Anatomical Study of *Nerium oleander* L. Leaves Found in Places Contaminated with H_2S

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Abstract. The research study the effect of H_2S on *Nerium oleander* leaves, the leaves were collected from the pollution places in the crowded streets with cars and factory areas from five different regions of Basrah city, The results showed that the average of highest concentration of hydrogen sulfide gas was 4. ppm in the Al-Amin Aldakhli area, while the lowest value of gas was recorded in Karmat Ali, it was 0.4 ppm of date palm plants and 0.8 ppm. Anatomy study of *N. oleander*leaves showed that the different changes in plant internal tissues with H_2S pollution which presented by difference in the thickness of epidermal cell walls of *N. oleander* leaves in the areas of Al-Dair, Manawi Pasha and Andal's, while the thickness of the cells was less in the area of Karmat Ali.Highest average length of cells of the upper epidermis was recorded in the Al-Dier and Karmat Ali region was 40 µm and 37.5 µm respectively, while the lower epidermis was 35.82 µmin Al-Dier. Stomata dimension found the lowest average was recorded in the Al-Amin Aldakhli area was 24 µm while in the upper epidermis have highest value, it was in Karmat ali was 30 µm. The number of stomata different between the stations, it was found that the highest average of stomata was found in the lower epidermis in Al-Amin Aldakhli station was 215.98, while the lowest average in Karmat Ali was 98. The upper epidermis had the highest average number of stomata in the Minawi Pasha station was 183.96 on lower epidermis and the lowest average in Andal's area was 66. Also, the highest average number of cells was in the Lower epidermis in Andal's region was 3159.96, and the lowest average was 919.98 in the Al-Dair.

Keywords. H_2S, Pollution, Leaves anatomy, *Nerium oleander*.

1. Introduction

Pollution occurs as a result of dumping waste for disposal, which leads to a change and imbalance in the balance that takes place between the constituent elements of the ecosystem so that it paralyzes the effectiveness of this system and loses the ability to self-dispose of pollutants through natural processes[1,2]. At the present time the most dangerous pollution process is air pollution[2].Air pollution is rapidly spreading, as its impact is not limited to the source area only but extends to neighboring and remote areas. Therefore, air pollution cannot be controlled after leaving the source, so it must be controlled and treated before leaving the atmosphere[3].

H_2Shas some dangerous air pollutants that, especially to human health, arise from the decomposition of organic compounds such as sewage water, stagnant water, dense swamps and mud. Hydrogen sulfide

(H_2S) is a colorless gas with a strong odor resembling the smell of rotten eggs. It occurs naturally in the environment, and they consist of two hydrogen and a sulfur atom, hydrogen sulfide can be formed and emitted in any place where bacteria attack waste containing sulfur compounds[3,4]. H_2s is spread in the sites of sewage networks, animal waste, and trucks that transport chemical compounds. It is also emitted from groundwater, especially in wells and areas near oil fields, or in areas where groundwater penetrates the limestone reservation layers. Hydrogen sulfide is also found in oil and natural gas. Natural gas contains up to 28% of hydrogen sulfide, and therefore it can become an air pollutant near natural gas production areas and oil refineries, and gas can also be emitted through industries that deal with sulfur landfills [5]. The gas is one of the most disturbing pollutants due to its foul smell, which resembles the smell of rotten eggs, in addition to its severe toxicity, which causes health damage to humans [6]. Hydrogen sulfide (H_2S) is an air pollutant present at high levels in various regions, H_2s is found naturally in the environment in volcanic gases, marshes, swamps, and sulfuric springs, It is found in all oil refineries, petroleum wells, gas units, and waste treatment units [7,8]. Hydrogen sulfide gas is generated naturally in different ways such asorganic matter decomposes, occurs as a result of the interaction of acidic water with an underground water reservoir that contains sulfur components and iron bacteria which use iron and manganese as part of their diet[8], in plants, H_2S is also produced in the photosynthetic sulfate assimilation pathway in the chloroplast [9]. H_2S has been shown to be essential in regulating a wide range of vital processes. It is allowing plant adaptability and viability, and its beneficial effects play a role in important aspects of development [10]. But different studies have shown that found many changes to the function of the proteins, physiological effects, stomatal closure, ethylene biosynthesis, and root hair growth[11].

2. Materials and Methods

2.1. H_2S Measurement

Samples of plant leaves close to sources of pollution were collected in streets crowded with cars and factory areas, from different regions in Basrah Governorate, and put in the refrigerator until it is used.Take 2 gm of leaves and washusing distilled water with a volume of 100 ml, by sensitive balance, in a homogeneous manner, then the washing water was filtered by filter paper to get rid of dust and others.The washing water is taken and acidified by adding 2 ml of HCL, thenthe sample is rubbed with iodine until the red color appears (Figure, 1), the volume of iodine does not exceed 3.5 ml) this volume is recorded, and the symbol is given as **a**.Add a few drops of starch to the sample and mixture. Then the mixture is filtered using sodium thiosulfate with continuous shaking until the blue color disappears. The volume of sodium thiosulfate is symbol **b**, then used the following equation to calculate H_2S concentration[12]:

$$H_2S \text{ concentration mg/L or ppm} = 400\ (a - b)/\text{Sample Volume}$$

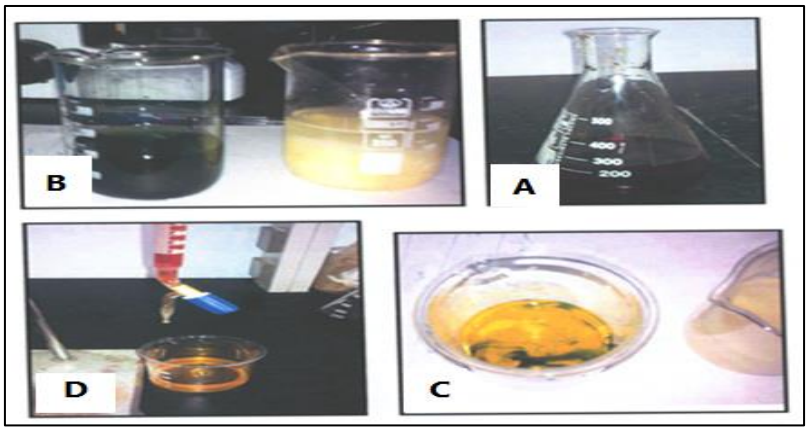

Figure 1. The work stages of the hydrogen sulfide method(A) After washing with iodine and the appearance of a red color (B) The starch solution is added to the solution to show the blue color (C) Appearance of a yellow color (D) The washing process.

2.2. Anatomical Study

For epidermis preparation using fresh leaves samples collected from the field andfixed for 48 hours in formalin-acetic acid-alcohol solution, FAA and preserved in 70% alcohol. The epidermal preparations were doneby taking the upper and lower epidermis of the leavesby macerating the leaves in Jeffrey's solution (equal parts of 10% chromium trioxidesolution and concentrated nitric acid), and thereafter mounted in safranin-stained glycerin jelly.Epidermal characters were examined by Olympus CH4 light andphotographed with Digital camera type DCE-2. Anatomical terms are cited from [13].

3. Results and Discussion

The study showed that there are significant differences between the different areas from which samples were collected from Basrah Governorate. The study showed that the Al-Amman aldakhli area has significant differences from the rest of the areas at the level of probability.0.05 as in (Table, 1). The highest concentration of H_2s was 4 ppm in Al-Amin Aldakhli region,while the lowest value of H_2s was recorded in the Karmat Ali region. The sense of smell is the first warning of the presence of hydrogen sulfide gas, as it is possible for the sense of smell to recognize the gas at a level 0.05 ppm [14], but the smell alone is not sufficient to determine the level of gas concentration and its toxicity, but rather indicates its quality or not [8].The highest value due to the large number of factories, including the oil and petrochemical plants, in that area and their proximity, and thus the rate of gas emissions is large, and exposure to this gas is permanent and continuous for workers and residents of this area Which causes inflammation in the eyes, nose, and throat, and prolonged exposure can disrupt the sense of smell [8, 14].Some authors showedthat when exposed to 1 ppm there are no significant physical effects and the sense of smell remains intact and can be detected and has the smell of eggs, but chronic (long-term) exposure to low concentrations (non-lethal) to hydrogen sulfide gas through the skin, breathing, and eyes (work and housing) will cause symptoms and risks such as shortness of breath accompanied by coughing, loss of appetite, bouts of nausea and dizziness[15]. explained that the current permissible level of exposure to H_2S gas in most cities and capitals ranges between 0.13 ppm and reaches at peak time, it was 0.33 ppm, which is much lower than the current study.

The value of H_2s in Al-deir, Al-Andlus and Minawi Pasha not significant (Table,1), This is due to the presence of sewage networks as well as water treatment units, as well as the presence of a paper mill in the Al-Deir or sampling near the places of electric power generation and caused pollutants that led to an increase in the value of H_2s [15]. that short-term exposure to H_2S gas may cause obstruction of the airways because it is a scratchy gas, and therefore it is not permissible to inhale it directly because it may cause internal (intestinal) poisoning and indirectly affect the nervous system [14]. It is possible for the sense of smell to recognize the gas, but there is no difference between the odors at the level of varying concentrations [16], but exposure to low concentrations of it for a long time leads to a dulling of the sense of smell, which makes relying on this sense to discover the gas in emergency conditions impractical [15]. It also found that effectH_2S effectson children more than adults, as children are more vulnerable than adults, because children breathe more quickly while they are busy with sports as well as activities, and this activity raises the respiratory rate, thus increasing the rate of inhalation of toxic substances, and because their bodies are immature, so exposure to H_2S causes damage to the brain Which leads to a weakness in mental abilities [16].

Table 1. The measurement of H_2Spollution in the studied areas.

Region	Volume of iodine	Volume of thiosulfate	H_2s (ppm)
Al-Amin Aldakhli	3	2	4
Andalus	3.5	3	2
Karmat Ali	3	2.8	0.8
Al-Deir	3	2.5	2
Minawi Pasha	3	2.5	2

3.1. Anatomical Study

The anatomical study of *N. oleander*leaves shows that the plant tissues were significantly and clearly affected, despite the presencesome of visible symptoms on the leaves taken for parts of this section. There are different changes in plant internal tissues with H_2S pollution (Table 2, Figure 2,3,4) which presented by difference in the thickness of epidermal cell walls of *N. oleander* leaves in the areas.The results showed that the highest average length of cells of the upper epidermis was recorded in the Al-Dier and Karmat Ali region was 40 µm and 37.5 µm respectively, while the lower epidermis was 35.82 µm in Al-Dier.

Stomata dimension found the lowest average was recorded in the Al-Amin Aldakhli area was 24 µm while in the upper epidermis have highest value, it was in Karmat ali was 30 µm. The number of stomata different between the stations, it was found that the highest average of stomata was found in the lower epidermis in Al-Amin Aldakhli station was 215.98, while the lowest average in Karmat Ali was 98. The upper epidermis had the highest average number of stomata in the Minawi Pasha station was 183.96 on lower epidermis and the lowest average in Andal's area was 66. Also, the highest average number of cells was in the Lower epidermis in Andal's region was 3159.96, and the lowest average was 919.98 in the Al-Dair..

Our results appeared clear difference was found in the thickness of the normal cells of the epidermis in*N. oleander*leaves in the regions of Al-Deir, Minawi Pasha and Andalus, while the thickness of the cells was less in the area of Karmat Ali, this may be due to the lack of pollution in Al-Karmat Ali (Table 2), and it is noted that the gaps are filled with the deposition of a dark substance, especially in highly polluted areas with hydrogen sulfide (Figures 2, 3, and 4).The variation in the size and dimensions of the normal cells of the epidermis is one of the interactions that occur between the plant and its surrounding environment. It was found that the highest rate of the longest cells in the upper epidermis was recorded in the Al- Al-Deir and Karmat ali, and it was 40 µm and 37.5 µm, respectively. The same was the case for the lower epidermis and it was 35.82 µm in the Al- Al-Deir

The number and dimension of stomata has good evidence of air pollution with various pollutants. Therefore, the plant makes many adaptations to bear the presence of high quantities of pollutants in the areas in which it is present.The dimensions of the stomata, the highest average of the stoma length was in Karmatali was 28.5 µm, and the lowest rate was recorded in the Al-Amin Aldakhli area was 24 µm, while in the upper epidermis, the highest rate was in the Karmat ali 30 µm, and the lowest 20.2 µm (Table 2), which agrees with [17], who mentioned that the decrease in the size of stomata is one of the adaptations carried out by plants that reduce the influence of pollutants and occurs as a result of a rapid response to external stimuli. Stomata is an adaptive way of air pollution and helps to reduce the uptake of polluted air.

Number of stomata on lower epidermis was highest rate in Al-Amin Aldakhli was 215.98 stoma, and the lowest rate of stomata in Karmatali was 98 stoma, whilein upper epidermis, the highest rate of stomata was in the Minawi Pasha area 183.96 stoma, and the lowest rate in Andalus was 66stomata, and the number of stomata is a means for plant survival in polluted conditions [18, 19], while [20]found that the abundance of helper cells surrounding the helper cells works on the speed of opening and closing of the stomata and vice versa, as noted the presence of Auxiliary cells reduce gases in greenhouses.

The highest average number of cells was found in the upper epidermis in the Andalus regionwas 3159.96 cells, and the lowest rate was found in the Al-Deir 919.98 cells. As for the lower epidermis, the highest was in Minawi Pasha 2239.98 cells, and the lowest was in the Al-Deir, and it was 1399.98 cells (Table 2; Figure 2,3,4).

Our results observed that the stomata closed in the area have higher concentration of H_2S which agree with[21,22]. A small number of plants could tolerate H_2S levels as high as 20 µl [21], but plants in higher H_2S levels quickly developed severe leaf necrosis and rapidly started wilting[21]. Stomata open or close is affected to H_2S responds by inducing stomata closure [22]. In guard cells caused complex interaction of H2S has been described, and the persulfidation of specific signalling components seems to be the underlying mechanism [23].

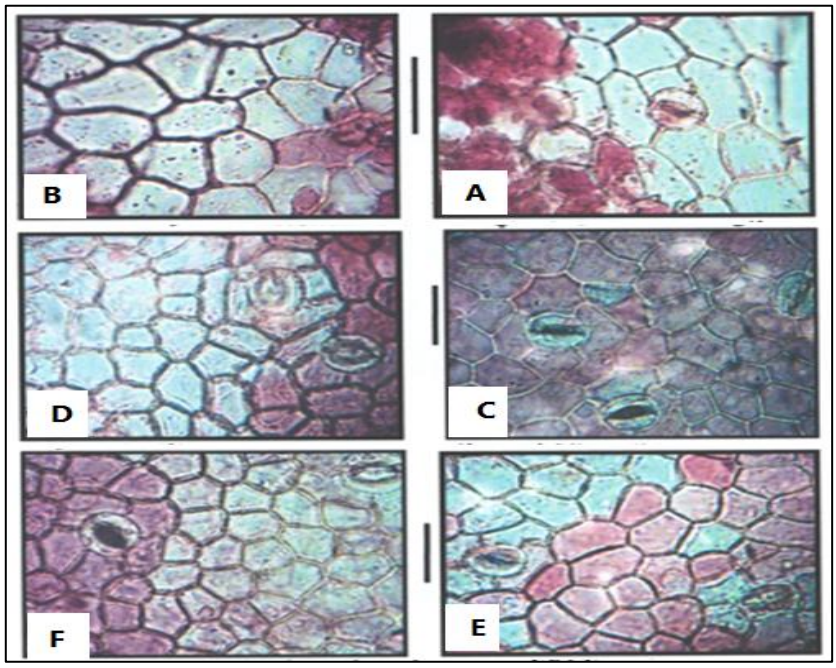

Figure 2. Variation in the epidermis of *N. oleander* leaves in the study regions: A, C, E, B, D, F.; A, lower epidermis, B upper epidermis of the Al-Amin Aldakhli region: C and D, the lower epidermis of the Andalus region,: E and F the upper epidermis of the Andalus region. (Scale 20 µm).

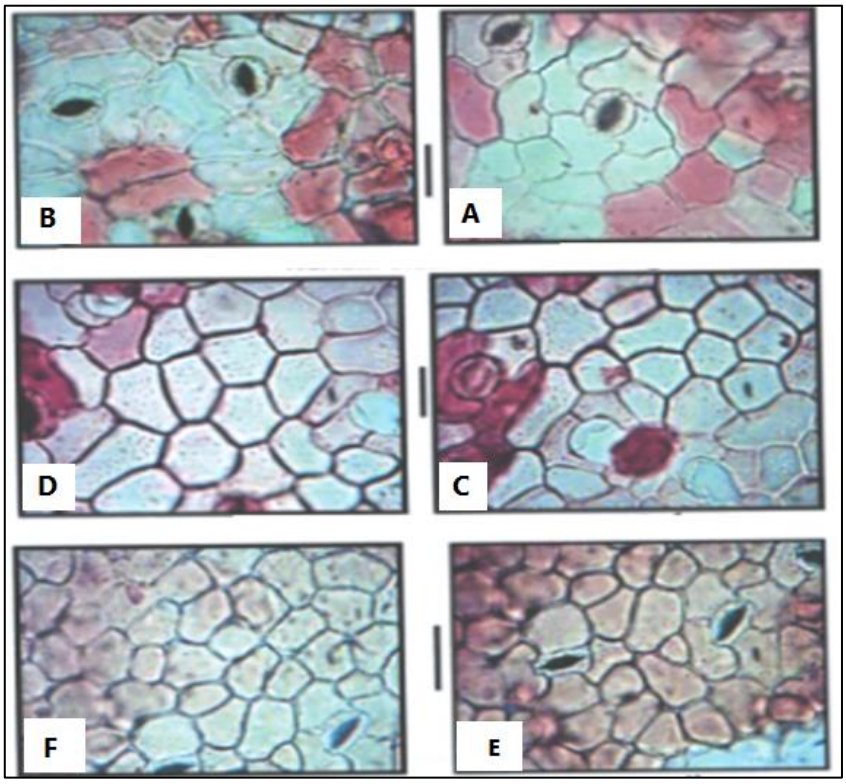

Figure 3. Variation in the epidermis of *N. oleander* leaves in the study region: A and B lower epidermis of Kermat Ali region, C and D the upper epidermis of Kermat Ali: E and F the lower epidermis of Al - Al-Deir region (scale 20 µm).

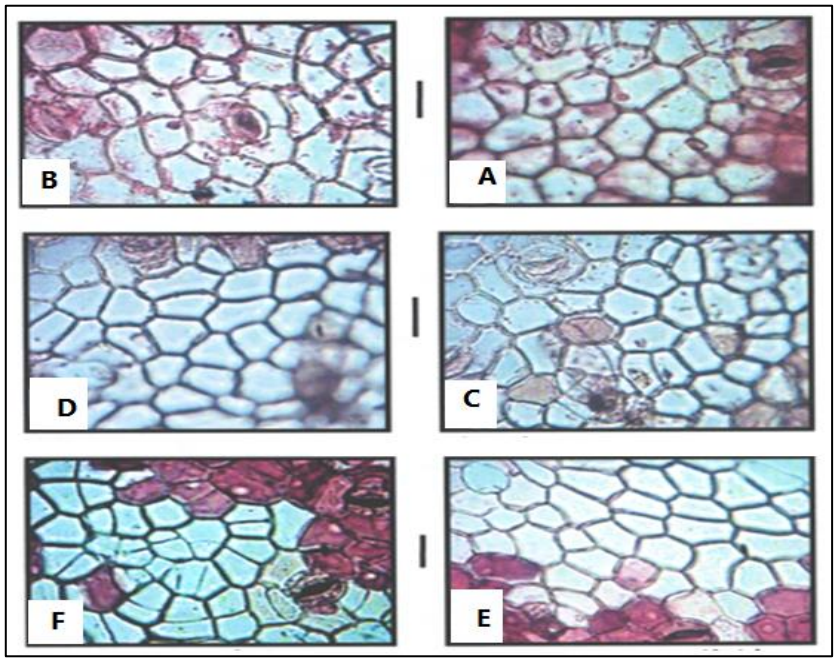

Figure 4. Variation in the epidermis of *N. oleander* leaves in the study areas: A and B upper epidermis of Al- Al-Deir area: C and D, the lower epidermis of Manawy Pasha area: E and F the upper epidermis of Manawi Pasha. (Scale 20 μm).

Table 2. Variation in the dimensions of epidermal cells and stomata of *N. oleander.*

Stations	Dimensions of normal cells of the epidermis				Dimensions of stomata				Number of cells		Number of stomata	
	Upper epidermis		Lower epidermis		Lower epidermis		Upper epidermis					
	Length	an offer	Length	an offer	Length	an offer	Length	an offer	Upper epidermis	lower epidermis	Upper epidermis	lower epidermis
Al-Amin Aldakhli	(12.5-32) 24.57	(15-27.5) 21	(20-40) 31.65	(12.5-27.5) 18.5	(22.5-25) 24	(17.5-20) 18.5	(25-27.5) 25.5	(20-22.5) 20.5	(2100-2400) 2220	(1440-2460) 1959.96	(144-225.33) 215.98	(108-150) 132
Andalus	(20-35) 28.5	(12.5-25) 21	(20-37.5) 28	(12.5-27.5) 18.5	(25-30) 26.5	(20-27.5) 23.5	(25-32.5) 27.5	(25-27.5) 26	(1200-1920) 1599.96	(2400-4200) 3159.96	(108-180) 165.98	(60-72) 66
Karma Ali	(25-50) 37.5	(17.5-30) 22.5	(22-37.5) 30	(12.5-20) 17	(22-30.5) 28.5	(17.5-25) 21	(27-32.5) 30	(20-25) 23.5	(1080-1860) 1579.98	(1020-1440) 1219.98	(92-100) 98	(100-192) 110
Al-Deir	(25-50) 40	(22-37) 29	(12.5-50) 35.82	(15-35) 23.5	(25-27.5) 26.5	(22.5-27) 25	(22-27.5) 20.5	(17.5-22.5) 19.5	(960-1800) 1399.98	(480-1440) 919.98	(156-198) 141.98	(90-139) 108
Minawi Pasha	(22.5-35) 25	(12.5-25) 17.5	(15-30) 24.5	(15-22.5) 19	(25-27) 27.5	(20-25) 23.5	(25-27.5) 26	(20-25) 21.5	(1440-2700) 2239.98	(2040-2640) 2280	(180-216) 198	(162-210) 183.96

Conclusion

The number and dimension of stomata has good evidence of air pollution with H_2S. So that, the plant makes many adaptations to bear the presence of high quantities of pollutants in the areas. The decrease in the size of stomata is one of the adaptations carried out by plants that reduce the influence of pollutants and occur as a result of a rapid response to external stimuli. Our results observed that the

stomata closed in the area have higher concentration of H_2S, stomata open or close is affected to H_2S responds by inducing stomatal closure.

References

[1] Abbasi, T.; Poornima, P.; Kannadasan, T. (2013). Acid rain: Past, present, and future. Int. J. Environ. Eng. 5:229–272.
[2] Clair, T.; Burns, D.; Rosas-Pérez, I.; Blais, J.; Percy, K. (2011). Ecosystems. In Technical Challenges of Multipollutant Air Quality Management, 1st ed.; Hidy, G.M., Brook, J.R., Demerjian, K.L., Molina, L.T., Pennell, W.T., Scheffe, R.D., Eds.; Springer: New York, NY, USA: pp. 139–229.
[3] Aroca, A.; Gotor, C.; Bassham, D.C. and Romero, L.C. (2020). Hydrogen sulfide: from a toxic molecule to a key molecule of cell life. Antioxidants 9: 621.
[4] Offre, P.; Spang, A. and Schleper, C. (2013). Archaea in biogeochemical cycles. Annual Review of Microbiology 67: 437–457.
[5] Robinson, E. and Robbins, R.C. (1970). Gaseous sulfur pollutants from urban and natural sources. Jour. Air. Poll.Control Assn. 20:233-235pp.
[6] Ausma,T. and Kok, L.J.D. (2019). Atmospheric H2S: Impact on Plant Functioning, Front. Plant Sci.,10:1-10.
[7] Bates, M.; Garrett,N.; Shoemack, P. (2002). "Investigation of health effects of Hydrogen Sulfide from ageothermal souring" Archives of Environmental Health, 57:405-411.
[8] Glass, DC 1990. "A Review of the Health Effects of Hydrogen Sulfide Exposure". Annals of Occupational Hygiene. 34(3): 323-327.
[9] Gotor, C.; García, I.; Aroca, Á.; Laureano-Marín, A.M.; Arenas-Alfonseca, L.; Jurado-Flores, A.; Moreno, I. and Romero, L.C. (2019). Signaling by hydrogen sulfide and cyanide through post-translational modification. Journal of Experimental Botany 70: 4251–4265.
[10] Zhou, M.; Zhang, J. and Shen, J. (2021a). Hydrogen sulfide-linked persulfidation of ABI 4 controls ABA responses through the transactivation of MAPKKK18 in Arabidopsis. Molecular Plant 14, 921–936.
[11] Zhou, M.; Zhou, H.; Shen, J.; Zhang, Z.; Gotor, C.; Romero, L.C.; Yuan, X. and Xie, Y. (2021b). H2S action in plant life cycle. Plant Growth Regulation 94, 1–9.
[12] Abdirakhimov, M.; Al-Rashed, M.H. and Wójcik, J. (2022). Recent Attempts on the Removal of H2S from Various Gas Mixtures Using Zeolites and Waste-Based Adsorbents. Energies, 15, 5391.
[13] Radford, A. E.; Dikison, W. C.; Massey, J. R. and Bell. C. R. (1974). "Vascular plants systematics", Harper and Row ،New York.
[14] Strtic, L. (2000). Hydrogen Sulfide, Oil and Gas, and People's Health, Master's of Science, May 2006, Energy and Resources Group. University of California, Berlcyley.
[15] Kilburm, Kaye H. (1999). "Evaluation of health effects from exposure to Hydrogen Sulfide: central nervous system dysfunction". Environmental Epidemiology and Toxicology 1: 207-216pp.
[16] Carman, N, (2003). Hydrogen sulfide and its health. from oil hog farms.
[17] Hetherington, A.M. and Woodward, F.I. (2003). The Role of Stomata in Sensing and Driving Environmental Change. Nature, 424: 901-908.
[18] Verma, R. B., Mahmooduzzafar, T. O. Siddiqi and M. Iqbal (2006). Foliar Response of Ipomea pes-tigridis L. to CoalSmoke Pollution, Turkish Journal of Botany. 30(5):413- 417.
[19] Gostin,I.N.(2009). Air Pollution Effects on the Leaf Structure of some Fabaceae Species. . Bot. Hort. Agrobot. Cluj. 37 (2):57-63.
[20] Abdulrahman, AA; Oladele, FA (2008). Global warming and stomatal complex type. Ethnobotanical Leaflet 12:333-338.
[21] Mudd, J. B. (1979). “Effects on vegetation and aquatic animals” in Hydrogen sulfide. ed. Assembly of Life Sciences (U.S.) Subcommittee on Hydrogen Sulfide (Baltimore: University Park Press): 67–79.
[22] Jin, Z.; Xue, S.; Luo, Y.; Tian, B.; Fang, H.; Li, H., and Pei, Y. (2013). Hydrogen sulfide interacting with abscisic acid in stomatal regulation responses to drought stress in Arabidopsis. Plant Physiol. Biochem. 62: 41-46.
[23] Aroca, A.; Zhang, J.; Xie, Y.; Romero, L.C. and Gotor, C. (2021). Hydrogen sulfide signaling in plant adaptations to adverse conditions: molecular mechanisms, Journal of Experimental Botany, Volume 72 (16): 5893–5904.