

PAPER • OPEN ACCESS

Effects of Zinc and Copper Stress on Antioxidant System of Olive Leaves

To cite this article: Jian Liang and Weifei Yang 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **300** 052058

View the [article online](#) for updates and enhancements.



IOP | ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

Effects of Zinc and Copper Stress on Antioxidant System of Olive Leaves

Jian Liang^{1,2,*}, Weifei Yang²

¹Aba Teachers University, Wenchuan, Sichuan 623002, China

²Xichang University, Xichang City, Sichuan Province, China

*Corresponding author e-mail: 583132434@qq.com

Abstract. The purpose of this experiment is to understand the effects of different concentrations of Cu (Cu) and Zn (Zn) heavy metals on the antioxidant index of olive oil and provide a theoretical basis for phytoremediation of heavy metal pollution. In this experiment, the annual olive seedlings were studied. In the experiment, the heavy metal solution was used to simulate the soil heavy metal pollution environment. The concentration of heavy metals Cu and Zn was studied. The active leaves of the olive oil were malondialdehyde (MDA) and soluble. Effects of physiological factors such as protein, superoxide dismutase (SOD), POD, and CAT. In this experiment, the pot experiment of stress oil olive seedlings was studied by setting different concentration gradients of heavy metal iron and Zn. The authors analyzed the antioxidant enzyme system and membrane lipid peroxidation of heavy metal Zn and Cu in olive seedlings. The link between changes in biochemical indicators. Finally, the experimental results showed that the activity values of CAT and POD in the leaves of olive oil showed a trend of decreasing first and then increasing within the experimental concentration range. Among them, the activity of SOD in the leaves was firstly increased, and then the concentration of the enzyme was increased first. The increase of the AMD dropped sharply; the content of MDA in the experiment was not changed under the low concentration treatment, but the high concentration of Zn and Cu in the experiment caused a sharp increase in the content of malondialdehyde; soluble protein content Continue to rise.

1. Introduction

Olea europaea L is also known as an evergreen tree of the genus Oleaceae. This is a cultivar with high edible value, which is rich in high-quality edible vegetable oil-olive oil. It is a famous subtropical fruit tree and an important economic forest, which is rich in the Mediterranean climate zone. This is a world-famous woody oil and fruit tree with a fruit oil content of up to 30%. Olive oil has high nutritional value and medicinal value, and its beauty and health functions have been widely recognized by the world.

The natural conditions in Liangshan Prefecture of Sichuan Province are superior. The temperature, sunshine, rainfall and air humidity in some areas are close to the Mediterranean region of the origin of olive oil. Xichang City in Liangshan Prefecture of Sichuan Province has also become one of the key bases for the development of olive oil in the country. At present, the Xichang Oil and Olive Industry



Technology Demonstration Park has built the only elite olive seed base in 226 national key forest tree breeding bases and China's first national-level olive germplasm resource bank, becoming the only provincial-level olive seed base and province in Sichuan Province. Grade olive oil research and development center [1].

As a protein and enzyme structure and as a cofactor for catalytic compounds, Cu is involved in the normal growth and development of plants, and is also one of the essential elements for physiological and biochemical processes. However, when the amount exceeds the required content of plants, it will produce a large amount. Free radicals and malondialdehyde (MDA) can cause the growth of plants to be inhibited, thus affecting the redox process of plants, which in severe cases will lead to disturbances in plant metabolism [2]. As an essential element of plant growth, Zn is very active in the biochemical process of plants. However, Zn is also easy to transfer, and when it is excessive, it will cause serious damage to the normal growth of plants [3]. In nature, people have begun to realize the impact of heavy metals on plants, and have begun to pay attention to the symbiosis of Cu and Zn and their behavioral characteristics in soil plant systems [4]. However, studies on the combined stress of Cu and Zn and their tolerance mechanisms have rarely seen news reports.

In this experiment, different concentrations of Zn and Cu were used to treat the olive seedlings. According to the understanding, the physiological indexes of the enzymes in the leaf protection system changed to some extent in the Cu^{2+} and Zn^{2+} environments. Coercion. By measuring the physiological and biochemical indexes of the protection system, a comparative study was carried out on the heavy metal complex stress olive oil, so as to further explore the physiological adaptation mechanism of olive oil to heavy metal Fe^{2+} and Cu^{2+} resistance, and provide reasonable restoration for itself, and provide some physiological theoretical basis. [5].

2. Materials and methods

2.1. Experimental materials

In this experiment, the annual olive oil was used, and the Foao variety cutting seedlings were used as materials (the leaves were collected from Lizhou in Xichang City in 2016). Plant height is about 60cm, and soil weight is about 3kg.

2.2. Experimental drugs and instruments

Experimental equipment: enzyme meter, HH-8 digital thermostat water bath, centrifuge, spectrophotometer.

Experimental drugs: $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, distilled water, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, H_2O_2 , ascorbic acid, EDTA- Na_2 , trichloroacetic acid, thiobarbituric acid (TBA), ethanol, L-methionine, Riboflavin, Coomassie Brilliant Blue, Bovine Serum Albumin, Nitro Blue Tetrazolium (NBT), Guaiacol

2.3. Experimental design

The experiment was conducted in Lizhou Town, Xichang City from September to December 2016. A total of 4 treatments and 1 control (Table 1) were set up, and each treatment was repeated 10 times. First, select 50 pots of annual olive seedlings with the same growth potential. The height of the plants is about 55~65cm, and the label is attached. The quality is weighed in turn, and the required drug is calculated according to the relative molecular mass of the drug and the weight of the soil weighed. The drug is weighed and weighed in an electronic balance, and then applied to each treatment.

After 40 days of heavy metal stress treatment, the physiological indexes of the leaves of the same leaf position were determined for each treatment. The olive leaves are deburred, shredded and mixed, and each sample is weighed to about 0.3g. 2ml of pH 7.8 phosphate buffer and a small amount of quartz sand were added to the pre-cooled mortar to grind it into a homogenate, and then the mortar was rinsed with pH 7.8 phosphate buffer pH 7.8 PBS to make a final volume of 6 ml. Centrifuge at

15000g for 10 minutes at 4°C to obtain the supernatant, and store it at a low temperature of 4°C. It must be stored on ice for shading and used as needed [6].

Table 1. Progressive complex concentration of Zn and Cu in soil

Processing number	Zn ²⁺	Cu ²⁺
CK	0	0
A	150mg/L	50mg/L
B	250mg/L	150mg/L
C	300mg/L	250mg/L
D	325mg/L	350mg/L

2.4. Determination method

In this experiment, the hydrogen peroxide method was used to determine CAT, guaiacol method for the determination of POD, SOD using nitroblue tetrazolium (NBT) method, and the determination of MDA and soluble sugar by arsenic barbituric acid (TBA) method. Coomassie blue staining was used to determine soluble protein.

3. Results and analysis

The experiment was repeated 3 times, the mean value±standard value was calculated, the average and standard deviation of the data were calculated by Microsoft Excel software, and the data was processed and differentially significant analyzed by IBM SPSS Statistics 19.

3.1. Effects of Zn and Cu complex stress on CAT activity in leaves of Olive

This experiment is to verify that CAT is ubiquitous in all tissues of plants. It is the terminal of a series of antioxidant enzymes in the process of biooxidation. CAT can remove excess H₂O₂ from cells, thus keeping cells at a normal level. Protect plant cell membrane structure [7]. The data in Table 2 shows that compared with CK and Cu/Zn, when compared with CK, when the stress concentration is Zn150+Cu50, the activity of CAT in olive leaves decreased significantly and reached the lowest value, with a decrease of 34%. However, with the increase of stress concentration, the activity of CAT in olive leaves increased gradually, with the increase rates of 103.06%, 26.08% and 21.28%, respectively, which were higher than CK (Table 2), and the activity of CAT in olive oil was in Cu350+Zn325 concentration. When the maximum value is reached, CK and A, B, C, and D are significantly different from each other.

Table 2. Effect of Cu-Zn compound stress on CAT content in leaves of olive oil (p<0.05)

deal with	Average data	Significant level
CK	116.667±4.226	d
A	76.167±4.475	e
B	154.667±9.208	c
C	195.000±8.322	b
D	236.500±11.056	a

3.2. Effects of Zn and Cu complex stress on POD activity of olive leaves

In this experiment, it is verified that POD is an enzyme containing Fe, which is widely present in plant tissues and is one of the important protective enzymes in plants. It can effectively remove excess free radicals in plants, thus enabling Cells are protected from poison. When an excess of Cu and Zn enter the plant body, the plant itself undergoes a series of physiological and biochemical reactions to produce peroxides that are harmful to itself, but as this peroxide increases, POD will gradually begin to utilize H₂O₂. To catalyze this peroxide, which is not conducive to plant growth, and begin to achieve oxidative decomposition. This effect cannot be ignored in plant respiration [8]. It can be seen

from Table 3 that as the concentration of the two heavy metal complex stresses increases, compared with CK, the POD activity of the olive leaves shows a tendency to decrease first and then slowly. The data in the table indicate that when the stress concentration is Zn150+Cu50, the POD activity of olive oil reaches the lowest value, compared with CK, the decrease is 45.38%. However, with the increase of the composite concentration, the activity of POD gradually increased, and the activity reached the maximum at Cu350+Zn325, which increased by 56.57%. Under the combined action of Cu+Zn, the complexes of Cu^{2+} and Zn^{2+} were treated from low to high. The POD content of A, C and D treatments was significantly higher than that of the control group ($P<0.05$), which were 0.55, 1.96 and 3.62 times of the control, respectively.

Table 3. Effect of Cu-Zn compound stress on POD content in leaves of olive oil ($p<0.05$)

deal with	Average data	Significant level
CK	47.330±1.333	c
A	26.000±3.055	d
B	53.330±3.528	c
C	92.670±3.712	b
D	171.330±5.812	a

3.3. Effects of Zn and Cu complex stress on SOD activity of olive leaves

In this experiment, it is necessary to verify that SOD is an important protective enzyme against oxygen free radical damage to the cell membrane system. It has an important role in delaying plant cell organ senescence, improving plant stress resistance, and resisting oxygen damage, and as a superoxide radical. Scavengers, the level of activity and plant growth resistance also have a certain correlation. Under moderate adverse conditions, the activity of SOD will increase. This is because under the adverse environment of heavy metal stress, the plant has an increasing tendency to resist the stress and survive in order to adapt to the adversity. This state is reached [9]. In the experiment, SOD peaked at Zn150+Cu50 treatment and decreased with increasing treatment concentration. The enzyme activities of treatment C and treatment D were 77.73% and 39.60%, respectively, and the enzyme activities decreased under the combined treatment. Obvious (as shown in Table 4). Under the stress of Cu and Zn, the active oxygen scavenging enzyme system and the physiological activities with resistance characteristics of olive oil were induced to accelerate [10], SOD was induced here, SOD activity decreased sharply or slowly, and Cu and Zn complexed. The effect is to make the SOD activity decrease more. The CK and A, B, C, and D treatments are significantly different from each other.

Table 4. Effect of Cu-Zn compound stress on SOD content in olive leaves ($p<0.05$)

deal with	Average data	Significant level
CK	117.176±1.334	c
A	173.277±1.763	a
B	146.803±5.414	b
C	91.076±4.070	d
D	46.390±2.098	e

3.4. Effects of Zn and Cu combined stress on MDA content in leaves of Olive

In this experiment, MDA is one of the most important products of membrane lipid peroxidation. Its production can also aggravate membrane damage. The content of malondialdehyde also indicates the strength of lipid peroxidation. The extent of damage to the membrane system can be indirectly determined by measuring the MDA content. The data in Table 5 showed that under the combined action of Cu/Zn, the MDA content began to increase with the increase of treatment concentration. The MDA of olive leaves under treatments A, B, C and D were higher than the control 25%, 64.13%, 177.17% and 298.91%, respectively. The increase of MDA content indicates that the membrane lipid

peroxidation level is increased, resulting in an increase in membrane permeability and a deeper damage to the membrane structure, which weakens the resistance of plants [11]. Compared with CK, the MDA content of olive oil increased by 43.92% under D treatment, which was 3.99 times of that of the control group. This indicates that with the increase of the concentration of Cu-Zn composite treatment, the level of MDA in the olive leaves increased, and the damage to the plants gradually deepened, which is consistent with the aggravation of the symptoms of olive oil under the combined treatment of high concentration of Cu and Zn [12].

Table 5. Effect of Cu-Zn compound stress on MDA content in leaves of olive oil ($p < 0.05$)

deal with	Average data	Significant level
CK	0.092±0.005	d
A	0.115±0.009	d
B	0.151±0.010	c
C	0.255±0.012	b
D	0.367±0.012	a

3.5. Changes in soluble protein content

In this experiment, it is necessary to verify that soluble proteins are important osmotic adjustment substances and nutrients. Their increase and accumulation can improve the water retention capacity of cells, protect the cells' vital substances and biofilms, and are therefore often used as screening resistance. One of the indicators [13]. It can be seen from Table 6 that the soluble protein content continued to increase in the stress treatment concentration range, and the soluble protein content reached the highest and was basically stable when D was treated. It can also be seen that after 40 days of treatment, soluble protein showed an increasing trend with the increase of two heavy metal concentrations. A~D treatment increased by 10.14%, 20.29%, 28.99% and 50.72%, respectively, compared with the control. ($P < 0.05$).

Table 6. Effect of Cu-Zn compound stress on soluble protein content in leaves of olive oil ($p < 0.05$)

deal with	Average data	Significant level
CK	0.069±0.000	d
A	0.076±0.001	c
B	0.083±0.004	b
C	0.089±0.000	b
D	0.104±0.001	a

4. Conclusions and discussion

4.1. Discussion

4.1.1. Effect of Zn and Cu Stress on SOD, POD and CAT in Olive Leaves. Under normal growth conditions, SOD, POD, CAT and other protective substances can maintain the dynamic balance of free radical production and removal in plants, and eliminate the damage of free radicals on cell membrane structure, and the maintenance of SOD, POD, and CAT activity. Improvement is also considered to be one of the material basis for plant tolerance to heavy metal stress [14]. In this experiment, when the low concentration of Cu and Zn were treated, the SOD activity of the leaves increased. This is because the active oxygen initiates the defense reaction of the cells, but as the treatment concentration increases, the accumulation of heavy metal ions in the body gradually increases. Large, the toxic effects of plants are also increasing. When the two heavy metal complex stresses make the clearance function of the protective enzyme system in the plant cell insufficient to withstand the active oxygen free radicals produced by the plant, the free radicals will accumulate in the leaf cells of the plant,

thereby inducing the passage of the plant cells. Oxidation damage. This also indicates that under the condition of heavy metal stress, the protective effect of the active oxygen scavenging system on plant cells in plants is not unlimited, and it has certain limits. On the other hand, the activity values of CAT and POD in the leaves showed a tendency to decrease first and then increase in the experimental concentration range. This is because the complex action of the experimental heavy metals stimulated and stimulated the activity of CAT and POD, combined with the permeability of the cell membrane. The trend of MDA obtained can be concluded that these two enzymes can alleviate free radical accumulation and membrane lipid peroxidation to a certain extent, and play a major antioxidant role in Cu and Zn stress. Therefore, in this stress process the effect is not obvious.

4.1.2. Effect of Zn and Cu stress on olive MDA. In this experiment, when the low concentration of Zn and Cu combined treatment, the content of malondialdehyde did not change substantially, but the high concentration of Zn and Cu compounded the content of malondialdehyde sharply. This indicates that when the two heavy metals exceed a certain concentration, the plant cell membrane system is damaged, and the degree of damage is directly proportional to the complex concentration. The increase in MDA content also shows that the level of membrane lipid peroxidation is increasing, the membrane permeability is also increasing, and the membrane structure is gradually weakened, which makes the plant's resistance weakened [15].

4.1.3. Effect on the soluble protein of olive oil. Soluble proteins are important osmotic adjustment substances and nutrients. Their increase and accumulation can improve the water retention capacity of plants, and play an important role in protecting the vital substances and biofilms of cells. In this experiment, the soluble protein content of the oily seedlings of Zn and Cu combined with heavy metals was significantly increased, which also indicated that under this stress, the plants were able to withstand stress and always protected the normal physiology and differentiation of the plants.

5. Conclusion

Heavy metal Cu and Zn combined stress caused the imbalance of the activity of the protective enzymes of plant cells, SOD, POD and CAT, which was highly destructive, which caused the disorder of normal physiological metabolism of plants and accelerated the senescence and death of plants. Furthermore, it was found that under the stress of two heavy metals with low concentration, the physiological activity indexes of olive oil changed in the direction favorable to the growth of olive oil, which improved the ability of antioxidant enzyme system to scavenge oxygen free radicals and reduced the oxidative stress. The damage is beneficial to alleviate the toxicity caused by high Zn stress on plants. In the case of high concentration of Zn stress, the olive oil will have physiological disorders, which will affect the healthy growth of the plant.

Acknowledgments

This work was financially supported by the key project of the Sichuan Provincial Department of Education fund, project number is (50180009); Xichang University two high talent project fund, project number is (50180062).

Liang Jian (1979-), female, Sichuanese, associate professor, mainly engaged in ecological environment research.

References

- [1] Li Jing, Gong Fayong, Peng Yin et al. Evaluation of Heavy Metal Pollution in Agricultural Products Producing Areas in Yuexi County, Sichuan Province. Xichang University Newspaper, Vol. 2 (2012) No. 26, pp.11-17.
- [2] Li Xiaojing, Li Desheng, Li Hairu et al. Effects of exogenous Cu stress on the growth and quality of woody vegetables. Environmental Pollution Dyeing and Control, Vol. 4 (2012) No. 35, pp.38-42.

- [3] Xu Xiaoyan, Yang Xiaoyu, Yang Yuaiet al. Research Progress in the Morphological and Physiological Mechanism of Zn in Plants. *Elemental Science*, Vol. 6 (1999) No. 11, pp.1-6
- [4] Wang Guanglin, Wang Lilong, Wang Yupeng et al. Soil Cu and Zn pollution and soil enzyme activity in sewage irrigation area of smelting plant. *Journal*, Vol. 6 (2005) No. 12, pp.328-332.
- [5] Zhao Shijie. *Experimental guidance of plant physiology*. Beijing: China Agricultural Science and Technology Press, (1998), pp152-154.
- [6] Gu Jiguang, Lin Qiuqi, Hu Ren et al. Approaches to the treatment of heavy metal pollution in soil-plant systems and their research prospects. *Soil Bulletin*, Vol. 1 (2005) No. 36, pp.128-133.
- [7] Jiang Xingyu, Zhao Kefu. Damage of Plant Heavy Metals and Its Resistance Mechanism. *Journal of Applied and Environmental Biology*, Vol. 1 (2005) No. 7, pp.92-97.
- [8] Xu Qinsong, Shi Guoxin, Zhou Hongwei et al. Chlorophyll content and active oxygen scavenging system of chlorophyll content before waterwheeling by Cd and Zn combined pollution Influence. *Journal of Ecology*, Vol. 1 (2003) No. 22, pp.5-8.
- [9] Wang Guanglin, Zhang Jinchi, Wang Li et al. Effects of Cu and Zn stress on physiological indexes of cloves. *Journal of Nanjing Forestry University*, Vol. 4 (2009) No. 33, pp.44-47.
- [10] Zhang Lina, Sun Hai et al. Effects of Fe²⁺ stress on some physiological indexes of American ginseng. *Southwest Agricultural Journal*. (2013), 25.
- [11] Yang Shuzhen, Gao Junfeng. Reactive oxygen, free radicals and plant senescence. *Journal of Northwest Botany*, Vol. 2 (2001) No. 21, pp.215.
- [12] Zhu Zheng, Jiang Jiayue, Jiang Changjun et al. SOD, soluble protein and soluble sugar content in tea leaves under low temperature stress Influence. *Journal of Anhui Agricultural University*, Vol. 1 (2011) No. 38, pp.24-26.
- [13] Hu Bingyi, Wu Anquan. Effects of Different Grinding Methods on Determination of Soluble Protein Content in Maize Leaves. *Journal*, Vol. 5 (2011) No. 28, pp.24-25.
- [14] Sha Cuiwei, Meng Qingrui, Wang Jing et al. Physiological responses of two colorful leaf plants to lead stress. *Journal of Northwest Forestry University Newspaper*, Vol. 4 (2011) No. 26, pp.36-40.
- [15] Liang Fang, Guo Jinping. Research progress on the mechanism of plant heavy metal toxicity. *Shanxi Agricultural Sciences*, Vol. 7 (2011) No. 35, pp.59-61.