Turkish Journal of Agriculture - Food Science and Technology, 10(12):2368-2372, 2022
DOI: https://doi.org/10.24925/turjaf.v10i12.2368-2372.4544

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Effect of CuO Nanoparticle on *Ceratophyllum demersum*

Hayder Hameed Alhamadani[1,a,*], Muhittin Dogan[1,b]

[1]*Department of Biology, Faculty of Arts and Sciences, University of Gaziantep, 27310 Gaziantep, Türkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 30/06/2021
Accepted : 29/07/2021

Keywords:
Ceratophyllum demersum,
CuO
Oxidative stress
Physiological effect
Nanoparticle

ABSTRACT

In this study, it was aimed to determine some physiological effects of CuO nanoparticle on *C. demersum*. The plants were collected from an uncontaminated pond in Gaziantep province. Different concentrations of CuO (0, 25, 50, 100 and 200 mg/L) were applied to the macrophytes after being acclimatized in controlled conditions. Some analyses were made on macrophytes harvested at the end of the application. The contents of protein, total carbohydrate, photosynthetic pigment, total phenolic compound, non-protein sulfhydryl groups of tissues increased with increasing CuO concentration under the influence of applied CuO concentrations. In addition, increases in hydrogen peroxide and MDA contents were also detected. As a result, it was determined that the applied CuO concentrations caused some physiological changes in *C. demersum* tissues.

[a] *hayder.en83@gmail.com* *https://orcid.org/0000-0002-2655-2546* | [b] *muhgan@gmail.com* *https://orcid.org/0000-0001-5400-8065*

Introduction

Nanotechnology refers to the interdisciplinary activities that research and develop the production, design, characterization, assembly and systems of nanoscale materials. Nanotechnology designs and synthesizes artificial structures by processing known molecules with different atoms and molecules. With nanotechnology, it has become possible to produce materials that are more functional, fast, take up less space, consume less energy, are more durable, inexpensive and have extraordinary new properties (Rao et al., 2005). Advances in nanotechnology and nanotechnology-based industries and applications are growing tremendously. With the studies in the field of nanotechnology, remarkable breakthroughs are made in many different fields such as materials, nanoelectronics, energy, biotechnology, medicine and health, national security and information technologies (Siddiqui et al., 2015).

Among the different types of nanomaterials, metal and metal oxide nanoparticles differ in their small size and large surface area, as well as their various physical and chemical properties such as surface loads, shapes, conductivity, differences in melting and freezing points (Dizaj et al., 2014; Falcaro et al., 2016). Due to its unique properties, many metals, metal oxides and alloy nanoparticles are produced and used in many fields (Karlsson et al., 2008). For example, metal nanoparticles such as TiO_2, Cu, Zn, Al and Ag nanoparticles are used as additives in consumer and industrial products.

In the toxicity and genotoxicity studies of metal nanoparticles, metal particles such as gold, silver, titanium and zinc are widely used. *In vivo* and *in vitro* studies conducted with these particles show that the toxic effects usually vary depending on the method, cell line and concentration used (Lanone et al., 2009). Many studies have been conducted on the environmental levels and effects of engineered nanomaterials on living organisms. However, the possible effects of these nanomaterials on ecosystems and plants are still largely unclear. Therefore, this study was carried out to determine some physiological effects of nano-CuO applications on *Ceratophyllum demersum*, an aquatic plant.

Materials and Method

Plant Material and Application

C. demersum was collected from Karapinar pond (Yavuzeli-Gaziantep, Turkey). The plants were acclimatized in 10% nutrient solution (0.88 mM K_2SO_4, 2 mM Ca $(NO_3)_2$, 0.25 mM KH_2PO_4, 1 mM $MgSO_4$, 0.11 mM KCl, 100 µM Fe-EDTA, 10 µM H_3BO_3, 5 µM MnSO4, 10 µM $ZnSO_4$, 2 µM $CuSO_4$ and 0.2 µM $(NH_4)6Mo_7O_{24}$), under 25±1°C, 16 h light (120 µE $m^{-2}s^{-1}$) and 8 h dark periods (Snijders Scientific, Netherlands). After the plants were acclimatized in the nutrient solution, they were cultured in jar (1000 mL) containing different concentrations of CuO (0, 25, 50, 100 and 200 mg/L). After 7-days of CuO treatments, the plants were harvested. They were washed three times with deionized water to remove any elements adhered to plant surface. All chemicals used in the study were prepared in analytical grade.

Physiological Analysis

100 mg of fresh plants were weighed for pigment determination and homogenized with 80% acetone in porcelain mortar. The final volume was then completed to 10 mL of 80% acetone and centrifuged at 3000 rpm for 5 minutes. Samples were read against acetone in a UV/VIS spectrophotometer at 662, 645 and 470 nm. pigment calculations were made according to Lichtentaler and Wellburn (1985). Protein contents of plant tissues were determined according to Lowry et al. (1951). Bovine serum albumin was used as a standard. Determination of non-protein –SH groups of the plant tissues was carried out according to Ellman (1959). Content of total phenolic compounds was determined by Ratkevicius et al. (2003). 50 µL of the supernatant of the centrifuged samples were taken at room temperature for 30 minutes by adding 3% sodium carbonate and 0.3 N Folin-Ciocalteau to a final volume of 1 mL. These samples were then read on the spectrophotometer at 765 nm. Gallic acid is used as standard. Anthron method was used to determine total carbohydrate contents (Plummer, 1998). Glucose was used as the standard. H_2O_2 content in the plant tissues was determined according to the method of Sergiev et al. (1997). The content of hydrogen peroxidewas calculated from a standard curve. Determination of malondialdehyde (MDA) of the plant tissues was carried out according to Zhou (2001).

Data Analysis

Statistical analysis of data was performed using SPSS 11.0. One-Way ANOVA LSD test was used to determine which group or groups were different. Pearson correlation analysis was used to determine the relationship between the data.

Results and Discussion

In this study, the physiological effects of different concentrations of CuO in *C. demersum* grown under controlled conditions were determined. The chl-a content of the plant is found to increase as 41.25%, 39.4%, 53.7%, and 57.3% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively (Figure 1a). Similarly, increases up to 52.6% were found in chlorophyll-b contents. In addition, there was no significant change in carotenoid contents (Figure 1a). It was determined that the chl-a and chl-b contents of *Solanum tuberosum* leaves increased the Cu nanoparticle effect (Mushinskiy and Aminova, 2019). However, there are conflicting data in the literature regarding the effect of nanoparticles on chlorophyll content. For example, some researchers talk about an increase in pigment content (Korotkova et al., 2017), while others talk about a decrease in pigment content (Lebedev et al., 2019). The reason for this is the nanoparticle type, nanoparticle diameter, concentration, plant variety, plant development period, application environment and conditions, etc. can be caused by many reasons.

The anthocyanin content of *C. demersum* is found to increase as 6.1%, 37.1%, and 48.9% at 50, 100, and 200 mg/L CuO, compared with control, respectively, but it is decreased as 3.4% at 25 mg/L CuO compared with control (Figure 1b). Similarly, significant increases in anthocyanin content were detected in Arabidopsis plants exposed to Ag nanoparticles (Qian et al., 2013).

The protein content of *C. demersum* was measured that which change by the effect of copper oxide nanoparticles (Figure 1c). The protein content of the plant is found to increase as 21% 32%, 29.7% and 21.2% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively. There are previous studies indicating an increase in protein content with concentrations of heavy metals, as the protein content of wheat plants treated with copper is lower compared to plants that have been treated with zinc (Singh et al., 2007). These results are also similar to Manivasagaperumal et al. (2011) who found that plants treated with low zinc concentration had a high protein and amino acid content in them, and this increase may be due to oxidative stress.

It was determined that the total carbohydrate content of the plant increased up to 22.1% (Figure 1d). Various results have been published regarding the effects of nanoparticles on macromolecules of plants. It has been reported that nano CuO at 50 and 100 mg/kg did not affect carbohydrate content in green pea seeds (Ochoa et al., 2018). On the other hand, nano Cu was found to reduce the carbohydrate content of thyme leaves (Du et al., 2015). However, carbohydrate synthesis was induced in tomato plant under the influence of TiO_2 nanoparticles (Song et al., 2013).

The phenolic content of the plant is found to increase as 11.2%, 23.1%, 28.6% and 46.9% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively (Figure 1e). Elzaawely et al. (2007) found that the total phenolic content in copper-treated plants increased significantly compared to untreated Alpinia plants. The non-protein SH groups content of the plant is found to increase as 11.6%, 18.6%, 25.3% and 24.7% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively (Figure 1f). Increased amounts of non-protein sulfhydryl groups were found in Cu-sensitive strains of *Silene cucubalus* (De Vos et al., 1992). In conclusion, plants under stress may have increased production of stress metabolites such as phenolic compounds and non-protein sulfhydryl groups compared to plants without stress.

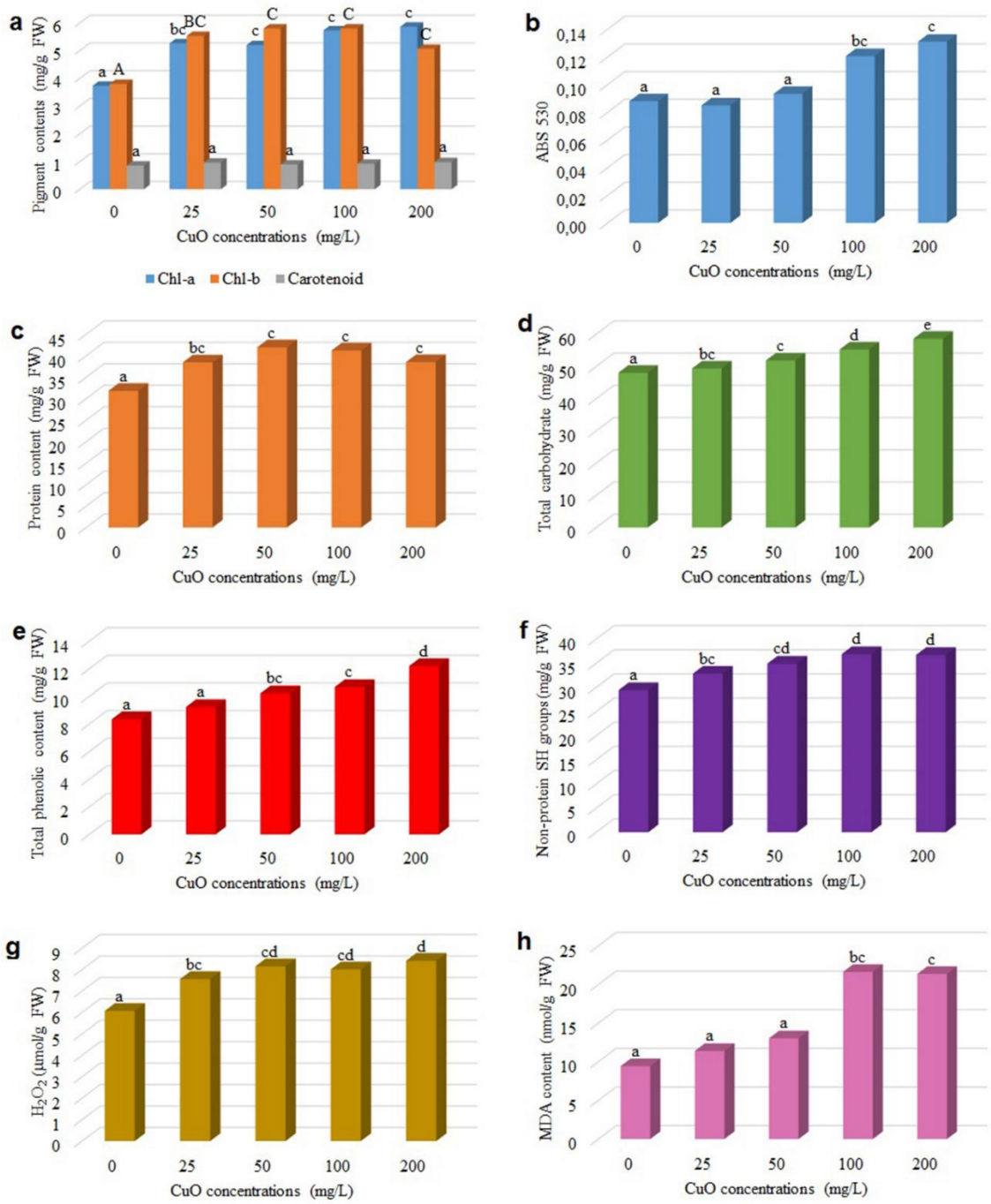

Figure 1. Effect of CuO on the contents of photosynthetic pigment (a), anthocyanin (b), protein (c), total carbonhydrate (d), total phenolic (e), non-protein sulphydryl groups (f), hydrogen peroxide (g) and MDA (h) in *C. demersum*. Means with different letters are significantly different from one another according to LSD test (P<0.05).

Table 1. Correlation analysis of the biochemical parameters obtained after CuO applications

	Chl-a	Chl-b	Car	Anth	Pro	TC	SH	TPC	H_2O_2	MDA
Chl-a	1									
Chl-b	0.647**	1								
Car	0.328	0.138	1							
Anth	0.457	0.302	0.237	1						
Pro	0.585*	0.517*	0.295	0.312	1					
TC	0.468	0.218	0.227	0.870**	0.426	1				
SH	0.673**	0.310	0.246	0.569*	0.695**	0.694**	1			
TPC	0.604*	0.406	0.251	0.813**	0.440	0.949**	0.718**	1		
H_2O_2	0.722**	0.468	0.074	0.512	0.733**	0.700**	0.828**	0.768**	1	
MDA	0.484	0.216	0.098	0.802**	0.345	0.908**	0.774**	0.852**	0.656**	1

Car: Carotenoid; Anth: Anthosiyanin; Pro: Protein; TC: Total Carbohydrate; Non-protein SH groups: SH; TPC: Total phenolic content; H_2O_2: Hydrogen peroxide; MDA: Malondialdehyde

There is an increase in reactive oxygen species (ROS) as a result of toxicity caused by nanoparticles (Melegari et al., 2013; Mohammed et al., 2021). As the amount of ROS in the cell increases, the negative effects of oxidative stress also increase (Sevindik et al., 2017; Mohammed et al., 2020; Pehlivan et al., 2021). The H_2O_2 content of the plant are found to increase as 24.5%, 34.2%, 31.9% and 38.5% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively (Figure 1g). MDA content, which is an important peroxidation product of polyunsaturated fatty acids, increased due to oxidative stress caused by high CuO concentrations. Based on the findings the MDA content of the plant are found to increase as 20.9%, 38.2%, 129.4% and 126.6% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively (Figure 1h). Similar to our findings, it has been shown in many previous studies that nano-Cu application causes an increase in the amount of H_2O_2 and MDA in plant tissues as a result of oxidative stress (Shaw and Hossain 2013; Nair and Chung 2015; Chung et al., 2019).

Nanotechnology is a recent area having many prospective to effect on the environment and plants. As a result, it was observed that CuO concentrations caused an increase in the content of protein, total carbohydrate, photosynthetic pigment, total phenolic compound, non-protein sulfhydryl groups of plant tissues. Also, the amounts of MDA and H_2O_2 in *C. demersum* tissues showed an increase under the effect of CuO, and was observed high concentrations of applied CuO cause oxidative stress. Depending on correlation analysis, relationships between oxidative stress parameters (H_2O_2 and MDA) and other analyzed parameters (Table 1) showed that these changes may be related to oxidative stress triggered by CuO.

References

Chung I, Rekha K, Venkidasamy B, Thiruvengadam, M. 2019. Effect of copper oxide nanoparticles on the physiology, bioactive molecules, and transcriptional changes in *Brassica rapa* ssp. *rapa* seedlings. Water, Air and Soil Pollution, 230: 48.

De Vos RCH, Vonk MJ, Vooijs R, Schat H. 1992. Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. Plant Physiology, 98: 853-858.

Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K. 2014. Antimicrobial activity of the metals and metal oxide nanoparticles, Materials Science and Engineering: C, 44: 278-284.

Du W, Gardea-Torresdey JL, Ji R, Yin Y, Zhu J, Peralta-Videa JR, Guo H. 2015. Physiological and biochemical changes imposed by CeO_2 nanoparticles on wheat: A life cycle field study. Environmental Science and Technology, 49(19): 11884-11893.

Ellman GL. 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics, 82:70-77.

Elzaawely AA, Xuan TD, Tawata S. 2007. Changes in essential oil, kava pyrones and total phenolics of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. Leaves exposed to copper sulphate. Environmental and Experimental Botany, 59(3): 347-353.

Falcaro P, Ricco R, Yazdi A, Imaz I, Furukawa S, Maspoch D, Doonan CJ. 2016. Application of metal and metal oxide nanoparticles@ MOFs. Coordination Chemistry Reviews, 307: 237-254.

Karlsson HL, Cronholm P, Gustafsson J, Möller L. 2008. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chemical Research in Toxicology, 21(9): 1726-1732.

Korotkova AM, Lebedev SV, Gavrish IA. 2017. The study of mechanisms of biological activity of copper oxide nanoparticle CuO in the test for seedling roots of *Triticum vulgare*. Environmental Science and Pollution Research, 24(11): 10220-10233.

Lanone S, Rogerieux F, Geys J, Dupont A, Maillot-Marechal E, Boczkowski J, Lacroix G, Hoet P. 2009. Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. Particle and Fibre Toxicology, 6: 1-14.

Lebedev SV, Gavrish IA, Galaktionova LV, Korotkova AM, Sizova EA. 2019. Assessment of the toxicity of silicon nanooxide in relation to various components of the agroecosystem under the conditions of the model experiment. Environmental Geochemistry and Health, 41: 769-782.

Lichtenthaler HK, Wellburn AR. 1985. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biochemical Society Transactions, 11: 591-592.

Lowry OH, Rosebrought NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193: 265-275.

Manivasagaperumal R, Vijayarengan P, Balamurugan S, Thiyagarajan G. 2011. Effect of copper on growth, dry matter yield and nutrient content of *Vigna radiata* (l.) Wilczek. Journal of Phytology, 3(3): 53-62.

Melegari SP, Perreault F, Popovic R, Costa RH, Matias WG. 2013. Evaluation of toxicity and oxidative stress induced by copper oxide nanoparticles in the green alga *Chlamydomonas reinhardtii*. Aquatic Toxicology, 143: 431-440.

Mohammed FS, Kına E, Sevindik M, Dogan M, Pehlivan M. 2021. *Datura stramonium* (Solanaceae): Antioxidant and Antimicrobial Potentials. Turkish Journal of Agriculture-Food Science and Technology, 9(4): 818-821.

Mohammed FS, Şabik AE, Sevindik E, Pehlivan M, Sevindik M. 2020. Determination of Antioxidant and Oxidant Potentials of *Thymbra spicata* Collected from Duhok-Iraq. Turkish Journal of Agriculture-Food Science and Technology, 8(5): 1171-1173.

Mushinskiy AA, Aminova EV. 2019 Effect of iron, copper and molybdenum nanoparticles on morphometric parameters of *Solanum tuberosum* L. plants. IOP Conf. Series: Earth and Environmental Science 341: 012195.

Nair PMG, Chung I M. 2015. Changes in the growth, redox status and expression of oxidative stress related genes in chickpea (*Cicer arietinum* L.) in response to copper oxide nanoparticle exposure. Journal of Plant Growth Regulation, 34(2): 350-361.

Ochoa L, Zuverza-Mena N, Medina-Velo IA, Flores-Margez JP, Peralta-Videa JR, Gardea-Torresdey JL. 2018. Copper oxide nanoparticles and bulk copper oxide, combined with indole-3-acetic acid, alter aluminum, boron, and iron in *Pisum sativum* seeds. Science of The Total Environment, 634: 1238-1245.

Pehlivan M, Mohammed FS, Şabik AE, Kına E, Dogan M, Yumrutaş Ö, Sevindik M. 2021. Some Biological activities of ethanol extract of *Marrubium globosum*. Turkish Journal of Agriculture-Food Science and Technology, 9(6): 1129-1132.

Plummer DT. 1998. An introduction to Practical Biochemistry. Third edition. Tata McGraw-Hill Publishing Company Ltd., New Delhi.

Qian H, Peng X, Han X, Ren J, Sun L, Fu Z. 2013. Comparison of the Toxicity of Silver Nanoparticles and Silver Ions on the Growth of Terrestrial Plant Model *Arabidopsis thaliana*. Journal of Environmental Sciences (China), 25(9): 1947-1956.

Rao CNR, Müller A, Cheetham AK. 2005. The Chemistry of Nanomaterials V.1, Wiley-Vch Verlag GmbH and Co. KgaA, Weinheim.

Ratkevicius N, Correa JA, Moenne A. 2003. Copper accumulation, synthesis of ascorbate and activation of ascorbate peroxidase in *Enteromorpha compressa* (L.) Grev. (Chlorophyta) from heavy metal-enriched environments in northern Chile, Plant, Cell and Environment, 26: 1599-1608.

Sergiev L, Alexieva E, Karanov E, 1997. Effect of spermine, atrazine and combination between them on some endogenous protective systems and markers in plants. Comptes Rendus de l'Académie Bulgare Des Sciences, 51: 121-124.

Sevindik M, Akgul H, Pehlivan M, Selamoglu Z. 2017. Determination of therapeutic potential of *Mentha longifolia* ssp. *longifolia*. Fresen Environ Bull, 26(7): 4757-4763.

Shaw AK, Hossain Z. 2013. Impact of nano-CuO stress on rice (*Oryza sativa* L.) seedlings. Chemosphere, 93: 906-915.

Siddiqui MH, Al-Whaibi MH, Mohammad F. 2015. Nanoparticles and their impact on plants. Nanotechnology and plant sciences. Springer, New York.

Singh D, Nath K, Sharma YK. 2007. Response of wheat seed germination and seedling growth under copper stress. Journal of Environmental Biology, 28: 409-414.

Song U, Jun H, Waldman B, Roh J, Kim Y, Yi J, Lee EJ. 2013. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO_2 and Ag on tomatoes (*Lycopersicon esculentum*). Ecotoxicology and Environmental Safety, 93: 60-67.

Zhou Q. 2001. The measurement of malondialdehyde in plants. In: Zhou Q. (Ed.): Methods in Plant Physiology. China Agricultural Press, Beijing: pp. 173-174.