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Complete List of Authors:	Rui, Mengmeng; China Agricultural University Ma, Chuanxin; Connecticut Agricultural Experiment Station, Department of Analytical Chemistry White, Jason; CT Agricultural Experiment Station, Analytical Chemistry Hao, Yi; China Agricultural University, College of Resources and Environmental Sciences Wang, Yaoyao; China Agricultural University Tang, Xinlian; Guangxi Normal University Yang, Jie; China Agricultural University Jiang, Fuping; China Agricultural University Ali, Arbab; China Agricultural University Yukui, Rui; china agricultural university, Department of Environmental Science and Engineering Cao, Weidong; Chinese Academy of Agricultural Sciences Chen, Guangcai; Research Institute of Subtropical Forestry, Chinese Academy of Forestry, ; University of Massachusetts, Xing, Baoshan; UMASS, Stockbridge School of Agriculture

Environmental Significance

Metal-based nanoparticles (NPs) and NP incorporated agrichemicals have great potentials to suppress disease and enhance crop growth. However, long-term evaluation of their impacts on crop quality and yield is rather limited. We investigated the effects of commonly used NPs on peanut yield and nutritional quality in a life cycle study. Our findings showed that not only metal-based NPs could reduce peanut grain production, but also they could significantly alter the nutritional levels as determined by amino acid and fatty acid content in peanut grains. Further investigations on how to safely apply NP or NP incorporated chemicals in agriculture are warranted.

Metal oxide nanoparticles alter peanut (*Arachis hypogaea* L.) physiological response and reduce nutritional quality: A life cycle study

Mengmeng Rui[1, 3, †], Chuanxin Ma[2, 4, †, *], Jason C. White[2], Yi Hao[1], Yaoyao Wang[1], Xinlian Tang[3], Jie Yang[1], Fuping Jiang[1], Arbab Ali[1], Yukui Rui[1, *], Weidong Cao[5], Guangcai Chen[6], Baoshan Xing[4]

[1]Beijing Key Laboratory of Farmland Soil Pollution Prevention and Remediation, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China

[2]Department of Analytical Chemistry, the Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504, USA

[3]College of Agriculture, Guangxi University, Nanning 530005, China

[4]Stockbridge School of Agriculture, University of Massachusetts, Amherst, Massachusetts 01003, USA

[5]Key Laboratory of Plant Nutrition and Fertilizer, Ministry of Agriculture of China/ Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China

[6]Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang, Zhejiang 311400, China

Corresponding authors:

Yukui Rui: ruiyukui@163.com; Phone: 8610−62733470;

Chuanxin Ma: chuanxin.ma@ct.gov; Phone: 203−974−8321.

[†]These authors contributed equally in this work.

Abstract

We investigated the effects of the metal oxide nanoparticles (NPs), iron oxide (Fe_2O_3), copper oxide (CuO), and titanium oxide (TiO_2) NPs at 50 and 500 $mg \cdot kg^{-1}$, on peanut (*Arachis hypogaea* L.) in a full life cycle study. In order to evaluate crop quality, the amino acid and fatty acid profiles in peanut grains were analyzed across all the NP treatments. After 145−day exposure, all the three NPs had no impact on plant growth in terms of biomass, shoot height, and per plant yield, with the exception of the 500 $mg \cdot kg^{-1}$ CuO NPs, where the fresh shoot biomass was significantly reduced by 44% ($p=0.0003$) relative to the control. However, exposure to the three NPs significantly decreased the 1000−grain weight by 10−31% ($p<0.05$), with the greatest reduction being in the 500 $mg \cdot kg^{-1}$ CuO NP treatment. The elemental analysis showed that the Cu contents in grains increased in a dose−dependent manner; however, exposure to Fe_2O_3 and TiO_2 NPs did not increase the Fe and Ti contents in the grain regardless of dose. The total amino acid content in all the NPs treated peanut grains was significantly reduced as compared to the control, but exposure to 50 $mg \cdot kg^{-1}$ TiO_2 NPs had no impact on the total amino acid content. Exposure to 50 and 500 $mg \cdot kg^{-1}$ CuO NPs resulted in 36.2% ($p=0.000004$) and 21.1% ($p=0.0001$) decreases in the total amino acid content, respectively. In comparison with the control, the resveratrol content at 50 and 500 $mg \cdot kg^{-1}$ CuO NP treated grains increased to 1.8 and 2.3 $mg \cdot kg^{-1}$, respectively, suggesting plant stress response. Taken together, our results suggest that metal−based NPs could alter peanut crop yield and more

importantly, nutritional quality. These findings raise concerns over how to safely and sustainably apply NP incorporated agrichemicals so as to protect food quality and security.

Keywords: *Arachis hypogaea* L.; Metal oxide nanoparticles; Phytotoxicity; Fatty acids; Amino acids; Food safety

Introduction

With the rapid development of nanotechnology, it has become possible to synthesize nanomaterials (NMs) with dramatically different types, sizes, and geometric shapes.[1,2] NMs are being widely used in agriculture, medicine, communications/electronics and environmental remediation. For example, titanium oxide nanoparticles (TiO_2 NPs) have been widely used as a carrier for drug delivery,[3] personal care products,[4] and for contaminant sorption.[5] Iron oxide nanoparticles ($\gamma-Fe_2O_3$ NPs) have been used in medical diagnostics, controlled drug release, separation technologies, and environmental engineering due to their superparamagnetism and inherent biocompatibility.[6, 7] Copper oxide nanoparticles (CuO NPs) have been used in gas sensors, photovoltaic cells, magnetic phase transitions, agrichemicals, and as catalysts and in semiconductors.[8, 9] Therefore, increasing amounts of NPs are entering the environment after use or by intentional release from agricultural production or remediation activities. The potential risk that these materials pose to food chain contamination remains largely unknown.[10, 11]

Although a number of studies have investigated the interactions between NPs and terrestrial plants,[12] few have focused specifically on the effects on nutritional quality. Metal−based NPs can accumulate in plant roots, and be transported to shoots via symplastic or apoplastic pathways.[12] Oxidative stress induced by NPs leads to the accumulation of reactive oxygen species (ROS), which unbalances the cell redox system and causes oxidative damage to cellular macromolecules such as lipids, proteins, and nucleic acids.[13] The presence of CeO_2 NPs decreased the content of prolamin, glutelin, lauric and valeric acids, and starch in exposed rice (*Oryza sativa* L.) grains.[14] Similarly, both ZnO and CeO_2 NPs altered the sugar, starch, and protein content in cucumbers (*Cucumis sativus*).[15] Castiglione et al. (2011) reported that TiO_2 exposure led to alterations in mitosis and chromosomal aberrations in narbonne vetch (*Vicia narbonensis* L.) and maize (*Zea*

mays L.), suggesting NP−induced genotoxicity.[16] Although metal−based NPs cause dose−dependent physiological and molecular damage in terrestrial plants, recent studies have also demonstrated the significant potential benefits of NP use in agriculture.[17] For example, γ−Fe_2O_3 NPs (2−1000 mg·kg^{-1}) use as nanofertilizers were shown to positively affect the growth of soybean (*Glycine max*) and peanut (*Arachis hypogaea*) plants.[18, 19]

Peanuts are considered an excellent source of protein, second only to soybean in terms of its quantity and nutrition. Peanut protein contains eight essential amino acids: isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Try), and valine (Val).[20] The most abundant amino acids in peanut protein, glutamic acid (Glu) and aspartic acid (Asp), can promote the development of brain cells and enhance memory. Additional important components in peanut grains are fatty acids (FA), which account for 40%–50% of the grains by weight. Up to 80% of the FA in peanuts are unsaturated (UFA), mainly being oleic and linoleic acids.[21] Resveratrol is a plant phenol that accumulates under biotic or abiotic stress, such as pathogen infection, ultraviolet irradiation, mechanical injury, salt damage, heavy metal exposure, and drought.[22, 23] Resveratrol has anti−cancer, anti−aging, anti−atherosclerosis and cardiovascular protective properties without causing significant cytotoxicity.[24] Therefore, due to its nutritional value and bioactive properties, peanut has been extensively used as a model to investigate abiotic stresses.

It is important to investigate the potential impacts of different NMs on edible portion of crops during full growth cycle studies. In the current work, peanut plants were grown in soil amended with 50 or 500 mg·kg^{-1} NP Fe_2O_3, TiO_2, and CuO for 145 days. At harvest, physiological parameters including biomass, and crop yield were measured. In order to evaluate the impact of different metal−based NPs on food quality, the content of amino acids, fatty acids, and

resveratrol in peanut grains were determined. The results provide important understanding on the overall responses of peanut exposure to different NPs and this exposure impacts overall food quality. Additionally, our findings provide important information to support the safe and sustainable use of nanomaterials in agriculture.

Materials and Methods

NP Fe_2O_3, TiO_2, and CuO characterizations

γ−Fe_2O_3 NPs, anatase−TiO_2 NPs, and CuO NPs were purchased from Shanghai Pantian Powder Material Co., Ltd. The morphology and particle size distribution of the three NPs were characterized by transmission electron microscopy (TEM; FEI Co., Tecnai G2 20 S−TWIN, USA). TEM images (**Figure S1**) show that Fe_2O_3 NPs, TiO_2 NPs, and CuO NPs were in spherical shape with average diameters of 20, 5, and 40 nm, respectively. The detailed information for three metal-based NPs is shown in **Table S1**.

Experimental design

Soil and sand were sampled from the Shangzhuang Experimental Station of China Agricultural University. The physical and chemical properties of soil are shown in **Table S2**. Prior to air drying, the sand was rinsed with tap water 3 times. Both sampled soil and sand were sieved through a 2 mm mesh. Sand was mixed with soil in a mass ratio of 1:5.5 (sand/soil) in order to ensure water drainage and root respiration.[19] Urea, superphosphate, and potassium sulfate were added to the soil−sand mixture in the ratio of N : P_2O_5 : K_2O= 0.25 : 0.3 : 0.25 $mg \cdot kg^{-1}$. Different amounts of NP powders were thoroughly blended with the above soil mixture to achieve designated concentrations of 50 and 500 $mg \cdot kg^{-1}$, according to our previous study.[25]

Individual pots were filled with 1.5 kg of different NP−amended soil (containing 75 or 750 mg corresponding NPs respectively) and were allowed to stabilize for 24 h prior to use. NPs−free replicates were set as controls.

Peanut seeds (Luhua No.11) were purchased from the Beijing Hongmei plantation, China. The seeds were sterilized with 5% hydrogen peroxide for 10 min, rinsed with deionized water for three times, then soaked in 50 °C deionized water for 4 h. Four seeds were sown in each pot. After 14 days, two seedlings with uniform size were kept in each pot and there were 10 pots in each treatment. The experiment lasted for 145 days.

Plant biomass and crop yield measurement

Peanut biomass was determined after grain formation. At harvest, plants were separated into aboveground (stem, leaf) and underground (root, grain) parts. All peanut tissues were thoroughly washed with tap water for 3 times following by additional 3 times with deionized water, according to a previous study.[26] Basic physiological parameters, including plant height, fresh and dry biomass, and the number of branches were measured for all replicates. In order to evaluate the NP impacts on the edible tissues, 1000−grain and per plant yield were measured across all the NP treatments.

Determination of elemental concentration

The oven−dried peanut plant tissues and grains were ground to fine powder. Plant tissues were digested using a microwave digestion method according to the national standard protocol, "Method for the determination of potassium, phosphorus, iron, calcium, zinc, aluminum, sodium, magnesium, boron, manganese, copper, barium, titanium, vanadium, nickel, cobalt, chromium contents in honey—Inductively coupled plasma atomic emission spectrometric method (GB/T

18932.11−2002).” Although the method is designed for honey, the QA/QC information above demonstrates adequate performance in our matrix. Briefly, approximately 1 g of dry tissue (accurate to 0.1 mg) was weighed into a microwave digestion tube containing 3 mL hydrogen peroxide (H_2O_2) and 3 mL nitric acid (HNO_3). All mixtures were predigested under the ambient conditions for 24 h prior to microwave digestion. The cap of each digestion tube was tightened before moving into the microwave digestion system (Mars6, CEM Corporation, USA). The heating program was: 120 °C for 5 min, 160 °C for 10 min, and 180 °C for 10 min. The working pressure of system was at approximately 15 Mpa. All digests were diluted to 25 mL with DI H_2O. The concentration of Fe, Ti, and Cu was measured using inductively coupled plasma mass spectrometry (ICP−MS; ICAP 6300, Thermo Scientific, USA). The recovery of the elements Ti, Fe and Cu in ICP-MS analysis was 95.5±1.0%, 103.1±4.7% and 100.1±1.7%, respectively.

Transmission electron microscopy (TEM)

The ultra−structure of fresh peanut grains was imaged using transmission electron microscopy (TEM) with energy dispersive X−ray spectroscopy (EDS) to observe the presence of NPs in the 500 $mg \cdot kg^{-1}$ CuO, Fe_2O_3, and TiO_2 NP treated tissues. The peanut grain was washed with 0.1 M phosphate buffer, immersed in 2.5% glutaraldehyde solution (pH=7.3), and dehydrated through a series of acetone concentration gradients.[27] The dehydrated samples were embedded in Suprr resin and were sectioned into sheets of 90 nm thicknesses using a microtome equipped with a diamond knife. The sectioned samples were then placed on Cu/Ni−based grids (CuO NP treated samples were mounted onto Ni grids) for TEM (JEM−1230, JEOL, Japan) observation at 80 kV. Any areas highlighted in red rectangles were qualitatively analyzed using EDS at 200 kV.[28, 29]

Amino acid profile

A portion of 0.1−0.2 g peanut grains was weighed into a hydrolysis tube containing 10 mL 6 mol·L^{-1} hydrochloric acid and three drops of phenol. The samples were frozen for 5 min and then the tubes were vacuumed infiltrated with nitrogen. This procedure was repeated three times prior to sealing the tubes. The samples were heated at 110 °C for 22 h in a water bath. After cooling to ambient temperature, the mixture was filtered into a 50 mL volumetric flask. One mL of filtrate was dried at 45 °C using a decompression drying procedure and then 1 mL of citric acid (pH2.2) was added into the tube. The mixture was passed through 0.22 μm membrane into a sample vial for amino acid analysis using an automatic amino acid analyzer (L−8900, HITACHI).[30]

Fatty acid profile

Sample peanut grains were freeze−dried in a lyophilizer for 48 hours, and then were ground to fine powder. An 80 mg sample was weighed into a 250 mL conical flask containing 4 mL chloroacetic methanol, 1 mL undecanoic acid methyl ester, and 1 mL n−hexane. The mixture was heated at 80 °C for 2 h and cooled to ambient temperature. Five mL of 7% potassium carbonate was added into the mixture prior to centrifugation at 1000 ×*g* for 5 min. The supernatant was filtered through 0.2 μm membrane into a sample vial. The fatty acid methyl ester content was analyzed by gas chromatography (GC, Agilent 6890) with a flame ionization detector (FID).[31]

Resveratrol content

The resveratrol content of peanut grains was determined by a high performance liquid chromatography (HPLC, L−2455, HITACHI) associated with a diode array detector at 306 nm. Approximately 2.5 g of freeze−dried grains without seed coat removal was weighed into a 150

mL conical flask containing 30 mL 85% ethanol solution. The container was capped and heated at 80 °C for 45 min in a water bath. The cooled mixture was filtered through a 0.2 μm membrane, and the residue in the conical flask was rinsed with 85% ethanol solution. Two mL of filtrate was centrifuged at 12000 ×g for 5 min, and the supernatant was used for resveratrol quantification by HPLC according to the national standard GBT 24903−2010.

Statistical analysis

Statistical significance was determined using a one−way analysis of variance (ANOVA) with SPSS 19.0 statistical software. The mean values for each treatment were compared using the Duncan's test at $p \leq 0.05$. All analyses were conducted with three replicates. Error bars represent the standard deviation (n=3). Different letters represent significant differences among treatments at $p \leq 0.05$.

Results and Discussion

Physiological responses of peanut

Phenotypic images of NP CuO, Fe_2O_3, and TiO_2 treated plants showed that NPs exposure caused no significant impact on either shoot or root systems, the exception being the treatment with 500 $mg \cdot kg^{-1}$ CuO NPs, where peanut growth was severely inhibited (**Figure 1**). The presence of all the three NPs had no impact on the fresh shoot biomass, with the exception of 500 $mg \cdot kg^{-1}$ CuO NPs, which significantly reduced the tissue mass by 44% (p=0.0003) relative to the control (**Figure 2A**). Similar results were evident in peanut shoot dry biomass (**Figure 2C**). None of the three NPs affected root biomass, regardless of dose (**Figure 2B and D**). Exposure to 500 $mg \cdot kg^{-1}$ CuO NPs resulted in a 25% decrease in shoot height (**Figure 2E**) but there was no

difference in the total number of branches upon exposure (**Figure 2F**). The result of 1000−grain weight was found to be reduced across all particles and doses (**Figure 2G)**. For example, exposure to 50 $mg \cdot kg^{-1}$ CuO, Fe_2O_3, and TiO_2 NPs resulted in 16.8% (p=0.003), 15.7% (p=0.000007), and 8.4% (p=0.002) reduction in peanut grain weight, respectively; in addition, 500 $mg \cdot kg^{-1}$ CuO NPs further decreased the 1000−grain weight by 30.9% (p=0.0001) relative to the control. Approximately 63.6% (p=0.00008) reduction in the per plant yield was found in the 500 $mg \cdot kg^{-1}$ CuO NP treatment as compared to the control.

Metal−based NP induced phytotoxicity has been widely reported.[12, 32, 33] However, NPs also have great potential for controlled release of targeted nutrients to crops as a novel plant fertilization strategy.[34, 35] Although applied dose is clearly critical here, differences in species response, particle characteristics, and environmental conditions will also be important. Thus, it is important to investigate under which conditions nutrient related NPs such as nanoscale Fe_2O_3, CuO, and ZnO could increase plant biomass and crop yield without causing damage. In the present study, CuO NPs caused greater phytotoxicity to peanut as compared to the other two NPs. Similar to our findings, Wang et al. (2012) showed that exposure to 10 $mg \cdot L^{-1}$ CuO NPs reduced maize shoot and root biomass by 60% and 34%, respectively, as compared to the control.[36] Rice exposed to 100 $mg \cdot L^{-1}$ CuO NPs exhibited a 27% reduction in plant height relative to the control; at 1000 $mg \cdot L^{-1}$, the fresh and dry biomass in shoots was decreased by 31% and 14%, respectively.[37] NP Fe_2O_3 and TiO_2 exhibited less toxicity or had no impact on peanut growth in terms of shoot and root biomass. In our previous study, exposure to Fe_2O_3 NPs at 2−1000 $mg \cdot kg^{-1}$ in soil increased peanut fresh biomass.[19] Sheykhbaglou et al. (2010) also reported that the foliar application of 500 $mg \cdot L^{-1}$ Fe_2O_3 NPs increased the dry biomass of pods and overall shoots (leaves+pods) in soybean by 7.3% and 31.2%, respectively, suggesting specific exposure

routes of NPs might significantly impact NP effects on plant growth.[38] One of possible explanation could be that iron stimulates chlorophyll biosynthesis, respiration, redox reaction process, and subsequently increases plant growth.[39] Due to its photocatalytic properties, TiO_2 NPs could also increase plant photosynthetic efficiency. Feizi et al. (2012) found that 100 and 500 $mg \cdot kg^{-1}$ TiO_2 NPs increased the dry biomass of wheat roots by 11% and 3%, respectively, and at 500 $mg \cdot kg^{-1}$, shoot dry biomass was increased by 8% relative to the control.[40] In a hydroponic study, Ma et al. (2017) noted that both 500 and 1000 $mg \cdot L^{-1}$ TiO_2 NPs had no impact on rice growth in terms of root and shoot fresh biomass.[41] Specifically regarding edible portion of crops, Zhao et al. (2015) reported that both ZnO and CeO_2 NPs significantly altered the numbers of corncobs and plant fresh biomass as compared to the control.[42] Similarly, 800 $mg \cdot kg^{-1}$ CeO_2 NPs significantly reduced cucumber yield by 31.6% relative to the control.[43] Conversely, Owolade et al. (2008) found that the seed yield of cowpea was notably increased upon exposure to TiO_2 NPs.[44] Hence, the use of metal−based NPs at certain doses clearly enhances crop growth in terms of biomass and yield of edible portions, demonstrating the significant potentials of NPs as a novel fertilization strategy in agriculture. Both the ionic and size effects could attribute to metal−based NP−induced toxicity. For example, 50 $mg \cdot L^{-1}$ CuO NPs (23−50 nm) was translocated from root to shoot, and significantly inhibited the fresh weight of water hyacinth (*Eichhornia crassipes*) by disrupting the root caps and meristematic zone; this effect was more pronounced than with the corresponding dissolved Cu^{2+} ions and CuO bulk particles, suggesting size was the main factor in toxicity.[45] However, others have demonstrated that the ionic effect could contribute greatly in NP−induce toxicity. For example, the negative effects of CuO NPs on germination rate and biomass of wheat (*Triticum aestivum* L.), were significantly alleviated by adding a novel Cu ion absorbent (rice husk derived biochar),

indicating the released Cu^{2+} ions was a main driver of toxicity.[46]

Elemental content in peanut

The Cu, Fe and Ti content were determined in the roots, shoots, and grains of treated peanuts **(Figure 3)**. Upon CuO NP exposure, the Cu content of peanut roots and shoots increased with increasing the CuO NP dose. The presence of 50 and 500 $mg \cdot kg^{-1}$ CuO NPs significantly increased the Cu grain content by 88 and 163% (16.93±1.3 $mg \cdot kg^{-1}$ and 23.7±0.61 $mg \cdot kg^{-1}$), respectively, relative to the control (**Figure 3A**). The addition of Fe_2O_3 NPs did not alter the Fe content in peanut tissues, with the exception of 500 $mg \cdot kg^{-1}$ Fe_2O_3 NPs, which decreased the root element content by 33% (p=0.012) (**Figure 3B**). Exposure to 50 $mg \cdot kg^{-1}$ TiO_2 NPs did not affect Ti accumulation in the peanut roots; however, at 500 $mg \cdot kg^{-1}$, the root Ti content was increased by approximately 44% (54.77±0.4 $mg \cdot kg^{-1}$) relative to the control (**Figure 3C**). In comparison with the control, no difference in Ti translocation to shoots or accumulation in TiO_2 NP treated grains was found.

The observed Fe reduction in plant tissues treated with the high dose of Fe_2O_3 NPs could be ascribed to the regulation of the iron−related transporters in the Fe deficiency environment. Ma et al. (2016) reported upregulation of ferric chelate reductase (FRO) and iron−regulated transporter (IRT) in NP CeO_2 and In_2O_3 treated *Arabidopsis thalia*na roots.[47] In addition, exposure to high dose of Fe_2O_3 NPs resulted in aggregation in the cell walls of *Capsicum annuum* and subsequently blocked the channels for the Fe uptake.[48] A large group of studies demonstrated that metal−based NPs could induce high level of ROS, and subsequently result in oxidative stress, which significantly contributed in the suppression of plant growth.[32] Another

possible explanation could be that root exposure of metal−based NPs in the rhizosphere could greatly alter the composition and total content of root exudates, which could in turn influence the NP transformation.[49] Thus, investigations on the compositions of root exudates as affected by metal−based NPs are warranted.

Micronutrients are required for plant metabolism and act primarily as enzyme activators to catalyze redox processes for electron transfer.[50] For example, both Fe and Cu play an important role in plant leaf photosynthetic systems, and their deficiency can result in electron transport impairment, leaf necrosis, stunted growth, and decreased crop yield.[51, 52] Previous studies have demonstrated that metal−based NPs could cause significant nutrient displacement in terrestrial plants. Wang et al. (2012) reported that CuO NPs could transport via phloem and significantly increase the Cu content in both shoots and roots of maize.[36] In a life cycle study, Cu accumulation in rice roots and stems increased in a dose−dependent manner with increasing the concentrations of CuO NPs. Aligning with our findings in the Cu content of peanut grains, exposure to 500 $mg \cdot kg^{-1}$ CuO NPs significantly elevated the Cu content in rice grains by 300% as compared to the control.[53] Similar findings were also reported in CuO NP treated cotton and spinach.[54, 55] Iron oxide nanoparticles have been used as a nanofertilizer in several studies. Rui et al. (2016) noted that the Fe content in peanuts upon Fe_2O_3 NPs exposure was significantly increased as compared to the control.[19] However, under the current life cycle study with longer exposure, it appears that Fe accumulation stabilizes over time. The possible explanations could be that Fe becomes unavailable to plants in high alkaline conditions and Fe_2O_3 NPs aggregated in the pore water in soils. Ti accumulation and translocation in TiO_2 NPs treated peanuts was unchanged upon exposure as compared to the other two NPs. Similarly, the Ti contents of TiO_2 NP treated basil (*Ocimum basilicum*) shoots and roots showed only slight increases as compared

to the control.[56] Other plant species such as lettuce and wheat also showed the similar minimal accumulation patterns.[57, 58] Synchrotron−based techniques further indicated that no biotransformation of TiO_2 NPs was evident in exposed cucumber fruit, suggesting potential negative consequences for food safety.[59]

TEM observation of peanut grains

Many dark spots were observed in the cells of NP treated peanut grains (**Figure 4C, E and G**), but not in the control samples (**Figure 4A**). Selected areas in each image were then analyzed by energy dispersive X−ray spectroscopy (**Figure 4B, D, F, and H**). In the control group, the weight percentage of Cu, Fe, and Ti was 0.10, 0.12, and 0.01%, respectively. In the CuO NP treatment, the weight percentage of Cu in the selected area was 2.43%, equivalent to an increase of 24%. Similarly, the Fe and Ti weight percentages were increased by 80.6% and 10% in the corresponding NP treatment. Further studies using synchrotron−based techniques could demonstrate whether the elevated levels of target elements in the edible portions of peanuts are in the NPs form or the result of metal biotransformation.

Previous studies have reported the presence of metal−based NPs in plant cells using TEM.[60, 61] For example, CeO_2 NPs preferentially accumulated in the chloroplasts and vacuoles of cotton.[29] In our previous long−term experiment, the presence of Ag NPs in treated peanut grains was evident; additionally, many starch particles were observed in the exposed grain cells, suggesting stress responses induced by Ag NPs.[62] In the current study, the presence of visible structures with increased elemental content corresponding to the NP exposure clearly suggests elemental nanoscale Cu, Fe and Ti in the exposed peanut tissues. Given the evidence for NP

accumulation in the edible portions of crops, further study to evaluate the toxicity and benefits of NPs in agriculture using full life cycle studies is clearly warranted.

Amino acid content

The amino acid content of peanut grains upon exposure to different concentrations of NP is shown in **Figure 5**, **Figure S2** and **Table S3**. The total amino acid content across all NP treated peanut grains significantly decreased as compared to the control, with the exception of the 50 $mg \cdot kg^{-1}$ TiO_2 NP treatment where no change was observed (**Figure 5**). For example, exposure to 50 and 500 $mg \cdot kg^{-1}$ CuO NPs resulted in 33.6% (p=0.000004) and 21.1% (p=0.0001) decreases in the total amino acid content, respectively. Similarly, 20.4% (p=0.001) and 12.0% (p=0.011) decreases in the total amino acid content were found in 50 and 500 $mg \cdot kg^{-1}$ Fe_2O_3 NP treated peanut grains, respectively. High dose of TiO_2 NPs caused 20.4% reduction in the total amino acid content of grains, while this change was insignificant as compared to the control (p=0.066). In addition to total amino acid contents, the amounts of five primary amino acids, including arginine (Arg), leucine (Leu), glycine (Gly), glutamate (Glu), and aspartate (Asp) are also shown in **Figure 5**. A common finding was that both NP CuO and Fe_2O_3 significantly decreased the content of all five amino acids. However, TiO_2 NPs had no impact on these amino acids, with the exception being the 500 $mg \cdot kg^{-1}$ TiO_2 NP treatment, in which the Arg content was reduced by 25% relative to the control. The content of remaining amino acids are given in **Table S3 and Figure S2**. Exposure to 50 and 500 $mg \cdot kg^{-1}$ CuO NPs significantly reduced the content of cysteine (Cys), glutamate (Glu), and glycine (Gly) in peanut grains; importantly, all of these molecules are key components in glutathione (GSH) biosynthesis in plants (**Figure S3**). Additionally, the

presence of metal−based NPs significantly altered the content of serine (Ser), leucine (Leu) and aspartate (Asp), all of which are involved in the glycolytic pathway and the citric acid cycle. For example, two doses (50 and 500 $mg \cdot kg^{-1}$) of CuO NPs lowered the Ser content by 37.0% ($p=0.000004$) and 19.2% ($p=0.0004$), respectively. Similarly, in comparison with the control, both NP Fe_2O_3 and CuO resulted in 13.0%−35.2% ($p<0.01$) and 14.2%−39.2% ($p<0.001$) reductions in the Leu and Asp content in peanut grains, regardless of dose (**Figure S3**). Across all the NP treatments, CuO NPs caused the greatest change in terms of the decreased amino acid content in peanut grains; TiO_2 NPs had the least impact on the amino acid profile, especially at 50 $mg \cdot kg^{-1}$.

Amino acids participate in many metabolic processes in plants, and play an essential role in defending against abiotic stresses.[63] For example, proline (Pro) is a reactive oxygen species (ROS) scavenger and an important component in plant cell walls.[64] Previous studies demonstrated that the elevated levels of Pro were evident in plants and algae in response to metal exposure, presumably for metal sequestration and metal−induced ROS scavenging.[65, 66, 67] Conversely, exposure to different metal−based NP in the current study notably reduced Pro content by 16.4−40% in peanut grains as compared to the control (**Figure S2**), suggesting that the Pro biosynthesis pathways were severely compromised. The GSH metabolic pathway is critical to the plants ability to counteract abiotic stressors. Approximately 43 and 50% increases in the content of cysteine, a precursor of GSH, were found in Ag NP and Ag^+ ion treated transgenic *Crambe abyssinica* by overexpressing bacterial γ−glutamylcysteine synthase as compared to wild type *C. abyssinica*.[68] In metal treated wild type *C. abyssinica*, Ag^+ ions lowered the cysteine content by approximately 15%, and no difference was found in the Ag NPs treatment.[68] Low doses of NP CeO_2 resulted in a significant increase in the free thiol contents in

rice roots, but with increasing CeO_2 NP concentrations, this value returned to that of the controls.[69] At the transcription level, exposure to 50 and 500 $mg \cdot L^{-1}$ indium oxide (In_2O_3) NPs significantly up−regulated the genes encoding cysteine synthetase and GSH synthetase in exposed *Arabidopsis thaliana* seedlings.[70] Similarly, TiO_2 NPs up−regulated the transcription levels of cysteine synthetase and GSH synthetase in *A. thaliana* roots.[71] The level of histidine (His) production directly influences select element accumulation (such as Ni, Cu, and Zn) in plants. The presence of NP CuO and Fe_2O_3 in peanut grains notably deceased the His content by 11−35% (**Figure S2** and **Table S3**), suggesting that metal−based NPs not only decreased select micronutrient contents, but also reduced overall nutritional quality at certain concentration levels. The lysine (Lys) and methionine (Met) contents in cucumber fruit of plants exposed to NPs CuO were decreased by 55%–61% and by 13%–25%, respectively.[72] FTIR results suggested that 500 and 750 $mg \cdot kg^{-1}$ TiO_2 NPs significantly decreased the amide band area in the exposed cucumber fruit relative to the control.[59] Priester et al. (2017) found that the content of protein carbonyl in soybean were significantly reduced by 51 and 60% upon exposure to 500 $mg \cdot kg^{-1}$ and 1000 $mg \cdot kg^{-1}$ CeO_2 NP treatments, respectively.[73] In *Brassica napus* L., exposure to CuO NPs decreased seedling protein content from 0.052 to 0.031 $mg \cdot g^{-1}$ dry weight.[74] In summary, exposure to metal−based NPs can significantly alter the content of amino acids in the edible portions of crops such as peanut, and the potential impacts on daily nutrient intake for human health should be further evaluated.

Fatty acids content

Fatty acids are important energy sources, essential components of membrane lipids, and also

play important roles in biotic and abiotic defenses in plants.[75, 76] Thus, the fatty acid content was measured in NP treated peanut grains to investigate potential alterations in fatty acid profile and fatty acid−derived signaling pathways, as well as decreased nutritional crop quality (**Figure 6**, **Figure S4** and **Table S4**).

Figure 6A shows the relative content of major saturated (C16:0, C18:0, C22:0, and C24:0) and unsaturated (C18:1n9c and C18:2n6c) fatty acids in control and NP treated peanut grains. Exposure to 50 $mg \cdot kg^{-1}$ TiO_2 NPs significantly decreased the relative content of C22:0 and C24:0 by 20.4% ($p=0.0045$) and 18.6% ($p=0.0084$), respectively; the other two NPs had no impact on the relative content of saturated fatty acids in peanut grains. For unsaturated fatty acids, TiO_2 NPs at 50 $mg \cdot kg^{-1}$ significantly elevated the relative content of C18:1n9c to 49.1% from 45.6% in the control; the relative content of C18:2n6c was also decreased in 50 $mg \cdot kg^{-1}$ TiO_2 NP treated grains. The relative content of the rest of the remaining saturated and unsaturated fatty acids (below 1%) in NP treated peanut grains are shown in **Figure S4** and **Table S4**. The presence of different metal−based NPs significantly altered the relative contents of C15:0, C17:0, C21:0, C20:1, and C20:2 in peanut grains. When comparing the ratios of saturated to unsaturated fatty acids upon NP exposure, an increasing but statistically insignificant trend at the lower CuO NP treatment level was evident, whereas both Fe_2O_3 and TiO_2 NPs caused a dose−dependent decreasing trend in the ratio (**Figure 6B**).

Many important fatty acid derived signaling molecules are localized in plant cell membranes and these molecules can act as intracellular or extracellular mediators in response to biotic and abiotic stresses.[77] Saturated fatty acids play essential roles in plant growth and unsaturated fatty acids determine the compositions of cell membrane and the integrity of specific cellular functions.[78, 79, 80] Under stress conditions, unsaturated fatty acids are converted to saturated fatty

acids. Exposure to 500 mg·L^{-1} CeO_2 NPs significantly lowered the contents of unsaturated fatty acids in rice roots, including (C18:1, C18:2, and C18:3), with the greatest reduction of the total unsaturated fatty acids evident at 500 mg·L^{-1} CeO_2 NPs.[81] Yuan et al. (2016) reported that C18:3, C16:3, and C18:2 were converted to C16:0 in 100 mg·L^{-1} CuO NP treated *Arabidopsis thaliana* roots, suggesting NP−induced oxidative stress.[82] Similarly, exposure to 0.5 mg·L^{-1} $NiCl_2$ NPs significantly increased the levels of C18:0, C20:0, and C22:0 in green microalgae.[83] Additional abiotic stressors could also result in the reduction in the contents of unsaturated fatty acids in plants.[80, 84, 85] Both 16− and 18−carbon fatty acids can function as chemical signals and help to maintain appropriate levels of phytohormones.[75, 76] For example, oxylipines, enzymatically oxygenated fatty acids, hexadecanoid (derived from 16:3) and octadecanoid (derived from 18:3) are involved in jasmonate (JA) biosynthesis.[76, 77] A previous study demonstrated that exposure to TiO_2 NPs (0−1000 mg·L^{-1}) had no impact on JA levels in rice, although slight increases in the NP treated plants were evident.[61] In a co−exposure scenario with cadmium, a 25% reduction in the JA contents was found, suggesting metal−induced abiotic stresses could disrupt phytohormone homeostasis.[61] However, studies on the relationship between phytohormones and fatty acid content in NP treated crops are lacking. The presence of CuO NPs significantly increased the fatty acid saturation degree in NP CuO exposed *Arabidopsis thaliana* roots, potentially reducing endocytosis and subsequently lowering NP translocation from roots to shoots.[82]

In addition to the alterations of the profiles of amino acids and fatty acids, previous studies also demonstrated that metal−based NPs could significantly affect carbohydrate synthesis pathways. For example, exposure to TiO_2 NPs (0−500 mg/L) severely disturbed starch and sucrose metabolic pathways, as well as glyoxylate and dicarboxylate metabolism, in rice, and

eventually resulted in yield loss and quality reduction.[86] In cucumber fruit, FTIR analysis showed that exposure to 250−750 mg·kg^{-1} TiO_2 NPs altered the composition of macromolecules, including lipids, amide, lignin, and carbohydrates.[59] Similarly, 50 mg·L^{-1} γ−Fe_2O_3 elevated the soluble sugar content and induced oxidative damage in watermelon at the earlier stage.[87] Thus, when using metal−based NPs as a novel fertilizer in agriculture, assessment of the potential negative consequences of NPs to crops in a life cycle should be carried out, as our present work suggests that high doses of metal−oxide NPs could lower the nutritional quality and cause yield reduction.

Resveratrol content

The resveratrol content in different NP treated peanut grains are shown in **Figure 7**. The resveratrol levels in both control and Fe_2O_3 NP treated grains were less than 0.1 mg·kg^{-1}. Exposure to CuO and TiO_2 NPs significantly increased the resveratrol content to 1.8 and 2.3 mg·kg^{-1} at 50 and 500 mg·kg^{-1}, respectively, while the resveratrol content in the control was less than 0.1 mg·kg^{-1} (**Table S5**). The presence of TiO_2 NPs significantly elevated the resveratrol content to 1.6 and 2.2 mg·kg^{-1} at 50 and 500 mg·kg^{-1}, respectively. No difference was found when comparing the levels of resveratrol between CuO and TiO_2 NPs at the same dose.

As a stilbene phytoalexin phenolic compound, resveratrol, produced in plants roots, shoots, and grains, played vital role in responding to biotic and abiotic stresses.[88, 89, 90] The resveratrol content in peanut increased almost 200−fold relative to the control upon exposure to ultraviolet radiation.[91] Other abiotic stressors in grape leaves such as ultraviolet C (UV−C) and calcium chloride ($CaCl_2$) also induced increases in the levels of resveratrol, ranging from 1.2 to 8.7−fold

of the control. For biotic stress, *Botrytis cinerea* infested tobacco exhibited disease resistance by producing up to 40 $\mu g \cdot g^{-1}$ resveratrol.[93] Phytohormones such as jasonmate also increased the resveratrol content of grape.[94] A number of other studies have shown that both abiotic and biotic stressor could significantly elevate the resveratrol contents.[95, 96, 97] Our findings demonstrate an increase in the resveratrol content in peanut grains as a function of CuO and TiO_2 NPs exposure. However, it seems that Fe_2O_3 NP had no impact on the resveratrol content as compared to that of the control. Further study is needed to determine the role of this compound as a signaling molecule to stimulate/activate plant defense related pathways.

Taken together, the greenhouse study suggests that the type and exposure dose of metal-based NPs could significantly determine the phytotoxicity to crop plants. Exposure to high dose of CuO NPs not only suppressed the peanut growth at the physiological level, but also it significantly altered the nutritional quality in terms of the amino acids content and saturation degree of SFA/UFA. In comparison with CuO NPs, other two metal-based NPs did not exhibit adverse impacts on peanut growth, but growth enhancement was observed in the treatments with certain exposure doses, indicating that use of metal−based NPs within appropriate doses as novel nanofertilizers might be possible for enhancing crop yield and nutritional quality. In addition, prior to widely apply NPs/NP incorporated agrichemicals, further investigation on evaluation of the safety and effectiveness of NPs to crops under both greenhouse and field conditions is warranted.

Associated content

Supporting information

Additional information includes TEM images of metal−based NPs, soil characterization, amino acid and fatty acid profile, figures of the contents of the remaining amino acids and fatty acids in grains, as well as summary of the amino acid contents involved in plant metabolic pathways.

Conflicts of interest statement

The authors declare no competing financial interest.

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Figure 1. Phenotypic images of peanut plants upon exposure to different concentrations of CuO (**A**, **B**), Fe_2O_3 (**C**, **D**), and TiO_2 (**E**, **F**) NPs for 145 days.

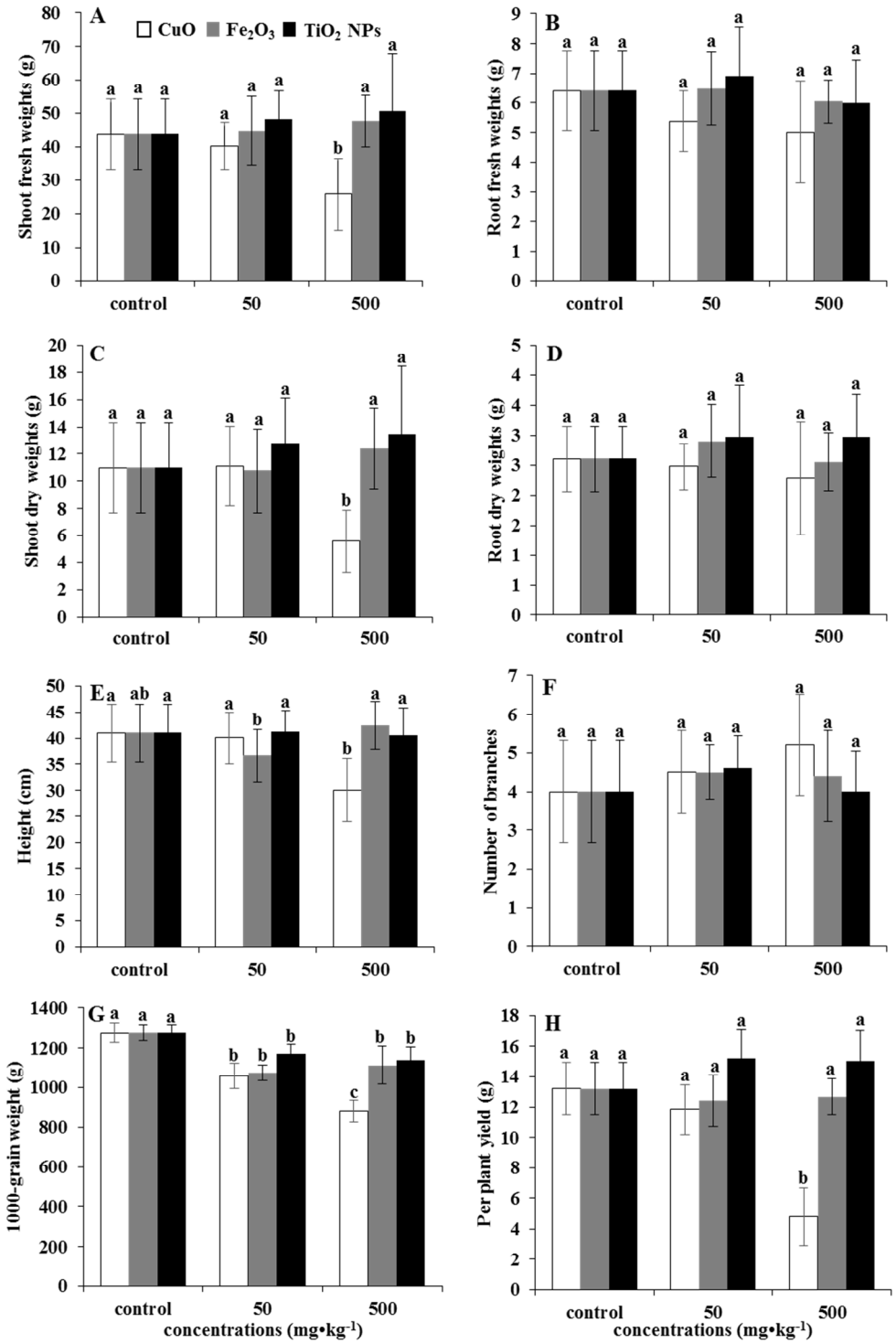


Figure 2. Physiological responses of peanuts upon exposure to different concentrations of different NPs. (**A**) – (**H**) represent plant height, fresh biomass, dry biomass, Number of branches, 1000-grain weight, as well as per plant yield, respectively. Error bars represent standard error (n=3), and different letters represent significant differences among treatments ($p \leq 0.05$).

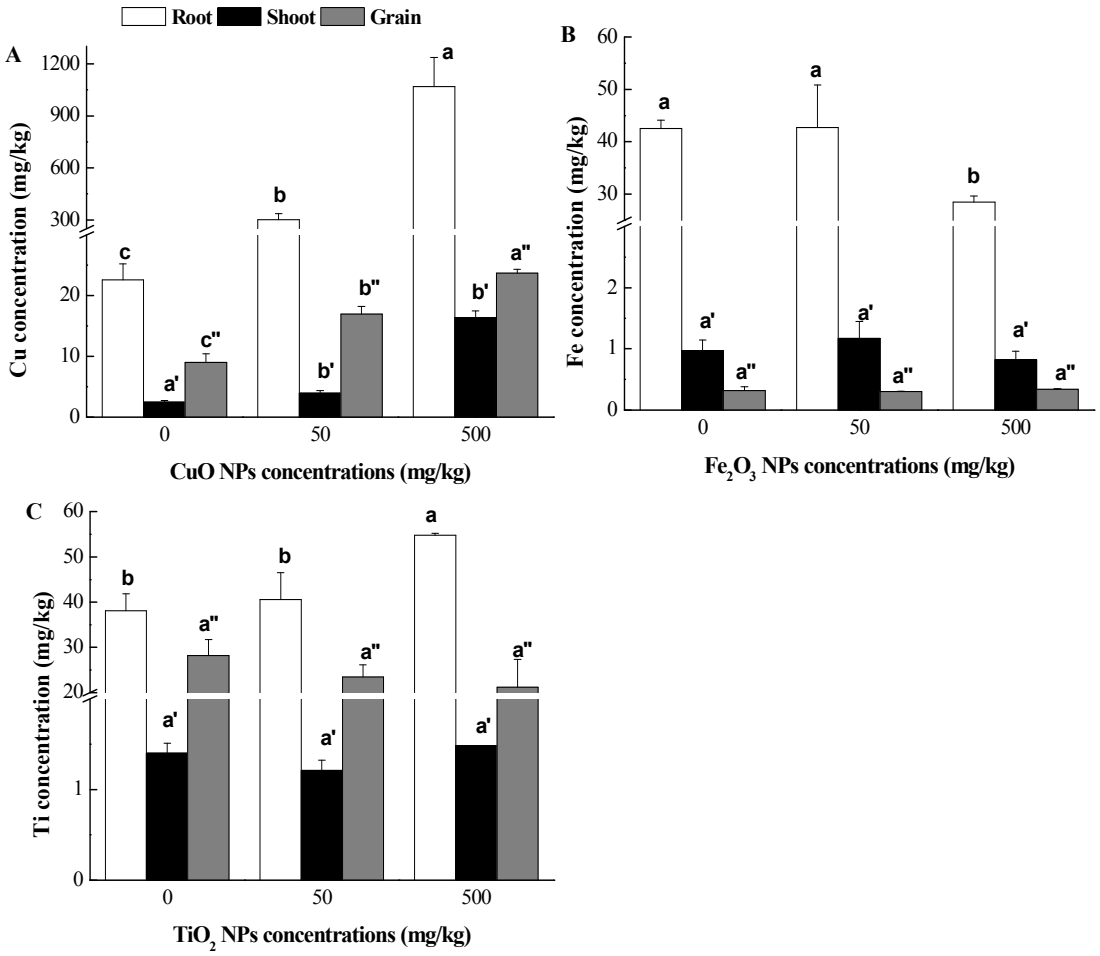


Figure 3 The contents of Cu, Fe, and Ti in the roots, shoots, and grains of peanuts treated with different concentrations of NP CuO, Fe_2O_3, and TiO_2, respectively. (**A**) The Cu contents in 50 and 500 mg/kg CuO NP treated peanut roots, shoots, and grains; (**B**) The Fe contents in element concentrations in 50 and 500 mg/kg Fe_2O_3 NP treated peanut roots, shoots, and grains; (**C**) The Ti contents in 50 and 500 mg/kg TiO_2 NP treated peanut roots, shoots, and grains. Error bars represent standard error (n=3), and different letters represent significant differences among treatments ($p \leq 0.05$).

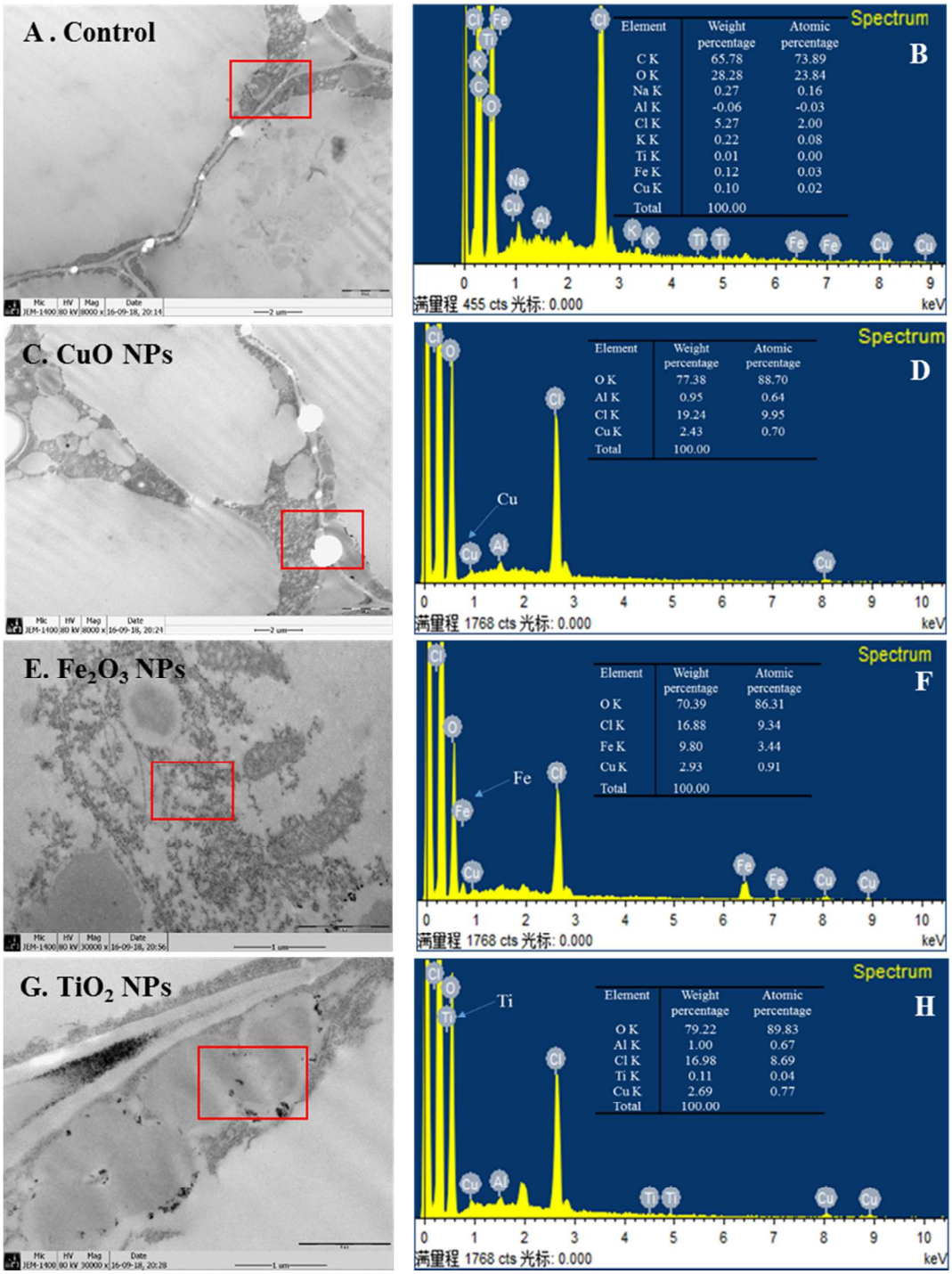


Figure 4. TEM images of NP observation in peanut grains treated with 500 mg/kg NPs. Figure **A** and **B**: control without NP treatment; Figure **C** and **D**: CuO NP treatment; Figure **E** and **F**: Fe_2O_3 NP treatment; Figure **G** and **H**: TiO_2 NP treatment. Figure **A**, **C**, **E** and **G** represent TEM images in NP treated peanut grain; Figure **B**, **D**, **F** and **H** represent the corresponding spectra in the TEM images.

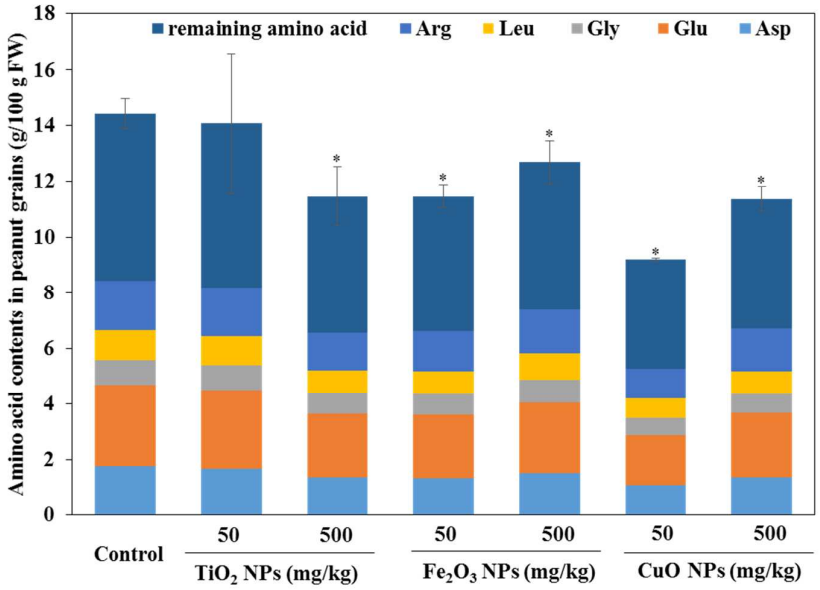


Figure 5. The contents of amino acids in different metal-based NP treated peanut grains.

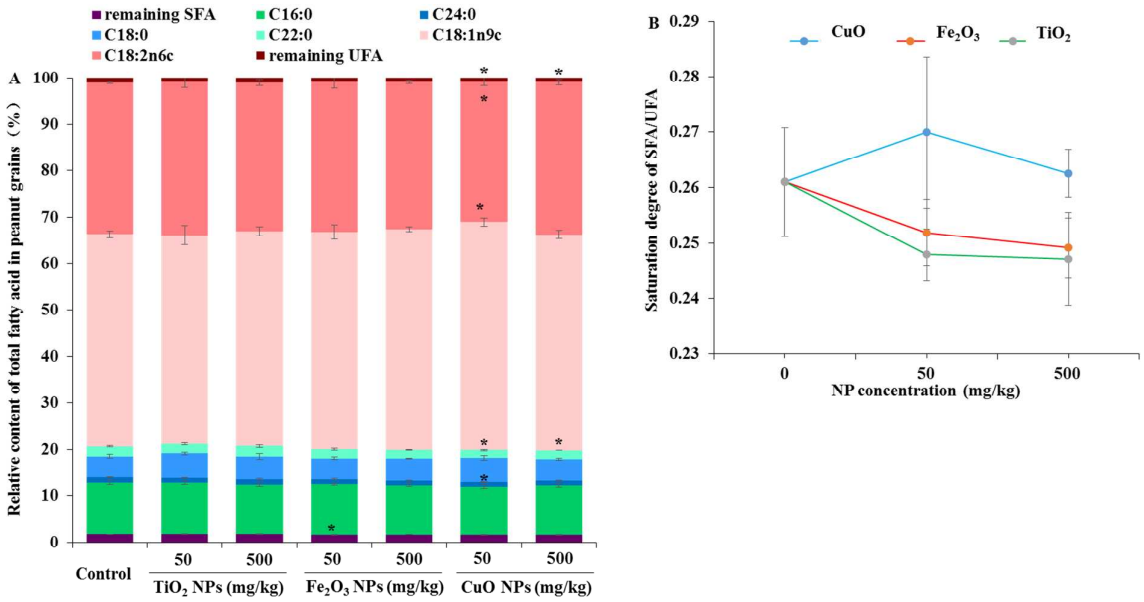


Figure 6. Fatty acid profiles (**A**) and dynamic variations of saturation degree (SFA/UFA) (**B**) in different NP treated peanut grains. The asterisks indicate the significant differences ($p \leq 0.05$) when compared with the control.

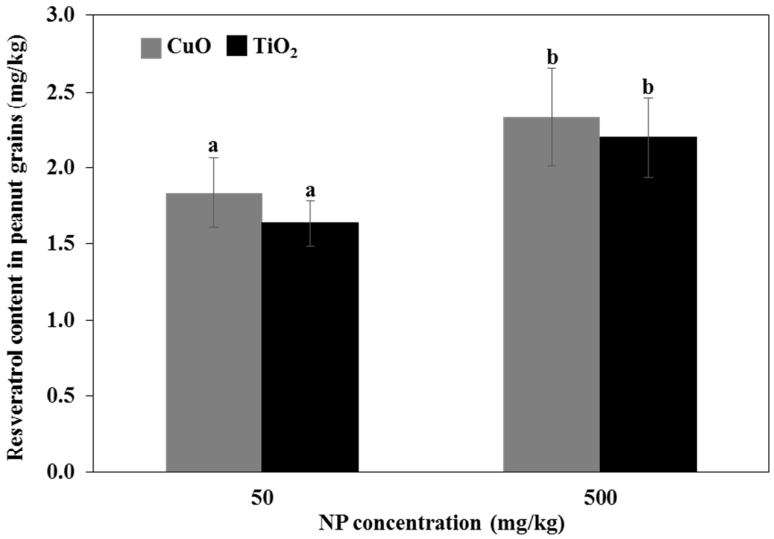
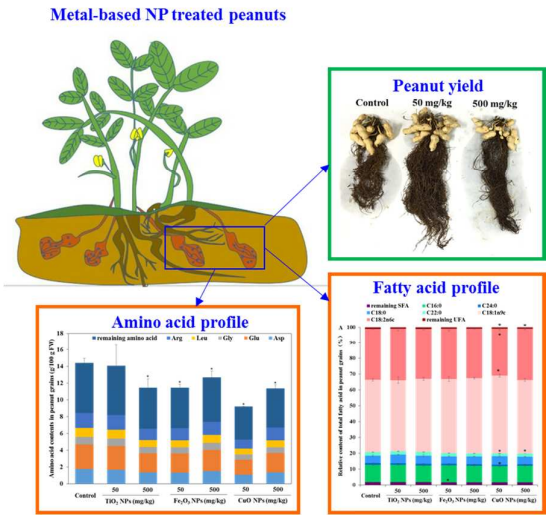


Figure 7. The contents of resveratrol in the NP treated peanut grains. Resveratrol contents in the control and both the Fe_2O_3 NP treatments were below detection limit.


Metal-based NP treated peanuts
Peanut yield
Control
50 mg/kg
500 mg/kg
Amino acid profile
Fatty acid profile

TOC