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DNA damage in *Halophila stipulacea* as pollution bioindicator in the gulf of Aqaba/Jordan

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Abstract

The aim of this study is to evaluate genotoxicity in *Halophila stipulacea* (Forsk.) Aschers. found in the Gulf of Aqaba as a pollution bioindicator. We used Comet assay to assess the degree of DNA damage in *Halophila stipulacea* (Forsk.) Aschers. Samples were collected from various regions in the Gulf of Aqaba. The results showed significant differences in DNA damage score, index and frequency among the studied sites (Tala Bay, Old Phosphate Port, Marine Science Station (MSS), Big Bay and the Industrial area). Significantly, the highest DNA damage score and damage index were found in Old Phosphate Port (1.78 and 49.7 respectively). The highest DNA damage frequency was reported in Old Phosphate Port and Industrial area (82.3% and 82.9%, respectively). Samples collected from the industrial area showed Moderate DNA damage score (1.62). Samples collected from Big Bay, MSS, Tala Bay and Industrial area showed similar DNA damage index (39.7, 41.5, 42.3 and 43.7 respectively). The lowest DNA damage score was observed in MSS, Big Bay, and Tala Bay (0.62, 0.72 and 0.73 respectively), and the lowest DNA damage frequency was in MSS (47.9%). The highest MDA content was found in Industrial area (0.277 nmole g $^{-1}$ FM), and the lowest MDA content was in MSS (0.158 nmole g $^{-1}$ FM). In addition, analysis of heavy metals showed that plants collected from Industrial area and Old Phosphate Port had the highest Al, Cr, Fe, Cu, Zn, Cd and Pb contents. Significant positive correlation was found between DNA damage index, DNA damage frequency, lipid peroxidation rate and heavy metal analysis, which indicates that pollution with heavy metals is the main cause of genotoxicity for *Halophila stipulacea* (Forsk.) Aschers in gulf of Aqaba. Our results indicate that Tala Bay, Big Bay and Marine Science Station (MSS) are the least polluted areas in the gulf of Aqaba based on plant comet assay.

Keywords: *Halophila stipulacea*; seagrass; heavy metals; pollution; Aqaba; genotoxicity.

1. Introduction

Aqaba is the sole maritime port in Jordan, located on the Red Sea, 330 kilometers south of Amman. Its strategic location is at the connection of three continents: Asia, Europe and Africa. One of the most diverse aquatic habitats and biodiversity was found in the Gulf of Aqaba, as well as one of the world's greatest coral groups. The Gulf of Aqaba in the Middle East area is one of the most heavily anthropogenically influenced coastlines because the increase in Gulf industrial and commercial activities has led to the degradation of naturally stressed coastal and marine ecosystems. Anthropogenic activities have led to environmental impacts on unique marine communities such as household sewage, industrial waste, ports, dredging and reclamation works, and coastal development (Al-Taani et al, 2020, Al-Najjar, 2008).

Coastal environments across the world are progressively being affected by a variety of human activities, many of which result in high quantities of trace metals (Abu Hilal and Ismail, 2008). In recent years, industrialization has resulted in an increase in water, air and soil pollution. Heavy Metal poisoning has become a problem in recent years due to its potential influence on human health as well as on the whole ecosystem (Siddiqui, 2015). Heavy metal bioaccumulation occurs at many structural levels in plants (cells, tissues and organs) (Chaplygin et al, 2022). Since the beginning of the industrial revolution, the quantity of metals produced by humans has increased. Metals can originate from a variety of sources in an urban area, including vehicle emissions, industrial discharges and weathered particles (Al-Khashman, 2007). These metals are harmful not only to the health of fish, turtles and other marine animals that feed on polluted seagrass tissues but also to human health because trace metals are ingested through contaminated seafood (Mohammadi et al, 2019).

The negative impacts of marine pollution on the environment influence coral reefs and seagrass ecosystems, human health, food security, and livelihoods (Riechers et al, 2021). The direct interaction of pollutants with DNA causes genotoxic consequences and genome instability (Theodorakis et al, 2001).

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Heavy metals can also interfere with DNA repair enzymes by modifying their protein structures or reducing their expression at the transcriptional level, potentially leading to chromosomal abnormalities (Whiteside et al, 2010). Therefore, bioindicators and biomonitoring systems are promising tools for studying the influence of external factors on ecosystem health and for pollution assessment. Genotoxicity is the term for the ability of chemical substances to change a cell's genetic information, possibly leading to genetic alterations. Genotoxicity is related to mutagenicity, with the exception that genotoxic impacts are often not linked to mutations.

Oxidative stress is a complicated process that involves chemical and physiological responses and occurs in all organisms including higher plants in response to excessive production and accumulation of reactive oxygen species. It occurs as a result of excess production and accumulation of reactive oxygen species (ROS). Genotoxicity refers to the capacity of certain substances to destroy genetic material. Genotoxicity refers to the ability of certain substances to harm genetic material. This does not only mean direct damage to DNA—it can also affect the proteins and structures that help DNA function properly inside the cell. For example, proteins involved in repairing DNA or regulating chromosomes condensation and separation are essential for healthy cell division. Likewise, structures such as the mitotic spindle can also be disrupted (Kumar et al, 2021).

A straightforward, popular, sensitive, adaptable and reliable technique for determining DNA damage is comet assay. The assay can be used to assess genotoxicity and detect the presence of genotoxins in the environment. Many researchers have used this technique to assess DNA damage since 1985. The genotoxic potential of heavy metals has been investigated using the plant comet assay. Plant comet assay is considered an extremely significant method used in ecotoxicology (Mishra, 2020). The comet test involves examining the electrophoretic mobility in a thin coating of agarose of extracted DNA from nucleoids following cell lysis. Sriussadaporn et al. (2003) evaluated DNA damage in Ginkgo trees. They found that environmental stress, increasing exposure time and roadside air pollutants lead to high degrees of DNA damage. In another study, Gómez-Arroyo et al. (2021) found that heavy metals lead to DNA damage in Moss (Hypnum Amabile). Liu et al. (2009) determined endosulfan's genotoxicity in Earthworms (*Eisenia foetida*) and White Clover (*Trifolium repens* L.) by using a comet assay. They found that exposure to different doses of endosulfan produces DNA strand breaks that were significantly higher than those in the control group. Al Khateeb (2018) used Comet assay to evaluate the level of DNA damage in a group of wild plants (*Citrullus colocynthis*, *Anabasis setifera*, *Prosopis juliflora*, *Prosopis farcta*, *Suaeda vermiculata*, *Ziziphus Spina-Christi*, *Capparis spinosa* and *Salsola vermiculata*) collected from different areas in Jordan. Results indicated that plants from the back roads of Aqaba and Ghour Assaal showed higher DNA damage levels. On the other hand, samples collected from Wadi Rum, Al Naqab Heights and Alshoneh showed low DNA damage levels. Although comet assay is widely applied in genetic toxicology for detecting DNA damage at the single-cell level, it does not provide information on long-term genetic effects or repair kinetics. Also, comet assay lack specificity, as it cannot distinguish between different types of DNA damage, such as single-strand breaks, double-strand breaks, or alkali-labile sites, without additional modifications (Olive & Banáth, 2006).

Pollution causes DNA damage in many plants, including *Nicotiana tabacum*, *Allium cepa*, *Vicia faba* and *Tradescantia*. It has been shown that growing *Tradescantia* plants in heavy metals contaminated soil results in a 10–15 fold increase in DNA damage levels in comparison to plants grown in uncontaminated soil (Jaskulak et al, 2019). Radziemska et al. (2021) used *Pinus sylvestris* L. as a pollution bioindicator for railway transport in Poland. They found that rail transport is a source of pollution that inhibits seed germination and root growth. Lumbreras et al. (2013) studied regional climate influence on the distribution of aquatic Ranunculus communities such as *R. peltatus Schrank*, *R. penicillatus* (Dumort.) Bab., *R. aquatilis* L. They found that Atlantic ranunculus species may be less adapted to habitat desiccation. Gomes et al. (2021) evaluated the effects of fluoride emissions on three plant species in Brazil: *Myrceugenia alpigena*, *Byrsonima variabilis* and *Eremanthus erythropappus*. They found that the phytotoxicity of fluoride depends on the stage of leaf development, genetic susceptibility and the concentration of the pollutant in the atmosphere. It has been shown that *Halophila stipulacea* (Forsk.) Aschers. grown on the phosphate mine dump in Aqaba had a substantial change in cell organization that resulted in leaf necrosis, swelling in the outer epidermal wall and chloroplast degradation (Wahsha et al, 2016).

Halophila stipulacea (Forsk.) Aschers. is a tiny tropical seagrass, native to the Red Sea. Seagrasses provide vital ecological functions such as the generation and storage of organic carbon, coastal protection through the stability of sediment and the creation of a necessary habitat for commercially valuable fish and crustaceans (Beca-Carretero et al, 2020). However, the loss of seagrass has been associated with a variety of negative outcomes such as decreased local fishing grounds, accelerated coastal erosion, destruction of related ecological populations and loss of primary productivity (Winters et al, 2020). The aim of this study was to evaluate using *Halophila stipulacea* (Forsk.) Aschers. as a biosensor for a potentially contaminated area of the Gulf of Aqaba by measuring DNA damage using a comet assay.

2. Materials and methods

2.1. Study area and sampling

Halophila stipulacea (Forsk.) Aschers. plants were collected from various regions in the Gulf of Aqaba (table 1) in March 2024. Samples were collected from Tala Bay, Old Phosphate Port, Marine Science Station (MSS), Big Bay and the Industrial area (figure 1). Plants with homogeneous sizes were chosen, and only plants without any apparent disease symptoms were collected. Before tissue examination, samples were washed with distilled water to get rid of unwanted debris. The youngest two leaves from each plant were used for comet assay analysis and the remaining leaves were used for heavy metals analysis and lipid peroxidation assay.

Table. 1: Collection sites and coordination for samples used in this study.

Sample number	Collection site	Coordinates
1	Tala Bay	Latitude: 29°31'45.67"N
		Longitude: 34°59'45.42"E
2	Old Phosphate Port	Latitude: 29°30'10.51"N
		Longitude: 34°59'30.02"E
3	MSS	Latitude: 29°27'31.51"N
		Longitude: 34°58'32.79"E
4	Big Bay	Latitude: 29°24'18.74"N
		Longitude: 34°58'26.86"E
5	Industrial area	Latitude: 29°22'22.26"N
		Longitude: 34°57'53.93"E

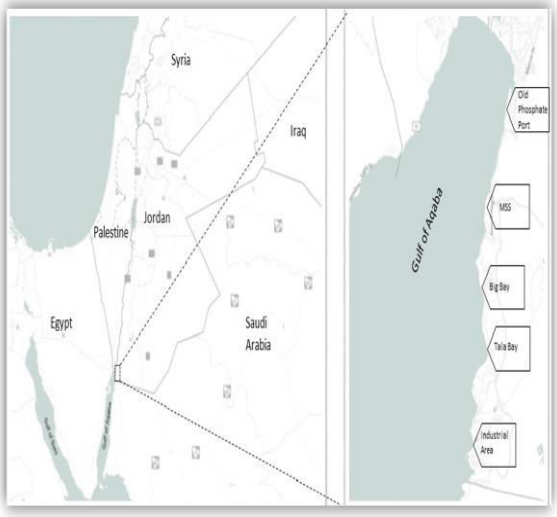

Figure 1: Samples collection sites in Gulf of Aqaba/Jordan.

2.2. Alkaline Comet assay

Plant samples were chopped with a razor blade in 1 ml of ice-cold Tris-$MgCl_2$ (pH=7.5). Then filtered and precipitated by centrifugation at 200 g for 5 min at 40°C to get the nuclei. The pellet was resuspended in 200 µl Tris-$MgCl_2$ buffer and kept on ice until used in the comet assay (Yıldız et al, 2009). To detect DNA damage in the plant cell, Oxiselect Comet Assay kit (cell Biolabs, Inc, san Diego, USA) was used. Lysis buffer, alkaline solution, and electrophoresis running solution were prepared and chilled at 4°C before performing the assay. Agarose was heated for 20 minutes at 90–95 °C before being cooled to 37 °C. Plant cell samples were prepared by centrifugation at 700 g, then 10 µl of cells were mixed with 90 µl of molten agarose, and then the mixture was placed onto the comet slide. Slides were set in the dark at 4°C for 15 min, then transferred to a basin containing pre-chilld lysis buffer (25ml/slide) and incubated in the dark at 4°C for 30–60 min, then the lysis solution was replaced with an alkaline solution for 30 min in the dark.

Prepared slides were transferred to the electrophoresis chamber and covered with cold electrophoresis buffer that was freshly prepared (300 mM NaOH, 1 mM EDTA, pH < 13). The run was started at 1 Volt/cm for 15–30 min, then slides were transferred carefully to the basin and washed twice with pre-chilled deionized water (DI) for 2 min for each wash, then left to dry. After the addition of 100µL / well Vista green DNA dye, slides were incubated at room temperature for 15 minutes. After that, slides were examined under a fluorescence microscope (EVOS ®) at 100x magnification (GFP filter, 470 nm excitation, 525 nm emission).

To evaluate DNA damage index, cells were grouped into five classes depending on tail length (0: no tail, 25%: short tail, 50%: medium tail, 75%: long tail, and 100% very long tail length), and then the average of all cells from each site was calculated.

$$\text{DNA damage index} = \frac{\text{Number of cells in each group}}{\text{Number of group}}$$

Damage frequency (%) was calculated by dividing number of cells that were grouped as 25, 50, 75, or 100% by the total number of cells.

$$\text{DNA damage frequency (\%)} = \frac{\text{Number of cells in 25, 50, 75 and 100}}{\text{Total number of cells}} * 100\%$$

The experiment was repeated three times.

2.3. Heavy metal analysis

Samples of *Halophila stipulacea* (Forsk.) Aschers. were cleaned of debris by washing with distilled water before being dried at 104°C for 24 hours. Samples were heated at 520°C for 5 hours to assess ash content. In a conical flask, one gram of each powdered sample was weighed. For samples digestion, 8 ml of $HN0_3$ and 2 ml of perchloric acid ($HCLO_4$) were mixed with samples (Arora et al., 2008). The samples were then placed in a water bath at 80°C for two to three hours, or until the solution was clear. Samples were digested, allowed to cool to room temperature, and then filtered using Whatman filter paper. Spectrophotometer was used for Heavy metals analysis depending on the atomic absorption. The experiment was repeated three times.

2.4. Lipid peroxidation content

To evaluate lipid peroxidation, malondialdehyde (MDA) content was measured following Yagi (1968). About 50 mg of fresh tissue was gently ground in a mortar and pestle in a solution of 0.5% 2-thiobarbituric acid (TBA) and 0.1% trichloroacetic acid (TCA). The mixture was then centrifuged at 4000 g for 15 minutes at 4 °C. The clear supernatant was heated in boiling water for 10 minutes and quickly cooled on ice bath. Then, the absorbance of the supernatant was measured at 532 and 600 nm using a UV–visible spectrophotometer. 0.1% TCA and 0.5% TBA were used as blank.

MDA content (expressed as nmol per gram of fresh tissue) was calculated using the formula:

$$\text{MDA content} = [((A532 - A600) \times v) \times 1000]/ (\varepsilon \times M).$$

where ε is the extinction coefficient (155 mM^{-1} cm^{-1}), *v* is the volume of the extraction solution, *M* is the sample's fresh weight, and A_{532} and A_{600} are the absorbance readings at 532 and 600 nm, respectively.

2.5. Statistical analysis

SPSS version 16 for Windows was used in the analysis of the data. Data were presented as mean ± standard error from five or more biological replicates. Analysis of variance (ANOVA) and Tukey's test were used to evaluate

the significant differences among the studied sites. P value of less than 0.05 was considered statistically significant.

3. Results

3.1. DNA damage

Comet assay, also known as single-cell gel electrophoresis, was used to evaluate DNA damage in collected samples. A range of DNA damage levels (0 - 4) was used for DNA damage score, where score 0 represents a cell with no damage, while score 4 represents cells that are severely damaged. The score of each sample was calculated using the following equation:

$$\text{Score of DNA damage} = \frac{0^* N0 + 1^* N1 + 2^* N2 + 3^* N3 + 4^* N4}{\text{Total number of cells}}$$

The letter N represents the number of cells in each level. Significant differences in DNA damage score ($p<0.05$) were observed between *Halophila stipulacea* (Forsk.) Aschers. samples collected from different sites in the Gulf of Aqaba. Old Phosphate Port showed the highest score among other groups (1.78). Samples collected from Industrial area showed 1.62 damage score. On the other hand, Marine Science Station (MSS), Big Bay, and Tala Bay showed the lowest score (0.62, 0.72 and 0.73 respectively) (figure 2 and 3).

Results showed significant differences in damage frequency and damage index ($p<0.05$) between *Halophila stipulacea* (Forsk.) Aschers. samples collected from different sites in Gulf of Aqaba (figure 4 and 5). The Old Phosphate Port showed the highest DNA damage index among other groups (49.7). On the other hand, (MSS), Big Bay, Tala Bay and Industrial area showed similar values (41.5, 39.7, 42.3 and 43.7 respectively). Samples collected from Old Phosphate Port and Industrial area showed the highest levels of DNA damage frequency (82.3% and 82.9% respectively). On the other hand, samples collected from MSS showed the lowest DNA damage frequency (47.9%).

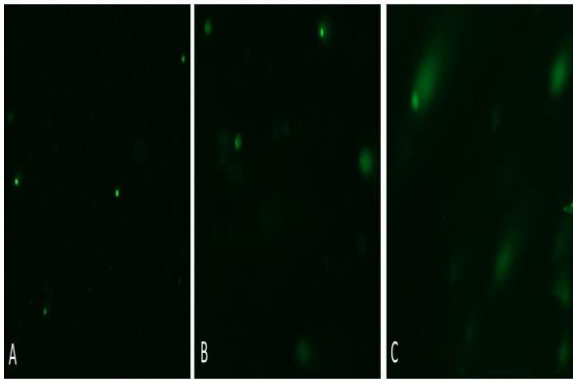

Figure 2: Comet assay for assessing DNA damage levels in *Halophila stipulacea* (Forsk.) Aschers. samples collected from the Gulf of Aqaba (No damage (A) intermediate damage (B) and high damage (C)).

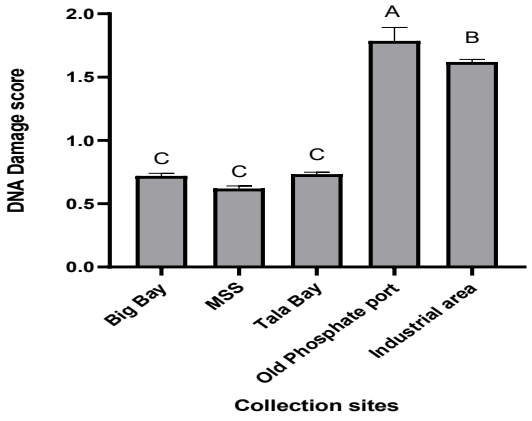

Figure. 3. DNA damage score of *Halophila stipulacea* (Forsk.) Aschers. samples collected from different locations in the Gulf of Aqaba. Means with different letters are significantly different at ($p<0.05$). Error bars represent standard error.

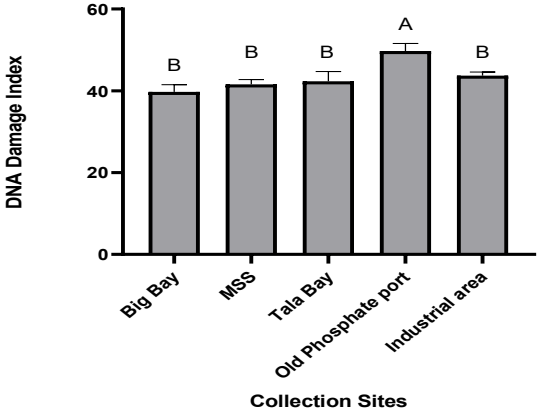

Figure. 4. DNA Damage Index of *Halophila stipulacea* (Forsk.) Aschers. samples collected from different locations in the Gulf of Aqaba. Means with different letters are significantly different at ($p<0.05$). Error bars represent standard error.

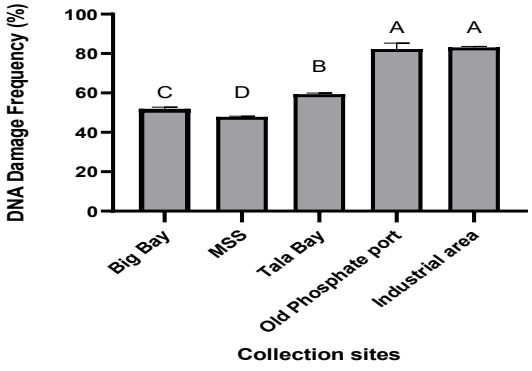

Figure. 5. DNA Damage Frequency of *Halophila stipulacea* (Forsk.) Aschers. samples collected from different locations in the Gulf of Aqaba. Means with different letters are significantly different at ($p<0.05$). Error bars represent standard error.

3.2. Lipid peroxidation level

Significant differences *($p<0.05$)* in Malondialdehyde (MDA) content were observed between *Halophila stipulacea* (Forsk.) Aschers. samples collected from different regions in Gulf of Aqaba (Figure 6). The

Industrial area showed the highest MDA content among other groups (0.277 nmole g $^{-1}$ FM). On the other hand, samples collected from Marine science station (MSS) showed the lowest MDA content (0.158 nmole g $^{-1}$ FM). Tala Bay, Old Phosphate Port and Big Bay samples showed intermediate values (0.217, 0.261 and 0.183 nmole g $^{-1}$ FM, respectively).

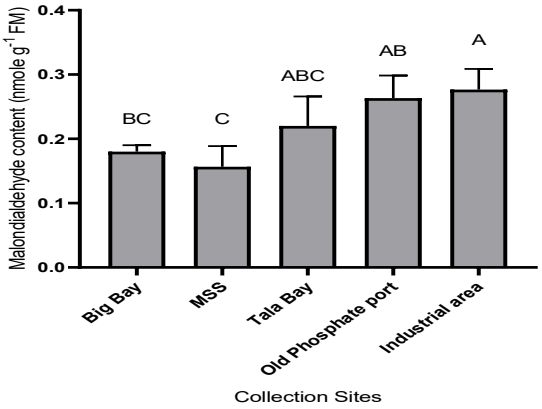

Figure 6. Total Malondialdehyde (MDA) content (nmole g $^{-1}$ FM) of *Halophila stipulacea* (Forsk.) Aschers. samples collected from different sites in the Gulf of Aqaba. Means with different letters are significantly different at ($p<0.05$). Error bars represent standard error.

3.3. Heavy metals analysis

Significant differences ($p<0.05$) in heavy metals content were observed between *Halophila stipulacea* (Forsk.) Aschers. samples collected from different sites in the Gulf of Aqaba (Figure 7). A high level of (Co) concentration in *Halophila stipulacea* (Forsk.) Aschers. was found in Old Phosphate Port samples (20.5 ppb), unlike Big Bay and MSS that showed the lowest concentrations (17.5 and 16.6 ppb respectively). Similar levels were found in Tala Bay and the Industrial Area samples (18.3 and 19.6 ppb respectively). A high level of (Cu) concentration in *Halophila stipulacea* (Forsk.) Aschers. was found in industrial areas (250.6 ppb), unlike in Big Bay and MSS that showed the lowest concentrations (136.9 and 104.4 ppb respectively). Similar content was observed in samples collected from Tala Bay and Old Phosphate Port (196.7 and 159.9 ppb respectively). A high level of (Fe) concentration in *Halophila stipulacea* (Forsk.) Aschers. was found in Old Phosphate Port, Tala Bay and the Industrial Area (186.2, 170.8 and 189.7 ppb respectively), whereas Big Bay and MSS were the lowest (136.2 and 129.7 ppb respectively). A high level of (Cr) was found in Old Phosphate Port and Industrial area samples (286.7 and 267.1 ppb respectively), samples collected from MSS and Big Bay showed the lowest content (103.3 and 97 ppb respectively). A high level of (Mn) was found in Tala Bay, Industrial area and Old Phosphate Port (375.1, 387 and 409.9 ppb respectively). On the other hand, low levels were found in MSS and Big Bay (304 and 317 ppb respectively). High levels of (Ni) were found in Old Phosphate Port and industrial area (128.6 and 117.1 ppb). Big Bay and MSS showed the lowest concentrations (66.5 and 64.4 ppb respectively). The highest level of (Th) was found in Old Phosphate Port (9.8 ppb), and the lowest concentration was found in MSS and Big Bay (3.3 and 3.5 ppb respectively). High levels of (Zn) were found in Old Phosphate Port, Tala Bay, and the Industrial Area (148.2, 148 and 146.4 ppb respectively), the lowest concentrations were found in MSS and Big Bay (96.2 and 113.2 ppb respectively). Similar Li levels were found in Big Bay, MSS, Tala Bay, Old Phosphate Port and Industrial area (39.2, 34.6, 41.2, 44.3 and 45.7 ppb respectively). A high level of (Mg) was found in Tala Bay and Industrial area (132206 and 137168.2 ppb respectively), and the lowest concentration was found in MSS (100371.1 ppb).

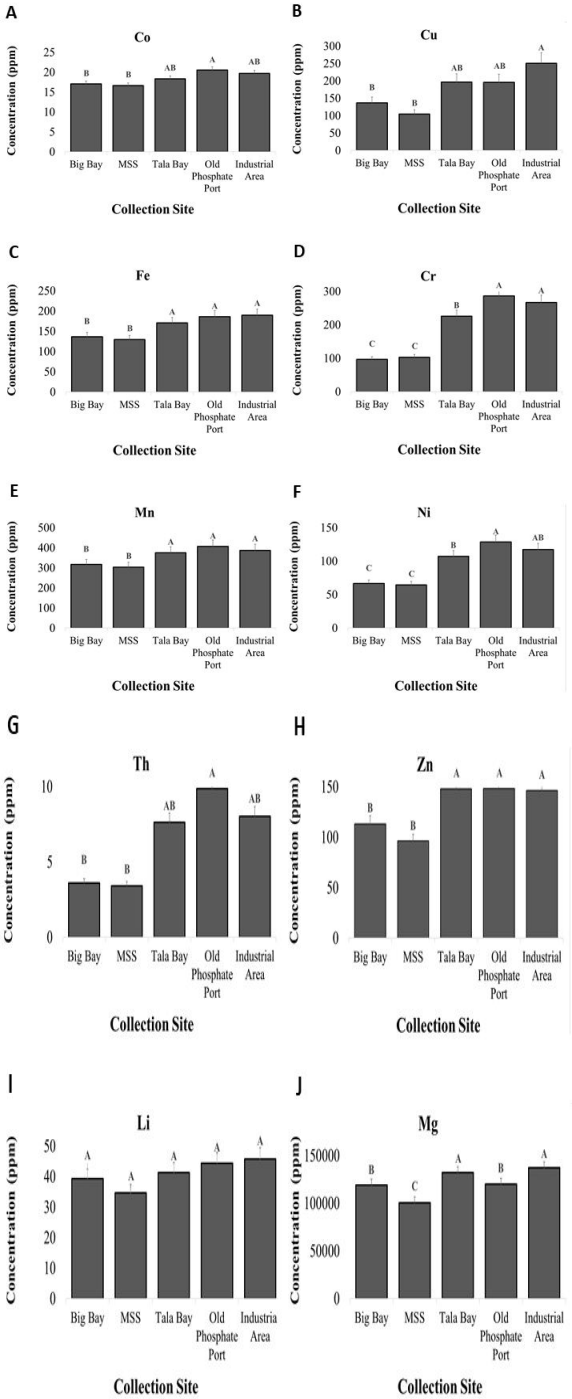

Figure 7. Heavy metals (Co, Cu, Fe, Cr, Mn, Ni, Th, Zn, Li and Mg) concentration (ppb) in *Halophila stipulacea* (Forsk.) Aschers. collected from different sites in the Gulf of Aqaba (A-J). Means with different letters are significantly different at ($p<0.05$). Error bars represent standard error.

4. Discussion

The aim of this study was to evaluate genotoxicity in *Halophila stipulacea* (Forsk.) Aschers. found in Gulf of Aqaba as a pollution bioindicator using DNA damage, heavy metals and the level of lipid peroxidation

Worldwide, levels of pollutants in the environment are rising alarmingly due to urbanization and industrial growth, which has a negative impact on ecosystem sustainability, organisms and humans (El-Gendy et al, 2021). Harmful environmental stressors can cause genotoxicity and apoptotic cell death, which can lead to a variety of pathological disorders. These stressors include cytotoxic agents, pollutants, and toxicants (Cortés-Eslava et al, 2018, Paulo Cardoso et al., 2024). Marine water makes up the majority of the planet's water supply, yet it must be processed by people in order to be used. Water pollution is defined as any alteration of water's natural qualities by anthropogenic contaminants to the level where it is either toxic to humans or unsuitable for supporting biotic organisms, such as fish, and those that depend on it. Since it causes the development of many fatal diseases, water pollution is a major cause of concern on a global scale (Anju et al, 2010). Because of the large size (local and global) and complex interactions of trace elements in the marine environment, contamination with heavy metals in such areas is a growing problem for ecosystem managers. Trace elements persist indefinitely in the environment. Accumulating in sediments, they enter the food chain and increase in concentration at higher trophic levels, ultimately posing risks to both human and wildlife health.

Environmental exposure to heavy metals (notably arsenic, cadmium, lead, mercury, chromium and nickel) is consistently associated with molecular and cellular markers of genotoxicity (Balali-Mood et al., 2021). Heavy metals generate genotoxicity by at least three interacting processes. First, many metals increase cellular reactive oxygen species (ROS) production and disturb redox homeostasis, yielding oxidative base lesions (e.g., 8-oxo-dG), single-strand breaks (SSBs) and, at higher or persistent exposures, double-strand breaks (DSBs)(Jomova et al., 2025). Second, several metals directly impair DNA-repair pathways. Cadmium and other metals can displace essential metal cofactors in repair enzymes, suppress repair-gene expression, or destabilize repair proteins, reducing capacity for base-excision repair (BER), nucleotide-excision repair (NER), mismatch repair (MMR) and DSB repair — an effect that amplifies the mutational consequences of otherwise reparable damage (Morales et al., 2016). Third, certain metals (notably hexavalent chromium and some nickel compounds) form direct adducts or crosslinks with DNA and DNA-binding proteins or perturb chromatin structure, creating bulky lesions and replication blocks that are intrinsically mutagenic and cytotoxic (Balali-Mood et al., 2021).

Significant differences in DNA damage score, DNA damage index and frequency were observed among *H. stipulacea* samples collected from the collection sites. Results indicate that the highest DNA damage (DNA damage score 1.78, DNA damage Index 49.7 and DNA damage Frequency 82.3%) and the highest lipid peroxidation rate (0.261 nmole g^{-1} FM) were observed in Old Phosphate Port. Similarly, heavy metal analysis showed high level of (Co) concentration (20.5 ppb), (Cu) concentration (159.9 ppb), (Fe) concentration (186.2 ppb), (Cr) concentration (286.7 ppb), (Ni) concentration (128.6 ppb), (Zn) concentration (148.2 ppb), (Mn) concentration (409.9 ppb), (Th) concentration (9.8 ppb), (Mg) concentration (120006.6 ppb) and (Li) concentration (44.3 ppb) in Old Phosphate Port. This could be explained by ship movement, oil and chemical leakage from ships in Development Corporation (ADC) and Aqaba Container Port. In addition, it could be due to the weak movement of water currents that lead to the accumulation of heavy metals in these regions.

Halophila stipulacea (Forsk.) Aschers. samples collected from the Industrial area showed DNA damage score of 1.62, DNA damage index of 43.7 and DNA damage frequency of 82.9%. These high values of DNA damage were accompanied with the highest lipid peroxidation rate (0.277 nmole g^{-1} FM), high levels of (Co) (19.6 ppb), (Cu) (250.6 ppb), (Fe) (189.7 ppb), (Cr) (267.1 ppb), (Ni) (117.4 ppb), (Zn) (146.4 ppb), (Mn) (387ppb), (Th) (8 ppb), (Mg) (132206 ppb) and (Li) (45.7 ppb). This could be due to the industrial activity such as container transportation, building activity and vehicle exhaust from chemical factories in this area such as KEMAPCO® Arab Fertilizers and others. In contrast, the lowest DNA damage levels were observed in *Halophila stipulacea* (Forsk.) Aschers. collected from MSS and Big Bay, these areas are far away from tourist and located away from industrial and ship traffic.

Bonanno and Raccuia (2018) used seagrass *Halophila stipulacea* as bioindicator, and found that *Halophila stipulacea* is considered as good bioindicator for a variety of heavy metals. Also, Lemos et al. (2005) used comet assay for the genotoxic assessment of water pollution and found significantly higher levels of DNA damage in *T. rendalli* in Lake Igapo II combared to the control group. Another study done by Al Khateeb (2018) used comet assay to assess pollution in different regions in Jordan; results showed that plants obtained from the back road/ Aqaba and Ghour Assaal had the highest DNA damage amount and high Al, Cr, Fe, Cu, Zn, Cd, and Pb levels, compared to plants obtained from Wadi Rum, Al Naqab Heights, Swaimeh/Deadsea, and Alshoneh Aljanobyeh.

Trace elements such as Cu, Zn, Mn, and Ni are considered as micronutrients for plants, but others, such as As, Cd, Cr, Hg, and Pb, are not necessary and may be toxic to many organisms even at low amounts. Li et al., (2016) assessed the relationship between levels of DNA damage of *Leymus chinensis* and heavy metals (Pb, Cd, Cr, Hg) content in China using Comet assay. Results showed that genetic damage (Tail DNA% and Tail Moment) in *L. chinensis* leaf cells under heavy metals was 77% compared to 35% in control samples. In addition, presence of trace elements in large amounts such as Cu and Zn can be hazardous. For example, plants require copper for proper plant development and growth, but a high level of Cu contamination leads to leaf chlorosis, oxidative stress and ROS, and it impacts different pigment secretions during photosynthesis and reduces plant growth. Also, zinc contributes in production of chlorophyll in plants, but high Zn concentrations prevent development of metabolic processes, limit root and shoot development, chlorosis in young leaves, decrease germination,

chlorophyll, carotenoid, sugar, amino acid and growth. In addition, Cadmium causes chlorosis, growth inhibition, browning of the root tips, abnormal membrane function and eventually death. Chromium is well-known for being poisonous and capable of harming both plants and animals (Bonanno and Raccuia, 2018). Chromium damages plant cell membranes and breakdown photosynthetic pigments, which leads to growth reduction. Numerous physiological changes, including chlorosis and necrosis, are driven by excessive Ni in the soil. Finally, plants utilize iron for a primary function in photosynthesis. An excess of iron causes a reduction in plant photosynthesis, and increase in oxidative stress leads to the creation of free radicals, which permanently alter the cellular structure and destroy proteins, DNA and membranes (Bonanno and Raccuia, 2018). Anbuselvan and Sridharan (2018) determined the distribution of heavy metals (Fe, Cd, Co, Cr, Cu, Ni, Zn, and Pb) in the Bay of Bengal as a sign of marine pollution and found that the area was extremely polluted with Cd and Pb contributions from anthropogenic activities in the nearby area. Furthermore, Zhang et al. (2020) studied heavy metals pollution in coastal areas of the East China Sea and found that Cd caused significant pollution and possible ecological hazards due to anthropogenic activities.

In this study, similarity coefficient matrix indicates a strong positive correlation between DNA damage index, DNA damage score, DNA damage frequency, LPO and heavy metals (table 2). The highest similarity coefficient value was observed between LPO and DNA damage frequency (0.979). Also, similarity coefficient value between LPO and Cu was (0.945), whereas similarity coefficient value between DNA damage frequency and Fe was (0.938) and similarity coefficient value between DNA damage score and LPO was (0.913), which indicates that heavy metal cause direct effect on DNA and lipid. However, the similarity coefficient value between the DNA damage index and Mg was (0.152), possibly because Mg is regarded as macronutrient for plants. Lukatkin et al. (2021) studied the effect of heavy metals on biochemical and physiological responses of *Amaranthus retroflexus,* they found a strong correlation between the accumulation rate of HMs and (MDA) content in *A. retroflexus* roots, stems and leaves. Our results indicate that heavy metals could be the main source of pollution in the gulf of Aqaba. In conclusion, our results indicate that plant comet assay is a reliable method for measuring genotoxicity in marine environment in general and specifically in the Gulf of Aqaba.

Table. 2: The Similarity coefficient matrix between studied parameter.

	frequencu	index	score	co	cu	fe	cr	mn	ni	th	zn	li	mg	lpo
frequencu	1													
index	0.790274	1												
score	0.975253	0.841248	1											
co	0.963261	0.853449	0.941556	1										
cu	0.865616	0.465421	0.737272	0.816707	1									
fe	0.938688	0.72011	0.847019	0.933949	0.94564	1								
cr	0.922737	0.800612	0.843996	0.933546	0.884787	0.986817	1							
mn	0.907135	0.802662	0.830663	0.953698	0.870624	0.978383	0.988204	1						
ni	0.914438	0.814102	0.838828	0.945285	0.870346	0.981955	0.996993	0.99663	1					
th	0.893597	0.846299	0.827048	0.944678	0.826507	0.961947	0.987777	0.994903	0.996011	1				
zn	0.807088	0.61143	0.681993	0.866599	0.909595	0.949465	0.931649	0.958881	0.943696	0.934073	1			
li	0.930786	0.608143	0.853875	0.931024	0.945734	0.944577	0.889957	0.909791	0.893932	0.867462	0.909549	1		
mg	0.61847	0.152018	0.448738	0.611374	0.911725	0.775268	0.680731	0.703555	0.679749	0.63478	0.852318	0.839785	1	
lpo	0.979052	0.696377	0.913025	0.949863	0.945722	0.975268	0.94097	0.933658	0.934113	0.906531	0.889697	0.977876	0.764218	1

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