

ISSN 2320-1355

JOURNAL OF
GLOBAL BIOSCIENCES

Volume 3, Number 7, 2014

Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

**STUDIES ON TOXICITY PREDICTION OF DIFFERENT SOIL SAMPLES BY
MITOTIC ACTIVITY AND CHROMOSOMAL BEHAVIOR IN ROOT
MERISTEMS OF *ALLIUM CEPA* L.**

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Abstract

Pollution of the agricultural area as a result of non degradable solid waste dumping turns to be a global problem for contemporary mankind today hence sustainable agriculture provides protection of the environment and requires a conversion period. Response of the *Allium cepa* root meristem to the presence of potential cytotoxic and genotoxic substances in the environment was used to evaluate the toxicity of soil collected from five different sites of Mercy College campus. Soil samples were collected from solid waste dumping site, open compost site, vermicompost, vermicompost amended with solid waste leachate (10; 1) and normal ground soil. For *insitu* monitoring of the cytotoxicity level, the inhibition of mitotic division in meristematic cells was assayed. For testing the genotoxicity of the collected samples chromosome aberration (CA) assay in mitotic cells and micronucleus assay in (MN) interphase cells were carried out. Mitotic index was normal in ground soil (15.50%) , higher in vermicompost (45.90%) ,minimum in solid waste dumping site (6.32%) and intermediate in vermicompost -solid waste amended soil (34.10%). Chromosome aberration (CA) and micronucleus formation was significantly high in solid waste soil, less in open compost and significantly nil in normal and vermicompost soil; intermediate in solid waste-amended vermicompost. The result indicates that soil of the solid waste dumping site and open compost site accumulates different toxic chemicals which are manifested through decreased mitotic index and significant increase of chromosome aberrations. Solid waste amended with vermicompost helped to decrease the toxicity indicated by increased mitotic index from 6.32% to 34.10% which is higher than control (15.50%).The present study indicates the feasibility of vermicomposting for the cleanup of toxic soil to mitigate the genotoxicity. Vermicompost application in the soil helps to replenish the toxic material and rejuvenate the soil, a boost for sustainable agriculture.

Key words: Mitotic index, chromosome aberration, genotoxicity, micronucleus, *Allium cepa*.

INTRODUCTION

The increasing discharge of hazardous chemicals into the environment has affected the balance of natural ecosystems and has consequently called the attention of several researchers and government agencies to the health of living organisms [1]. It is well established that pollution lowers the quality of life in various aspects. Besides the direct health effects, the subtle danger of pollutants lies in the fact that they may be mutagenic or toxic and lead to several human afflictions like cancer, cardiovascular diseases and premature ageing [2]. Agricultural soil is often contaminated with genotoxic chemicals [3]; [4]. The soil has been traditionally the site for disposal of genotoxic chemicals which need to be treated. Unlike organic compounds, metals cannot be degraded [5] and their cleanup requires conventional remediation techniques [6]. Soil conditions can be defined by physical, chemical and biological methods depending on the functional aspects of the investigation. Bioassays provide a means of assessing the toxicity of complex mixtures like soil, without prior knowledge about their chemical composition [7]. Accumulation of solid waste in the environment causes environmental pollution, toxic for all living organisms [8]. When some toxic chemicals accumulated within the food chain to a toxic level, these chemicals affect directly the public health [9].

Cytogenetic tests in plants are relatively inexpensive and can easily be handled. Due to their size of their chromosomes, higher plants are suitable to cytological analysis and they have shown good correlation with other bio-testing systems [10] plant roots are extremely useful in biological testing. The root tips are often the first to be exposed to chemicals dispersed naturally in soil or in water. *Allium cepa* also enables the evaluation of different endpoints [11]. The objective of this study was to perform a comparative evaluation of the genotoxicity of five different soil types from Mercy College Campus using *Allium cepa* chromosome aberration (CA), micronucleus assays (MN) and calculation of mitotic index to prove best soil type for sustainable agriculture.

MATERIALS AND METHODS

Onion (*Allium cepa* (2n=16)) were used as test organism. Ten clean and healthy bulbs of *Allium cepa* were chosen for each treatment groups. Soil samples were collected from 5 different sites of Mercy College campus- solid waste dumping site (SWL), open compost site (OC), vermicompost (VC), vermicompost amended solid waste (SWL+VC) (10:1) and ground soil (GS) as control. Before starting the experiment dry scales of bulbs were removed and allowed to germinate in ground soil for first 24 hrs at 25 °C with regular light cycle. The healthy bulbs were selected and transferred to experimental plots. Ten bulbs with well developed roots were chosen from each experimental plot. Seed germination percentage, shoot length and root length were observed in different treatment groups. For mitotic studies, root tips of *Allium cepa* were fixed in farmers fluid, and hydrolysed in Acetic acid: HCl solution (45% Acetic acid: 1 M HCl) for 10 minutes and heated for 5 min. at 50°C. Root tip squashes were prepared in 2% acetocarmine solution. At least 1000 cells of each meristem were analysed. Different phases of mitosis were counted and chromosomal abnormalities were observed to calculate mitotic index, phase indices and total abnormality percentage at different phases. The mitotic index was calculated as ratio between the cells in mitosis and the total number of analysed cells in percents. The microscopic analysis includes mitotic index, micronuclei presence in interphase cells and chromosomal aberrations in different stages. The index of each phase of mitotic division was calculated as a ratio between the cell number in the respective period and the number of dividing cells in percents. The frequency of aberrant cells was calculated as percentage of the total number of analysed cells. The chromosome aberrations were characterized and classified in the following categories: bridges, fragments, laggards, micronuclei and disturbed metaphase etc.

The results were expressed as the Mean \pm SE and statistical comparisons were done by using student's t-test with $P < 0.05$ indicating significance.

RESULTS

Seed germination was hundred percentages in vermicompost site where as less in open compost and solid waste leachate in comparison with the control [Fig.4]. Germination potential enhanced in vermicompost amended solid waste sample. Morphological studies of *Allium cepa* indicated coiled and wavy roots in the plots of Solid waste leachate and open compost soil but no root abnormality was reported in VS [Fig.2]. Mitotic index was normal in ground soil (15.50%) , significantly higher in vermicompost (45.90%) ,minimum in solid waste dumping site (6.32%) and intermediate in vermicompost -solid waste amended soil (34.10%) [Table.1; Fig.3]. Microscopic observations of squashes of *Allium cepa* root meristem cells showed solid waste leachate induced a number of mitotic abnormalities when compare with control. The most common abnormalities were metaphase bridges, clumbed chromosome, laggards, disturbed anaphase, polypliod and micronucleus. The types and percentage of these abnormalities are given in Table-2 and Fig.1. Chromosome aberration (CA) and micronucleus formation was significantly high in solid waste soil ($90.6 \pm 0.18\%$), less in open compost ($29.28 \pm 2.08\%$) and significantly nil in contol and vermicompost soil; intermediate in solid waste-amended vermicompost ($1.73 \pm 0.05\%$) (Table.1.). The result indicates that soil of the solid waste dumping site and open compost site accumulates different toxic chemicals which are manifested through decreased mitotic index and significant increase of chromosome aberrations. Solid waste amended with vermicompost helped to decrease the toxicity indicated by increased mitotic index from 6.32% to 34.10% which is higher than control (15.50%). It could be concluded that solid waste dumping at the campus is toxic to the plants and animals in the near vicinity. So the better solution for sustainable agriculture is to reduce the toxic potential by vermicomposting or use the vermicompost amended solid waste in the ratio 10:1.

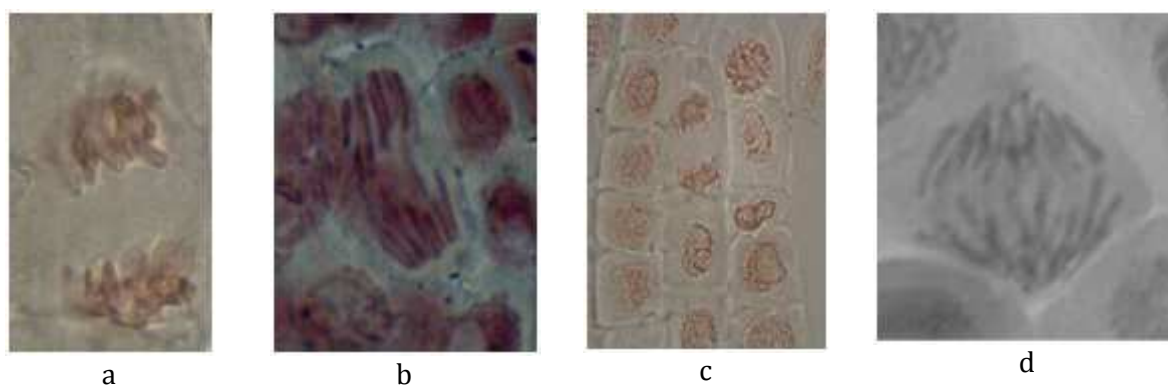


Fig.1- Some mitotic abnormalities observed in the root tips of *Allium cepa* L.

(a) Disturbed anaphase (b) Mitotic bridge (c) Micronucleus (d) Diagonal anaphase



Fig. 2.Morphological difference in root lengths

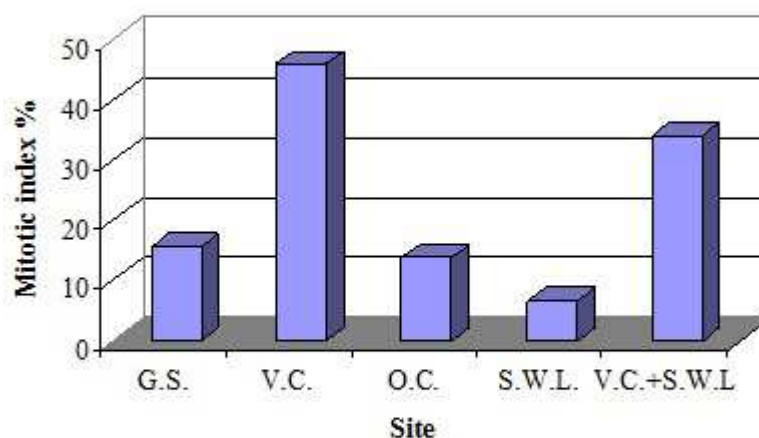


Fig. 3. Comparison of mitotic index between sites



Fig.4. Germination of *A. cepa* in different soil types.

(a) SWL (b) SWL+VC (c) VC (d) GS

Table.1. Mitotic index test in *Allium cepa* root cells grown in five different soil samples at Mercy College campus.

| Site | Total cells | Dividing cells | Mitotic index | Phase Index % | | | |
|------------|-------------|----------------|---------------|---------------|------------|------------|------------|
| | | | | Prophase | Metaphase | Anaphase | Telophase |
| G.S. | 1000±0.8 | 155±0.8 | 15.5±0.29* | 63.22±0.12 | 14.83±0.16 | 7.74±0.08 | 14.19±0.13 |
| V.C. | 1002±1.4 | 460±1.2 | 45.90±0.18* | 48.35±0.18 | 15.18±0.08 | 11.6±0.15 | 24.81±0.06 |
| O.C. | 1449±1.22 | 199±0.95 | 13.73±0.21* | 41.9±0.35 | 21.9±0.18 | 12.69±0.12 | 23.49±0.11 |
| S.W.L. | 1088±0.9 | 71±0.5 | 6.52±0.12* | 35.0±0.26 | 10.0±0.09 | 10.0±0.06 | 45.0±0.28 |
| V.C.+S.W.L | 1000±1.71 | 341±0.82 | 34.1±0.82* | 42.52±0.19 | 28.73±0.17 | 13.48±0.07 | 17.59±0.06 |

*P< 0.05. GS-Ground soil; V.C.-Vermicompost; O.C.-Open compost; SWL-Solid waste leachate

Table-2. Chromosome aberration and micro nuclear assay in *Allium cepa*

| Site | Total cells | Dividing cells | Chromosome aberrations (%) | | | | | | Total Abnormality % |
|--------------|---------------|----------------|----------------------------|--------------------|---------|--------------------|-----------|------|---------------------|
| | | | C. Bridge | Clumbed chromosome | lagards | Disturbed anaphase | polypliod | MNC | |
| G.S. | 1000 ± 0.8 | 155± 0.8 | 0.0 | 1.2 | 0.0 | 0.0 | 0.0 | 0.0 | 1.20 ±0.11* |
| V.C. | 1002 ±1.4 | 460± 1.2 | 0.0 | 0.25 | 0.0 | 0.0 | 0.0 | 0.0 | 0.25±0.03* |
| O.C. | 1449 ±1.22 | 199± 0.95 | 8.31 | 6.24 | 2.06 | 9.20 | 3.01 | 0.47 | 29.28±2.08* |
| S. W. L. | 1088 ±0.9 | 71±0.5 | 6.7 | 25.0 | 7.1 | 45.5 | 3.0 | 4.0 | 90.6±0.18* |
| V.C. +S. W.L | 1000 ±1.71 | 341± 0.82 | 0.0 | 1.25 | 0.15 | 0.08 | 0.0 | 0.25 | 1.73±0.05* |

*P< 0.05GS-Ground soil; V.C.-Vermicompost; O.C.-Open compost; SWL-Solid waste leachate

DISCUSSION

Allium cepa test has often been used for the determination of cytotoxic or genotoxic effects of various substances [1]. It is considered to be a standard procedure for quick testing and detection of toxicity and pollution levels in the environment [12]. The cytotoxicity levels can be determined by the decreased rate of mitotic index. Mitotic index decrease below 22% of the control causes lethal effects on test organisms [13]. Significantly high mitotic index in vermicompost site compared control site was due to the fact that earth worm activity enhances soil fertility. While decrease of mitotic index below 50% usually have sublethal effects [14] and is called cytotoxic limit value [1]. In the present study, solidwaste leachate sample and open compost sample showed a low mitotic index 6.52% and 13.73% respectively. Significantly low mitotic index of solid waste leachate indicate sublethal effect of soil [Table.1]. The vermicompost is a rich source of beneficial microorganisms and nutrients and is used as a soil conditioner [16]. Increased rate of mitotic index in vermicompost amended solid waste was in agreement with the findings of [17]. Earthworm activity enhances soil organic matter, improves nutrition and reduces toxicity [18]. It is obvious from the result of the present investigation that solid waste leachate is cytotoxic on meristematic cells of plant tests (Fig.1 & Table-2). The cytotoxic effect has been evaluated at micro and macroscopic levels. Macroscopically we have observed reduction of root growth in SWL and OC. The cytogenic analysis showed that inhibition of root growth was due to the toxicity of SWL through disturbances of mitotic process and induction of chromosome aberrations and cell death [1]. The inhibition of mitotic index can also be attributed to be the effect of environment chemicals on DNA/protein synthesis of the biological system [19]. It is evident from the results that the mitotic index varied considerably in different treatment groups. The drop in mitotic index is very steep in the solid waste leachate site. It showed the mitodipressive activity of solid waste. Similar results were obtained by [20] in *A. cepa*. The reduction in mitotic activity may result from a blocking of G1 stage suppressing DNA synthesis [21]. SWL was toxic to a remarkable extent but vermicomposting of sludge might be beneficial for bioremediation and recommended before land filling [22]. *A. cepa* has been used to evaluate DNA damages, such as chromosome aberrations and disturbances in the mitotic cycle [23].

Genotoxic activities of the solid waste samples induced micronuclei in the roots of *Allium cepa* indicates the efficiency of *Allium* MN system in detecting clastogenic potential of soil pollution, these observations are in agreement with [24]; [25]. The induction of micronuclei in root meristems of *A. cepa* is the manifestation of chromosome breakage and disturbance of the mitotic process due to spindle abnormalities [26]; [27]. Micronuclei were considered as an indication of a true mutation effect [28], thus, the high percentage of the micronuclei induced in solid waste leachate soil sample indicates the mutagenic effect of them. On the other hand, the percentage of aberrant metaphase as well as anaphase cells for solid waste leachate and open compost soil sample indicates genotoxicity of the soil samples. High genotoxicity of the solid waste samples may be attributed to the accumulation of heavy metals and other mutagenic substances [25]. It was shown that metals could induce clastogenic and aneugenic effect including mitosis and cytokinesis disturbances [29]. The mitotic index and chromosomal abnormalities are used to evaluate genotoxicity and micronucleus analysis used to verify mutagenicity of different chemicals. The clastogenic effects were noticed in the form of chromatin bridge/s, chromatin break/s and ring chromosomes. Ring chromosomes are the result of loss of chromosomes from the telomeric side. Chromatin bridges could happen during the translocation of the unequal chromatid exchange and cause structural chromosome mutation [30].

The present study indicated a decrease of the soil genotoxicity after amending the solid waste leachate with vermicompost. *Allium cepa* test might be used for cytogenetic monitoring of soils without preliminary extraction of the chemicals they contain [31]. The present study indicates the feasibility of vermicomposting for the clean up of toxic soil to mitigate the genotoxicity. Use of vermicompost help to replenish the toxic material and rejuvenate the soil, a boost for sustainable agriculture. Bioremediation of solid waste disposal site with vermicompost in the ratio 10:1 was ideal before starting agriculture.

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