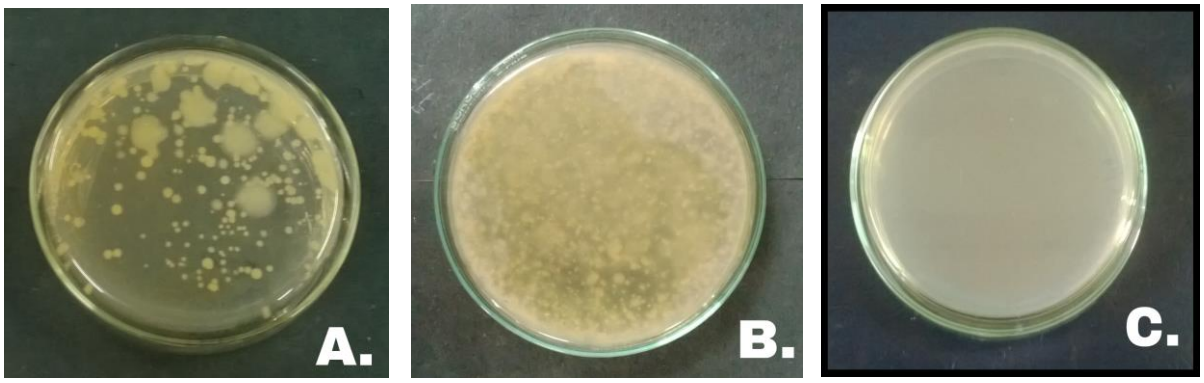


Figure 1: Mutagenicity assay for *Salmonella typhimurium* TA100; (A) Ultratech Cement Ltd. with S-9, 184 colonies; (B) BEC without S-9 286 colonies and (C) control.

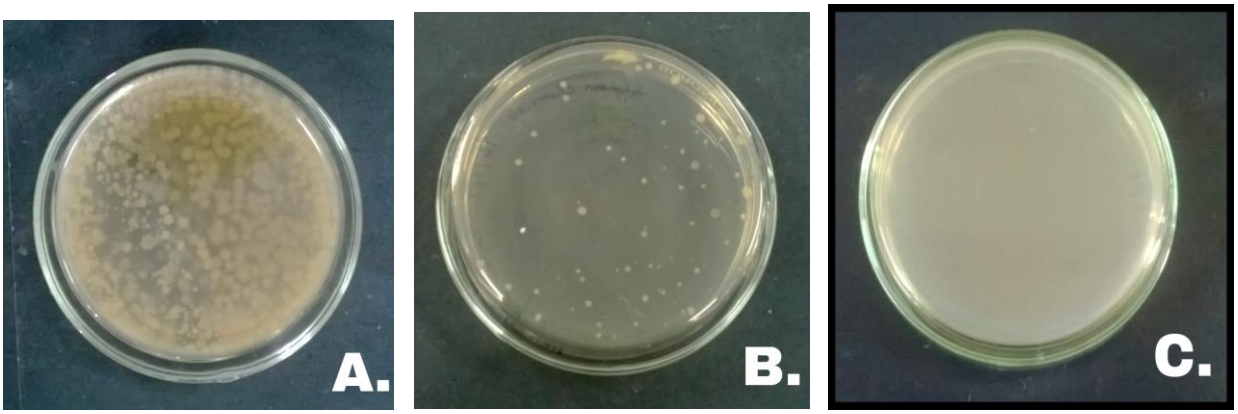
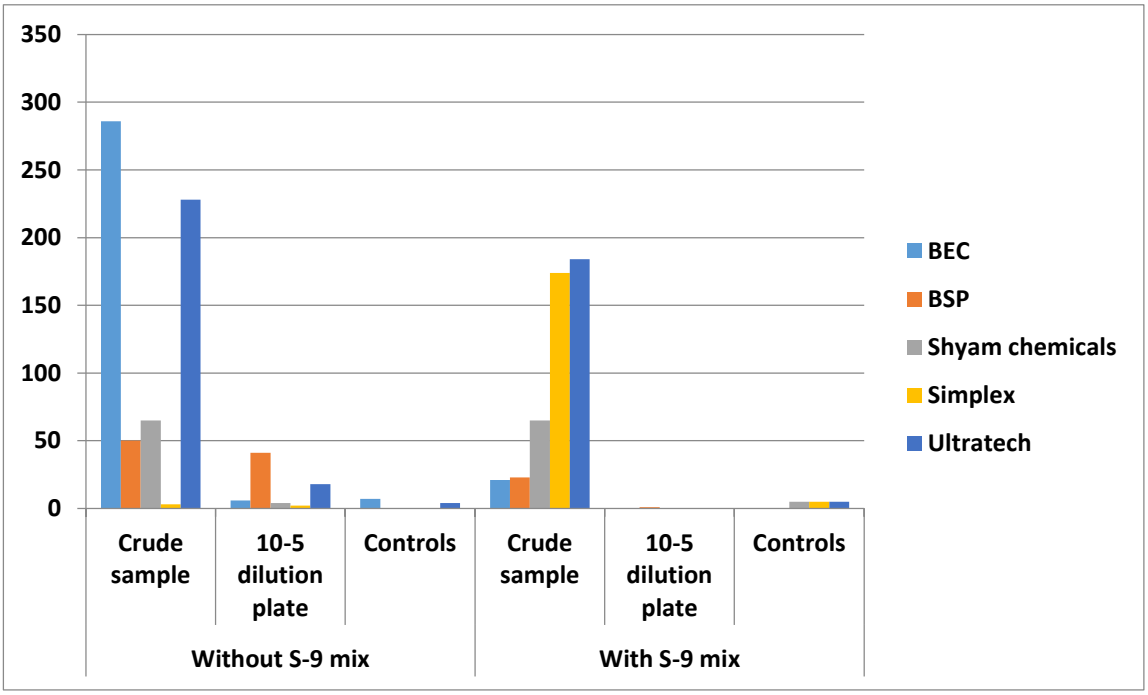
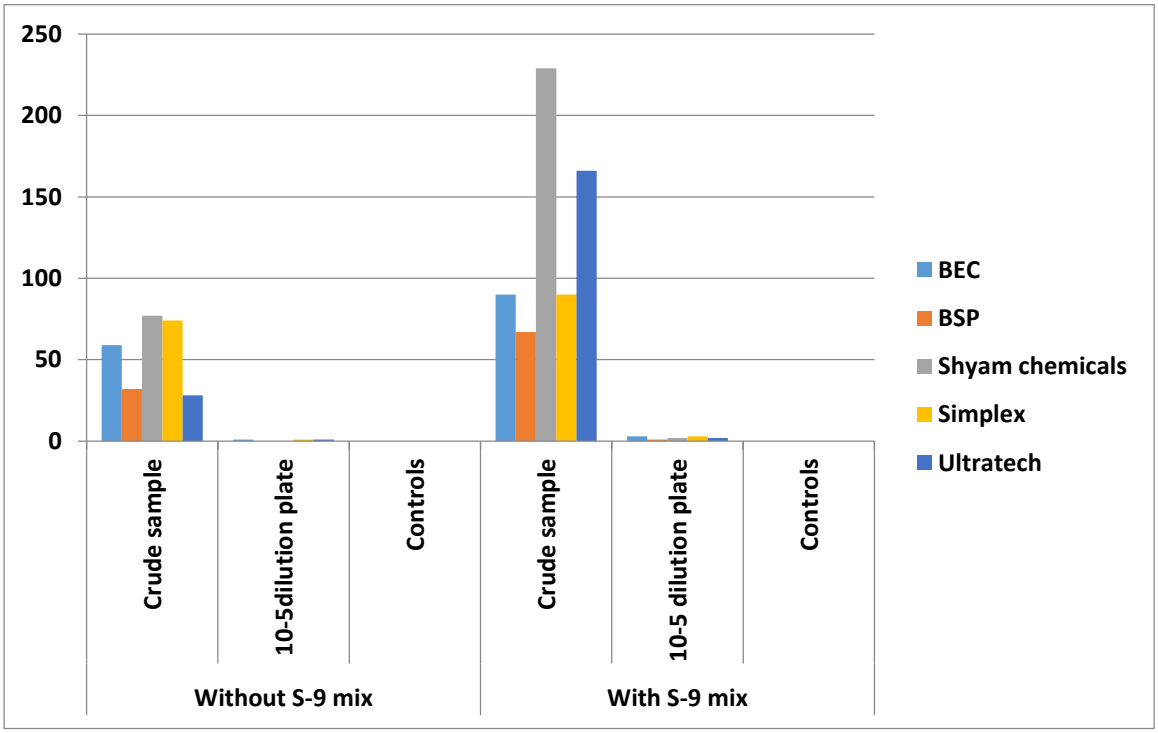


Figure2: Mutagenicity assay for *Escherichia coli* JW1254-2; Shri Shyam Chemicals (A) 229 colonies with S-9; (B) 77 colonies without S-9 and (C) Control.

Graphs:



Graph 1: Revartant colonies for *S. typhimurium* TA-100



Graph 2: Revartant colonies for *E. coli* JW1254-2

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