Asian Journal of Water, Environment and Pollution, Vol. 19, No. 4 (2022), pp. 33-39.
DOI 10.3233/AJW220053

Influence of Copper Amendments on Soil Properties, Growth and Metal Accumulation by *Mentha arvensis* L.

Mamta Bisht[1,2]*, Chitra Pande[2], Geeta Tewari[2] and Sonal Tripathi[3]

[1]Department of Chemistry, School of Applied and Life Sciences, Uttaranchal University, Dehradun – 248007, India
[2]D.S.B. Campus, Kumaun University, Nainital, Uttarakhand, India
[3]Department of Agricultural Chemistry and Soil Science, Navsari Agriculture University, Gujarat – 396450, India
✉ mamtabisht15@gmail.com

Received March 19, 2021; revised and accepted October 25, 2021

Abstract: A polyhouse study was conducted to assess the influence of copper amendments on the soil properties, plant growth parameters (fresh weight and plant height) and metal accumulation in *Mentha arvensis* L. The amendments of copper were 270, 500, 700 and 900 mg kg^{-1} in triplicate along with an unamended control. After 90 days of plant growth, the aerial parts of the plant were harvested and the physicochemical properties of the soil such as pH, EC, %OC, %OM and metal content in plant and soil were recorded. The pH and EC values were higher for the amendments as compared to the control. The pH (8.22) and EC (0.80 dS m^{-1}) values were the highest for Cu_{270} mg kg^{-1} and Cu_{500} mg kg^{-1} amendments, respectively. The percentage of OC and OM was the highest (1.16%, 2.00%) for Cu_{500} mg kg^{-1}. The plant height was maximum (36.30 cm) for the control plant while the fresh weight was maximum for Cu_{700} mg kg^{-1} amendment. The copper accumulation was observed to be the highest (19.3 mg kg^{-1}) for the Cu_{500} mg kg^{-1} amendment and least (13.7 mg kg^{-1}) for the higher amendment (Cu_{900} mg kg^{-1}) showing that for all the amendments along with control, copper accumulation was within the permissible limit. Thus, growing medicinal and aromatic plants in the metal-rich soils can be a sustainable and environment-friendly approach to obtaining metal-free commercially important end products.

Key words: *Mentha arvensis* L., pH, electrical conductivity, organic carbon, organic matter, growth, copper, atomic absorption spectroscopy.

Introduction

Soil is one of the most important environmental factors which influences many aspects of human life. It is the key factor for sustainable agricultural productivity and also maintains human, and animal health and enhances environmental quality (Arshad and Marin, 2002; Doran and Zeiss, 2000; Halecki et al., 2015; Solgi, 2016). Hence, the quality of soil must be maintained and preserved throughout the course of plant growth. The chemical, biological and physical factors like pH, electrical conductivity (EC), organic carbon (%OC), organic matter (%OM), cation exchange capacity (CEC), water holding capacity (WHC) and soil texture affect the soil quality. Besides nutrient imbalance, the application of excessive fertilisation and pesticides for enhancing crop productivity reduces soil health and fertility (Ali et al., 2015; Weber et al., 2018). An increase in the metal ion concentration in the soil showed toxic and adverse effects (Garbisu and Alkorata, 2003). At higher concentrations, metals like copper (Cu), zinc (Zn), chromium (Cr), mercury (Hg), arsenic (As), lead (Pb) and cadmium (Cd) are toxic to the plants and other organisms (Wang et al., 2021).

*Corresponding Author

Metal contamination also influences the ecological balance. A high amount of metal stimulates the reactive oxygen species (ROS) which can cause the fish and other aquatic organisms to die, and it also decreases the activity of soil microorganisms (Woo et al., 2009). Copper and zinc are important metal nutrients, but at higher concentrations, they cause toxicity (Broadley et al., 2007). Similarly, mercury, arsenic, chromium and cadmium are also toxic at higher levels, and they adversely affect the metabolism and growth (Divan et al., 2009). In plants, an excessive amount of metal reduces the photosynthetic pigments (Radic et al., 2010) and enhances oxidative stress and cell death (Chang et al., 2005). Further, to avoid such stressful situations, plants have developed defense mechanisms including restriction of metal ion uptake, metal export from plants or a chelation process by which they balance the nutrients in the plants (Komal et al., 2015).

Copper is widely used for making electrical wires, industrial machinery, rings and containers. The high level of this metal is unsuitable for the cultivation of crops as it leads to the contamination of the food chain. Some aromatic plant species used for essential oil extraction have the ability to grow and develop in metal-rich soils near mining sites (Scora and Chang, 1997; Zheljazkov and Neilson, 1996). These aromatic plants are seen to be metal tolerant and the metal uptake by them can be utilised to clean metal polluted sites (Zheljazkov et al., 2008). To the best of our knowledge, no such study was undertaken for the analysis of copper uptake by *Mentha arvensis* L. The aim of this study was to evaluate the influence of copper amendments on the physicochemical properties of soil, growth and metal accumulation by an essential oil-bearing plant (*M. arvensis* L).

Materials and Methods

Collection of Plant Material

The collection of plant material was done from CIMAP, Pantnagar, Uttarakhand (Accession number was 116656) (Bisht et al., 2021).

Preparation of Soil Samples

The surface (0-20 cm) soil sample was collected from a field of Ramnagar (29°39′0″ N and 79°12′0″ E) district Nainital, Uttarakhand (28°43′-31°27′ N and 77°37′-81° 02′ E). The soil samples (composite surface soil sample; *n*=20; 0-20 cm) were taken and air dried in shade, processed and then different physicochemical properties (pH, electrical conductivity; EC, organic carbon; OC and organic matter; OM) of the soil samples before and after harvest were evaluated in the Phytochemistry and Soil Analysis Lab, D.S.B. Campus, Kumaun University, Nainital.

Polyhouse Experiment

About 2.5 kg of the soil was filled in the earthen pots (20 cm in diameter). The inorganic salt of copper was added to the soil to provide different levels of copper (control (0), Cu_{270}, Cu_{500}, Cu_{700}, Cu_{900}, mg kg^{-1}). A basal dose of primary macronutrients nitrogen, phosphorus and potassium (NPK) was also applied to all the pots and then mixed with the soil. After amendments, the soil of each pot was thoroughly mixed on a polythene sheet, transferred to pots in a completely randomized design set up and kept for one month incubation period. After 90 days of the plantation, *M. arvensis* L. was harvested and at the same time, soil samples were air dried and processed for determining variation in the physicochemical properties of the soil such as pH, EC, organic carbon by the Walkley and Black method and copper accumulation in soil and plant (Walkley and Black, 1934). A transfer factor was also calculated. The growth parameter was also measured at the time of harvest.

Digestion of Soil Samples for Copper Content in Soil

For soil digestion, the process given by Lokeshwari and Chandrappa (2006) was adopted. Soil samples were also extracted with diethylene triamine pentaacetic acid (DTPA) for assessing available copper content in the soil following the procedure developed by Lindsay and Norvell (1978).

Digestion of Plant Samples for Copper Content in *M. arvensis* L.

After drying the properly washed samples upto constant weight, 0.1 g of plant material was used for the detection of copper. The plant samples were digested with 10 mL of triacid (HNO_3:H_2SO_4:$HClO_4$ 10:1:4) (Piper, 1942).

Statistical Analysis

All the data were expressed as means with standard deviation (SD) and were statistically analyzed using SPSS 16.0 software and the results were calculated by variance analysis (ANOVA) with three replications. The statistical significance was tested by Duncan´s multiple range test (DMRT) at a probability level of $p<0.05$.

Results and Discussion

Preliminary Physicochemical Properties of Experimental Soil

The pH of the experimental soil before the experiment was 7.65 being slightly alkaline in nature with electrical conductivity of 0.38 dS m^{-1}. The percentage of organic carbon and organic matter was 1.67 and 2.87, respectively. The texture class of the soil showed that the soil was sandy loam in nature. The cation exchange capacity (44.09 mili eq/100g) was also good. The macronutrients including nitrogen, phosphorous and potassium were in the optimum range (Table 1). The total copper content in the experimental soil was 50 mg kg^{-1}.

Physicochemical Properties of the Soil After Sowing

After 90 days of planting time, the main physicochemical properties of soil were recorded which are listed in Table 2. The pH of the control soil was 7.97. The pH value increased after amendments and was highest (8.22) for the Cu_{270} mg kg^{-1} amendment and for other amendments viz. Cu_{500}, Cu_{700}, Cu_{900} mg kg^{-1}, it was 8.12, 8.12 and 8.13, respectively. After amendments, an increase in the pH values might be due to the plants drawing anions from the deep soil layer to the soil surface or a higher amount of the plant residue which possesses high nitrogen (Butterly et al., 2013). Furthermore, as the copper level increased, the pH value decreased. Our results were similar to Alva et al. (1999) reported that increasing copper concentration decreased the pH value of the soil.

The electrical conductivity of the experimental soil was 0.38 dS m^{-1}. After harvesting the plant, the electrical conductivity values increased as copper amendments increased except for the Cu_{900} mg kg^{-1} amendment. For the control soil, the value of electrical conductivity was 0.30 dS m^{-1}. It was maximum (0.80 dS m^{-1}) for Cu_{500} mg kg^{-1} amendment and minimum (0.30 dS m^{-1}) for Cu_{900} mg kg^{-1} amendment. For other amendments, such as Cu_{270} and Cu_{700} mg kg^{-1}, it was 0.50 and 0.40 dS m^{-1}, respectively. The increase in the EC values might be due to the decomposition of organic matter by soil microorganisms and the decrease in the EC value of Cu_{900} mg kg^{-1} amendment might be due to either precipitation or poor irrigation of water (Cetin and Kirda, 2003). Our results were similar to the Brazil and Canada reports which checked the soil EC values after increasing the organic waste rate in different crops like basil and tomato (Zheljazkov et al., 2003).

The organic carbon value of the experimental soil was 1.67%. It decresed after amendments along with control. For control soil, the value of organic carbon

Table 1: Physicochemical properties of soil before sowing

pH±SD	*EC (dS m^{-1}) ±SD*	*OC (%)±SD*	*OM (%)±SD*	*Sand (%)*	*Silt (%)*	*Clay (%)*	*Soil texture*	*CEC (mili eq/100 g) ±SD*	*N (kg/h) ±SD*	*P (%)±SD*	*K (%)±SD*	*Cu (mg kg^{-1}) ±SD*
7.65± 0.13	0.38±0.01	1.67±0.10	2.87±0.20	74	16	10	Sandy Loam	44.09±0.64	410.19 ±1.54	0.0096 ±0.00	0.0259 ±0.00	50±0.00

EC = Electrical conductivity; OC = Organic carbon; OM = Organic matter; CEC = Cation exchange capacity

Table 2: Effect of copper amendments on physicochemical properties of soils cultivated with *Mentha arvensis* L.

Amendments (mg kg^{-1})	*pH Mean±S.D*	*EC Mean±S.D*	*%O.C Mean±S.D*	*%O.M Mean±S.D*
Cu_0	7.97^{a}±0.12	$0.3^{a,b}$±0.00	1.15^{b}±0.02	1.98^{b}±0.04
Cu_{270}	8.22^{b}±0.03	0.5^{b}±0.00	1.12^{b}±0.02	1.93^{b}±0.03
Cu_{500}	8.12^{b}±0.06	0.8^{c}±0.05	1.16^{b}±0.02	2.00^{b}±0.04
Cu_{700}	8.12^{b}±0.02	$0.4^{b,c}$±0.10	1.06^{a}±0.03	1.78^{a}±0.06
Cu_{900}	8.13^{b}±0.06	0.3^{a}±0.05	1.17^{b}±0.04	2.02^{b}±0.07

S.D = Standard Deviation; Mean values followed by different alphabets (a-d) at superscript are significantly different at $p<0.05$

was 1.15%. It was the highest (1.17%) for the Cu_{900} mg kg^{-1} amendment and for other amendments; Cu_{270}, Cu_{500}, and Cu_{700} mg kg^{-1}, it was 1.12, 1.16 and 1.06% respectively. As soil pH increases, the bioavailable fraction of heavy metals can undergo complexation or co-precipitation with hydroxide ions and might decrease the bio-accessibility of metals in soil (Kumpiene et al., 2008). As per an old report, lower organic carbon value was due to the nutrient imbalance by the use of excess inorganic fertilisers (Natsheh and Mousa, 2014). Like organic carbon, organic matter also affects the soil fertility (Kononova, 1996) and follows similar trends and was the highest (2.02%) for Cu_{900} mg kg^{-1} amendment which might be due to either a large amount of root residue or plant biomass on the surface (Kaur et al., 2008).

Effect of Copper Amendments on Growth Parameters and Metal Accumulation in Soil and Plant

Copper amendments affected plant growth parameters. From Table 3, it was clear that as copper concentration increased, the height of the plants gradually decreased. The plant height was recorded as maximum for the control (36.30 cm) plant while it decreased at the highest concentration (Cu_{900} mg kg^{-1}) which was 27.04 cm. For other amendments; Cu_{270}, Cu_{500}, Cu_{700} mg kg^{-1}, the height of the plant was 31.63, 30.52 and 27.04 cm, respectively. A negative correlation was observed between plant height and total copper content in the soil ($r^2 = –0.952$; $p<0.05$) (Table 4). Due to the excessive use of copper fertilizers, plant growth could be reduced and

Table 3: Effect of copper amendments on growth and copper accumulation in soil and plant

Amendments (mg kg^{-1})	*Survival Rate (%)*	*Plant height (cm) Mean± SD*	*Fresh weight (g) Mean± SD*	*Total metal in soil (mg kg^{-1}) Mean± SD*	*DTPA extractable metal in soil (mg kg^{-1}) Mean± SD*	*Total metal in plant (mg kg^{-1}) Mean± SD*	*Transfer factor Mean± SD*
Cu_0	100	$36.30^a±6.09$	$230^a±10$	$58.75^a±2.29$	$5.29^a±0.20$	$18.1^d±0.7$	$0.30^b±0.10$
Cu_{270}	100	$31.34^a±5.33$	$300^b±13$	$376.75^b±5.88$	$32.02^b±0.50$	$16.6^c±0.4$	$0.04^a±0.03$
Cu_{500}	100	$31.63^a±4.79$	$230^a±10$	$483.75^c±6.00$	$48.38^c±1.18$	$19.3^e±0.6$	$0.03^a±0.01$
Cu_{700}	100	$30.52^a±8.76$	$340^c±17$	$744.00^d±5.10$	$81.84^d±1.16$	$15.2^b±0.5$	$0.02^a±0.01$
Cu_{900}	100	$27.04^a±5.02$	$220^a±5$	$925.75^e±5.50$	$97.2^e±0.43$	$13.7^a±0.2$	$0.01^a±0.00$

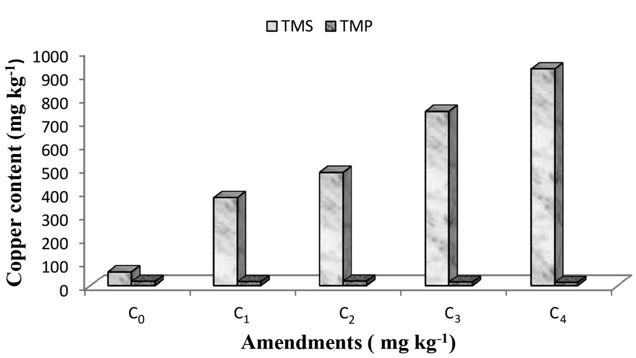
S.D = Standard Deviation; Mean values followed by different alphabets (a-d) at superscript are significantly different at $p<0.05$

Table 4: Correlations between soil properties, growth parameters, metal accumulation in soil, plant and transfer factor

	pH	*EC*	*OC*	*OM*	*Height*	*Weight*	*TMS*	*TMP*	*DTPA*	*T.F*
pH	1	0.354	-0.221	-0.176	-0.661	0.432	0.488	-0.318	0.412	-0.850
EC		1	0.120	0.130	0.015	-0.050	-0.081	0.663	-0.116	-0.381
OC			1	0.997**	-0.012	-0.968**	-0.140	0.235	-0.172	0.214
OM				1	0.002	-0.946*	-0.174	0.249	-0.213	0.214
Height					1	-0.073	-0.952*	0.739	-0.918*	0.873
Weight						1	0.153	-0.256	0.161	-0.331
TMS							1	-0.762	0.995**	-0.820
TMP								1	-0.762	0.437
DTPA									1	-0.776
T.F										1

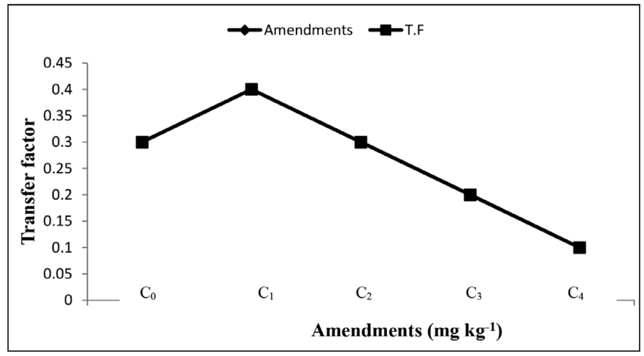
Where EC - Electrical conductivity, OC - Organic carbon, OM - Organic matter, TMS - Total metal content in soil, TMP - Total metal content in plant; DTPA - Extractable of DTPA; T.F - Transfer factor; **Correlation is significant at a probability level of $p<0.01$ (2 tailed); *Correlation is significant at a probability level of $p<0.05$ (2 tailed) it also reduces root system, and plant productivity and affects important cellular processes like photosynthesis and respiration (Narula et al., 2005; Atanassova and Zapryanova, 2009). The reduction in the plant height might be due to the negative effect of copper on some physiological processes such as photosynthesis, water relation and mineral nutrition (Anjum et al., 2016; Hu et al., 2015). There is one report in which copper toxicity reduced the root growth of rhodes grass (Sheldon and Menzies, 2005) and biomass production of *Polygonum convolvulus* (Kajaer and Elmegaard, 1996). For the control plant, the fresh weight of the plant was 230g. The weight was maximum (340g) for Cu_{700} mg kg^{-1} amendment and minimum (220g) for the highest amendment (Cu_{900} mg kg^{-1}). For amendments; Cu_{270} mg kg^{-1} and Cu_{500} mg kg^{-1}, it was 300g and 230g, respectively (Table 3).

On increasing copper concentration from control to higher amendments, the total copper content in the soil gradually increased i.e. it was maximum for Cu_{900} mg kg^{-1} amendment and the lowest for the control soil (Figure 1). A similar trend was also observed for DTPA extractable copper. Copper accumulation for the control plant was 18.1 mg kg^{-1} while for the plant grown in the upper permissible limit of copper was 16.6 mg kg^{-1}. As copper concentration increased, the copper accumulation in the plant decreased. For the highest concentration (Cu_{900} mg kg^{-1} of copper), it was 13.7 mg kg^{-1}. Copper accumulation for plant grown in Cu_{500} mg $kg^{-1}$and Cu_{700} mg kg^{-1} amendments was 19.3 mg kg^{-1} and 15.2 mg kg^{-1} respectively. At higher concentrations, the metal availability decreased which might be due to the adsorption of copper onto the soil particles, reducing their availability to the plants (WHO,1998). The transfer



where C_0 = Control, $C_1 = Cu_{270}$, $C_2 = Cu_{500}$, $C_3 = Cu_{700}$, $C_4 = Cu_{900}$ mg kg^{-1} and TMS means total metal in soil while TMP means total metal in plant

Figure 1: Comparative study of copper in soil, and plant with elevated amendments.



where C_0 = Control, $C_1 = Cu_{270}$, $C_2 = Cu_{500}$, $C_3 = Cu_{700}$, $C_4 = Cu_{900}$ mg kg^{-1} and T.F means transfer factor

Figure 2: Graphical representation of transfer factor with elevated copper concentration.

factor was also calculated and it was observed that as copper concentration increased, the transfer factor also decreased being the lowest (0.01) for the highest amendment Cu_{900} mg kg^{-1} (Figure 2). For control plant, it was 0.30 and for other amendments; Cu_{270} mg kg^{-1}, Cu_{500} mg $kg^{-1}$and Cu_{700} mg kg^{-1}, it was 0.04, 0.03 and 0.02, respectively. A copper level above 20 µg g^{-1} on the dry weight basis shows a toxic effect due to this inhibition effect in roots, shoots and chlorosis in younger leaves occurs (Yruela, 2005; Hossain et al., 2012). In the present study, all copper accumulation concentrations in the plants were within the permissible limits. So, we can promote the cultivation of such types of aromatic plants to prevent food contamination.

Conclusion

From the above study, it can be concluded that the cultivation of *Mentha arvensis* can be a good alternative for the copper polluted soils as metal accumulation in plants is below the permissible limit. The essential oils obtained from the aromatic plants are free from metal toxicity and are used in perfumery, food and pharmaceutical industries. So, we can promote the cultivation of aromatic plants for the income generation aspect of the farmers as well as for the prevention of food contamination.

Acknowledgements

The authors are thankful to the GBPNIHESD, Kosi Katarmal, Almora for financial support and Head, Department of Chemistry, D.S.B. Campus Kumaun University, Nainital for providing necessary facilities.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of article.

References

Ali, Z., Kazi, A.G., Malik, R.N., Naz, M., Khan, T., Hayat, A. and A.M. Kazi (2015). Heavy Metal Built-Up in Agricultural Soils of Pakistan: Sources, Ecological Consequences and Possible Remediation Measures. *In:* Sherameti I., Varma A. (eds) Heavy Metal Contamination of Soils. Soil Biology, (Vol. 44). Springer, Cham. https://doi.org/10.1007/978-3-319-14526-6_2

Alva, A.K., Huang, B., Prakashand, O. and S. Paramasivam (1999). Effects of copper rates and soil pH on growth and nutrient uptake by citrus seedlings. *Journal of Plant Nutrition*, **22(11):** 1687-1699. doi.org/10.1080/01904169909365747

Anjum, S.A., Ashrf, U., Khan, I., Tanveer, M., Saleem, M.F. and L. Wang (2016). Aluminum and chromium toxicity in maize implications for agronomic attributes, net photosynthesis, physicochemical oscillations and metal accumulation in different plant parts. *Water Air Soil Pollution*, **227(9):** 326-340.

Arshad, M. and S. Marin (2002). Identifying critical limits for soil quality indicators in agrosystem. *Agriculture Ecosystem and Environment*, **88(2):** 153-160.

Atanassova, B. and N. Zapryanova (2009). Influence of heavy metal stress on growth and flowering of Salvia splendens Ker.-Gawl. *Biotechnology Biotechnological Equipment*, **23(1):** 173-176.

Bisht, M., Pande, C. and G. Tewari (2021). Effect of copper amendments on the quality of essential oils extracted from the aerial parts of *Mentha arvensis* L. *J. Essent. Oil-Bear. Pl.*, **24(2):** 193-200.

Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I. and A. Lux (2007). Zinc in plants. *New Phytologist*, **173:** 677-702.

Butterly, C.R., Baldock, J.A. and C. Tang (2013). The contribution of crop residues to changes in soil pH under field conditions. *Plant and Soil*, **366(1):** 185-198.

Cetin, M. and C. Kirda (2003). Spatial and temporal changes of soil salinity in a cotton field irrigated with low-quality water. *Journal of Hydrology*, **272(1-4):** 238-249.

Chang, H.B, Lin, C.W. and H.J. Huang (2005). Zinc induced cell death in rice (*Oryza sativa* L.) roots. *Plant Growth Regulation*, **46(3):** 261-266.

Divan, A.M., Jrde-Oliveira, P.L., Perry, C.T., Atz, V.L., Azzarini-Rostirola, L.N. and M.T. Raya-Rodriguez (2009). Using wild plant species as indicators for the accumulation of emissions from a thermal power plant, Candiota, South Brazil. *Ecol. Indic.*, **9:** 1156-1162.

Doran, J.W. and M.R. Zeiss (2000). Soil health and sustainability managing the biotic component of soil quality. *Applied Soil Ecology*, **15(1):** 3-11.

Garbisu, C. and I. Alkorata (2003). Basic concepts on heavy metal soil bioremediation. *The European Journal of Mineral Processing and Environmental Protection*, **3(1):** 58-66.

Halecki, W. and M. Ga-Siorek (2015). Seasonal variability of microbial biomass phosphorus in urban soils. *The Science of the Total Environment*, **502:** 42-47.

Hossain, M.A., Piyatida, P., da Silva, J.A.T. and M. Fujita (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: Central role of glutathione in detoxification of reactive oxygen species and methyl glyoxal and in heavy metal chelation. *The American Journal of Botany*, **872875:** 1-37.

Hu, J., Deng, Z., Wang, B., Zhi, Y., Pei, B., Zhang, G., Luo, M., Huang, B., Wu, W. and B. Huang (2015). Influence of heavy metals on seed germination and early seedling growth in *Crambe abyssinica*: A potential industrial oil crop for phytoremediation. *American Journal of Plant Science*, **6(1):** 150-156.

Kajaer, C. and N. Elmegaard (1996). Effect of copper sulphate on black bindweed (*Polygonum convolvulus* L.). *Ecotoxicology and Environmental Safety*, **33(2):** 110-117.

Kaur, T., Brar, B.S. and N.S. Dhillon (2008). Soil organic matter dynamics as affected by long-term use of organic and inorganic fertilizers under maize-wheat cropping system. *Nutrient Cycling in Agroecosystems*, **81(1):** 59-69.

Komal, T., Mustafa, M., Ali, Z. and A. Gul (2015). Heavy metal uptake and transport in plants. *In:* Heavy Metal Contamination of Soils. Vol.44, Springer, Cham.

Kononova, M.M. (1996). Soil Organic Matter its Nature its Role in Soil Formation and in Soil Fertility. Oxford U.K Pergamon Press. pp, 544.

Kumpiene, J., Lagerkvist, A. and C. Maurice (2008). Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments-A review. *Journal of Waste Management*, **28:** 215-225.

Lindsay, W.L. and W.A. Norvell (1978). Development of a DTPA micronutrient soil test for zinc, iron, manganese, and copper. *Journal of Soil Science Society of America*, **42(3):** 421-428.

Lokeshwari, H. and G.T. Chandrappa (2006). Impact of heavy metal contamination of Bellandur lake on soil and cultivated vegetation. *Current Science*, **91(9):** 622-627.

Narula, A., Kumar, S. and P.S. Srivastava (2005). Abiotic metal stress enhances diosgenin yield in *Dioscorea bulbifera L.* cultures. *Plant Cell Reports*, **24(4):** 250-254.

Natsheh, B. and S. Mousa (2014). Effect of organic and inorganic fertilizers application on soil and Cucumber (*Cucumis Sativa* L.) plant productivity. *International Journal of Agriculture and Foresty*, **4(3):** 166-170.

Piper, C.S. (1942). Soil and Plant Analyses: A laboratory manual of methods for examination of soils and the determination of inorganic constituents of plants. The University of Adelaide, Adelaide, Australia. pp. 368.

Radic, S., Babic, M., Skobic, D. and V. Roje (2010). Ecotoxicological effects of aluminium and zinc on growth and antioxidants in *Lemna minor L. Ecotoxicology and Environmental Saftey*, **73(3):** 336-342.

Scora, R.W. and A.C. Chang (1997). Essential oil quality and heavy metal concentrations of peppermint grown on a municipal sludge-amended soil. *Journal of Environmental Quality*, **26:** 975-979.

Sheldon, A.R. and N.W. Menzies (2005). The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Kunth) in resin buffered solution culture. *Plant and Soil*, **278(1, 2):** 341-349.

Solgi, E. (2016).Contamination of two heavy metals in topsoils of the Urban Parks Asadabad. Archives of hygiene sciences, **5:** 92-101.

Walkey, A. and I.A. Black (1934). An examination of the digestion method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science*, **63:** 251-263.

Wang, K., Ma, J., Li, M., Qin, Y., Bao, X., Wang, C., Cui, D., Xiang, O. and L.Q. Ma (2021). Mechanisms of Cd and Cu induced toxicity in human gastric epithelial cells: Oxidative stress, cell cycle arrest and apoptosis. *The Science of the Total Environment*, **756:** 143951.

Weber, J., Dradrach, A., Karczewska, A. and A. Kocowicz (2018). The distribution of sequentially extracted Cu, Pb, and Zn fractions in Podzol profiles under dwarf pine of different stages of degradation in subalpine zone of Karkonosze Mts (central Europe). *Journal of Soil Sediments*, **18:** 2387-2398.

World Health Organisation (WHO) (1998). Environment Health Copper.

Woo, S., Yum, S., Park, H.S., Lee, T.K. and J.C. Ryu (2009). Effects of heavy metals on antioxidants and stress-responsive gene expression in Javanese medaka (*Oryzias javanicus*). *Comparative Biochemistry and Physiology, Part C,* **149:** 289-299.

Yruela, I. (2005). Copper in plants. *Brazilian Journal of Plant Physiology*, **17(1):** 145-156. https://doi.org/10.1590/S1677042020050001000012

Zheljazkov, V.D., Cracker, L., Xing, B., Nielson, N.E. and A. Wilcox (2008). Aromatic plant production in metal contamination soils. *The Science of the Total Environment*, **395:** 51-62.

Zheljazkov, V.D. and N.E. Neilson (1996a). Effect of heavy metals on peppermint and cornmint. *Plant and Soil*, **178(1):** 59-66. doi.org/10.1007/BF00011163

Zheljazkov, V.D. and P.R. Warman (2003). Application of high copper compost to swiss chard and basil. *Science of the Total Environment*, **302(1,3):** 13-26. doi: 10.1016/s0048-9697(02)00390