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Assessment of Genotoxic Effects of Soil From Open Landfill in Daet, Camarines Norte, Philippines

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ABSTRACT

Soil degradation caused by improper management of wastes is becoming rampant and is considered one of the major concerns in the environment. Thus, this study primarily aimed to assess the genotoxic effect of polluted soil on *Allium cepa*. The present study involves genotoxicity of soil samples collected from Bibirao open dumpsite, employing chromosomal aberration assay in root tip cells of *Allium cepa* using root dip mode of treatment. The samples were computed via one-way ANOVA and independent samples t-test to determine the difference of mitotic index and chromosomal aberrations among the treatments. Mitotic Index (MI) was higher in control samples at 24 h and 48 h than the rest of the treatment groups. However, the difference of the Mitotic Index in treatment is significantly varied in different concentrations but this was not dose and duration-dependent. The occurrence of chromosomal aberrations was more prominent in control treatments in 24 h (14.7 ± 3.57) and 48 h (9.09 ± 2.45) than in treatment groups. The preparations from root tip cells of treated *Allium cepa* bulbs showed different types of chromosomal aberrations which were apportioned into physiological aberrations (c-mitosis, delayed anaphases, stickiness, laggards, vagrants) and clastogenic aberrations (chromosomal breaks, chromatin bridge and ring chromosomes). The frequency of cells with c-mitosis was found to be maximum followed by laggards which were found in metaphase and anaphase. Moreover, the Chromosomal Aberration (CA) between the treatment groups and the control treatment is not dose and duration-dependent. The greater number of aberrations in the control treatment might be considered to occur during its root development prior to root tip treatment. The study indicates a potential genotoxicity in the soil samples but further studies should be conducted focusing on soil quality assessment.

Keywords: *Allium cepa, chromosomal aberration, Genotoxicity, mitotic index, soil contamination*

1 Introduction

The continuous industrial development is accompanied by the escalation of pollution in the environment. One of these is the elevating concerns on soil pollution which is becoming more pervasive and profound nowadays (Garbuio 2012; Datta et al. 2018). Anthropogenic activities are one of the main reasons for this intensifying pollution, especially in the degradation of soil composition which varies from organic and inorganic pollutants including heavy metals (Poonam et al. 2014; Jones and Rowe 2017). These industrial wastes are often deposited to municipality landfills which

are unregulated and no proper treatment controls are being imposed. Because of limited treatment measures to address possible pollution of the wastes, it often leads to leaking and seepage of pollutants to the soil. Consequently, it highly affects the soil quality and fertility (Ali et al. 2014).

Soil pollutants such as heavy metals, organic wastes, pesticides and other chemicals may significantly alter chromosomes structures which lead to various aberrations like c-metaphases, sticky chromosomes and breaks, bridges, laggards, binucleate and multinucleate cells (Matsumoto et al. 2006; Cherednichenko et al. 2020). These pollutants can directly influence the survival of plants within the vicinity since it posed danger and disturbance on the chromosomal composition of organisms (Ali et al. 2014; Datta et al. 2018)

The province of Camarines Norte encounters an increasing problem with waste disposal. Bibirao municipal landfill in Daet, Camarines Norte is one of the open landfills around the province. This has served as the site for the disposal of wastes of many municipalities. Due to lack of proper management of waste segregation in the area and its accumulation, the area reaches a saturation point which resulted in a closure order of the landfill issued by the Department of Environment and Natural Resources (DENR) last 2018 (Bicol News Paper 2018). These increasing wastes concern has a high potential of affecting neighboring communities particularly soil pollution due to the possible seepage of pollutants to nearby agricultural areas.

Allium cepa bioassay is one of the techniques used to determine genotoxicity of various chemicals. This test is considered efficient and has been used to different genotoxic endpoints like chromosomal aberrations, root growth inhibition and mitotic index alterations in many organisms specially plants (Datta et al. 2018; Wijeyaratne and Wadasinghe 2019). Datta et al. (2018) made use of this bioassay in assessing the genotoxic effect of pesticide and vermicompost treated soil. The same with Cherednichenko et al. (2020), where water extract of soils were analysed using the same method to determine its genotoxic potentials. In addition, Bonciu et al. (2018) evaluated flexible use of *Allium cepa* test on assessing genotoxicity of various chemicals, pollutants in both water and soil.

As a consequence of the rampant soil degradation caused by improper management of wastes, this study aimed to assess the genotoxic effect of dumpsite soil on *Allium cepa*. Specifically, this focused on determining the genotoxic effects of polluted soil in increasing concentrations and the length of exposure to different treatments.

2 Materials and Methods

Sampling site

The soil samples were obtained from the open landfill in Barangay Bibirao, Daet, Camarines Norte. Three sampling sites were identified randomly and were marked as sampling site 1 (S1), sampling site 2 (S2) and sampling site (S3). Sampling sites were located 25 m from the center of the landfill. The three sites were situated five m apart, north of the landfill. The remaining parts of the dumpsite were inaccessible to people due to the physical condition of the landfill and other safety precautions imposed by the landfill management.

Figure 1. Location of three sampling locations in the Open Landfill in Daet, Camarines Norte, Philippines.

Soil Collection

Soil samples were taken from three (3) sampling sites in the Month of March 2021. The samples were collected by digging soil to depth of 15-20 cm. All of the soil samples gathered was pooled and mixed together to make a single sample. Afterwards, two sets of two kg of soil were taken from the mixed soil samples, air dried and sealed in a Ziploc bag and brought to the laboratory for soil and heavy metal analysis, respectively (Waoo et al. 2014; Datta et al. 2018; Afolagboye et al. 2020).

Soil Extract Preparation

The soil extracts were prepared by homogenizing soil in distilled water in ratio of 1:2 (w/v) on a commercial blender for one h and then filtered. The filtrate was immediately tested for genotoxic effects. Various concentrations (20%, 40%, 60%, 80%) of extracts along with control (distilled water) were used for treatment (Afolagboye et al. 2020).

Soil Analyses

Five soil parameters namely pH, electrical conductivity (EC), Nitrogen (N), Phosphorus (P), and Potassium (K) tests were analyzed at the Department of Agriculture Regional Soils Laboratory Region 5. The pH of the soil was measured using potentiometric method and electrical conductivity was determined using EC meter device. The macronutrients N and P were measured using the Walkey-Black technique and the Olsen P method, respectively. Meanwhile, K was detected using the Soil Test Kit (STK).

On the other hand, soil samples for heavy metal (Cd, Pb Cr and Ni) analysis were analyzed using the Atomic Absorption Spectrophotometer (AAS) at the Department of Agriculture Regional Office III. Concentrations of these heavy metals were considered as basis for the genotoxicity test of onion roots (Addis and Abebaw, 2017).

Test organism

The genotoxicity test employed three replicates per treatment of roughly equal-sized, untreated bulbs (25–30 mm) of a commercially available variant of onion (*Allium cepa* L.). Before the experiment, the onions were kept cold and dried in a plastic drying tray. The bulbs' outer scales were carefully discarded just before use, and the brownish bottom plates were scraped away without crushing the root primordia. The experiment was conducted in a laboratory setting that was kept from direct sunlight exposure to ensure a constant rate of cell division. The bulbs were placed in distilled water and kept undisturbed for 5 days when roots of 1-2 cm length have emerged (Datta et al. 2018).

Genotoxicity studies

Onion bulbs were first placed in distilled water-filled 50 mL beakers in the dark for 24 hours. Following that, three bulbs of approximately equivalent root lengths were transferred to each of the test concentrations, with one set placed in distilled water as a control. The roots were exposed to various amounts of soil samples which served as treatments such as treatment 1 (T1) containing 20% soil extract, treatment 2 (T2) with 40%, treatment 3 (T3) having 60 % and treatment 4 (T4) with 80% concentration extract. The root tips were exposed to various concentrations for 24 and 48 h. After the exposure time, roots were obtained separately during the morning hours between 11 A.M. and 12 P.M. Roots were immediately fixed in chilled Carnoy's solution (ethanol: glacial acetic acid=3:1) after collection (Datta et al. 2018).

The root tips were hydrolyzed in 1 N HCl and washed with distilled water and added with a drop of acetocarmine. Finally, the slide was heated quickly and squashed gently and covered with a cover slip. Triplicate slides were prepared for each of the treatments for the first 24 h and another set of triplicates for the next 48 h. The specimens were then viewed using 600x magnification (Datta et al. 2018).

Genotoxic effects were analyzed by determining the mitotic index (MI) and chromosomal aberration (CA). Mitotic index (MI) was calculated on 1000 cells for each concentration and duration of treatment with the following formula (Balog 1982; Datta et al. 2018):

$$MI = \frac{\text{Total number of dividing cells x 100}}{\text{Total number of cells}}$$

Chromosomal aberrations were determined as either physiological aberration such as stickiness, c-mitosis, laggard and vagrant chromosomes or clastogenic aberrations namely formation of chromatin bridges and appearance of chromosomal breaks (Datta et al. 2018). The chromosomal aberration frequency (CA) was also obtained for each concentration and duration of treatment with

the following formula:

$$CA = \frac{\text{Total number of aberrated cells} \times 100}{\text{Total number of dividing cells}}$$

Statistical Analysis

The level of significance in the MI and CA between control and treatment groups were evaluated by One-way ANOVA. The difference between the MI of each concentration after 24 h and 48 h were analyzed by Independent t-test. Statistical analysis was done using IBM SPSS version computer software program. The data was also presented as mean ± S.E. of replicate experiment.

3 Results and Discussion

Soil Properties

To elucidate the possible factors of the genotoxicity of the soil samples, its basic properties, and concentration of selected heavy metals were determined (Table 1). Soil pH is slightly acidic at 5.74 ± 0.48, while its electrical conductivity is 0.626 ± 1.35 mS/cm, which is non-saline. The macronutrients found in the samples were low or deficient except Phosphorus. Concurrently, the concentrations of heavy metals Cd, Cr, Pb, and Ni are still within the limits set by European Union Standards (EU) and the United States Environmental Protection Agency (US EPA) Standards (Table 2).

Mitotic Index

Root tip treatment was employed to estimate the genotoxicity of soil samples. MIs that are considerably lower than the negative control might suggest changes in the growth and development of exposed organisms as a result of potential genotoxicity of the test compounds (Caritá and Marin-Morales 2008; Liman et al. 2011). The MI of the control treatment after 24 h is 13.34 ± 0.77 which is higher than in samples with soil extract (Table 3). MIs that are greater than the negative control, on the other hand, are the consequence of an increase in cell division, which can be damaging to the cells, resulting in disorderly cell proliferation and even tumor tissue development (Lutterbeck et al. 2015).

Chromosomal Aberrations

Different kinds of aberrations were assigned into physiological aberrations and clastogenic aberrations. The physiological aberrations include c-mitosis, delayed anaphase, laggards and vagrants while clastogenic aberrations include chromosomal breaks, and chromatin bridges (Fig. 2). The frequencies of aberrations were induced by the treatments are given in Table 3. The occurrence of chromosomal aberrations was more prominent in control treatments in 24 h (14.7 ± 3.57) and 48 h (9.09 ± 2.45) than in treatment groups (Table 4). The frequency of cells with c-mitosis (Figure 2E) was found to be maximum followed by laggards (Figure 2G) which were found in metaphase and anaphase. Cells with delayed anaphase (Figure 2F), stickiness (Figure 2H), and vagrants (Figure 2I-2J) were also seen. Chromosomal breaks (Figure 2K) and revealed that the frequencies of chromosomal aberrations were only statistically different in 40% (3.06 ± 1.50) and 60% (3.39 ± 1.68) concentrations compared to control treatment in 24 h. Moreover, the CA between the treatment groups and the control treatment is not dose and duration-dependent.

Discussion

A vast range of farming and manufacturing activities have influenced the environmental components in various ways. Soil has acquired tons of contaminants including wastes which

Table 1. Soil Properties of the Sample from Bibirao Open Landfill

	Value (mean ± S.E)	Indication
pH	5.74 ± 0.48	Slightly acidic
EC (mS/cm)	0.626 ± 1.35	Low
N (%)	1.67 ± 0.50	Low
P (ppm)	157.22 ± 2.31	High
K	-	Deficient

Table 2. Heavy metal concentration of soil samples from Bibirao Open Landfill

Metal	Concentration (mg/kg)	Soil Limit (mg/kg)	
		EU Standards*	US EPA**
Cadmium (Cd)	2.27	20–40	85
Lead (Pb)	21.45	750–1,200	420
Chromium (Cr)	11.48	NA	3000
Nickel (Ni)	2.54	300–400	75

*Source: Alloway (2013)
** Source: USDA NRCS (2000)

Table 3. Mitotic index of the root meristem cells of A. *cepa* exposed to various concentrations of soil extracts after 24 h and 48 h

Exposure time (h)	Concentration	% Mitotic index (mean ± S.E.)
24 h	**Control**	**13.34 ± 0.77**
	20%	8.56 ± 0.47[a]
	40%	10.28 ± 0.73
	60%	5.92 ± 1.23[a,b]
	80%	7.56 ± 1.04[a]
48 h	Control	15.62 ± 1.56
	20%	12.44 ± 2.25
	40%	7.33 ± 1.38[a]
	60%	16.32 ± 2.37[b]
	80%	6.68 ± 0.50[a]

[a] One-way ANOVA p≤0.05
[b] T-test p≤0.05

Table 4. Chromosomal aberrations of the root meristem cells of A. *cepa* exposed to various concentrations of soil extracts after 24h and 48 h

		Chromosomal Abberations										
Exposure Time (h)	Treatment	Physiological abberations (PA)							Clastogenic abberations (CA)			Total Abberations (% ± S.E.)
		Cm	Da	Lg	St	Vg	Aa	Am	Br	Bk	Rc	
24 h	**Control**	3	1	5	-	2	1	3	4	1	-	14.7 ± 3.57
	20%	3	-	1	1	-	-	1	-	-	-	7.62 ± 2.45
	40%	-	1	-	1	1	-	-	-	-	-	3.06 ± 1.50*
	60%	-	1	2	-	-	-	-	-	-	-	3.39 ± 1.68*
	80%	-	-	2	-	1	-	3	1	-	-	7.29 ± 2.95
48 h	Control	3	1	2	3	-	1	3	2	-	-	9.09 ± 2.45
	20%	3	1	2	-	1	-	-	-	-	-	5.44 ± 2.34
	40%	3	1	2	1	1	-	1	-	1	-	12.16 ± 2.70
	60%	4	3	2	1	1	-	3	2	-	-	6.94 ± 3.18
	80%	2	-	-	-	-	-	-	1	-	-	2.39 ± 1.56

Cm - C-mitosis; **Da** - Delayed anaphase; **Lg** - Laggards; **St** -Stickiness; **Vg** - Vagrants; **Aa** –abnormal anaphase; **Am** - abnormal metaphase; **Bg** - Chromatin bridges; **Bk** - Chromosomal breaks; **Rc** - Ring chromosomes
* One-way ANOVA p≤0.05

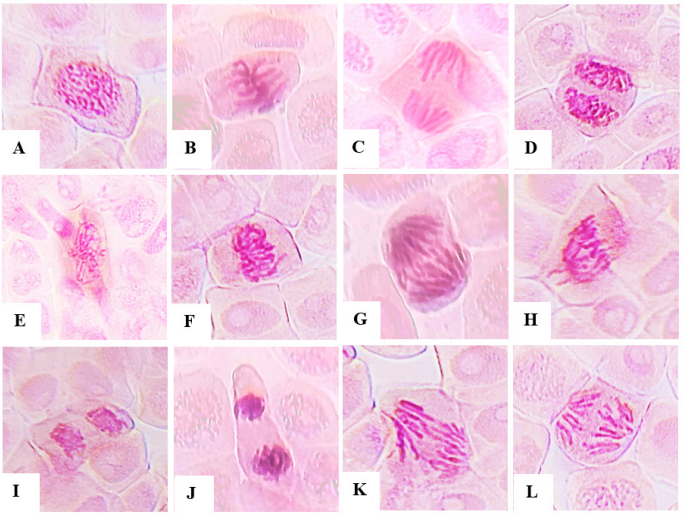

Figure 2. Representative photomicrographs of root tip cells of A. *cepa* showing normal cells (A-D): prophase (A), metaphase (B), anaphase (C), telophase (D); physiological chromosomal aberrations (E-I): c-mitosis (E), delayed anaphase (F), laggard (G), stickiness (H), vagrant chromosome (I-J) and clastogenic aberrations(K-L): chromatin bridge (K), chromosomal breaks (L).

consequently results in alteration of its structure and making it potentially harmful (de Souza Pohren et al. 2013). Several studies implicated that dumpsites produce a significant impact on the environment (Ali et al. 2014; Cortez and Ching 2014; Ojekunle et al. 2015; Agbeshie et al. 2020). The most commonly observed in these studies represent the higher concentrations of micronutrients, exchangeable ions (sodium and potassium), alkaline pH, and heavy metals (Parameswari et al. 2015). The results elucidated a 5.74 pH which is slightly acidic. Low pH by itself was responsible for most of the growth restrictions in the roots However, further chemical analysis will be required if other factors might affect the genotoxicity of substances (Fiskesjö 1993). On the other hand, an elevated concentration of heavy metals in soil may impose potential risks when these were spread through leachates (Alabi et al. 2019; Baderna et al. 2019). The levels of heavy metal composition were below the standard limit, which may explain the low MI and CA of the onion root meristem. Meanwhile, distilled water is used in the study, which is a demineralized water lacking some nutrients like Magnesium (WHO 2005). This defies the possible the relationship of genotoxicity of Magnesium in the study of Liu et al. (1995). To date, it can be noted that the direct effects of the CA to the control setup has not been fully documented as evidenced by the lack of pertinent literatures.

The results of the assay may indicate the presence of other certain cytotoxic, genotoxic, or mutagenic substances other than was mentioned above. The presence of aberrant like c-mitosis, laggards, stickiness, and anaphase bridges especially visible in 48 h may indicate possible contamination of other substances in the soil. Metal ions can disrupt the proper alignment of nucleolar organizing regions on chromosomes during mitosis (Bonciu et al. 2018). In addition, a significant induction of c-metaphases may cause complete inactivation of mitotic spindle fibers. Chromosomal bridges and incomplete separation of chromosomes during anaphase might be a result of cross-links between proteins and chromosomes (Martins et al. 2016). The relationship of acidic pH, and wide concentration of heavy have been positively associated with the genotoxicity studies by Chahal et al. (2014) and Soodan et al., (2014). The study also suggests that the lack of observance in micronucleus in the *A. cepa* cells might be an indicator that the soil samples have high concentrations of organic matter with reference to a study conducted by Kong et al. (1999). However, the results in both MI and CA are not dose-dependent. The genotoxic effects observed were attributable to the complex mixture that may be present in the soil extracts. Therefore, further studies should be conducted focusing on soil quality assessment.

4 Conclusion and Recommendations

The study indicates a potential genotoxicity of the soil samples of Bibirao dumpsite. The results showed that the MI was greater in the control setup in comparison with the treatment groups. The preparations from root tip cells of treated *Allium cepa* bulbs showed different types of chromosomal aberrations which were apportioned into physiological aberrations and clastogenic. The frequency of cells with c-mitosis was found to be maximum followed by laggards which were found in metaphase and anaphase. Moreover, the MI and CA between the treatment groups and the control treatment were not dose and duration-dependent. It is highly suggested to perform more intensive tests especially for the Phosphorus test and with other elements that were not included in the study. The study also suggests conducting experiments to identify the possible biological effects of the interaction between the inorganic and organic compounds presents in this complex mixture. In general, *Allium cepa* genotoxicity studies can indicate a soil contamination to evade potential risks with associated food chains supported by numerous researches that were mentioned in the study.

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Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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