Environment Protection Engineering

Vol. 47 2021 No. 2

DOI:10.37190/epe210201

KAINING SUN[1], NING YANG[1], YARU CAO[2], KEAN WANG[1*], XUHUA LI[2]

IMPROVEMENT OF PHYTOREMEDIATION POTENTIAL OF INDIAN MUSTARD BY SULFUR TREATMENT OF CADMIUM-CONTAMINATED SOIL

The phytoremediation potential of Indian mustard (*Brassica juncea*) on the cadmium-contaminated soil was investigated under the treatment of sulfur (15, 30 and 60 g/kg soil). The effects of the sulfur treatment were evaluated by measuring the biomass and root vitality of the plants, enzymatic activities, and the content of malondialdehyde and Cd. The results show that the biomass and root vitality of the plants were significantly increased, and the activities of superoxide dismutase and catalase were improved when the soil was treated with 30 g sulfur /kg soil, while the activities of peroxidase and malondialdehyde were decreased. The total Cd in the plants treated with 15 g/kg soil was 2.8 times higher than that in the control plants. In summary, the results indicate that the addition of sulfur could promote the growth of Indian mustard and promote the uptake of Cd. As such, the treatment of cadmium-contaminated soil with sulfur can be used as a strategy for the removal of cadmium contamination by improving the phytoremediation potential of Indian mustard.

1. INTRODUCTION

Heavy metal contamination has been a problem to the environment, posing a great threat to the health of animals and humans [1]. In China, about 20% of arable land has been contaminated with heavy metals, among which, cadmium has even a higher level than others [2]. Cadmium contamination originates from two different sources. First, Cd can be found from a natural source, such as sedimentary rocks [3]. Second, serious

[1]Shandong Key Laboratory of Greenhouse Vegetable Biology, Shandong Branch of National Improvement Center for Vegetables, Huang-Huai-Hai Region Scientific Observation and Experimental Station of Vegetables, Ministry of Agriculture and Rural Affairs, Institute of Vegetables and Flowers, Shandong Academy of Agricultural Sciences, Jinan, Shandong, 250100, China.

[2]National Engineering Laboratory for Efficient Utilization of Soil and Fertilizer Resources, College of Resources and Environment, Shandong Agricultural University, Tai'an, Shandong, 271018, China, corresponding author X. Li, email address: lixh@sdau.edu.cn

Cd contamination is from anthropogenic sources, such as commonly applied fertilizers, pesticides, manures and industrial wastes. Cd does not play any essential biological function but is known for its adverse effects on cell metabolism and oxidative stress [4]. Cd accumulation in the edible parts of plants causes a health concern when the plants are consumed by humans or used as feed in the animal husbandry industry [3].

Several approaches have been proposed for the remediation of Cd, either by *in situ* or *ex situ* treatment technologies. Among these, phytoremediation has been developed by utilizing vegetation to absorb and sequester cadmium [5]. As one of many known plants with high ability of metal hyperaccumulation [6, 7], Indian mustard (*Brassica juncea*) is highly tolerant toward Cd (up to 400 mg/kg). In addition, Indian mustard has a fast growth rate, high biomass, and short harvest time, making it a great candidate for phytoremediation of Cd. Many studies have demonstrated that Indian mustard is capable of remediating Cd from contaminated soil [8, 9]. The high accumulation of Cd in Indian mustard may be due to the formation of the phytochelatin–cadmium–sulfide complex [10]. Glutathione may play a critical role in heavy metal toxicity and tolerance [11].

In this study, we attempted to improve the phytoremediation ability of Indian mustard in the Cd-contaminated soil. Instead of genetic modification of Indian mustard, we changed the physical and chemical properties of the soil to increase the total accumulation of Cd in Indian mustard. Our results show that by adding an appropriate amount of sulfur to the contaminated soil, the plants grew much bigger and the total amount of Cd accumulated in the plants is 2.8 times higher than that in the control, indicating that changing the soil properties by adding sulfur is a potential route to improve the phytoremediation ability of Cd. Further experiments need to be performed to fine-tune the conditions for plant growth, biochemical parameters and metal accumulation by Indian mustard.

2. MATERIALS AND METHODS

Plant materials. Seeds of Indian mustard (*Brassica juncea*) were germinated in pots (14 cm in diameter and 15 cm high) which contained 1.5 kg of soil collected from a passive solar greenhouse constructed in 2012 in the experimental field of the Institute of Vegetable and Flower, Shandong Academy of Agricultural Sciences, Jinan, Shandong, China. Cd in the soil was determined with a graphite furnace atomic absorption spectrometry (GF-AAS, model Vario 6) by dissolving in an acidic mixture of nitric and perchloric acid (HNO_3:$HClO_4$); the total concentration was measured to be 0.26 mg/kg. pH of the soil was between 6.5 and 7.5. According to the Environmental Quality Evaluation Standard for Farmland of Greenhouse Vegetable Production in China (HJ 333--2006), the limit value of Cd is 0.30 mg/kg in soil when the soil pH is between 6.5 and 7.5.

Three experiments were carried out. The soil was treated with different concentrations of sulfur (Licheng TanDang Co. Ltd., Jinan, Shandong, China). The control pot (T0) was set up with the untreated soil. Sulfur was added to three pots (1.5 kg soil) to a final concentration of 15, 30 and 60 g/kg (T1, T2 and T3, respectively), and thoroughly mixed with the soil. For each treatment, three replicates were performed. The pots were placed in a passive solar greenhouse during the experiments. During the growth period, appropriate treatment conditions were applied before the plants were harvested. The physiological and biochemical indexes, such as the leaf and root length, fresh weight and dry biomass (oven-dried at 85 °C for 36 h) were measured.

Determination of the root vitality. The root vitality was evaluated by the measurement of the respiratory activity with triphenyltetrazolium chloride (TTC) [12]. TTC was reduced to triphenyl formazan (TF) by dehydrogenases, the concentration of which can be calculated by measuring the absorbance at 485 nm.

Antioxidant enzyme activity and soluble protein content. Lipid peroxidation was evaluated by measuring the malondialdehyde (MDA) content. The soluble protein content was also measured. The activity of SOD was determined by monitoring the reduction of nitrogen blue tetrazolium (NBT) (purchased from Sinopharm Chemical Reagent Co., Ltd., China) at 560 nm, as described elsewhere [13]. The activity of POD was measured by the method described elsewhere [14]. 50 μL extract was added to an assay mixture containing 3% hydrogen peroxide and 0.15 cm^3 of 4% guaiacol (freshly prepared), and the POD activity was calculated by monitoring the absorbance at 470 nm. The activity of CAT was evaluated by measuring the absorbance of H_2O_2 at 240 nm [15]. The MDA content was determined by a thiobarbituric acid (TBA) method [16]. MDA can react with 2-thiobarbituric acid to produce a pink compound, which can be detected by measuring the absorbance at 532 nm. The soluble protein content was evaluated by the Bradford assay, by measuring the absorbance shift of Coomassie Brilliant Blue G-250 at 595 nm [17].

Cadmium content. The cadmium content in leaves and roots was analyzed on the dried samples dissolved in a mixture of nitric and perchloric acid (HNO_3:$HClO_4$) by a graphite furnace atomic absorption spectrometry (GF-AAS, model Vario 6) [18].

Soil enzyme activity and pH. Four enzymes were tested for the activity in the soil after sulfur was added, including urease, phosphatase, sucrase and CAT. The urease activity was measured by the colourimetric sodium phenol-hypochlorite method [19]. Urease is an amidase specifically hydrolyzing urea. The determination of urease activity in soil is based on the amount of ammonia produced by the enzymatic reaction. The phosphatase activity was measured by the disodium phenyl phosphate method [20]. The sucrase activity was evaluated by the 3,5-dinitrosalicylic acid colourimetry [21]. Sugar produced by sucrase can react with 3,5-dinitrosalicylic acid to form an orange-colored 3,5-dinitrosalicylic acid which can be used to represent the activity of sucrase. The activity of CAT in soil was

evaluated by the same method as for the measurement of CAT in plants [15]. Soil pH was closely monitored.

Statistical analysis. The experimental data were processed using Origin 7.5 (Microcal Software, Inc., Northampton, MA, USA) and presented as means±standard error (SE) of three replicates.

3. RESULTS

The presence of sulfur clearly showed an impact on the growth of Indian mustard plants (Fig. 1). At 15 g/kg soil (T1), sulfur promoted the growth of the plants, which were much bigger than those without the sulfur treatment. However, the growth was inhibited when sulfur was increased to 30 g/kg soil (T2). When sulfur was further increased to 60 g/kg soil (T3), the plants died 7 days after planting. Hence, only plants in T0, T1 and T2 were subject to further analyses in the following content.

Fig. 1. Indian mustard grown in the soil with various concentrations of sulfur

The changes of the plant dry biomass were reflected by the changes of both the leaves and roots (Fig. 2). The average weight of fresh leaves in T1 was 22.01 g (Table 1), which was more than five times higher than that in T0. Similarly, the fresh roots were about five times higher than that of T0. The average weight of the leaves was 10 times higher than that of roots, which indicates that Indian mustard is a great plant for phytoremediation.

Various parameters of leaves and roots were measured, including the total leaf area, circumference, total root length, and root surface area (Tables 2 and 3). Plants in T1 were significantly higher and bigger than those in T0 and T2, indicating that the appropriate amount of sulfur can promote the growth of Indian mustard. The growth of the plants in T2 was poor compared with those in T0 and T1, indicating high concentration of sulfur inhibited the growth of Indian mustard.

The root vitality was evaluated by measuring the activity of dehydrogenase of the mitochondrial respiratory chain, which can be reflected by the respiratory activity with

triphenyltetrazolium chloride. The results show that the plants in T1 had significantly higher vitality that those in the T0 with an average TTC of 18.2 μg/(g·h) (Table 4). However, the TTC is at the same level in T2 as in T0, though the growth of Indian mustard was very poor compared with T0.

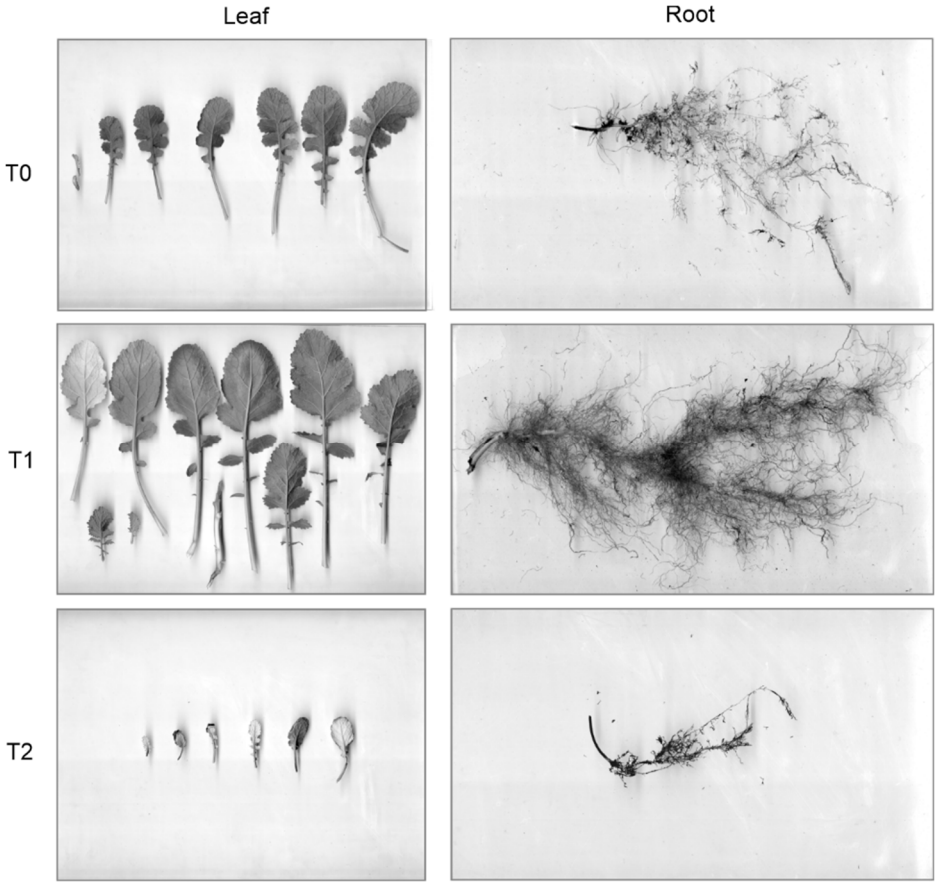

Fig. 2. The leaves and roots of Indian mustard plants under different growth conditions

Table 1

Fresh biomass and dry biomass of the leaves and roots of Indian mustard [g/plant]

Sample	Fresh leaf	Fresh root	Dry leaf	Dry root
T0	4.31±1.22	0.35±0.18	0.38±0.09	0.04±0.01
T1	22.01±1.76	1.95±0.71	1.64±0.16	0.18±0.03
T2	0.53±0.20	0.08±0.05	0.08±0.03	0.01±0.005

All the data are presented as mean±standard error.

Table 2

Characteristics of leaves

Sample	Leaf area [cm^2]	Circumference [cm]	Horizontal length [cm]	Vertical length [cm]	Horizontal length /vertical length	Shape coefficient
T0	122.19±37.82	263.94±52.47	47.09±8.20	27.53±4.48	4.50±0.78	1.37±0.17
T1	360.04±93.81	646.37±216.56	55.95±10.06	55.95±10.06	6.61±1.01	1.59±0.59
T2	7.31±2.44	105.17±31.14	12.84±9.32	6.83±1.90	4.59±0.12	1.28±0.50

All the data are presented as mean±standard error.

Table 3

Characteristics of roots

Sample	Length [cm]	Surface area [cm^2]	Diameter [cm]	Volume [cm^3]?	Number of root tips
T0	214.08 ± 95.01	30.16 ± 16.16	0.44 ± 0.09	0.35 ± 0.25	2115.17 ± 893.78
T1	686.87 ± 205.74	156.50 ± 46.96	0.76 ± 0.28	3.13 ± 1.72	5642.67 ± 882.24
T2	66.70 ± 39.63	7.83 ± 5.29	0.36 ± 0.03	0.07 ± 0.06	371.33 ± 114.50

All the data are presented as mean±standard error.

Table 4

Root vitality

Sample	TTC([μg/(g·h)]
T0	14.39±1.29
T1	18.18±1.09
T2	14.60±1.33

Determined by the measurement of respiratory activity with triphenyltetrazolium chloride.

All the data are presented as mean±standard error.

Table 5

Antioxidant enzyme activity, MDA content and soluble protein content

Sample	SOD [U/g]	POD [ΔOD_{470}/(min·g)]	CAT [ΔOD_{240}/(min·g)]	MDA [μmol/g]	Protein content [mg/g]
T0	220.88±49.58	34.87±7.06	0.42±0.22	6.07±3.31	27.18±6.92
T1	263.45±49.39	27.35±5.21	0.90±0.30	4.90±1.76	33.00±1.93
T2	282.16±19.18	21.49±4.32	0.77±0.41	2.85±0.54	25.10±5.90

All the data are presented as mean±standard error.

Activities of antioxidant enzymes, including SOD, POD and CAT were measured, and the MDA and soluble protein content were determined. The results show that the

activities of SOD and CAT were increased with the addition of sulfur, while the activity of POD was decreased in T1 compared with that in T0 (Table 5). The activity of CAT in T1 was more than 2-fold higher than that in T0. The protein content was slightly increased in T1 compared with T0.

Table 6

Cadmium contents in tissues of Indian mustard

Sample	Leaves		Roots		Total Cd in a plant [μg]
	Concentration [mg/kg]	Total content [μg/plant]	Concentration [mg/kg]	Total content [μg/plant]	
T0	2.63±0.22	1.16±0.32	2.44±0.21	0.07±0.04	1.21±0.36
T1	1.94±0.04	2.70±0.66	2.12±0.23	0.34±0.09	3.35±0.55
T2	2.48±0.26	0.20±0.08	2.73±0.08	0.03±0.001	0.23±0.08

All the data are presented as mean±standard error.

All plants showed an accumulation of Cd in the leaves and roots. The concentration of Cd was comparable between the leaf and root (Table 6). However, the total amount of Cd in leaves is about 10 times higher than that in the root, indicating the leaves are the main organ for Cd absorption and storage. The concentration of Cd was slightly decreased in T1 compared with that in T0. However, because the dry weight of the plants in T1 was much higher than that in T0, the total amount of Cd was 2.8 times higher than that in T0. The concentration of Cd in T2 was comparable with that in T0, while the total amount of Cd was much lower than that in T0.

Table 7

Effects of sulfur on soil pH and its other physical and chemical properties

Sample	pH	Total nitrogen [g/kg]	Effective phosphate [mg/kg]	Potassium [mg/kg]
T0	6.88±0.16	2.93±0.08	11.96±1.41	470.98±58.55
T1	5.58±0.21	2.83±0.17	19.27±0.75	282.00±47.24
T2	4.73±0.36	3.08±0.07	27.89±1.33	630.75±11.69
T3	4.50±0.20	3.11±0.22	27.94±3.41	656.08±14.71

All the data are presented as mean±standard error.

The effect of phosphate is generally influenced by soil acidity. When pH exceeds 7.5 or is below 6, phosphoric acid interacts with calcium, iron and aluminum, and the effectiveness is reduced (Table 7). Our results showed that, by adding sulfur, the pH of the soil was decreased from 6.88 in T0 to 5.58, 4.73 and 4.5 for soils in T1, T2 and T3, respectively. The total nitrogen was not changed. However, the effective phosphate was increased with the addition of sulfur. The potassium concentration was decreased in T1, but increased in T2 and T3.

Table 8

Soil enzyme activity

Sample	Urease [mg NH_3-N/(g·d)]	Phosphatase [mg phenol/(kg·d)]	Sucrase [mg glucose /(kg·d)]	CAT
T0	5.88±0.76*	69.63±11.08	1151.38±29.55	0.09±0.001
T1	4.65±0.12	91.41±10.21	996.60±52.57	0.08±0.01
T2	1.30±0.10	35.97±0.83	394.28±36.28	0.01±0.003
T3	1.01±0.17	20.96±2.32	364.37±33.49	0.01±0.002

All the data are presented as mean±standard error.

Soil acidity affects soil microbial activity. The most suitable pH for soil microbes is within a neutral range of 6.5–7.5. Peroxyacid or overbased additives severely inhibit the activities of soil microbes, which affect the transformation and supply of nitrogen and other nutrients (Table 8). Four enzymes were tested for the activity in soil after sulfur was added, including urease, phosphatase, sucrase and CAT. The activity of phosphatase was increased in T1, but all other enzymes had a lowered activity. In T2 and T3, the decrease of the enzyme activity was even higher. For example, the activity of urease in T3 was more than 5 fold lower than that in T0 (Table 8).

4. DISCUSSION

Vegetation such as Chinese cabbage [7] and wheat seedlings [6] have been used as a way to remove or absorb heavy metals in farmland. In this study, we present the improvement of the phytoremediation ability of Indian mustard in the Cd-contaminated soils by changing the physical and chemical properties of soil. By adding an appropriate amount of sulfur to the contaminated soil, the plants grew much bigger and the total amount of Cd accumulated in the plants was 2.8 times higher than that in the control, indicating that changing the soil properties is a potential route to improve the Cd phytoremediation ability of Indian mustard.

Cd contamination in soil normally has a higher concentration of Cd than that used in this study [22]. In our study, the Cd concentration is only about 0.26 mg/kg soil. We did not purposely add additional Cd in the soil used in the experiments; rather, we collected the soil in a greenhouse built in 2012 in Jinan, Shandong, China. This kind of greenhouses was commonly built in the north part of China. Studies using this soil serve a practical purpose for remediation of Cd in this region of China. The agricultural production depends on the soil, the vast majority of which are neutral, slightly acidic or slightly alkaline with a pH range of 5.5–8.5 [23]. Due to the differences between the north and south of China, the soil studied in this study is slightly acidic with a pH of 6.8.

Our results show that the addition of sulfur changed the acidity of the soil which may be one of the main reasons for the improvement of the growth of Indian mustard.

The plants grew much better at pH 5.58 than that at either 6.8 or below 4.73. The amount of sulfur should be further fine-tuned to find the optimal condition for Indian mustard. Other than the effect of sulfur on soil acidity, sulfur may have a direct impact on the growth of Indian mustard, as indicated by the higher dry weight of the leaves and roots in T1. One study shows that sulfur can improve leaf ascorbate and glutathione and hence protect Indian mustard from cadmium toxicity [24]. A proper application scheme and risk assessment of the sulfur treatment should be carried out before it can be applied on a large scale. Other than sulfur, other reagents, which can improve the properties of the soil, should be tested in future studies.

The phytoremediation of Cd should use a strategy combining different methods. Other than the method we developed, many attempts have been documented in the literature. For example, eleven isolated bacteria, *Variovorax paradoxus*, *Rhodococcus* sp. and *Flavobacterium* sp., offer promising strategies for improving the growth of Indian mustard and for the development of a phytoremediation system of polluted soils [8]. Genetic manipulations have been carried out to improve the detoxification of heavy metals by altering reduced glutathione (GSH) and phytochelatin (PC) levels in plant and hence increase the phytoremediation ability.

5. CONCLUSIONS

W developed a strategy to improve the phytoremediation ability of Indian mustard by treating the soil with sulfur (30 g/kg soil). The biomass and root vitality of the plants were markedly improved. The total amount of Cd in the plants was 2.8 times higher than that in the untreated control. In summary, the results indicate that the addition of sulfur could promote the growth of Indian mustard and promote the uptake of Cd. Our study provides a new way to improve the phytoremediation potential of Indian mustard.

ACKNOWLEDGEMENTS

This work was supported by the Agriculture Industrial Technology System Funding of Shandong Province of China (grant No. SDAIT-05-07); Agricultural Scientific and Technological Innovation Project of Shandong Academy of Agricultural Sciences; Prospect of Shandong Seed Project, China(2016LCGC035); Jinan Top Ten Agricultural Characteristic Industry Science and Technology Innovation Team, and China Agriculture Research System of MOF and MARA.

REFERENCES

[1] JOHN R., AHMAD P., GADGIL K., SHARMA S., *Heavy metal toxicity. Effect on plant growth, biochemical parameters and metal accumulation by Brassica juncea L.*, Int. J. Plant Prod., 2012, 3, 65–76. DOI: 10.22069/IJPP.2012.653.

[2] WEI B., YANG L., *A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China*, Microchem. J., 2010, 94, 99–107. DOI: 10.1016/j.microc. 2009.09.014.

[3] WUANA R.A., OKIEIMEN F.E., *Heavy metals in contaminated soils. A review of sources, chemistry, risks and best available strategies for remediation*, Int. Sch. Res. Not., 2011, 1–20. DOI: 10.5402 /2011/402647.

[4] JAISHANKAR M., TSETEN T., ANBALAGAN N., MATHEW B.B., BEEREGOWDA K.N., *Toxicity, mechanism and health effects of some heavy metals*, Interdisc. Toxicol., 2014, 7, 60–72. DOI: 10.2478/intox-2014-0009.

[5] GERHARDT K.E., HUANG X.-D., GLICK B.R., GREENBERG B.M., *Phytoremediation and rhizoremediation of organic soil contaminants. Potential and challenges*, Plant Sci., 2009, 176, 20–30. DOI: 0.1016/j. plantsci. 2008.09.014.

[6] LIN R., WANG X., LUO Y., DU W., GUO H., YIN D., *Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (Triticum aestivum L.)*, Chemosphere, 2007, 69, 89–98. DOI: 10.1016 /j.chemosphere.2007.04.041.

[7] LIU W., ZHOU Q., AN J., SUN Y., LIU R., *Variations in cadmium accumulation among Chinese cabbage cultivars and screening for Cd-safe cultivars*, J. Hazard. Mater., 2010, 173, 737–743. DOI: 10.1016/j. jhazmat.2009.08.147.

[8] BELIMOV A.A., HONTZEAS N., SAFRONOVA V.I., DEMCHINSKAYA S.V., PILUZZA G., BULLITTA S., GLICK B.R., *Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern.)*, Soil Biol. Biochem., 2005, 37, 241–250. DOI: 10.1016/j.soilbio.2004.07.033.

[9] SU D., WONG J.W.C., ZHANG F., *The absorption and activation of insoluble Cd in soil by Indian mustard (Brassica juncea)*, Zhon. Huan. Kex., 2002, 22, 342–345.

[10] SPEISER D.M., ABRAHAMSON S.L., BANUELOS G., OW D.W., *Brassica juncea produces a phytochelatin–cadmium–sulfide complex*, Plant Physiol., 1992, 99, 817–821. DOI: 10.1104/pp.99.3.817.

[11] HOSSAIN M.A., PIYATIDA P., DA SILVA J.A.T., FUJITA M., *Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation*, J. Bot., 2012, 1–37. DOI: 10.1155 /2012/872875.

[12] THALMANN Y.A., THALMANN O., *Methodology for the determination of dehydrogenase activity in soil using triphenyltetrazoli-umchloride (TTC)*, Landwirtsch. Forsch., 1968, 21, 249–258 (in German).

[13] BEAUCHAMP C., FRIDOVICH I., *Superoxide dismutase. Improved assays and an assay applicable to acrylamide gels*, Anal. Biochem., 1971, 44, 276–287. DOI: 10.1016/0003-2697(71)90370-8.

[14] SADASIVAM D.S., MANICKAM A., *Biochemical methods*, 2nd Ed., New Age International (P), Ltd., New Delhi 1996.

[15] AEBI H., *Catalase in vitro*, Methods Enzymol., 1984, 105, 121–126. DOI: 10.1016/s0076-6879 (84) 05016-3.

[16] SCHMEDES A., HØLMER G., *A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation*, J. Am. Oil Chem. Soc., 1989, 66, 813–817. DOI: 10.1007/BF02653674.

[17] BRADFORD M.M., *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding*, Anal. Biochem., 1976, 72, 248–254. DOI: 10.1016/0003-2697 (76)90527-3.

[18] RICE E.W., *Standard methods for the examination of water and wastewater*, 23rd Ed., American Water Works Association, Washington, D.C., 2017.

[19] WEATHERBURN M.W., *Phenol-hypochlorite reaction for determination of ammonia*, Anal. Chem., 1967, 39, 971–974. DOI: 10.1021/ac60252a045.

[20] LUCHTER-WASYLEWSKA E., *Continuous assay for acid phosphatase using phenyl phosphate*, Anal. Biochem., 1996, 241, 167–172. DOI: 10.1006/abio.1996.0394.

[21] GE Y., WANG Q., WANG L., LIU W., LIU X., HUANG Y., CHRISTIE P., *Response of soil enzymes and microbial communities to root extracts of the alien Alternanthera philoxeroides*, Arch. Agron. Soil Sci., 2018, 64 (5), 1–10. DOI: 10.1080/03650340.2017.1373186.

[22] KABATA-PENDIAS A., *Trace elements in soils and plants*, 4th Ed., CRC Press, Taylor & Francis Group, London 2010.
[23] AGARWAL S.K., *Water pollution*, APH Publishing, New Delhi 2005.
[24] ANJUM N.A., UMAR S., AHMAD A., IQBAL M., KHAN N.A., *Sulphur protects mustard (Brassica campestris L.) from cadmium toxicity by improving leaf ascorbate and glutathione*, Plant Growth Regul., 2008, 54, 271–279.