

Chromium Phytotoxicity Test of Metal Coating Wastewaters

Şükrü Dursun¹, Zeynep Cansu Ayturan^{1,*}

¹ Environmental Engineering Department, Engineering Faculty, Selcuk University, Konya, Turkey

E-Mail: sdursun@selcuk.edu.tr, zcozturk@selcuk.edu.tr

Received April 18, 2018; Accepted May 25, 2018

Abstract: Metals are deformed by some mechanisms such as oxidation and corrosion. In order to protect metals from deformation and effect of some agents, metal coating method may be used. Hot galvanizing, thermal spraying, electroplating and sherardizing are some of these methods and they depend on the type of metal which will be coated to protect to metal materials. Zinc, nickel, brass, chrome, gold, cadmium, copper, brass, and silver are the main materials used for metal coating process. Chromium may be the most deteriorating one within these metals because it has negative impacts on both human and other livings. It is found in the environment in many forms and mined in different countries. Especially chromium +6 is considered as carcinogenic and may lead to health problems such as allergic reactions, nose irritations and nosebleed, stomach ulcer, weakened immune system, genetic material alteration, stomach ulcer, kidney and liver function disorders and death. Therefore, metal coating wastewaters including high concentrations of chrome should be cleaned and treatment is necessary. In this study wastewater containing chrome produced by metal coating industry was analysed by using some plant species. In this study, the reaction of some plant species was investigated against different concentrations of chrome solution. Main plants used for the tests are *Lepidium sativum* and *Lemna minor*. Examining the negative effects of chrome which may harm plants and offering more accurate wastewater treatment techniques against chromium wastewater is possible with this method. There is significant relation between chromium concentration and toxic level on plant germination and growth rate. Moreover, variance analysis was applied to the results taken from experimental study in laboratory condition with selected level of chromium level and the significance importance was determined with our results.

Keywords: Metal Industry Wastewaters, Chromium, Lemna minor, Phytotoxicity Test

INTRODUCTION

Aquatic plants are known as absorbent plants and macrophytes species that happen in numerous sorts of living spaces. These plants are the significant sources for wildlife and aquatic environment. Moreover, stabilization of sediment, water quality control, and nutrient cycles are affected positively from them ^[1]. Main plant species which are used as an energy source are micro and macro algae species, macrophytes, phytoplankton. Any sudden or slow change in the aquatic plant species affect both other animal and plant species and water environment such as drinking water quality, usage of water body as recreational purposes.

Aquatic ecosystems are debilitated by diffuse, ceaseless, and various contaminations that impact life forms' working and unfavourably influence environment wellbeing and security. Living beings are persistently presented to various blends of contaminations made from different compound structures, metabolites, and buildings that regularly are available at low focuses ^[2]. In toxicity researches, heavy metals are mostly used constituent due to their negative effects on all living forms.

The separation of the dissolved heavy metals from water can be achieved with aquatic plants and two fundamental mechanisms may be used for this separation. First one is fast metabolism reaction with unconnected surfaces and the second one is slow metabolism uptake with connected cells ^[3]. Modelling of the first mechanism has been made according to diffusion process which is completed after absorption of metal ions in to the outer surface of cell of biomass. Furthermore, modelling of the second mechanism has been made with respect to transfer of the mass from outer to inner cell wall. While first mechanism helps the separation of huge amount of metals within very short time, second mechanism takes very long time ^[4].

*Corresponding E-mail: zcozturk@selcuk.edu.tr

This paper has been presented as an oral presentation at International Congress of Health and Environment, 2017, Adana – Turkey.

The aquatic plant, namely *Lepidium sativum*, is a specie of Brassicaceae family cultivated in India and widespread called as "garden cress". It is mainly cultivated as a vegetable in Europe and America while the seeds of it are used as a food source in different areas in the world. Besides, this plant is considered as land cress, Haliy and common cress in India [5].

Lemna minor which is also called duckweed is very small aquatic plant with free-floating ability. It propagates in shallow and stagnant water environments and it enters the family of Lemnaceae which include the group of plants capable of floating and flowering. These plant species also grow very fast and distributed over whole water body when the circumstances are appropriate. Due to these special features, these plants are mainly used as test plants for the several studies related to aquatic environment and wastewater treatment. For example, constructed wetlands are one of the mostly used systems which is capable to treat several types of wastewaters in low cost and simple environment without high technological equipment. According to the previous studies, these plants have high tolerance against toxic materials. However, there are other species which are tolerant to the toxicity as the duckweed plants. Main usage area of duckweed plants is the testing toxic pollution in several environments such as lake, river, sediment. When the algae and duckweed are compared, their complexity are different from each other. They are used in phytotoxicity test together because they complement each other [6-7].

The main purpose of this study is assessing the toxicity of chromium with *Lepidium sativum* and *Lemna minor* species.

MATERIAL AND METHOD

Experiments

Three experiments were performed for this study. For first and second experiments *Lepidium sativum* specie were used and third experiment with *Lemna minor* was used as plant. The details of experiments are given below:

Experiment 1

In this experiment, five sets were prepared that each set was included four Petri dishes. In Table 1 summary of these sets and their denominations were given. There is one control set which is used to control and minimize experiment errors. Also, four other sets were prepared for comparison purposes.

Table 1. The experiment design replicates

Control	1 st Set	2 nd Set	3 rd Set	4 th Set
01	11	21	31	41
02	12	22	32	42
03	13	23	33	43
04	14	24	34	44

Four sets were investigated with different chromium concentrations and for control set chromium concentration is zero. In this experiment petri dishes were used as media and nutrient solution was prepared for growth of the plants. Concentrations of chromium addition in petri dishes were given in Table 2.

Table 2. Concentrations of Chromium addition to each set

Name of Set	Chromium Solution Concentration (mg/L)
Control Set	0
1 st Set	15.075
2 nd Set	30.15
3 rd Set	60.3
4 th Set	120.6

Preparation of growth media content of *Lepidium sativum* and *Lemna minor* as given as following:

Chemicals amount used for 1L of Nutrient Solution

- Ca(NO₃)₂ → 0.63g
- NH₄NO₃ → 0.32 g
- MgSO₄.7H₂O → 0.80 g
- H₃BO₃ → 0.04g
- Na₂MoO₄.2H₂O → 0.004g

After preparation of nutrient solution, 20 seeds were added to each of 20 clean petri dish and different dilutions of chromium solutions were added to sets indicated in Table 2. One mL chromium solution was added to each petri dish and it was completed to 10 mL with nutrient solution. chromium concentration of each petri dish is given in Table 3. Then, they were left for the growth for one week period in a dark place. After one-week period, germination and/or growth of seeds were measured for a root and body part. Figure 1 shows the petri dishes at the end of one-week period.

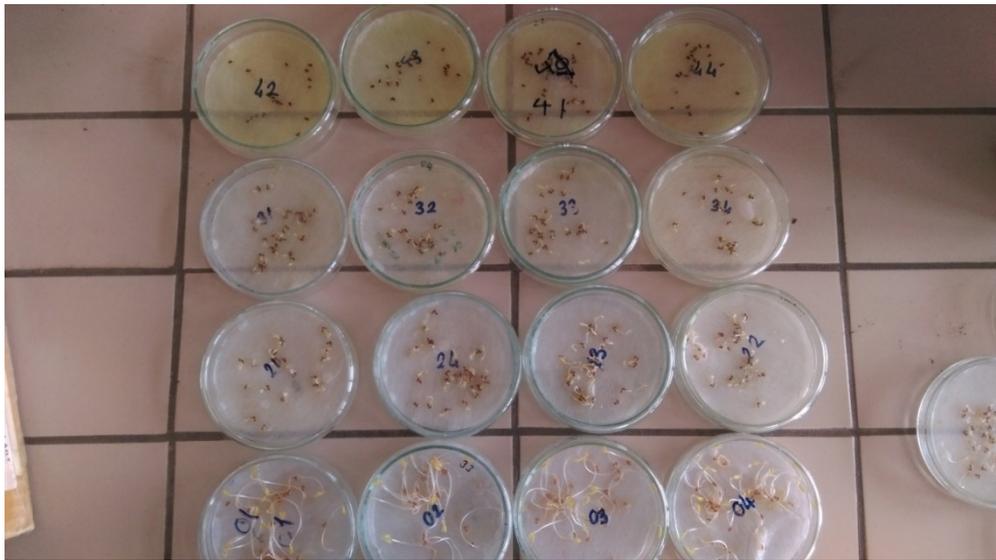


Figure 1. Petri dishes after growth period

Table 3. Chromium concentration of each petri dish

Control Set	Cr Conc. (mg/L)	1 st Set	Cr Conc. (mg/L)	2 nd Set	Cr Conc. (mg/L)	3 rd Set	Cr Conc. (mg/L)	4 th Set	Cr Conc. (mg/L)
01	0	11	1.5075	21	3.015	31	6.03	41	12.06
02	0	12	1.5075	22	3.015	32	6.03	42	12.06
03	0	13	1.5075	23	3.015	33	6.03	43	12.06
04	0	14	1.5075	24	3.015	34	6.03	44	12.06

Experiment 2

The summary of experiment 1 given in Table 1 was repeated. In this experiment only growth place was changed that tubes were used instead of petri dishes. Moreover, the chromium concentrations given in Table 2 were used in the second experiment. 20 tubes were cleaned and 3 seeds were added to each tube. Nets and little pipes were used in order to prevent drowning of *Lepidium Sativum* seeds into nutrient solution. In Figure 2 and 3 pictures of experiment is seen.



Figure 2. Tubes after seeding



Figure 3. Body and root growth of the seeds

Different dilutions of chromium solutions were added to sets same with first experiment. 1 mL chromium solution was added to each tube and completed to 20 mL with nutrient solution. In Table 4 chromium concentration of each tube is given. Tubes were checked every day and diluted water added to tubes for preventing dry of seeds. After one-week period, growth of seeds was measured as a root and body.

Table 4. Chromium concentration for root elongation experiment

Control Set	Cr Conc. (mg/L)	1 st Set	Cr Conc. (mg/L)	2 nd Set	Cr Conc. (mg/L)	3 rd Set	Cr Conc. (mg/L)	4 th Set	Cr Conc. (mg/L)
01	0	11	0.753	21	1.5075	31	3.015	41	6.03
02	0	12	0.753	22	1.5075	32	3.015	42	6.03
03	0	13	0.753	23	1.5075	33	3.015	43	6.03
04	0	14	0.753	24	1.5075	34	3.015	44	6.03

Experiment 3

Similar with first and second experiments, four groups were prepared with different dilutions of chromium solution and one group as control. The summary of third experiment is in Table 1 and chromium concentrations used in this experiment is given in Table 2. 50 mL beakers were used as growth place and *Lemna minor* was used in this experiment as a plant which will give reaction against

chromium. 20 beakers were cleaned and 10 *Lemna minor* were added to each beaker with 20 ml nutrient solution. 1 mL chromium solution was added to each beaker. Also, the concentration of each beaker is same with concentrations of tubes in experiment 2 and given in Table 4. Beakers were checked every day and diluted water added to beakers to prevent dry of plants because of evaporation. After one-week period, the number of survived plants was counted. Figure 4 shows the beakers after addition of chromium solution.

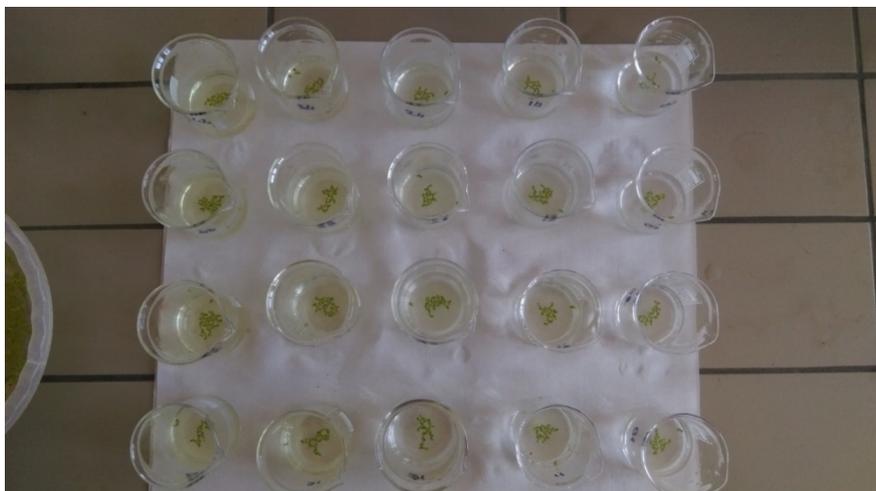


Figure 4. Beakers after chromium addition

Variance Analysis

According to these experiments, variance analysis was conducted. For experiment one and two, growth of the body and the root were analysed separately and for experiment three survival number of plants was counted. One-way ANOVA variance analysis was used for analysing the data taken from phytotoxicity experiments by SPSS Software Statistic programme. The analysis of variance (ANOVA) is the most powerful tool in order to test the statistical significance between mean values of one or more groups. The ANOVA assumes that the dependent variable is affected from the independent variables. The main advantage of variance analysis is capability of testing the influence of more than one independent variable [8]. In this study, only effect of one independent variable which is chromium addition was investigated against growth rate of each species separately.

RESULTS AND DISCUSSION

ANOVA of Experiment 1

ANOVA analysis was applied to the data of the root and body separately. There is a significant relationship between chromium addition and growth of the plant as root and body for first experiment according to the results of the tests. Table 5 gives the comparison of independent variables with respect to body and root growth. With the help of this table, it can be understood that the possibility of the similarity between two different concentrations of independent variable is coincidence or not.

Results of Table 5 indicate that chromium addition has some effects on body and root growth of the plant. There is significant relationship between different concentrations of Cr. With respect the P value, the extent of the significance can be understood.

Table 5. Comparison of independent variables between each other for Experiment 1

Cr Conc. (mg/L)	Cr Conc. (mg/L)	P values for body growth	Significance for body growth	P values for root growth	Significance for root growth
0	1.5075	0.000	***	0.000	***
	3.015	0.000	***	0.000	***
	6.03	0.000	***	0.000	***
	12.06	0.000	***	0.000	***

1.5075	0	0.000	***	0.000	***
	3.015	0.288	NS	0.169	NS
	6.03	0.002	**	0.001	**
	12.06	0.000	***	0.000	***
3.015	0	0.000	***	0.000	***
	1.5075	0.288	NS	0.169	NS
	6.03	0.038	*	0.063	NS
	12.06	0.010	*	0.010	*
6.03	0	0.000	***	0.000	***
	1.5075	0.002	**	0.001	**
	3.015	0.038	*	0.063	NS
	12.06	0.608	NS	0.470	NS
12.06	0	0.000	***	0.000	***
	1.5075	0.000	***	0.000	***
	3.015	0.010	*	0.010	*
	6.03	0.608	NS	0.470	NS

Which is $P < 0.05 \rightarrow *$, $P < 0.01 \rightarrow **$, $P < 0.001 \rightarrow ***$, Not Significance $\rightarrow NS$ [9]

ANOVA of Experiment 2

ANOVA analysis was applied to the data of the root and body separately like experiment one. There is a significant relationship between chromium addition and growth of the plant as root and body for second experiment according to the results of the tests. Table 6 gives the comparison of independent variables with respect to body and root growth.

Table 6. Comparison of independent variables between each other for Experiment 2

Cr Conc. (mg/L)	Cr Conc. (mg/L)	P values for body growth	Significance for body growth	P values for root growth	Significance for root growth
0	0.753	0.009	**	0.000	***
	1.5075	0.000	***	0.000	***
	3.015	0.000	***	0.000	***
	6.03	0.000	***	0.000	***
0.753	0	0.009	**	0.000	***
	1.5075	0.204	NS	0.169	NS
	3.015	0.144	NS	0.115	NS
	6.03	0.010	*	0.049	*
1.5075	0	0.000	***	0.000	***
	0.753	0.204	NS	0.169	NS
	3.015	0.843	NS	0.838	NS
	6.03	0.179	NS	0.541	NS
3.015	0	0.000	***	0.000	***
	0.753	0.144	NS	0.115	NS
	1.5075	0.843	NS	0.838	NS
	6.03	0.250	NS	0.684	NS
6.03	0	0.000	***	0.000	***
	0.753	0.010	*	0.049	*
	1.5075	0.179	NS	0.541	NS
	3.015	0.250	NS	0.684	NS

Which is $P < 0.05 \rightarrow *$, $P < 0.01 \rightarrow **$, $P < 0.001 \rightarrow ***$, No Significance $\rightarrow NS$ [9]

ANOVA of Experiment 3

For Experiment 3 ANOVA was applied to the data including the number of plants which survived at the end of the time period. There is a significant relationship between chromium addition and survival of the plant according to results of the test. The comparison of independent variables with respect to survival rate is given in Table 7.

Table 7. Comparison of independent variables between each other for Experiment 3

Cr Conc. (mg/L)	Cr Conc. (mg/L)	P values	Significance
0	0.753	0.157	NS
	1.5075	0.000	***
	3.015	0.000	***
	6.03	0.000	***
0.753	0	0.157	NS
	1.5075	0.009	**
	3.015	0.000	***
	6.03	0.000	***
1.5075	0	0.000	***
	0.753	0.009	**
	3.015	0.000	***
	6.03	0.000	***
3.015	0	0.000	***
	0.753	0.000	***
	1.5075	0.000	***
	6.03	0.000	***
6.03	0	0.000	***
	0.753	0.000	***
	1.5075	0.000	***
	3.015	0.000	***

Which is $P < 0.05 \rightarrow *$, $P < 0.01 \rightarrow **$, $P < 0.001 \rightarrow ***$, No Significance $\rightarrow NS$ [9]

According to these results from the variance analysis for each experiment, it is clearly showed that chromium concentration on the growth rate affects significantly important. Changing the growth place for first and second experiments was very helpful for understanding the effect of growth place and changing the concentration was helpful for understanding the effect of different concentrations clearly.

Moreover, owing to these results, making comment on different concentrations of chromium and their effects on plant growth is possible. For example, there is no significant difference between concentrations 3.015 mg/L and 6.03 mg/L in Table 5. This indicates that these two concentration did not create any difference on the plant growth. If the concentrations and range between them were increased, the significance amount would be risen.

CONCLUSION

Lepidium sativum and *Lemna minor* was used in this study as experiment plants. Both of them gave reaction against the chromium addition. Chromium is one the most dangerous carcinogenic metal found in n rocks, animals, plants, and soil in different forms. Metal coating industry wastewaters include chromium metal mostly. The amount of chromium in the wastewater is very important to understand its negative effects on both animal and human life. With the help of this study, the concentrations which are dangerous for some plants were investigated and their relationships with each other were understood. Chromium addition to the wastewaters with high concentrations may create negative effects on the plant growth and survival rate of some species may be decreased. Therefore, wastewaters of metal coating industry should be analysed carefully, and chromium pollution should be removed before discharging the water bodies.

REFERENCES

- [1] Welch, E. B., 1992, *Ecological Effects of Wastewater*, E&FN Spon, NY.
- [2] Lam P.K.S., Wut P.F., Chan A.C.W., Wu R.S.S., 1999, Individual and combined effects of Cd and Cu on the growth response of *Chlorella vulgaris*. *Environ Toxicol* 14:347–353.
- [3] Cho D.Y., Lee, S., Park, S., Chung, A., 1994) Studies on the biosorption of heavy metals onto *Chlorella vulgaris* J. *Environ. Sci. Health A*, 29 (2) pp. 389–409.
- [4] Sternberg, A. and Claussen, K., 2003, Lead and nickel removal using *Microspora* and *Lemna minor*. *Bio. Technol.*, 89: 41-48.
- [5] Bermejo J.E., 1994, Neglected horticultural crops. In: Hernando Bermejo JE, Leon J (eds), *Neglected Crops: 1492 From a Different Perspective*, Plant Production and Protection Series No. 26. Rome, Italy: FAO, pp 303–332.
- [6] Rahmani, G.N.H., Sternberg, S.P.K., 1999, Bioremoval of lead from water using *Lemna minor*. *Biores. Technol.*, 70: 225–230.
- [7] American Public Health Association, American Water Works Association, Water Environment Federation, 1992, *Standard Methods for Examination of Water and Wastewater*, Section 8211, Toxicity Test Procedures Using Common Duckweed, *Lemna minor*, 18th edition, Washington DC.
- [8] Miller, R.L., Brewer, J.D., 2003, *The A-Z of Social Research: A Dictionary of Key Social Science Research Concepts*, SAGE Publications Ltd., pp 09-12.
- [9] Dursun, S., 1994, *The Effects of Sulphur Pollution On Soil Fungi and Decomposition of Tree Leaf Litters*, Thesis of Philosophiae Doctor, University of Wales, Collage of Cardiff, 53.