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ARTICLE

Cytogenotoxic Effects of Stormwater on Allium cepa L.: A Future Perspective

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Urbanization has fundamentally altered the way water moves through landscapes. When rainwater cannot soak into the ground, it runs along streets and parking lots and picks up pollutants such as debris, sediments, pesticides, automotive fluids, fertilizers, and herbicides (Ryan, 2019). Furthermore, construction sites, power plants, illegal discharges, and improper sewer connections all contribute substantial amounts of pollution to runoff (Ryan, 2019). This polluted runoff can flow into rivers and streams or overwhelm local infrastructure to cause sewage overflows. This stormwater is then eventually discharged into the ocean without going through any treatment plan. Henceforth, stormwater contributes to environmental problems threatening humans, animals, and the ecological balance (Ryan, 2019).

This pollution can also adversely affect other equally important activities such as commercial and recreational fishing, swimming, and public health. Irrigation of agricultural crops is also affected by toxic chemical compounds found in the stormwater in creeks and rivers (Capital Regional District, 2013). To extend, if these plants are consumed as food, it could lead to serious health implications in humans and in animals feeding on these plants, leading to bioaccumulation. Currently, in Victoria, British Columbia, Canada, there is no fundamental treatment plan for stormwater, thus the water has a direct impact on the aquatic wildlife life living in the waterways containing the stormwater, as well as the surrounding plants and animals that depend on the water for their development (Capital Regional District, 2013).

Many synthetic and natural substances in the aquatic environment should be tested regularly due to the carcinogenicity of the pollutants present in stormwater. However, such tests require long periods of time with expensive equipment and in-depth analysis of the complex stormwater components (Fiskesjo, 1988). Therefore, due to the highly conserved structure of plants' genetic material, the common onion, Allium cepa L., makes a convenient test system for projecting the effects of stormwater contaminants using various genotoxicity parameters (Fiskesjo, 1988). Additionally, onions have a small chromosome number, 16, which is useful for microscopic analysis as the few, yet relatively large, chromosomes allow for the detection of morphological change (Rank & Nelson, 2004). Also, the main cell structures of an onion are easy to see when viewed with a microscope, as one can see the nucleus of each cell easily, and effectively notice any abnormalities present in the cells. Thus, onions may act as efficient models for looking at the effects of stormwater on their cells. Moreover, the macroscopic examination of plant roots is also useful to this experiment, due to the fact that root tips are the first to be exposed to the chemical compounds present in water and they are sensitive to genetic damage (Rank & Nelson, 2004). Finally, onions have a low cost and are fast growing vegetables, allowing one to get results very quickly and cost-effectively.

The objective of this study is to investigate effects of stormwater on the root growth and chromosomal aberrations of Allium cepa L. Data obtained from this study may reveal the possible health implications of stormwater on marine life and assist policy makers on creating a water treatment plan for its discharge into the ocean.

METHODS

Stormwater Collection

The stormwater was collected from Bowker Creek, in Victoria, British Columbia, during mid-February of 2019. That time of year is significant for plant growth, and rainfall continues to contribute to stormwater runoff. The creek that provided the sample is 8 km in length and the stormwater collected was taken near the discharge location into the ocean in the Oak Bay municipality. The stormwater was kept in plastic containers at 4°C prior to the analysis, which occurred 48 hours later.

Onion Preparation

Small white Allium cepa L. bulbs of a uniform diameter (3.5-4 cm) were procured from the local Fairway Market, in Victoria. The onion roots were dry and intact with no decay or other forms of mechanical damage, and the bulbs were free from mushes and other physical abnormalities. After removing the loose outer scales and dry roots carefully with a sharp razor blade to expose the fresh meristematic tissues, the bulbs were immersed in mason jars filled with deionized water (the control) and the various concentrations of the effluent (i.e., stormwater). The stormwater was

diluted with deionized water to 25%, 50%, and 100% concentrations to allow for examination of any root growth trends. To account for a number of bulbs that would be naturally slow or poor growing, three replicate bulbs were used for each concentration of stormwater and for the control (i.e., 12 bulbs in total).

The exposure period of the onion bulbs was 96 hours, and the jars were kept in a warm room with direct artificial light at 22 ± 1°C. Observations were recorded every 24 hours, and the water was filled to the original level as needed, as some water was absorbed by the onion roots. The temperature of the room was also checked every 24 hours in order to maintain an optimal environment for the onions to grow in. At the end of the exposure period, the roots of the onion bulbs with the best growth for each concentration were removed with forceps, and their lengths were measured with a ruler. The macroscopic parameters that were tested include the root length, the number of roots, the root form, and the root colour after the 96-hour cultivation.

Micr*oscopic Evaluatio*n

For the evaluation of chromosomal aberrations, the root tips that were cut were then fixed in Carnoy's fluid (a 3:1 alcohol and acetic acid fixative) for a period of 24 hours and then stored in 70% ethyl alcohol until slide preparation (Grover & Kaur, 1999). Carnoy's fluid acts as a fixative, which is a stabilizing or preservative agent, that preserved the onion roots until microscopic examination. The fixative also softens the roots allowing them to be easily squashed under a microscope slide. In effect, the squash technique described by Kurelec and Al-Sabti (1985) was used for the preparation of chromosomes of the onion root tip cells. For microscopic examination, the root tips were placed into a watch glass, where 9 drops of acetocarmine stain and 1 drop of 1M HCl were added (Al-Sabti & Kurelec, 1985). This was warmed over an alcohol burner for 2-3 minutes, allowing the roots to retain the stain into their cells, specifically into the nuclei. These root tips were then kept at room temperature for 15-20 minutes to further absorb the stain. The stain gives the nucleus of the onion root tip cells a pinkish colour, allowing the cells to be easily visible under the microscope. After carefully removing the root caps of the stained root tips using a sharp blade, 1 mm of the mitotic zone was placed on a slide and squashed under a cover slip, in order to spread the cells evenly, and so that the chromosomes of individual

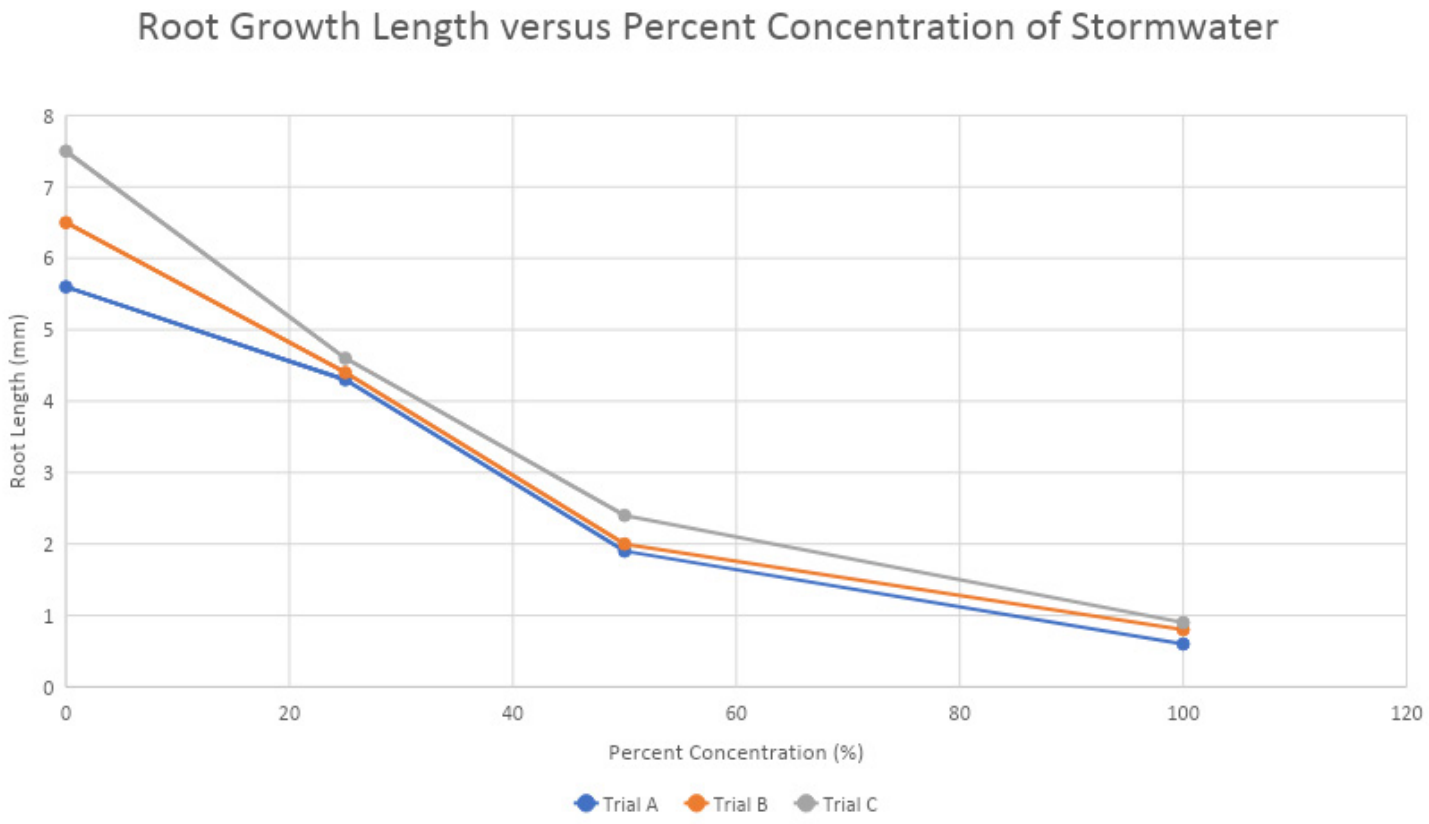

Figure 1: Root Growth Length versus the Percent Concentration of Stormwater. The length of the longest root for each onion (3 replicates per treatment group) was recorded after the 96-hour cultivation period.

cells can be observed (Grover & Kaur, 1999). Microscopically, the roots' tip cells grown in the various concentrations of stormwater were compared with the those from the control group to see any changes present in the cells' nuclei and other important structures.

The mitotic zone, also known as the apical meristem, is the area of the onion where cell division takes place at a rapid rate. The mitotic zone has all the diving root tip cells, thus one should clearly see any effects the roots' environment has on mitosis (a form of asexual cell division) (Kusumaningrum et al., 2012). For instance, if the cells are fixed during prophase (the first stage of mitosis) the effects of the stormwater contaminants on the chromosomal separation may be viewed and recorded. As a result, the slides of each concentration and the control were analyzed at 1000x magnification with an oil immersion lens for the observation of any mitotic abnormalities.

RESULTS

Examination of the root length, illustrated in Figure 1, showed that the concentration of the stormwater was inversely associated with root growth. Root growth and length was highest in the control (Figure 2) with a mean of 6.5 mm and the 25% concentration jars (Figure 3) with a mean of 4.4 mm. On the contrary, root growth in the 50% concentration group (Figure 4) was approximately 30% shorter compared to control, and root growth present in the 100% concentration group (Figure 5) was approximately 80% shorter than the control. The longest root of each onion at each concentration was recorded and plotted on a graph (Figure 1). In the control, the roots were whitish in color, unbroken, and straight, while in the various concentrations the roots were brownish in colour, as seen in Figures 2-5.

Microscopic examination of the Allium cepa L. root tip meristem cells showed that, compared to the control, there appeared to be mitotic abnormalities. These included micronuclei (Figure 6), as fragments of the nuclei were not intact, an effect of chromosomal instability (Olorunfemi et al., 2012). Also, some cells had elongated nuclei (Figure 7). To extend, the number of abnormally shaped cells present in each group were 1-2 cells, 3-4 cells, 5-8 cells, and 10-12 cells in the control, 25%, 50%, and 100% groups, respectively.

DISCUSSION

Stormwater is a source of direct and continuous input of pollutants that slide from streets and parking lots into the runoff. These contaminants have long term implications on the ecosystem functioning and well-being (Wijeyaratne, 2019). The present study provides evidence that stormwater effluent was negatively associated with root growth and may have caused growth retardation in the roots of Allium cepa L. The stormwater also appeared to have an effect on the dividing cells present in the root tips, causing mitotic abnormalities, which may lead to further genomic damage to the surrounding cells. This micronucleus assay is a test used in toxicological screening for potential genotoxic compounds. Micronuclei are usually a sign of genotoxic events and genetic damage (Luzhna

Figure 2: Control Group Onion. There were 10 white roots growing from this bulb of the control group after the 96-hour cultivation period.

Figure 3: Onion from the 25% Stormwater Concentration Group. There were 5 brownish roots growing from this bulb of the 25% treatment group after the 96-hour cultivation period.

Figure 4: Onion from the 50% Stormwater Concentration Group. There were 4 brownish roots growing from this bulb of the 50% treatment group after the 96-hour cultivation period.

Figure 5: Onion from the 100% Stormwater Concentration Group. There were 3 brownish roots growing from this bulb of the 100% treatment group after the 96-hour cultivation period.

et al., 2013). Since onion root tip cells divide using mitosis, the abnormal cell characteristics can be passed on to their daughter cells, having an overall exponential effect on the nearby cells and the root itself (Kusumaningrum, 2012). Overall, the decline in root growth and increase in mitotic abnormalities in Allium cepa L. as the concentration of the stormwater increases reflects cytogenotoxicity.

The results of the Allium test may indicate the presence of certain cytotoxic/genotoxic substances in the environment, which represent direct risks for all living organisms (Olorunfemi et al., 2012). Thereupon, the stormwater entering the ocean through Bowker Creek may be adversely affecting the marine environment. Based on the Capital Regional District (CRD) report from 2019, there are high amounts of heavy metal ions, E. coli bacteria, and nitrates in the stormwater running through Bowker Creek, Victoria, B.C. which can have an immense impact on the aquatic marine life as it continues to make its way to the ocean without treatment (Capital Regional District, 2019). Using stormwater in irrigation of crops may lead to health implications in humans that consume these crops and animals that depend on these crops. In order to prevent this, people need to reduce stormwater pollution, by using less fertilizers and pesticides on lawns, washing their cars on grass instead of driveways, preventing oil drips from cars, picking up animal waste, and not dumping anything into stormwater drains (Wijeyaratne, 2019).

Results of this study have also shown that Allium cepa L. is a useful model for monitoring the genotoxic effects of effluents and stormwater, due to its low cost, accessibility, and the efficiency in obtaining results. Since the roots are sensitive to genetic damage and their roots are the first structures to be exposed to all of the chemicals in the water and soil, onions can act as a fast and reliable water analysis test system for both macroscopic and microscopic parameters. Fiskesjo and Levan (1993) reported that the Allium test has high correlation with other test systems (e.g., medical testing with mice or rats) and proposed that it could be used as an alternative to laboratory animal in some toxicological research (Fiskesjo, & G.L.van, 1993). In places of the world that do not have access to expensive and time-consuming water analysis equipment, the Allium test can be employed for an elementary analysis of water safety. This provides a short-term analysis where the macroscopic results can be seen within just a couple of days.

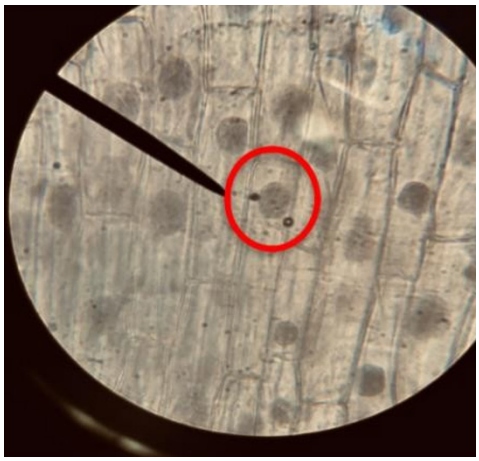

Figure 6: Micronucleus from the 100% Stormwater Treatment Group. The micronucleus, indicated by the red circle, is seen in a meristematic cell of a root from an onion of the 100% treatment group (viewed at 100x magnification).

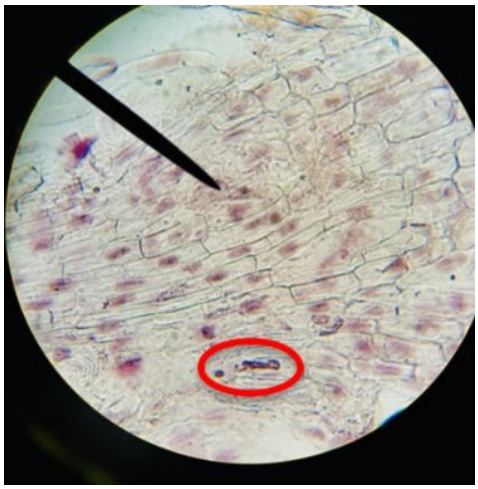

Figure 7: Elongated Nucleus from the 100% Stormwater Treatment Group. The elongated nucleus, indicated by the red circle, is seen in a meristematic cell of a root from an onion of the 100% treatment group (viewed at 100x magnification).

Individuals living in Canada should understand the problems with stormwater and continue to prevent stormwater runoff pollution. The government should also regulate factories and construction sites to reduce the release of chemicals, dyes, electronics, oils, etc. into local waterways which can make its way into the ocean and other large discharge locations. All in all, results from this experiment should be considered by policy makers as onions act as model for efficient effluent analysis, and it is recommended that stormwater should be treated before being released into the ocean so that marine life is protected.

In the future, more trials should be conducted in order to view more chromosomal aberrations and their effects on the root growth. Certain heavy metals present in the stormwater can also be isolated to test their individual effects on root growth and mitotic abnormalities. This may clarify which pollutants precisely affect the stormwater and their potential impacts on marine life.

CONCLUSION

Allium cepa L. makes an effective model test system for examining the effects of stormwater using various genotoxicity parameters. Due to the onion's small chromosome number, large visible nuclei, sensitivity to genetic damage, root exposure to the water, fast growth, and low price makes them an excellent choice for a short-term water analysis test. Macroscopic examination of the root growth showed that as the concentration of the stormwater increased, root growth declined and a noticeable colour change occurred. Microscopic examination of the onions root tip meristem cells showed that the stormwater effluent induced mitotic abnormalities in the 50% and 100% stormwater treatment groups, compared to the control, which showed no mitotic abnormalities. Therefore, the Allium test may be a useful, cost-effective method in places that do not have access to water analysis equipment to test the safety of their water resources for drinking and irrigation. Overall, prevention and management of stormwater pollutant runoff is a significant way to help maintain the safety of humans, marine life, and ecosystems that surround these water pathways.

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My name is Rohan Gupta, and I am a current first year medical student at the University of St Andrews. I have always been interested in the stormwater running through nearby creeks, and how the water can have an effect on the surrounding ecosystems. Furthermore, during a visit to India, I had seen severely contaminated water running through the streets and contributing to heavily polluted creeks. I fell in love with scientific research about stormwater and started taking part in science fairs, winning gold and the top environment project for seniors at the Canadian Wide Science Fair. In my free time, I am a part of the St Andrews Surgical Society and regularly volunteer at local hospitals.