

Effect of Heavy Metals with Mycorrhiza on Vegetative Growth and Chemical Composition of Maize

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تأثير العناصر الثقيلة مع الميكوريزا على النمو الخضري والتركيب الكيميائي لنبات الذرة

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Abstract:

In order to assess the impact of mycorrhizal fungi on the growth and metal contents in maize plants growing in soil contaminated with heavy metals (lead and cadmium) at different concentrations, a pot experiment was carried out in the city flower garden in Tobruk during 2024. In a randomized complete block design (RCBD), the experiment included 13 treatments: Pb (25, 50, 75, 100, 150 mg/l +200g myco.), Cd (50, 75, 150, 200, 250 mg/l +200g mycorrhiza), and negative control (without mycorrhiza). The findings indicated that, in comparison to the positive control (plus 200g mycorrhiza), which recorded the greater of seed germination, plant height, leaf area, total chlorophyll, total fresh and dry weight, and the negative control (without mycorrhiza), higher concentrations of Cd (250 mg/l cd +200g myco.) and Pb (150 mg/l Pb +200g myco.) recorded the lower germination percentage, plant height, leaf area, total chlorophyll, and total fresh and dry weight with increasing concentrations of Cd and Pb. However, in contrast to the positive control (plus 200g mycorrhiza), which recorded the highest percentage of N, P, and K, and the negative control (without mycorrhiza), higher concentrations of Cd (250 mg/l cd + 200g myco.) and Pb (150 mg/l Pb + 200g myco.) recorded the lowest percentage of N, P, and K. Furthermore, greater Pb (150 mg/l Pb +200g myco.) and Cd (250 mg/l cd +200g myco.) concentrations yielded the greatest values of Pb, whereas Pb content yielded higher values with Pb (150 mg/l Pb +200g myco.) and Cd (250 mg/l cd +200g myco.), respectively. In conclusion, the majority of the time, the stress caused by heavy metals decreased the fresh and dry weights of maize shoots, their phosphorus content, and their mycorrhizal levels. Comparing similar non-mycorrhizal plants to maize plants grown in heavy metal-contaminated soil, mycorrhizal colonization generally boosted the growth response of the former. According to this study, cultivating maize with AM inoculum can boost growth and NPK uptake while reducing the toxicity of heavy metals. The AM fungus is useful in the phytoremediation of heavy metal contamination in soil because it plays a partially protective role for the host plant in this way.

Keywords: Maize, Mycorrhiza, Heavy Metals, Vegetative Growth, Chemical Composition.

أجريت تجربة الأصص في مشتل ز هور المدينة بمدينة طبرق خلال منتصف شهر مارس موسم 2024 لتقييم تأثير فطر الميكوريزا على نمو ومحتوى المعادن في نباتات الذرة الصفراء المزروعة في تربة ملوثة بالمعادن الثقيلة)الكادميوم

الملخص

والرصاص) بتركيزات مختلفة. تكونت التجربة من 13 معاملة، وهي الكنترول الإيجابي (مع 200 جم فطر الميكوريزا)، الكنترول السلبي)بدون فطر الميكوريزا(، الكادميوم بتركيز (،50 ،75 ،150 ،200 250 مجم/ لتر + 200 جم ميكوريزا) والرصاص (25، 50، 75، 100، 150 مجم/ لتر + 200 جم ميكوريزا) في تصميم القطاعات العشوائية الكاملة)RCBD). أظهرت النتائج أن القيم األعلى إلرتفاع النبات والمساحة الورقية وإجمالي الكلوروفيل وإجمالي الوزن الطازج والجاف كانت مع زيادة تركيز الكادميوم والرصاص)250 مجم/ لتر كادميوم *+* 200جم ميكوريزا(والرصاص)150 مجم/ لتر رصاص 200+ جم ميكوريزا(سجل انخفاض نسبة اإلنبات و إرتفاع النبات، مقارنة بالكنترول الموجب)مع 200 جم ميكوريزا(الذي سجل أعلى إنبات للبذور، ارتفاع النبات، المساحة الورقية، الكلوروفيل الكلي، الوزن الطازج والجاف الكلي، يليه الكنترول السلبي (بدون ميكوريزا)، على التوالى. من ناحية أخرى، سجل التركيز الأعلى من الكادميوم)250 مجم/لتر كادميوم200*+* جم ميكوريزا(والرصاص)150 مجم/ لتر رصاص 200+ جم ميكوريزا(انخفاض نسبة النيتروجين والفوسفور والبوتاسيوم مقارنة بالكنترول الموجب)مع 200 جم من الميكوريزا(التي سجلت النسبة المئوية الأعلى للنيتروجين والفوسفور والبوتاسيوم تليها الكنترول السلبي (بدون ميكوريزا). بالإضافة إلى ذلك، سجل التركيز الأعلى من الكادميوم (250 مجم/ لتر كادميوم + 200 جم ميكوريزاً) أعلى قيمة للكادميوم يليه الرصاص (150 مجم/ لتر رصاص + 200 جم ميكوريزا)، بينما سجل محتوى الرصاص القيم الأعلى مع الكادميوم (250 مجم/ لتر رصاص + 200 جم ميكوريزا). ومع الرصاص (150 مجم/ لتر رصاص +200 جم ميكوريزا)، على التوالي. نستنتج من ذلك أن الإجهاد بالمعادن الثقيلة أدى في معظم الحالات إلى خفض الأوزان الطازجة والجافة لنبات الذرة ومحتوى الفوسفور والنيتروجين والبوتاسيوم مقارنة بالكنترول على النباتات غير المعاملة. أدى اضافة الميكوريزا إلى زيادة استجابة النمو، بشكل عام، لنباتات الذرة المزروعة في التربة الملوثة بالمعادن الثقيلة مقارنة بالنباتات غير الميكوريزا. توضح هذه الدراسة أن زراعة الذرة باستخدام لقاح الميكوريزا يمكن أن يقلل من سمية المعادن الثقيلة ويزيد من النمو وامتصاص NPK. في هذا الصدد، يلعب فطر الميكوريزا دو ًرا وقائيًا، إلى حد ما، للنبات المضيف، وبالتالي فهو ذو قيمة في المعالجة النباتية للمعادن الثقيلة في تلوث التربة.

الكلمات المفتاحية: الذرة، الميكوريزا، المعادن الثقيلة، النمو الخضري، المحتوى الكيماوي.

1. Introduction

With a production of over 1.1 million tons on an area of 194 million hectares, maize is one of the most widely grown product groups worldwide [1]. It is an accumulator crop, meaning that it can accumulate heavy metals and has a high resistance to heavy metal pollution [2] One of the most significant crops grown worldwide is maize, which interacts strongly with AM fungi [3]. Furthermore, since AM fungal species differ in their capacity to produce glomalin, there has been a close correlation observed between fungal-associated soil C accumulations and AM fungal communities [4].

Because of anthropogenic activities like mining, smelting, fertilizer and pesticide application, and sewage sludge, heavy metals (HMs) are major sources of contamination on Earth [5]. Because HMs accumulate in soils and are absorbed by plants, they pose a serious threat to agriculture (e.g., Cd, Pb, Cr, As, Hg, Ni, Cu, and Zn) [6]. While plants do not require some metals, like cadmium (Cd), even at low concentrations, others, like nickel (Ni), are necessary in small amounts but can be toxic to plants at higher concentrations, these metals are resistant to deterioration and can remain in the soil for years if not absorbed by plants or leached [6],[7].Human health and safe crop production are seriously threatened by heavy metal (HM) contamination, and different inbred lines of maize react differently to cadmium (Cd) stress [8],[9]. Because of their higher toxicity, recalcitrance, and persistent nature, heavy metal pollution has gained global attention. The environment's stability and the wellbeing of all living things are at risk due to these hazardous metals. Eating tainted food is another way that heavy metals enter the human food chain and have harmful effects on health [10].

Plant life, human health, and the world's food supply are all seriously at risk from heavy metal (HM) poisoning of agricultural soils. Crop health and yield are negatively impacted when HM levels in agricultural soils reach hazardous levels [11]. Heavy metal pollution is a major problem these days. Due to their toxicity and environmental persistence, they are the primary cause of soil pollution [12]. The presence of heavy metals (HMs) in soils is caused by a number of factors, including rapid industrialization, air deposition, farmyard manure, sewage sludge, and widespread use of synthetic fertilizers [12],[13].Heavy metals in contaminated soil can be removed using chemical and physical techniques, but these are both very costly and inefficient. Most plant roots are home to mutualistic symbionts known as arbuscular mycorrhizal fungi (AMF). Moreover, AMF are the key mycorrhizae for phytoremediation because of their vast hyphal network, which can improve soil absorption of water, heavy metals, and micro- and macronutrients. On the other hand, heavy metals can be segregated within plant roots by AMF hyphae that have colonized those roots [14],[15]. To determine the impact of allochthonous Arbuscular Mycorrhizal Fungi (AMF), which were inoculated, on the growth and stability

of soil aggregates, it is crucial to understand native AMF and their relationship to the edaphic features in their habitat [16]. AMF can create a symbiotic relationship with the majority of land plant species, pierce the cortical cells of plant roots, and develop branched structures called arbuscules for the exchange of nutrients. To maintain their growth and development under a variety of abiotic stressors, plants have evolved a comprehensive plant–AMF symbiosis system [17].

The most common type of mycorrhizae in nature are thought to be arbuscular mycorrhizas (AMs), which are present in about 72% of land plants and have significant functions in the rhizosphere of plant [18]. Mucoromycete's Glomeromycotan is home to arbuscular mycorrhizal fungi (AMF) [19]. AMs are essential to ecosystems and have a major impact on how plants grow, use water, absorb nutrients, and balance hormones in response to biotic and biotic stressors. Enhancing plant biomass accumulation is one of AMs' most significant ecological roles [20],[21]. AMF create a direct link between the soil and the roots, which can enhance plant uptake of water and nutrients as well as photosynthetic capacity. This lessens the detrimental effects of abiotic stresses like drought, salt, and nutrient shortage [22]-[24]. It is well known that heavy metals can build up in plants, where they can have a detrimental effect on physiological and biochemical processes, leading to significant reductions in yield [25]. By adversely influencing germination-related processes, HMs decrease seed germination and subsequently decrease overall stand establishment [26]. By producing excessive amounts of hydrogen peroxide (H2O2) and malondialdehyde (MDA), HMs also disrupt the water status of plants, compromise the stability of their membranes, and increase the loss of significant osmolytes. Moreover, HMs causes an overabundance of reactive oxygen species (ROS), which harms DNA, lipids, and proteins [27]. This work's primary goal is to find out how mycorrhizal fungus affects the growth and metal contents of maize plants grown in soil containing various concentrations of heavy metals, such as lead and cadmium.

2. Material and Methods

Pot experiments was conducted in the city flower nursery in the city of Tobruk 2024 to evaluate the effects of mycorrhizal fungi on the growth and metals contents in maize plants grown in soil contaminated with heavy metals (cadmium and lead) at different concentrations. The experiment consisted of 13 treatments i.e. positive control (plus 200g mycorrhiza), negative control (without mycorrhiza), Cd (50, 75, 150, 200, 250 mg/l +200g myco.) and Pb (25, 50, 75, 100, 150 mg/l +200g myco.) in a randomized complete block design (RCBD). A seed germination test was performed in the laboratory at room temperature (30°C). To prevent fungal infection during the experiment, the selected seed material was thoroughly washed with 2% sodium hypochloride for 5 min and then rinsed with distilled water. Seeds were imbibed in distilled water for 30 min and then were air-dried. Petri plate (10 cm in diameter) was employed with two filter papers and 10 seeds following three replicates per treatment. Each Petri plate was moistened with 10 mL of the metal solution while the control treatment continued with distilled water. Overall, the plates were observed daily for moisture/treatment requirements. Germinated seeds with one mm radicle were counted daily till the final germination day (day 10). Percent seed germination was determined following the study [28] using the formula:

(Germination $(\%)$ = Total seeds germinated / total seeds arranged \times 100). Healthy seeds of uniform size were sown 1–2 cm deep in the topsoil of the pots. After ten days, the seedlings were thinned by removing weak seedlings, and metal treatment was simulated for up to four weeks. Different growth attributes i.e., plant length, leaf area, SPAD value, plant fresh and dry weight [29].

Data recorded:

A) Vegetative growth

- Plant height (cm): It was measured with a tape measure from the base of the stem at the contact with the soil to the shoot tip of the marked plants.
- Number of leaves/ plant: The average number of the total leaves was calculated for the selected plants.
- Leaf area (dm²/plant): It was calculated by taking 30 known-area disks from three leaves of three plants and drying them in an electric oven at 65 °C until their weight stabilized. Then, the leaf area was calculated according to the following equation [30].
- Fresh weight (g/plant): The average weight of the shoot system of five plants from each experimental unit was calculated.
- Dry weight (g/plant): After estimating the fresh weight, the plants were dried with an electric oven at 70°C for 48 hours until the weight stabilized, and then the average dry weight was calculated.

• Total Chlorophyll Content (SPAD unit): Total chlorophyll content in fresh leaves was determined by using Minolta meter according to [31].

B) Chemical composition

- $N(%)$
- $P (%)$
- $K(%)$

Determination of Pb^{2+} and Cd^{2+} Anodic stripping voltammetry was performed to determine the concentrations of Pb²⁺ and Cd²⁺in the samples of this study. The optimal electrode and optimized parameters were used to determine the heavy metal concentrations in each of the samples. The calibration curves of Pb^{2+} and Cd^{2+} were used to compute for the concentrations present in the sample. Statistical analysis was performed via histograms for the optimization of the ASV parameters and linear regression for the calibration curves.

3. Statistical Analysis

The data were statistically analyzed according to the design used in the statistical program (GenStat12) and the statistical averages were compared according to the L.S.D test under the probability level of 5% [32].

4. Results and Discussion

A) Vegetative growth

Cd and Pb treatments suppressed the maize seed germination, plant height, leaf area, total chlorophyll, total fresh and dry weight with increasing concentration of Cd and Pb Table (1) and Figure (1). Results showed that higher concentration of higher concentration of Cd (250 mg/l cd +200g myco.) recorded the lower germination (31 90%), plant height (78.90 cm), leaf area (52.51 cm²), total chlorophyll (34.00 cm), total fresh (339.67g) and dry weight (75.48 g), also, higher concentration of Pb (150 mg/l Pb +200g myco.) decrease all of germination (35.85%) , plant height (71.84 cm) , leaf area (53.00 cm^2) , total chlorophyll (34.52 cm), total fresh (323.73 g) and dry weight (71.94 g), respectively, as compared to positive control (plus 200g mycorrhiza) which recorded the greater of seed germination, plant height, leaf area, total chlorophyll, total fresh and dry weight, followed by negative control (without mycorrhiza), respectively.

Treatments	Germination (%)	Plant height (cm)	Leaf area $\rm \left(cm^2 \right)$	Total chlorophyll (SPAD)	Total fresh weight (g)	Total dry weight (g)
Positive control (Plus, 200g mycorrhiza)	97.5	136.79	69.91	47.20	590.24	131.16
Negative control (Without mycorrhiza)	95	107.21	65.63	45.41	543.03	120.67
50 mg/l Cd $+200g$ myco.	90	103.82	63.22	43.18	481.82	107.07
75 mg/l Cd $+200g$ myco.	86.50	95.48	60.83	39.43	443.28	98.51
150 mg/l Cd $+200g$ myco.	75.00	87.84	58.40	37.23	395.09	87.80
200 mg/l Cd +200g myco.	62.63	80.27	55.25	34.55	388.28	86.28
250 mg/l Cd +200g myco.	3190	78.90	52.51	34.00	339.67	75.48
25 mg/l Pb $+200 \text{g}$ myco.	82.55	103.78	64.00	44.35	498.63	110.81
50 mg/l Pb $+200g$ myco.	72.25	92.16	61.55	43.00	466.79	103.73
75 mg/l Pb $+200g$ myco.	62.50	85.87	58.50	39.40	429.45	95.43
100 mg/l Pb $+200g$ myco.	52.51	78.80	55.67	37.35	381.73	84.83
150 mg/l Pb $+200g$ myco.	35.85	71.84	53.00	34.52	323.73	71.94
LSD (0.05)	7.19	1.10	2.83	2.51	2.90	0.64

Table (1): Effect of Cd, Pb and mycorrhiza on vegetative growth of maize.

Figure (1): Effect of Cd, Pb and mycorrhiza on vegetative growth of maize.

Research on the characteristics of seed germination revealed that it was inhibited by lead (Pb) toxicity, even at low or micro-molar concentrations [33]. Though Elsholtzia argyi has been the subject of a few reports regarding the inhibition of radical/hypocotyl length and the progression of seed germination [34], these findings were not made during the current investigation. It was discovered that all of the Cr and Pb concentrations inhibited maize germination, and that this inhibition increased as the metal concentrations in the medium increased. According to arguments made by [35] germination was inhibited because Pb and Cr interfered with the enzymes that are necessary for seed germination (amylase and protease). Furthermore, Study [36] showed that during Cicer arietinum (chickpea) germination, there was an inhibition of GA³ (gibberellic acid) and an activation of ABA (abscisic acid). Metals become lodged in plant roots and obstruct the uptake of nutrients from the soil, which is why they have toxic effects on various plant growth attributes [37],[38]. On the other hand, heavy metals impact the biosynthesis of the electron transport system and change the structure of the chloroplasts. This is because they cause an increase in the activity of chlorophyllase, which results in a decrease in chlorophyll.

The main environmental pollutants that have a negative impact on all living things, including plants, are thought to be heavy metals [39],[40]. Metals become lodged in plant roots and obstruct the uptake of nutrients from the soil, which is why they have toxic effects on various aspects of plant growth [41]. Pb doses of 0.6 mM–1.2 mM significantly reduce several agronomic parameters of rice plants, including plant length, tiller count, and dry weight biomass. Results showed that a Pb dose of 0.6 mM was less toxic than a Pb dose of 1.2 mM. When rice plants were exposed to the highest Pb dosage, their length decreased by 13% and their dry weight decreased by 61% in the cultivar Ilmi [42]. The primary factors influencing plant growth are the properties of leaves and the efficiency of photosynthesis in plants. Pb is a well-known biotic stressor that reduces intact plants' capacity for photosynthetic activity, which in turn affects plant growth and biomass yield [43].

By causing chlorosis, water and nutritional imbalances, decreased activity of Calvin cycle enzymes, CO² deficiency, protein denaturation, and potentially plant death, high concentrations of heavy metals hinder plant growth and biomass production [44]. Associated nickel (Ni) with decreased photosynthetic pigments, decreased growth, and decreased sodium (Na) and phosphorus (P) concentrations in maize (*Zea mays*, L.) [45]. Cd-treated maize plants showed decreased photosynthesis, decreased biomass production, significant ultra-structural damage, and reduced growth [46]. A leaf is a vital photosynthetic organ that is essential to a plant's ability to grow. Pb and Cr have a negative impact on *Lycopersicon esculantum, Pisum sativum*, and *Zea mays* leaf growth and development [47]. Research revealed that heavy metals inhibited rice plant leaf growth and development by producing reactive oxygen species, or ROS [48]. The growth attributes of plants exposed to lead (such as wheat, maize, barley, sunflower, mustard, and soybean) are significantly impacted by Pb. It also inhibits various growth attributes such as plant height, root-shoot length, fresh-dry weight of seedlings, tolerance index, leaf number, and photosynthesis [49]. When cadmium enters a plant's body, it builds up and eventually prevents the plant from respiring and photosynthesizing. This causes the plant's body to have less enzyme activity and to contain less soluble sugar and protein. This leads to slow growth, yellowing of the leaves, a delayed climacteric period, and other issues that have a negative impact on crop yield, quality, and safety [50]. The most noticeable effect of Cd stress on maize seedlings is the inhibition of root growth, which results in yellowing and curving leaves, a significant reduction in aboveground biomass, and slowed plant growth [51]. The plant's inability to perform regular transpiration and photosynthesis prevents it from growing and developing normally [52]. These enzymes offer protection to the plant up to a certain Cd concentration. High levels of Cd, however, can also be detrimental to these enzymes. Plants' soluble proteins, soluble sugars, and proline function as antioxidants and osmoregulatory, reducing the harm that heavy metals can do to plant cells [53],[54].

As the amount of Cd in the substrate and/or soil increased, the growth traits decreased [55],[56]. It's possible that Cd toxicity, nutrient inequality, decreased water rates, and decreased plant nutrient uptake are the causes of this decline in fresh and dry biomass as well as the length of maize plants [57],[58]. Furthermore, the disruption of the transpiration process in metal hassle, particularly Cd, may have resulted in a decrease in xylem transport [59]. The ability to extract maize shows that it can accumulate heavy metals, particularly Cd, and that it can be utilized for phytoextraction [60].

A) Chemical composition

Results in Table (2) and Figure (2) showed that higher concentration of Cd (250 mg/l cd +200g myco.) recorded the lower percentage of N (1.62 %), P (0.50 cm), K (1.30 cm2), also, higher concentration of Pb (150 mg/l Pb +200g myco.) decrease percentage of N (1.45 %), P (0.55 cm), K (1.35 cm2), respectively, as compared to positive control (plus 200g mycorrhiza) which recorded the greater of percentage of N (2.82 %), P (0.82 cm), K (2.54 cm2), followed by negative control (without mycorrhiza) which recorded N(1.93 %), P(0.56 cm), K (2.25 cm2), respectively. On the other hand, higher concentration of Cd (250 mg/l cd +200g myco.) recorded the highest value of Cd (1.56 ug/g), followed by Pb (150 mg/l Pb +200g myco.) which recorded (1.30 ug/g), while Pb content recorded the higher values (12.44 ug/ g) with Cd (250 mg/l cd +200g myco.) and (15.20 ug/g) with Pb (150 mg/l Pb +200g myco.), respectively.

Table (2): Effect of Cd, Pb and mycorrhiza on chemical composition of maize.

Figure (2): Effect of Cd, Pb and mycorrhiza on chemical composition of maize.

Because of the leaching mechanism of toxic metal ions, heavy metals degrade land by causing acidification of the soil. Low pH profiles in soils make metals available to growing plants, which lowers crop yield [61],[62]. Even though soils serve as a buffer and are resistant to pH changes, heavy metals applied over time can cause subsequent soils to become acidified. In a solution of these soils, heavy metals hydrolyze, produce H⁺ ions, and reduce pH [63]. Thus, crop plants are impacted by soil acidification, which causes nutrient depletion [64]. Because of the presence of phosphorus in the contaminated soil, the efficiency of soil enzymes (phosphatase, urease) to recycle nutrients increases [65]. Comparably, bioremediation's microbial component might be an additional option for recovering contaminated soils. Currently, irrigation with municipal and industrial waste water ought to be prohibited, or if irrigation with the waste water is permitted, it ought to be recycled through waste water treatment facilities in order to stop additional heavy metal addition to agro-soils [66]. The study [67] found that plants absorbed more heavy metals when the concentration of the metals in the soil increased. When it comes to movement within the plant, HMs and nutrients may compete. Because cadmium competes with other minerals for the same transporters or binding sites, it can impede the absorption and utilization of vital minerals like iron, zinc, calcium, and magnesium. Growth and biomass production may noticeably decline as a result [68]. When Pb concentrations reach 30 mg/kg or higher, they typically inhibit the growth mechanism [69]. However, certain plant species have the capacity to withstand Pb stress levels as high as 1,000 mg kg−1 (70).

The involvement of AMF on heavy metals uptake from contaminated sites is a significant phenomenon, even though mycorrhizal plants have a greater tolerance to toxic metals, root pathogens, and stresses like drought, salinity, high soil temperature, adverse pH, and transplant shock [71]. In soil and water contaminated with metal, they can colonize plant roots. Moreover, AMF has the ability to boost plant phytoremediation potential through a number of different mechanisms. As a result, we can increase the phytoremediation potentiality of plants by introducing them as inoculums to heavy metal-contaminated sites [72]. Metal ions from the soil are first bound by the walls of the root cells, after which they are taken up through the plasma membrane. Secondary transporters (like channel proteins and/or H+ coupled carrier proteins) are responsible for the uptake of metal ions [73].

5. Conclusion

The germination of seeds, plant height, leaf development, biomass, and chlorophyll content are all negatively impacted by both heavy metals. Furthermore, under metal stress, the affected plant growth mechanism demonstrated its affinity with the affected soil characteristics, with a greater emphasis on higher metal levels. Data have shown that heavy metals have an acidic effect on the soil and have an impact on soil respiration through altering soil microbial activity. Similarly, declining soil enzyme levels have shown altered soil nutrient recycling. Apart from the harmful effects of metals, maize plants demonstrated significant ability to partition and accumulate Cd and Pb from the rhizosphere pot soils that followed.

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