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Effect of Some Heavy Metals Accumulation on the Proteins Content of *Zea mays* L. Plant

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Abstract

This study aims to determine the potential impact of heavy metal pollution on plant cultivation and its proteins content. This study presents the effect of pollution with some heavy metals (Pb, Ni, Cd, Zn) on total protein content during the vegetative growth stages of maize (*Zea mays* L.) plant via environmental indicators.

Pb, Ni, Cd, and Zn were measured in deposited dust, surface soil, and Maize plant (*Zea mays* L.) samples collected from two sites in Benha city, Egypt, as well as proteins in maize plant were evaluated. Additionally, Ecological indices [geo-accumulation index (I_{geo}), pollution load index (PLI) and contamination factor (CF)] were determined.

The obtained data showed that, the average rate of dust falls in Benha city, Egypt reached to 15.41 g/m^2 /month. The concentration levels of metals are arranged in the order of: Zn > Ni > Cd > pb in the deposited dust and maize plant, while they are arranged in the order of: Zn > Ni > pb > Cd in the surface soil. The wind rose of the meteorological parameters in Benha city shows that site 1 is on up wind direction, while site 2 is located down wind. Consequently, site 2 had higher deposition rates and higher levels of heavy metals than site 1. Furthermore, the total protein content was extremely affected as a result of the heavy metals stress, where the protein content significantly decreased in Site 2 plants compared to those of Site 1. Ecological indices showed that deposited dust and surface soils at site 2 were moderately to heavily pollute with Cd, while they were unpolluted with other metals (Pb, Ni, and Zn).

Keywords: Heavy metals, deposited dust, Surface soil, Ecological indices, Total protein content.

1. Introduction

Air pollution is one of the main concerns in developing countries from the standpoint of environmental safety and public health [1]. Air pollution can directly affect human, plants, animals, and soil, which can have an impact on ecosystems' structure and function and, ultimately, quality of life [2].

Particulate matter (PM) is released into the atmosphere by several processes, such as the burning of biosolids, transportation, and industrial and agricultural processes [3-5]. PM has an impact on the vegetation and climate, which frequently results in contamination of the surface soil and ecosystem [6]. PM can also result in mist, haze, and photochemical smog [7]. PM often contains some heavy metals in various forms that can affect human health through ingestion, adhering to the skin, or inhalation, leading to extremely devastating health risks [8-10].

Falling dust is thought to be a common and significant portion of atmospheric particles. The diameter of these particles is greater than 10 µm and less than 100 µm, they fall to the ground due to their gravity. Dust fall, as an important component of atmospheric aerosols, can impact the quality of air, climate, and urban environment [11-14], and is therefore considered one of the most important indicators of environmental quality [15]. Heavy metals, polycyclic aromatic hydrocarbons, Black carbon, and other compounds suspended in the atmosphere are all present in dust fall [16].

The primary cause of soil and airborne dust contamination with heavy metals is anthropogenic activities [17-18]. These activities include industrial discharges, combustion of fossil fuels, pesticide usage, fertilizer application, etc. which may be a factor in their existence in both urban and rural soils [19-20]. Furthermore, both dry and wet deposition allows atmospheric heavy metals to accumulate in soil and plant leaves, increasing their concentrations in both matrices [21]. Heavy metals in soil are readily accumulated in plants and then transported through the food chain, posing a major threat to human health [22]. Numerous researches have employed trees to track atmospheric elemental deposition [23]. The most polluting metals in urban environments are like Cd, Ni, Zn, and Pb. These metals can have several negative effects on human health because of their unique characteristics, which include toxicity, non-biodegradation, long biological half-lives, and potential for bioaccumulation [24-26]. Due to the significant environmental concerns associated with heavy metals, further research has been urged [27-29].

Various studies have shown that the presence of heavy metals in plants depends on the types of plants and their growth environment, such as the presence of these metals in particles dispersed in the atmosphere in addition to their presence in the

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soil [30]. Heavy metal contamination of plant growth media can alter the biological pathway of secondary metabolites, leading to notable alterations in the amount and quality of these metabolites. Certain elements, such as micronutrients or trace elements (Fe, Zn, Ni, Cu, and Cr), are essential for plant metabolism. But certain elements become toxic to plants once their concentration in the growing environment exceeds the normal level [31]. Furthermore, several additional elements possess an unclear biological function and have considerable toxicity to plants, such as Cd and Pb [32-33]. Chibuike and Obiora [34] summarize some impacts of heavy metals on maize plants. Cadmium can reduce plant shoot growth and cause inhibition of root growth. Nickel causes a decrease in chlorophyll content and reduces enzyme activity. Lead causes reduction in the percentage of germination, a decrease in plant biomass, decreased plant protein content, and growth inhibition. Zinc causes a decrease in chlorophyll content and reduces plant growth. Mineral accumulations and their impact on plant growth were the most important points of interest in many studies that addressed the effects of air quality on vegetation in Egypt. Emberson et al. [35] and Abdel Latif [36] addressed the effect of ambient air in the Greater Cairo region and around the main traffic routes within the Nile Delta region; it was conducted under real field conditions and revealed that the atmosphere of many areas is very harmful to Egyptian plants.

Proteins are responsive macromolecules that have roles in membrane fusion, structural support, protection, intra- and intercellular transport of nutrients and other molecules, regulation, catalysis, and cellular signaling [37]. The structure of a protein is essentially what determines its function; it is obtained after the amino acid chain undergoes ribosome synthesis. Furthermore, the physical and chemical conditions of the protein environment, which are impacted by high temperatures, reactive molecules, heavy metal (HM) ions, and other stressors, have a significant impact on the conformation of proteins. These conditions not only cause misfolding in proteins that are already formed, but also interfere with the folding process of newly synthesized proteins [37-39]. Since proteins are functionally the major workhorses of living cells, they utterly reflect the healthy status of the plant cell. Heavy Metals stress also dramatically affects the protein content in *Zea mays* L. crop.

Therefore, the objectives of this study were to evaluate: I) the levels of heavy metal contamination (Pb, Ni, Cd, and Zn) in deposited dust and surface soil during the vegetative growth stage of maize plants, as well as the concentrations of these metals accumulated in the plants at two sites in the Benha city, Egypt. III) Their influence on plant total protein content.

2. Materials and Methods

Description of Study area

Benha is located in Qalyubia Governorate, 48 km (30 miles) north of Cairo with coordinates: 30°27′39″N and 31°11′15″E; city area about 10.9 km2 (4.2 sq mi); Population about 182,254 people (City population, 2023). It is situated on the east bank of the Nile River's Damietta Branch (Figure 1). The city of Benha contains large areas of agricultural land, well-irrigated by canals leading off the Delta Barrage, in addition to industrial areas; nowadays, Benha is thought to be one of Egypt's major industrial sectors for electronics. Furthermore, it serves as a major center for the rail network that runs north from Cairo and has Egypt's sixth-largest train station.

Samples were collected from two sites in Benha city, Egypt, which are Site 1 (Al-Shamout village) and site 2 (Kafr Al-Arab village). Site 1 (Al-Shamout village) is a rural area, characterized by calm life, with a population of about 20,450 people according to the official census of 2006. Most of them practice agricultural activities, as there are no factories or companies in this village. The village of Al-Shamout contains many agricultural fields that are planted with the seasonal crop determined by the agricultural association of the village.

Site 2 (Kafr Al-Arab village): is one of the villages belonging to the Qalyubia Governorate in Egypt, (downwind direction) with population of about 8,235 people according to the official census of 2006, in which a rural life prevails (Fig. 1). There are many barns for raising cows and buffaloes, and poultry farms adjacent to the area under study; The waste of these barns is burned from time to time. Kafr Al-Arab village also has many public roads and means of transportation, in addition to an upper bridge passes through it, that follows the Shubra-Benha free road, lower than one kilometer away from the study area.

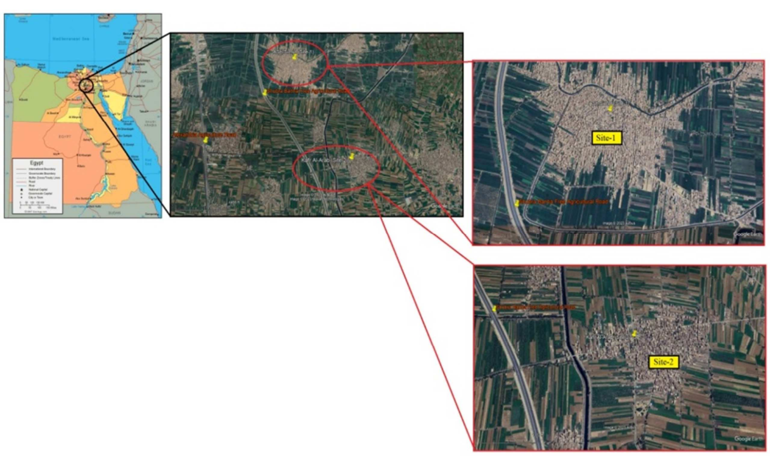

Figure 1: The sampling sites in Benha city, Egypt

Sample collection and analysis
Maize plant cultivation
Biological material

Visually Healthy and uniform sized seeds of *Zea mays* L. (Maize) plants were acquired from the Centre of Agriculture Research, Giza, Egypt. The seeds were grown in the two chosen sites in Benha city. Firstly, Seeds, with 0.01% mercuric chloride, were sterilized for 5 minutes, and then washed thoroughly with distilled water. After that the seeds were sowed at a rate 5 seeds/ bag then thinned to one plant/ bag. Eighty plastic bags with a mixture of soil (sand: clay, 1:2 v/v) were used to set up the pot trials under completely randomized block design (CRD) in every site. Super phosphate and urea fertilizers were added to the bags as regular fertilization practice after they were irrigated with water-holding capacity. In order to investigate the impact of heavy metal contamination on the plants growing in the two sites, samples were collected every 15 days after sowing (15 DAS) through the three months of the vegetative stage. The plant samples were tested for their content of accumulated concentrations of Pb, Ni, Cd, and Zn, as well as total protein content.

Determination of heavy metals in deposited dust (dustfall)

Monthly samples of deposited dust (dustfall) were collected from the two chosen areas in Benha City for three months (April - July): the period of planting Maize (*Zea mays* L.) until vegetative growth. Dust fall collectors were used for collecting the deposited dust [40-44]. Cylindrical glass beakers with a height of 17 cm and a diameter of 8 to 9.5 cm make up the collectors (Figure 2). To prevent the collected dust from being re-entrained, the glass beakers were half filled with distilled water and were placed on iron tripods at a height of 1.2 m above the surface level to prevent surface dust collection. Dust collectors were replaced monthly. The monthly rate of deposition at the different sites was calculated in gm/m².30 days. 1 g of the dried dust samples was weighed accurately and transferred with acid mixture of HNO_3 and HCl (3:1) into clean vessel. Then, the vessel was transferred in an ultrasonic water bath (Ultrasons H, J.P., Selecta, Spain) for 30 min at $70^{O}C$ till digestion. After that, 10 ml of distilled water was added and the mixture that results in was filtered with filter paper (Whatman no. 42). Following that, the solution was diluted to 25 ml in a volumetric flask. Before being used, all the glassware and plastic vessels were treated for 24 hours with diluted (1:1) nitric acid and then rinsed with water [12]. Metals (Cd, Zn, Ni and Pb) were determined in the dustfall samples by analytical method using inductively coupled plasma (ICP – Perkin Elmer).

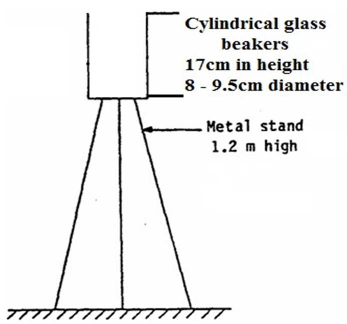

Figure 2: Sketch of deposited dust collectors (source: Mohammed et al. [12])

Determination of heavy metals in surface soil

Samples of surface soil have been taken from places in the investigated sites. By using a hand auger, the soils were sampled at a depth of 10 cm below the surface and stored in plastic bags at room temperature. 0.5 gm of soil samples were weighed and were extracted into 20 ml of 1M HNO_3 contained in glass vials and agitated within an ultrasonic water bath (Ultra-sons–H, J.P., Selecta, Spain). Then, the extracted solution was filtered using Whatman no. 41 filter, completed to 25 ml with HNO_3 (1M) and kept in pre-cleaned bottles in the refrigerator until analyzed [12, 45-47]. Metals (Cd, Zn, Ni and Pb) in surface soil samples were detected by analytical method using inductively coupled plasma (ICP – Perkin Elmer).

Determination of heavy metals in maize (*Zea mays* L.) plant

Maize (*Zea mays* L.) plant samples were collected every 15 days at the two chosen sites in Benha City, for three months (April - July) which is the period of planting Maize (*Zea mays L.*) until vegetative growth (6 growth stages), and before the formation of fruits. Wet ashing method was used for determination of heavy metals bioaccumulation in maize (*Zea mays* L.) plant [48]. Plant leaves were dried in an oven at $80^{O}C$ until constant weight. The resulting oven-dried materials were digested using the method of Ruiz-Huerta et al. [48], One gram of ground leaves was weighed in a flask (250 ml) which was previously washed with acid and distilled water. 10 ml of acid mixture (HNO_3 and $HClO_4$, 4:1 (V/V) was added, and the mixture was thoroughly digested using a hot plate in a fume hood until white vapors appeared, demonstrating that the plant samples had completely digested. After allowing the samples to cool, they were quantitatively transferred into a 50 ml volumetric flask and diluted with distilled water. Then, filtration was carried out using filter paper Whatman No.42. The volume was completed to 50 ml with distilled water and kept in pre-cleaned bottles in the refrigerator until analyzed. Metals (Cd, Zn, Ni and Pb) in plant samples were determined by analytical method using inductively coupled plasma (ICP – Perkin Elmer) [48].

Extraction and estimation of total protein in *Zea mays* L. plant

Proteins were extracted by homogenizing *Zea mays* L. leaves with liquid nitrogen in cold 0.05 M Tris buffer [49]. Proteins were determined using Bradford method [50]. Absorbance was recorded at 595 nm using Spectrophotometer (Carl Zeiss

Spekol, USA), and bovine serum albumin was used as a standard protein. All data were calculated on a dry matter basis as mg 100 g^{-1} dry matter.

Determination of ecological indices

Ecological indices (geo-accumulation Index (I_{geo}), contamination factor (CF) and pollution load index (PLI)) were determined to examine the effects of metals pollution detected during this study as indicators.

Geo-Accumulation Index (I_{geo})

The geo-accumulation index (I_{geo}) was applied to evaluate the pollution level of heavy metals. The I_{geo} was calculated as follows [43-44, 51-55]:

$$\mathbf{I_{geo} = \log_2 [C_i / (1.5B_i)]} \text{ -------------------------- } \mathbf{(Eq.\ 1A)}$$

Where:

C_i: is the concentration of heavy metal i in deposited dust, and surface soil (mg/kg)

B_i: is the local background concentration of metal i in soil (mg/kg), (Ni: 20 mg/kg; Zn: 67 mg/kg; Cd: 2 mg/kg; and Pb: 80 mg/kg [10, 56])

1.5: is the coefficient in the equation, it is used to minimize the effect of possible variations in the background values

The I_{geo} for each metal is classified using seven (0–6 grades) enrichment classes (Muller 1969) (Table 1).

Contamination factor (CF)

A contamination factor (CF) is used to express the degree of metal contamination in soil dust and calculated as [57]:

$$CF = [Cm_{\text{sample}} / Cm_{\text{Background}}] \text{ -------------------------- } \mathbf{(Eq.\ 1B)}$$

Where: Cm_{sample}: is the concentration of metal in soil dust. $Cm_{\text{background}}$: is the background value for the metal. If the contamination factor CF < 1 refers to low contamination; 1 ≤ CF < 3 means moderate contamination; 3 ≤ CF ≤ 6 indicates considerable contamination and CF > 6 indicates very high contamination [57].

Pollution load index (PLI)

The integrated pollution load index (PLI) was calculated by finding the n-root from the n-CFs for all heavy metals [58-60].

$$\mathbf{PLI = \sqrt[n]{CF1 \times CF2 \times CF3 \times \ldots \times CFn}} \text{ -------------------------- } \mathbf{(Eq.\ 1C)}$$

Where: n is the number of metals. The classification of PLI was as follows: PLI ≤ 1, low level; 1 < PLI ≤ 2, middle level; and PLI > 2, high level [18, 61-62].

Table 1: The geo-accumulation index (I_{geo}) for heavy metals [10, 51]

I_{geo}			
Grade	**Level**	**Grade**	**Level**
≤ 0	Unpolluted	3–4	Heavily polluted
0–1	Lightly polluted	4–5	Heavily to extremely polluted
1–2	Moderately polluted	> 5	Extremely polluted
2–3	Moderately to heavily polluted		

Wind Rose Plots for meteorological data (WRPLOT View)

To plot meteorological data (WRPLOT View) for Benha city, Egypt, Wind-Rose version 4.41 was used. It provides visual wind rose plots and wind speed classes for a given location and time [63].

3. Results and Discussion

Meteorological data

Figure 3 shows the wind rose of the meteorological parameters in Benha city, Egypt, during the cultivation of maize (*Zea mays* L.) plant. The results showed that most of the prevailing wind directions in the study area were directed from the north and northwest by 67%, which indicates that Site 1 is upwind, and Site 2 is downwind.

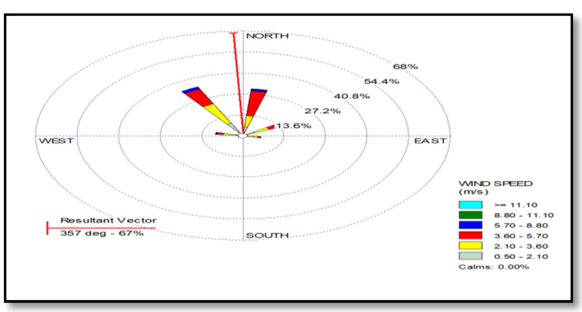

Figure 3: Wind rose of meteorological parameters in Benha city, Egypt.

Deposited dust (dust fall)

Figure 4 shows the average deposition rate of dust fall at the two selected sites in Benha city, Egypt, during maize plant cultivation. The results showed that higher deposition rates were detected at site 2 (with an average of 12.86, 9.82 and 15.41 g/m^2. month) for the first, second, and third months from cultivation. These high deposition rates at site 2 may be attributed to the burning of waste resulting from barns for raising cows and buffaloes and emissions from poultry farms adjacent to this area, in addition to the presence of many unpaved public roads, which cause re-suspension and deposition of dust by means of transportation. Moreover, site 2 is located downwind (as shown by the Wind rose of meteorological parameters in Figure 3) and adjacent to the main road (Shubra-Benha free road).

Table 2 shows a comparison of the average dust fall deposition rate at the two selected sites in Benha city, Egypt, with that in other countries around the world. This table showed that the average dust fall deposition rates in the city of Benha, are almost similar to the results found in Iraq (ranging between 4.86 and 8.01 mg/m^2 per 30 days) [15] and lower than the results found in Kharagpur, India, (20.00 mg/m^2 per 30 days) due to the different meteorological factors as high wind speed, rainfall and low humidity [64]. Dust fall rate has no Air Quality limit values. However, western countries typically declared that the area may be considered clean when dust fall values are less than 10 g/m^2 per 30 days (month) [65].

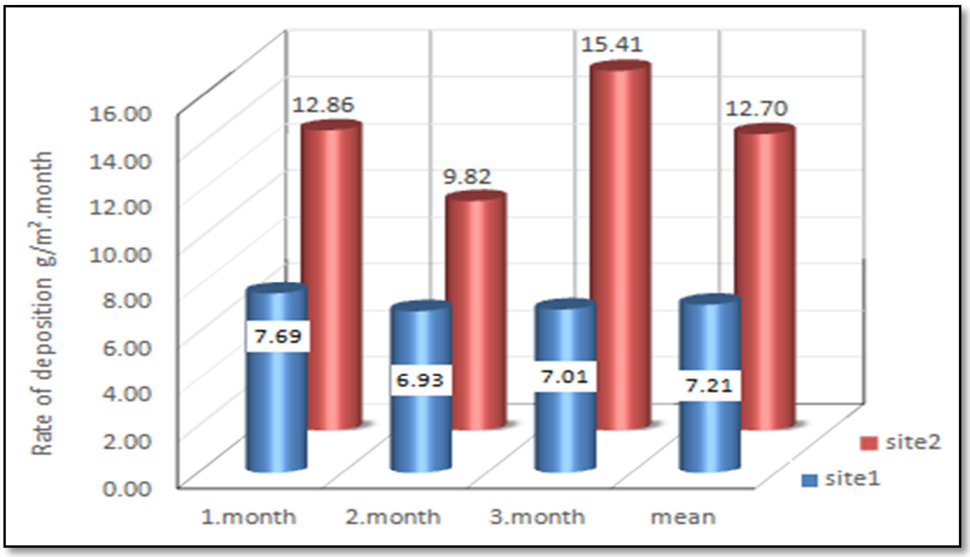

Figure 4: Average deposition rate of dust fall at the two selected sites in Benha city, Egypt, during maize plant cultivation

Table 2: A comparison of dust fall deposition rates in the current study with others in the world

Country		Rate of deposition (g/m^2.30day)	References
Egypt	Benha, site-1	7.21	The current study
	Benha, site-2	12.70	
Iraq	Duhok,	5.01	[15]
	Koya	4.86	
	Pirmam	5.04	
	Makhmur	5.34	
	Erbil	8.01	
India	Kharagpur	20.0	[64]

Heavy metals concentration levels in dust fall, surface soil, and maize (*Zea mays L.*) plant

Table 3 shows average concentration levels of heavy metals in dust fall collected from the selected sites in Benha city, Egypt, during the growing period of maize. This table shows that the average concentration of Cd, Ni, Pb, and Zn were 3.4, 9.8, 0.7, and 11.8 mg/kg, respectively, in dust fall samples collected from site 1, and 9.5, 15.2, 2.3 and 18.6 mg/kg, respectively, in dust fall samples collected from site 2. The results indicate that the investigated metals in the dust fall over site 2 were higher than site 1. This is consistent with the results which found that Site 2 had a higher deposition rate compared to Site 1. The concentration levels of metals in dust fall were arranged in the following order: Zn > Ni > Cd > pb as shown in Figure 5.

Table 3 also shows the concentration of heavy metals in surface soil samples collected from the selected sites in Benha city, Egypt, over growing period. The table shows that the concentration levels of Cd, Ni, Pb, and Zn increased in surface soil with the time of growth, reached to 2.1, 23.9, 9.1 and 49.2 mg/kg, respectively, in the surface soil samples collected from site 1, and 2.4, 25.08, 10.03 and 51 mg/kg, respectively, in surface soil samples collected from site 2 after 90 days of cultivation. The results indicated that the concentration levels of selected heavy metals in the surface soil at Site 2 were slightly higher than

those at Site 1. The results showed that zinc has a higher concentration than other metals in the surface soil. The concentration levels of metals in the surface soil were arranged in the following order: Zn > Ni > pb > Cd as shown in Figure 4.
Table 3 also indicates concentration levels of heavy metals in leaves of maize plant that cultivated at the two sites in Benha city, during the vegetative growth period. The table shows that the concentration levels of the selected heavy metals increased in leaves of maize plant with the period of growth. The concentration levels of Cd, Ni, Pb, and Zn, after 90 days of cultivation, reached to 3.18, 4.12, 2.62 and 14.4 mg/kg, respectively, in leaves of maize plant samples collected from site 1, and 4.31, 4.43, 2.85 and 15.12 mg/kg, respectively, in leaves of maize plant samples collected from site 2. The results demonstrated that specific heavy metal concentrations in the leaves of the maize plant grown at Site 2 were higher than those at Site 1. These results are consistent with the concentrations of those metals in the surface soil and dust fall. The arrangement of heavy metals found in the leaves of maize plant was similar to their arrangement in the dust fall samples as shown in figure 5.

Table 3: The average concentration levels of heavy metals in dust fall, surface soil, and maize leaves (*Zea mays* L.) collected from Benha city, Egypt.

Heavy metals	Sampling sites	Sampling period	Cd	Ni	pb	Zn
Dust fall (mg/kg)	Benha, site-1	1	1.7	1.7	0.6	8.2
		2	3.9	15.9	0.7	13.6
		3	4.5	11.8	0.9	13.8
		Average	**3.4**	**9.8**	**0.7**	**11.8**
	Benha, site-2	1	2.7	8.4	4.2	11.5
		2	14.2	18.2	1.1	24.6
		3	11.5	19.0	1.5	19.8
		Average	**9.5**	**15.2**	**2.3**	**18.6**
Surface soil (mg/kg)	Benha, site-1	15days	1.5	16.4	4.3	29.9
		30days	1.5	17.4	4.5	30.5
		45days	1.6	19.2	4.7	34.2
		60days	1.9	20.7	6.4	41.6
		75days	2.02	23.6	7.9	48.4
		90days	2.1	23.9	9.1	49.2
		Average	**2.0**	**22.7**	**7.8**	**46.4**
	Benha, site-2	15days	1.6	16.8	4.5	30.9
		30days	1.6	18.5	4.7	33.2
		45days	1.8	20.2	5.6	36.8
		60days	2.07	22.5	6.8	45.9
		75days	2.24	24.8	8.2	48.9
		90days	2.4	25.08	10.03	51
		Average	**2.2**	**24.1**	**8.3**	**48.6**
Maize plant (mg/kg)	Benha, site-1	15days	1.17	2.04	0.95	5.58
		30days	1.26	2.23	1.11	6.87
		45days	1.64	2.65	1.55	9.19
		60days	1.76	2.78	1.63	12.67
		75days	2.58	3.65	2.00	13.45
		90days	3.18	4.12	2.62	14.40
	Benha, site-2	15days	1.29	2.15	1.09	9.46
		30days	1.45	2.31	1.28	9.96
		45days	1.78	2.59	2.05	12.24
		60days	2.16	3.14	2.11	13.70
		75days	2.92	3.88	2.21	14.23
		90days	4.31	4.43	2.85	15.12

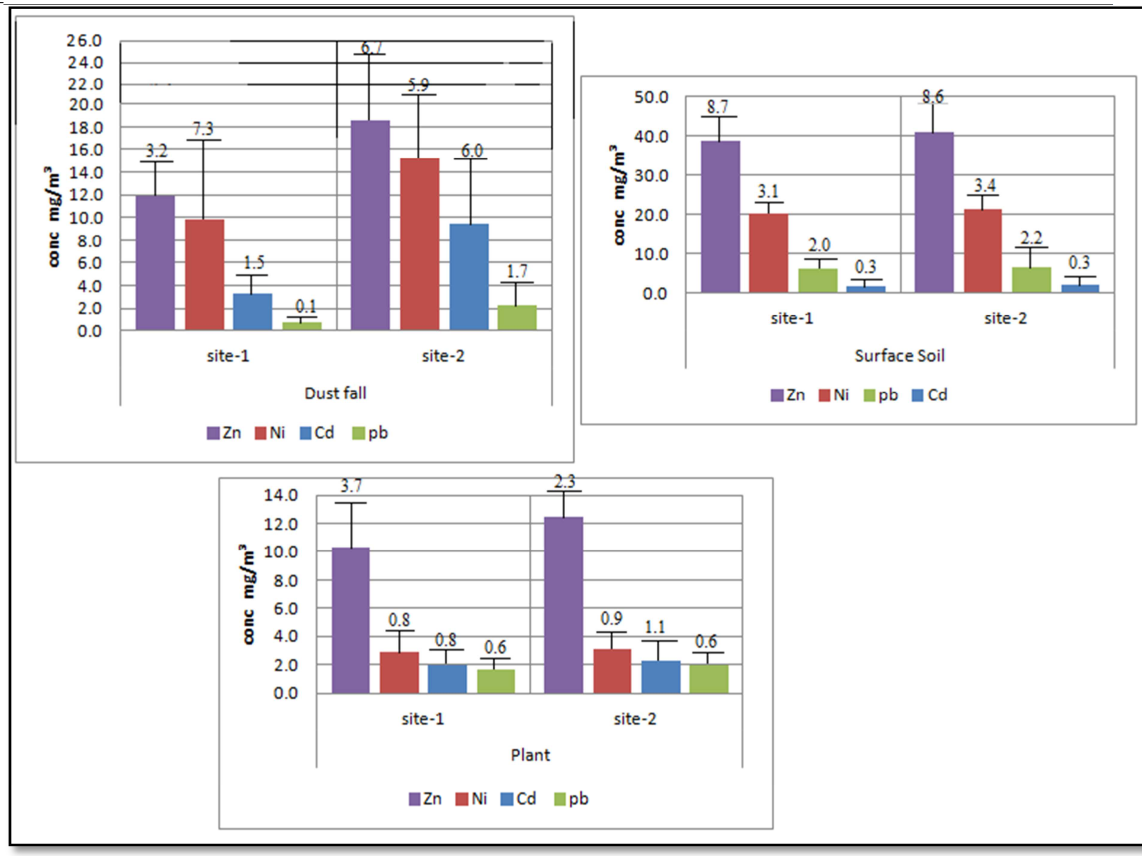

Figure 5: The arrangement of heavy metals found in dust fall, surface soil, and maize (Zea mays L.) plant collected from Benha City.

Table 4 shows a comparison of the recorded levels of heavy metals in dust fall, surface soil, and maize plant of Benha city with those in other countries around the world. This table shows that, except for cadmium in the surface soil, the selected metals concentrations found in dust fall, surface soil, and maize plant at the two investigated sites were much lower than those found in most other countries around the world.

Table 4: A comparison of concentration levels of heavy metals in dust fall, surface soil, and maize plant (Zea mays L.) with countries around the world

Country		Heavy metals concentration levels				References
		Pb	**Ni**	**Cd**	**Zn**	
Dust fall (mg/kg)						
Egypt	Benha, site-1	0.7	9.8	3.4	11.8	The current study
	Benha, site-2	2.3	15.2	9.5	18.6	
	Assiu	22	-	4.5	235	[66]
	Aswan	-	-	3.7	-	[25]
China	Zhanjiang	86	188	39.5	482	[67]
	Baotou city	36	25	0.3	49	[68]
India	West	160	-	8.5	288	[66]
Poland	Urban	470	36	12.5	318	
Iran	Ahvaz	74	12	0.5	554	[69]
	Khorramabad	32	60	11.3	-	[25]
Saudi Arabia	Urban	23	-	-	-	
UK	Urban	-	43	2.0	-	
Australia	Urban	-	49	2.5	-	
USA	Urban	-	23	-	-	
Iraq	Duhok	2	0.2	0.2	1.5	[15]
Turkey	Istanbul	28	236	0.8	832	[69]
Australia	Sydney	76	15	1.6	372	
Malaysia	Urban	31	9	-	148	

Country		Heavy metals concentration levels Pb	Ni	Cd	Zn	References
KSA	Urban Riyadh	5	4	0.012	13	[18]
	Suburban Riyadh	4	6	-	11	
	Jeddah	141	51	-	488	
Angola	Luanda	351	10	1.1	317	[70]
Jordan	Petra	177	-	1.0	129	
Surface soil (mg/kg)						
Egypt	Benha, site-1	6.2	20.2	1.8	39.0	The current study
	Benha, site-2	6.6	21.3	2.0	41.1	
	Abu-Qir	28.2	71.9	2.5	115.8	[44]
	Alexandria	81.7	-	-	198.1	[71]
	Luxor	39.8	13.7	0.5	128.6	[72]
	Aswan	42.7	15.9	0.8	72.2	
	Habo	11.4	83.1	0.7	135.2	
	Edfo	9.5	71.2	0.5	113.1	
	Fiyla	15.1	23.3	0.4	410	
KSA	Riyadh (Urban)	0.4	0.2	0.1	0.2	[18]
	Riyadh (Suburban)	0.3	0.1	-	0.2	
India	Delhi	120.7	36.4	2.65	284.5	[73]
Korea	Seoul	245	-	3	296	
Norway	Oslo	180	41	-	412	
China	Hong Kong	181	-	3.77	1450	
	Beijing	69.6	41.10		248.5	[74]
Ghana	Konongo	18.4	-	1.04	9.30	[75]
Nigeria	Jos Metropolitan	18.1	0.47	0.63	26.3	[76]
Jordan	Baqa'a area	10	90	0.10	87	[77]
Mexico	San Luis	36.9	3.2	0.95	-	[20]
Bangladesh	Tangail	12.1	5.8	1.35	-	[78]
India, Background		13.1	27.7	0.9	22.1	[73]
China, Guidelines		300	50	0.3	250	
Canada, Guidelines		600	50	22	360	
Australia, Guidelines		300	60	3.0		[79]
WHO/FAO		50		0.8	300	[75]
WHO		100	50	3	300	[80]
Maize plant (mg/kg)						
Egypt	Benha, site-1	1.6	2.9	1.9	10.4	The current study
	Benha, site-2	1.9	3.1	2.3	12.4	
Pakistan	Peshawar	49.1	-	10.9	-	[81]

Impacts of heavy metals pollution on Protein contents in maize (*Zea mays L.*) plant

Fig. 6 shows the total protein content in leaves of maize plant (*Zea mays L.*) collected from the two studied sites in Benha city, Egypt, during the vegetative growth stage. This figure shows that the total proteins increased with the stages of vegetative growth from 4235 to 14712 mg/100g d.wt at site 1 and from 3518 to 13983 mg/100g d.wt at site 2. The results indicated that the total proteins significantly decreased in the more polluted site with heavy metals (Site 2), compared to the less polluted site (Site 1) at 15, 30, 45, 60, and 75 days. This decrease in total protein content is in consistency with higher accumulated concentrations of the examined heavy metals (Pb, Ni, Cd, and Zn) in dust fall, surface soil and maize leaves. These conflicting levels of proteins and heavy metals may be attributed to the consumption of the protein content found in the plant tissues in certain metabolic processes to counteract the deleterious effects of the heavy metals on the cellular activities [82].

Furthermore, the declined levels of total protein content as a result of the elevated levels of heavy metals in Site 2 may be attributing to the removal and degradation of proteins when these proteins failed to restore their normal conformation after disruption owing to the metal toxicity [83].

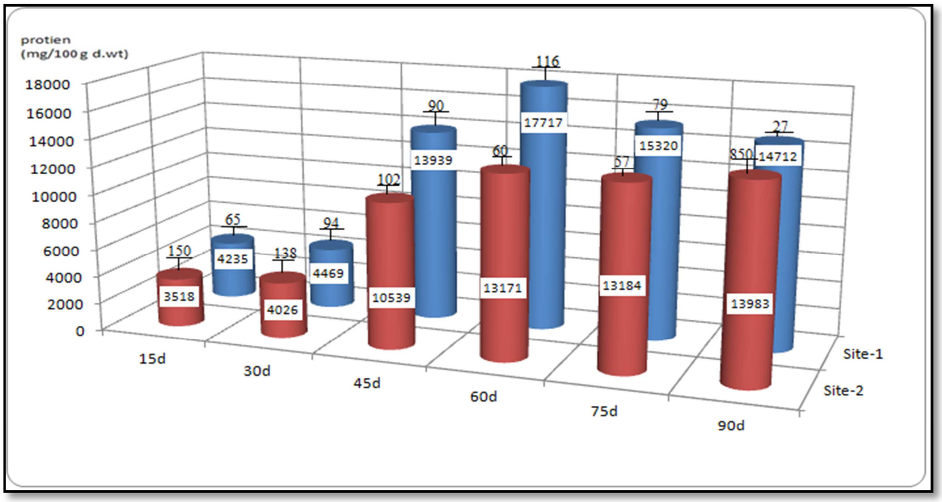

Figure 6: Total protein content (mg/100g d.wt) in Maize plant (*Zea mays L.*) leaves at the two studied sites.

Table 5 shows the Pearson Correlation of heavy metals in maize plant with metals contents in dust fall and surface soil of both sites at Benha city. This table showed that there is a higher significant positive correlation between heavy metals (Cd, Ni, Pb, and Zn) in dust fall and surface soil vs in plant. Concerning the relation between maize contents of metals and proteins for each stage, there was a significant negative correlation in all growth stages, except at the last stage as this negative relation was not significant. Heavy metals ions were found to inhibit the cellular protein homeostasis and, consequently cell viability, through the physiological interferes with protein folding and aggregation of nascent or non-native protein. It is evident that plant responses to heavy metals and other environmental stresses could induce gene expression which affect protein encode. However, cellular protection mechanism systems of plant can efficient enhancement the tolerance against such stresses impacts. Plants tolerance respond mechanisms to metals toxicity include chelation and compartmentalization of the metals ions as well as repair or removal of damaged proteins [83-84].

Table 5: The Pearson correlation of heavy metals contents in maize plants with the corresponding concentrations in dust fall and surface soil as well as protein contents

	Dust fall	Surface soil	Maize	Protein[1]
	Cadmium (Cd)			
Dust fall	1	0.869**	0.965**	
Surface soil		1	0.934**	
Maize			1	- 0.677
Protein[1]				1
	Nickel (Ni)			
Dust fall	1	0.913**	0.934**	
Surface soil		1	0.964**	
Maize			1	- 0.740
Protein[1]				1
	Lead (Pb)			
Dust fall	1	0.879**	0.951**	
Surface soil		1	0.936**	
Maize			1	- 0.772
Protein[1]				1
	Zinc (Zn)			
Dust fall	1	0.646*	0.592*	
Surface soil		1	0.930**	
Maize			1	- 0.614
Protein[1]				1

[1]: Protein contents of the last growth stage (90 days)
*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

Ecological indices

Geo-Accumulation Index (I_{geo})

The geo-accumulation index (I_{geo}) of heavy metals was evaluated in dust fall and surface soil collected from the two investigated sites in Benha city during the cultivation of maize (*Zea mays* L.) plant (Fig. 7). The I_{geo} values for each metal are classified, according to Muller [55], into seven enrichment classes (0–6 grades) (Table 1). The results in figure 7 shows that dust fall is lightly polluted with Cd at site 1, and moderately to heavily polluted with Cd at site 2, while it is unpolluted with other metals (Pb, Ni, and Zn). Furthermore, the surface soil is unpolluted with metals (Cd, Pb, Ni, and Zn) at the two sites.

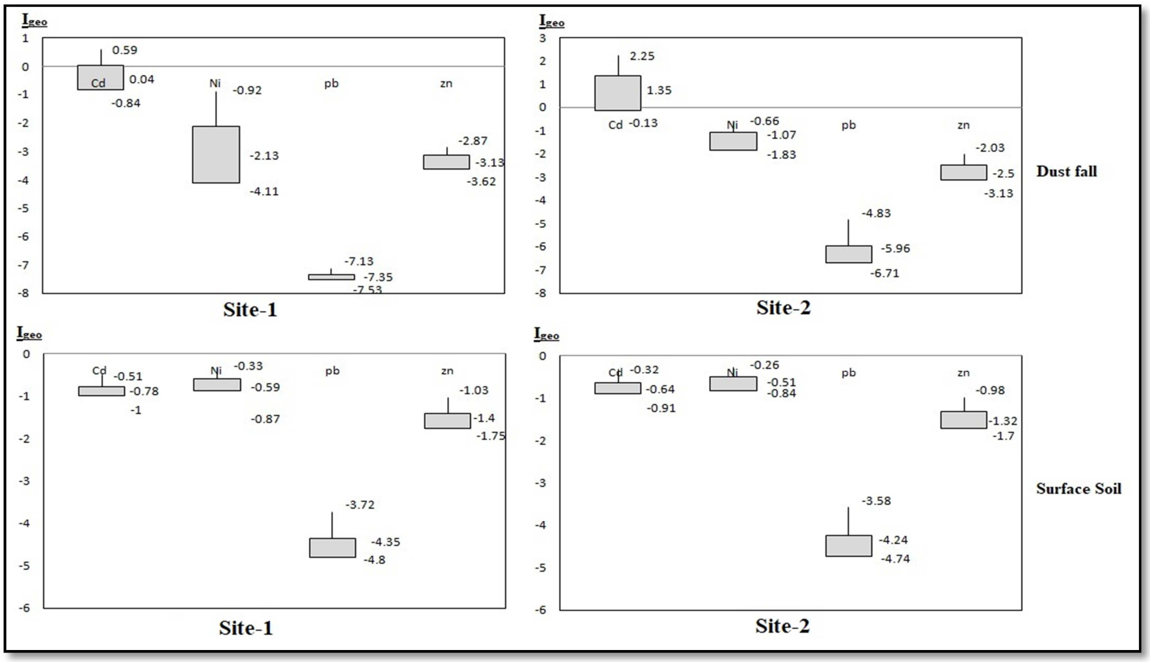

Figure 7: The geo-accumulation index (I_{geo}) of heavy metals (Pb, Ni, Cd, and Zn) in dust fall and surface soil collected at the two studied sites.

Contamination factor (CF) and pollution load index (PLI)

The contamination level was evaluated in terms of contamination factor (CF) and integrated pollution load index (PLI) for heavy metals in dust fall and surface soil collected from the two selected sites in Benha city, Egypt during maize (Zea mays L.) cultivation (Table 6). CF values for each metal were classified according to Gao et al. [57] into four categories. The results indicated that the dust fall in site 1 is low to moderate contaminated with Cd, but it is low contaminated with other metals. While in site 2 it is moderate to very high contaminated with Cd, low to moderate contaminated with Ni and low contaminated with Pb and Zn. Furthermore, Surface soil is low to moderate contaminated with Cd and Ni, but low contaminated with Pb and Zn in the two sites.

In addition, the integrated pollution load index (PLI) was calculated for all heavy metals according to Soares et al. [58], Alghamdi et al. [59], Thomilson et al. [60]. The results showed that dust fall and surface soil are low loaded with metals (Cd, Pb, Ni, and Zn) at the two sites.

Table 6: Contamination factor (CF) and pollution load index (PLI) of heavy metals in dust fall and surface soil collected at the two studied sites.

Heavy metals			CF				PLI
			Cd	Ni	Pb	Zn	
Dust fall	Site-1	1.month	0.8 ^{a}	0.1 ^{a}	0.0 ^{a}	0.1 ^{a}	0.01 ^{i}
		2.month	1.9 ^{b}	0.8 ^{a}	0.0 ^{a}	0.2 ^{a}	0.05 ^{i}
		3.month	2.3 ^{b}	0.6 ^{a}	0.0 ^{a}	0.2 ^{a}	0.05 ^{i}
	Site-2	1.month	1.4 ^{b}	0.4 ^{a}	0.1 ^{a}	0.2 ^{a}	0.07 ^{i}
		2.month	7.1 ^{d}	0.9 ^{a}	0.0 ^{a}	0.4 ^{a}	0.18 ^{i}
		3.month	5.8 ^{c}	1.0 ^{b}	0.0 ^{a}	0.3 ^{a}	0.17 ^{i}

Surface soil	Site-1	15days	0.8 [a]	0.8 [a]	0.1 [a]	0.4 [a]	0.12 [i]
		30days	0.8 [a]	0.9 [a]	0.1 [a]	0.5 [a]	0.13 [i]
		45days	0.8 [a]	1.0 [b]	0.1 [a]	0.5 [a]	0.15 [i]
		60days	1.0 [b]	1.0 [b]	0.1 [a]	0.6 [a]	0.22 [i]
		75days	1.0 [b]	1.2 [b]	0.1 [a]	0.7 [a]	0.29 [i]
		90days	1.1 [b]	1.2 [b]	0.1 [a]	0.7 [a]	0.32 [i]
	Site-2	15days	0.8 [a]	0.8 [a]	0.1 [a]	0.5 [a]	0.13 [i]
		30days	0.8 [a]	0.9 [a]	0.1 [a]	0.5 [a]	0.15 [i]
		45days	0.9 [a]	1.0 [b]	0.1 [a]	0.5 [a]	0.19 [i]
		60days	1.0 [b]	1.1 [b]	0.1 [a]	0.7 [a]	0.26 [i]
		75days	1.1 [b]	1.2 [b]	0.1 [a]	0.7 [a]	0.32 [i]
		90days	1.2 [b]	1.3 [b]	0.1 [a]	0.8 [a]	0.38 [i]

Where:
[a] CF < 1 means low contamination
[b] 1 ≤ CF < 3 means moderate contamination
[c] 3 ≤ CF ≤ 6 indicates considerable contamination
[d] CF > 6 indicates very high contamination

[i] PLI ≤ 1, refers to low level
[ii] 1 < PLI ≤ 2, means middle level
[iii] PLI > 2, indicates high level

4. Conclusion

Plants often encounter a diverse range of biotic and/or abiotic stresses, such as heavy metal stress. In the current study heavy metals were evaluated in deposited dust, surface soil, and maize plants (*Zea mays* L.), which were planted in two locations in the city of Benha, Egypt, during the vegetative growth stage. Total proteins content and some Ecological indices (such as Geo-accumulation Index (I_{geo}), Contamination Factor (CF) and Pollution Load Index (PLI)) were determined to clarify the implication of heavy metals stress on total protein content in maize plants.

The average deposition rate of dust in Benha City, Egypt, ranged between 6.93 and 15.41 grams/m2. month. Dust deposition rates have increased in the place where waste from cattle and buffalo farms is burned and due to emissions from poultry farms, in addition to the presence of unpaved public roads, which causes resuspension and deposition of dust by means of transportation. Dust deposition rates also increased in the area located downwind and adjacent to the main road. Zinc is the dominant element in dust fall, surface soil, and maize plant in the samples collected from Benha city. The concentration levels of metals were arranged in the order of Zn > Ni > Cd > pb in dust fall and maize plant, while they are arranged in the order of Zn > Ni > pb > Cd in surface soil.

The concentration levels of heavy metals increased in surface soil and maize leaves with the stages of growth in both sites. The total proteins significantly decreased in the more polluted site with heavy metals, compared to the less polluted site. However, the total protein content in maize leaves decreased with increasing heavy metal concentration in maize plant, which may be attributed to the inhibitory effect of heavy metals on protein metabolism. It is believed that heavy metals attach to proteins and change their natural structure, impairing their ability to perform biological tasks by chelating the metal and partitioning it into a vacuole or even removing and degrading plant proteins that fail to regain their original conformations after heavy metals toxicity. This autolysis of proteins as a result of metal toxicity consequently leads to diminished protein levels in the more polluted site plants. A highly significant positive correlation was found between heavy metals (Cd, Ni, Pb, and Zn) in dust fall and surface soil vs in plant. While there was a significant negative correlation between maize contents of metals and proteins in all growth stages, except at the last stage.

The geo-accumulation index (I_{geo}) values of heavy metals concluded that the deposited dust in the investigated sites of Benha City is lightly polluted to heavily polluted with Cd, while they are unpolluted with other metals (Pb, Ni, and Zn) according to Muller (1969). Furthermore, the surface soil is unpolluted with Cd, Pb, Ni, and Zn metals. The values of Contamination factor (CF) concluded that the deposited dust in Benha city is low to very high contaminated with Cd, low to moderate contaminated with Ni and low contaminated with Pb and Zn, according to Gao et al. (2023). In addition, Surface soil is low to moderate contaminated with Cd and Ni, but low contaminated with Pb and Zn. Dust fall and surface soil in Benha city were low loaded with metals (Cd, Pb, Ni, and Zn) according to (Soares et al., 1999; Alghamdi et al., 2022; Thomilson et al., 1980). The results of the study reflect the need to more search to counteract the negative effects of air contamination on such socioeconomic crops to achieve sustainable development.

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