Bull. Environ. Contam. Toxicol. (2003) 71:1011–1018
© 2003 Springer-Verlag New York Inc.
DOI: 10.1007/s00128-003-0227-6

Environmental Contamination and Toxicology

Effects of Sewage Irrigation on the Growth and Scavenging System of Activated Oxygen of Crop Plants

L. Dengyi, W. Youbao

College of Life Science, Anhui Normal University, Wuhu 241000, Anhui, People's Republic of China

Received: 31 August 2002/Accepted: 24 June 2003

Since 1950s, there have been dramatic developments in industry and the increasing of urban population, because of which, the amount of disposal of industrial and domestic wastewater has increased with the passing of each year. Many farmers in the suburban area get to irrigate their land with such disposing wastewater. So far, the total sewage irrigated land in the whole country exceeds 1.33×10^6 hm (Shao 1983). It is shown that the sewage irrigation will increase the organic content of the soil. However, it may also cause some negative effects as decreasing enzyme activity of soil, accumulating heavy metal in soil and crops, lowering poorer quality of the agricultural products, and reducing crude protein and amino acid of the seeds (Shao 1983; Wang 1983; Gao et al. 1997; Qin et al. 1997). Up to now, there have been many publications about the effects of unfavorable conditions on the metabolism of activated oxygen of crop (Khelghati-Bana et al. 1999; Chen et al. 2000; Imahori et al. 2000; Jiao et al. 2000; Kurilenko et al. 2000; Lee et al. 2000; Pritchard et al. 2000; Ye et al. 2000). However, there is no report so far concerning the effects of sewage irrigation on the scavenging system of activated oxygen of crop. This paper explores the effects of irrigation with the waste water from the city on the growth and scavenging system of activated oxygen of crop, and hence provides us the basis for establishing the system of irrigation for the crops.

MATERIALS AND METHODS

Phaseolus radiatus L. and *Raphanus sativus* L. seeds were purchased from Wuhu Seeds Company, Anhui. Full and even quality seeds of *P. radiatus* and *R.. sativus* were select, disinfected in 0.5% NaClO for 10min, and soaked in moving water until the roots and buds show up, then were put them in 25℃ and dark place for 2 days for quick sprouting. Cultivating the seeds treated as above with running water under the conditions of 23C/17C, 9000-12000lx, which was exposed to 14h light a days until the sprouts reached 3.5cm tall.

The experiments were carried out with waste water from Wuhu Iron and Steel

Correspondence to: L. Dengyi

Plant (Waste water I) and that from the Coke Plant nearby (Waste water II), with water from Lifeng section of the Qingyi River as comparison. The seedlings were cultivated according to the methods mentioned above such as the treatments of light exposures and temperature control. Each treatment contained 15 individual seedlings, with 3 repetitions. The seedlings were cultivated for 14 days, changing the cultivating water every 2 days.

Data were taken as follows: 1) Growth of the seedlings: Count the number of the roots, test the total and average length of the roots and the height of the vegetation above the soil, and weigh the fresh and dry weight of the roots as well as the vegetations above soil. 2) Leaf cell membrane's permeability: Weigh 0.25g sample of plant leaf, without main vein, cut it into pieces sized 0.5cm x0.5cm, and vibrate them in 20ml double distilled water for 1h, and then test the conductivity with DDS-12 conductivity tester. 3) Leaf pigment content: Grind and extract the sample in 80% acetone, determine light density by spectrophotometry at the places of 633nm, 645nm and 440nm ,with the unit as mg/g • FW (Zhu et al. 1990). 4) MDA: Determine by Li Zhifang's TBA method. The unit is μ mol/g FW. 5) POD activity: Determine light density at 470nm by spectrophotometry, with traumatin phenol as the substrate of enzyme peroxide. The unit is △D470/mg • FW • min. 6) CAT activity: By hydrogen peroxide decomposing measurement, the unit is units/g • FW • min. 7) SOD activity: With reference to Chen Yizhu's method, take 0.5g sample and freeze-centrifugalize the enzyme liquid for 20 min at 12000/min, and determine light density at 560 nm with △560/g • FW • h as unit (Chen and Pateson 1988).

RESULTS AND DISCUSSION

Fourteen days later following the treatment of sewage irrigation, the *P. radiatus* and *R. sativus* plants appeared harmful symptoms in various degrees. There are no significant differences between the treatments and the controls for the effects of Waste Water I on the growth of the crops (Table 1). However, for the plants treated with Waste Water II, the fresh and dry weight of the vegetation above the ground and the roots decreased 20.6%-43.5% and 51.8%-74.3%, respectively, compared to the control plants (Table 1).

The leaf pigment contents of different crops vary under a certain environment intimidation, and even opposite results may be turned out as different crops may have different strategy to stand for the environment intimidation. We found that there existed significant differences of the chlorophyll content between the sewage-irrigated plants and the control ones (Table 2). The contents of chlorophyll a/b and carotenoid of the sewage-irrigated plants were lower than those of the control ones. For *R. sativus*, the chlorophyll a and b contents of the sewage-irrigated plants decreased 31.61%-47.85% and 21.31%-38.45%, respectively, and the chlorophyll a/b value went down to 10.83%-15.70%,

Table 1. Effects of sewage irrigation on growth.

Crop species	*Phaseolus radiatus*			*Raphanus sativus*		
Treatments*	CK	WWI	WWII	CK	WWI	WWII
Fresh weight above ground part (g)	0.2160	0.2071	0.1385	0.1676	0.1454	0.1159
Dry weight above ground part (g)	0.0201	0.0108	0.0117	0.0105	0.0083	0.0079
Fresh weight of root (g)	0.0301	0.0285	0.0143	0.0137	0.0109	0.0063
Dry weight of root (g)	0.0048	0.0044	0.0018	0.0017	0.0012	0.0006
Number of root (pieces)	9.2	6.1	5.3	8.8	7.3	3.6
Average length of root (cm)	2.6	1.8	1.4	2.0	1.6	0.5
Length of Main root (cm)	4.1	3.7	3.4	3.5	1.9	0.8
Length of above ground part (cm)	9.0	8.5	6.8	8.1	7.1	6.6

* CK: Control, WWI: Waste Water I, WWII: Waste Water II

compared to the controls. However, also for *R. sativus*, the carotenoid content of sewage-irrigated plants increased 12.95%-21.16%, compared to the control ones. The sensitivity of the above three pigments to sewage irrigation ranked as: chlorophyll a > chlorophyll b > carotenoid, which is in accordance with Woolhouse and Yan Zhonglin etc'findings (Woolhouse 1974; Yan et al. 1997). For *P. radiatus*, the chlorophyll a, b and carotenoid contents of the sewage-irrigated plants decreased to 14.6%-19.4%, 6.8%-27.3% and 9.6%-22.4%, respectively, compared to the control ones. The chlorophyll a/b value increased 5.85%-10.31% compared to the controls. The sensitivity of the three pigments to the sewage irrigation ranked as: chlorophyll b > carotenoid > chlorophyll a, which is in accordance with Shu Jianmin etc' findings (Shu et al. 1987).

Membrane, as an important structure of the crop to adjust and control the movement and exchange inside and outside the cell, its permeability is normally one of the methods of testing the crop reaction to pollution. The seepage conductivity of the cell is in positive correlation with the level of pollution (Li et al. 1992; Qin et al.1997; Ma 1998). The higher the conductivity, the higher the peroxidation of the membrane fat (Woolhouse 1974), which will cause the membrane structure to damage, the permeability to enlarge and the solvable substance inside the cell to seep, resulting in serious damage of crops. In our study, the electric conductivity of sewage-irrigated crop was apparently higher than that of the non-sewage-irrigated crop (Fig.1), with average increasing range of 18.03%-175.3%. The conductivity of the crop irrigated with Waste Water II

Table 2. Effects of sewage irrigation on pigment content of the crop leaf.

Crop species	*P. radiatus*			*R. sativus*		
Treatments*	CK	WWI	WW II	CK	WWI	WW II
Chlorophyll a	0.827	0.809	0.661	1.409	0.636	0.485
Chlorophyll b	0.407	0.397	0.297	0.489	0.375	0.301
Carotenoid	0.445	0.402	0.345	0.372	0.447	0.420
Chlorophyll a/b value	2.017	2.135	2.225	1.902	1.696	1.600

* CK: Control, WWI: Waste Water I, WWII: Waste Water II

increased to 2.5-2.8 times higher than that of the control plants.

It was shown that the sewage irrigation resulted in an accumulation of MDA in crops (Fig.2). The MDA content of the sewage-irrigated plants increased and reached 1.12-3.40 times as much as that of the control ones. Being a major product of peroxidation of the membrane fat, MDA interacts with the activated materials as protein, nucleic acid, amino acid etc. and produces unsolvable compound sediment (fat pigment), which disturbs the normal life of the plant cel (Xu et al. 2001). The increase of MDA content results in the rise of membrane fat peroxidation, the enlargement of the membrane permeability and the aggravation of the damage of the membrane structure, which weakens the resistance of the crops against environment intimidation.

Compared to the controls, the POD, SOD and CAT activities of the sewage-irrigated plants droped 37.19%-72.03%, 7.53%-33.57% and 25.00%-61.70%, respectively (Table 3). Scandalios et al. found that the POD-H_20_2 decomposing system participated in the degradation of chlorophyll, and the activity of POD is negatively related to the content of chlorophyll (Paula et al. 1984; Scandalios 1993; Yan et al. 1997). However, it was shown that in this study the activity of POD was positively related to the content of chlorophyll, i.e., under the intimidation of the sewage irrigation, happened the reduction of both the activity of POD and the content of chlorophyll. The possible reason for the above contrasting results is that certain environment intimidation will cause the activity of POD and the content of chlorophyll negatively related. However, beyond certain extreme, the serious intimidation will possibly cause the reduction of both the activity of POD and the content of chlorophyll (Xu et al. 2001). In our study, the sewage irrigation apparently slowed down the crop growth and caused serious damage to crops, so that the reduction happened to both the POD activity and the chlorophyll content.

Table 4 shows the chemical properties of the waste water. It can be drawn from table 4 that, due to the high content of COD, BOD and EC, the two types waste water can not directly used to irrigate crop. Otherwise, they will cause serious damage to the crop. So, these sorts of waste water must be treated before being used. However, the proper irrigation standard of such waters and maximum quantity of these waters to be used need to be further studied. What needs to be

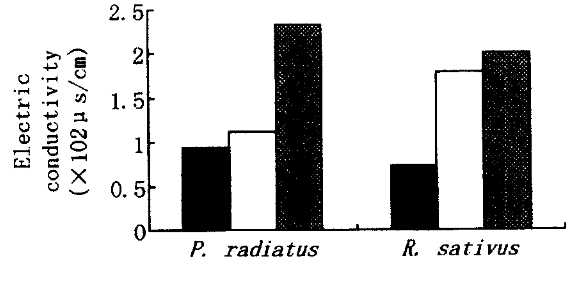

Figure 1. Effect of sewage irrigation on membrane permeability of the crop leaf

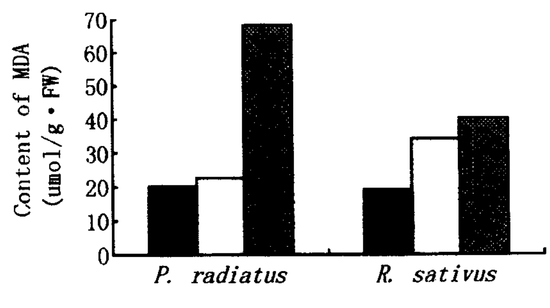

Figure 2. Effect of sewage irrigation on MDA content of the crop leaf

mentioned is that the chemical properties of sewage must be analyzed before being used to irrigate.

The environment intimidation disturbs the balance in the process of the formation and clearance of activity oxygen in crop individuals, and causes heavy accumulation of the free radical, which leads to the peroxidation of the membrane fat and the destruction of the membrane structure of the crops (Yan et al. 1997). POD, SOD, CAT jointly constitutes an effective eliminating system of activated oxygen, which eliminates effectively free radical and peroxidation substances (Paula et al. 1984; Chen et al. 1988; Zhou et al. 1993; Yan et al. 1997; Xu et al. 2001). SOD activity is somewhat related to the crop resistance (Zhou et al. 1993; Yan et al. 1997; Ye et al. 2000; Zhu et al. 2000; Xu et al. 2001). SOD makes O_2 into H_2O_2 and H_2O, and reduces the level of the free radical of the crop individuals. In a certain scope, SOD and CAT can work together to transform O_2 and H_2O into H_2O and O_2, and therefore reduce the formation of ·OH which is in poison and high activation. POD and CAT can catalyze H_2O_2 into H_2O, which prevents O_2 and H_2O_2 from accumulating, and restricts these free radicals from activating the

Table 3. Effects of sewage irrigation on the activity of POD, SOD, CAT of the crops.

Crop species	*P. radiatus*			*R. sativus*		
Treatments*	CK	WWI	WWII	CK	WWI	WWII
POD	121	76	38	118	52	33
SOD	1007	703	669	1010	934	869
CAT	48	36	25	45	32	18

* CK: Control, WWI: Waste Water I, WWII: Waste Water II

Table 4. The chemical properties of the waste water (Unit: mg • L^{-1}).

Water	DO	BOD5	CODCR	pH	EC (μ s • cm^{-1})
Control	7.3	1.6	3.8	7.63	$0.05x10^3$
Waste Water I	4.9	48	164	8.32	$1.01x10^3$
Waste Water II	3.2	163	406	7.67	$1.25x10^3$

peroxidation of the membrane fat (Paula et al. 1984; Yan et al. 1997; Xu et al. 2001).

After sewage irrigation, the activated oxygen eliminating system of the crops was damaged, and the activities of POD, SOD and CAT were greatly reduced, which led to the reduction of the eliminating ability of the crops to activated oxygen, and the accumulation of free genes and peroxidized substances as O_2 and H_2O in crops. This resulted in peroxidation of the membrane fat, destruction of the membrane structure and aging of plant cells and tissues. It came clear that the destruction of the eliminating system of activated oxygen in crop was the major harm of the sewage irrigation to the crops, which resulted in increasing free radical content, high accumulation MDA, peroxidating membrane fat, enlarging membrane permeability, and destroying inner enzyme and original metabolism zone in cell. These effects accelerated the aging process of the plant cells, reduced the content of the leaf pigments, slow the growth of crop and, further, cut down the crop yield.

Acknowledgments. We thank Dr. Zhou Q X (Institute of Applied Ecology, CAS) for comments on the manuscript. We thank Mr. Zheng J Z for corrections of English. The research was supported by the Key Research Fund of Science and Technology of the Education Commission of China. Presented at the First International Conference on Pollution Eco-Chemistry & Ecological Processes, Shenyang, China, Aug. 26-31, 2002.

REFERENCES

Chen YZ, Pateson B (1988) Effects of low temperature on the SOD, CAT activity and H_2O_2content in plant leaves. Acta Phytophysiolog Sinica 14: 323-328.

Chen ZL, Zhu YG (2000) Capillary gel electrophoretic separation of superoxide dismutases in leaf extracts of *Triticum aestivum* L. Phytochem Anal 11: 362-365.

Chis B, Marc V, Dirk I (1992) Superoxide dismutase and stress tolerance. Ann Rev Plant Physiol Plant Mol Biol 42: 83-116.

Gao LX, Wang AM, Yuan ZF, Liu XF (1997) The effect of wastewater on ecology-physiological and cyto-toxicology of vegetables. China Environ Sci 17(5): 443-445.

Imahori Y, Kanetsune Y, Ueda Y, Chachin K (2000) Changes in hydrogen peroxide content and antioxidative enzyme activities during the maturation of sweet pepper (*Capsicum annuum* L.) fruit. J Japanese Soc Hort Sci 69: 690-695.

Jiao HJ, Wang SY (2000) Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. J Agri Food Chem 48: 5672-5676.

Khelghati-Bana F, Alizadeh A (1999) A study on the genetic diversity of Iranian populations of *Tilletia indica* using isozyme analysis. Iranian J Plant Pathol 35: 52-55.

Kurilenko IN ,Pauadina TA (2000) NaCl effect on lipid peroxidation and antioxidant systems in corn seedlings. Plant Physiol Biochem 38:178-194.

Lee DH, Lee CB (2000) Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: In gel enzyme activity assays. Plant Sci, 159: 75-85.

Li Y, Wang HX, Wu YS (1992) Effects of cadmium and iron on the some physiological indication in leaves of tobacco. Acta Ecolog Sinica,12: 147-154.

Ma CC (1998) Hg harm on cell membrance of rape leaf and cell endogenous protection effect. Chinese J App Ecol 9: 323-326.

Paula KP, Thompson JE (1984) Evidence for the accumulation of peroxidized lipids in membranes of senescing cotyledons. Plant Physiol 75:1152-1157

Pritchard SG, Qiu JS, Weaver DB, Prior SA, Rogers HH (2000) The influence of elevated CO_2 on the activities of antioxidative enzymes in two soybean genotypes. Australian J Plant Physiol 27: 1061-1068.

Qin TC, Wu YS, Huang QY, Hu HQ (1997) Effects cadmium, Lead single and combination pollution on the contents of ascorbic acid in *Brassica chinensis* L. Chinese J Ecol 16:31-34.

Scandalios JG (1993) Oxygen stress and superoxide dismutase. Plant Physiol 101: 7-12.

Shao YL (1983) Sewage irrigation in China. China Environ Sci 3: 53-57.

Shu JM, Cao HF, Gao YX, Liu YY (1987) Effects of long-term exposure to low concentration of SO_2 on growth and yield of *Phaseolus vugaris*. China Environ Sci 7: 9-14.

Wang AG (1983) The study on SOD of soybean seed. Acta Phytophysiol Sinica 9: 77-84.

Woolhouse HW (1974) Longevity and senescence in plant. Sci Prog Oxford, 61: 23-28.

Xu QS, Shi GX, Du KH (2001) Effects of Cd^{2+} on antioxidase system and ultrastructure of *Ottelia alismoides*(L.). Rural Eco-Environment 17: 30-34.

Yan CL, Hong YT, Fu SZ, Fang CH, Lei JX, Shen Q (1997) Effect of Cd, Pb stress on scarenging system of activated oxygen in leaves of tobacco. Acta Ecolog Sinica 17: 488-492.

Ye L, Gao HY (2000) Responses of the antioxidant systems and xanthophyll cycle in *Phaseolus vulgaris* to the combined stress of high irradiance and high temperature. Photosynthetica 38: 205-210.

Zhou JY, Dai ZK, Wu ZL, Yu F, Xu YG (1993) The effects of SO_2+NO_2 on superoxide dismutase activity and leaf injury of tomato plant. China Environ Sci 13:429-432.

Zhu YJ, Wang CY, Ma YX, Xia GJ, Wang YH (2000) Effects of arsenic stress on the growth and metabolism of the wheat root systrm. Acta Ecolog Sinica, 20: 707-710.

Zhu GL, Zhong HW, Zhang AQ (1990) Plant physiology experimentation. Peking: Beijing University Press, 51-53, 126-130.