

The effect of Saharan dust on the copper uptake by *Lemna minor*

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Abstract: In this study, the aim was to model the toxic effect of copper (Cu) and analyse the removal of Cu in aqueous Saharan and non-Saharan mediums by *Lemna minor*. Two separate test groups were formed: with Saharan dust (S) and without Saharan dust (WS). These test groups were exposed to 3 different Cu concentrations (0.05, 0.50 and 5.00 ppm). Time, concentration, and group-dependent removal efficiencies were compared using the non-parametric Mann-Whitney U test and statistically significant differences were found. The optimum removal values were tested at the highest concentration 79.6% in the S medium and observed on the 4th day for all test groups. The lowest removal value (16%) was observed at 0.50 ppm on the 1st day in the WS medium. When the S medium and WS medium were compared, in all test groups Cu was removed more successfully in the S medium than the WS medium contaminated by Cu in 3 different concentrations of (0.05 ppm, 0.50 ppm, 5.00 ppm). The regression analysis was also tested for all prediction models. Different models were performed and it was found that cubic models show the highest predicted values (R^2). The R^2 values of the estimation models were found to be at the interval of 0.939–0.991 in the WS medium and 0.995–1.000 in the S medium.

Introduction

Nowadays, increasing concentrations of heavy metals in the ecosystem are an important problem. Heavy metals influence the quality of the atmosphere and the aquatic environment (Verma and Dwivedi 2013). Although some metals are essential nutrients, it is known that excessive concentrations of all heavy metals cause various toxic effects (Hejna et al. 2018). Copper (Cu) is an essential element in terms of the physiology of plants, and is responsible for vitamin, carbohydrate and protein synthesis, as well as photosynthesis and respiration (Hansch and Mendel 2009). Nevertheless, it is highly toxic because it is non-biodegradable and carcinogenic. Electroplating and metalworking industries have the potential to discharge large amounts of Cu into the aquatic environment. Cu effluents have been reported as a significant problem for the aquatic environment (Parmar and Thakur 2013).

There are numerous removal studies regarding contaminants, using various methods such as granular activated carbon/nanoscale zero valent iron (Majlesi and Hashempour 2017), electroless copper plating process (Thomas et al. 2018a), sodium trithiocarbonate (Thomas et al. 2018b), zeolite bearing tuff (Zendelska et al. 2018), and UV-Fenton method (Thomas et al. 2018c). Various contaminants such as textile dye (Deshmukh

et al. 2016), persistent organic pollutants (Chakraborty and Das 2016), metals (Sidhoum and Fortas 2019), nanoparticles (Fikirdeşici-Ergen and Üçüncü-Tunca 2018) and polycyclic aromatic hydrocarbons (Kuppusamy et al. 2016) were tested by removal studies. Phytoremediation is a cost-effective technology that is useful for treating contaminated water. Recently, the use of aquatic plants has been at the forefront of the removal of contaminated waters. *Lemna minor* has a high breeding capacity and easily adapts to laboratory conditions, therefore it is a very important phytoremediation agent (Mkandawire and Dudel 2007, Üçüncü et al. 2013a). There are many studies on the removal of heavy metals by *L. minor* (Basile et al. 2012, Bokhari et al. 2016). However, there are no studies on the efficiency of Saharan dust for *L. minor* in the removal of Cu.

The Sahara Desert in the north of the African continent, which is the largest desert in the world with an area of 9,149,000 km², is the source of much of the desert dust that is prevalent in and around Turkey. It is known that this desert contributes to 1–1.5 billion tons of atmospheric dust spreading in various directions every year (Laity 2008). The dust coming from the desert does not make any difference on the ground or in the air, but as a result of its contact with clouds during the transport process, the precipitation induces a series of reaction mechanisms resulting in the reduction of

iron, various elements and amino acids (Saydam 2014). It was reported that macronutrients such as Ca, K, Mg, Na, P, S and micronutrients such as Zn, Mn, Cu, and Fe are present in the rain composition containing the Saharan dust (Rizzolo et al. 2017). The iron (Fe^{+2}) transport system (YSL1 and YSL3), which plays an active role in plant metabolic activities, has also been shown to be effective in transporting Cu in plants (Grillet et al. 2013).

There are studies in the literature showing that Saharan dust has positive effects on the vegetative organs of plants and their growth, flowering and fruit-giving processes (Özsoy and Örnektekin 2008, Yücekutlu et al. 2011). Because of the positive growth effects of Saharan dust on plants, a matter of curiosity for us has been its contribution to the remediation capacity and the growth rate of *Lemna minor* plants. For this reason a study was carried out on the capacity of *L. minor* plants, with Saharan dust (S) and without Saharan dust (WS), to remediate Cu polluted water. The toxicity effects of Cu on the selected organism were also modeled for each concentration.

Materials and methods

The test species

The experiment was conducted in the Toxicity Laboratory of the Biology Department of Ankara University. Before the beginning of the experiment, *L. minor* was put into a culture. *L. minor* was cultivated and reproduced in the pool of the greenhouse at Ankara University, and was kept in 50 L glass aquariums for 8 weeks (OECD 2006). The temperature and lighting of the aquariums were maintained at the natural conditions of the greenhouse. The temperature (T), pH, dissolved oxygen, and electrical conductivity were measured for the physicochemical parameters of the culture using a multiparameter Hanna HI-9828. The parameters of the culture were listed as follows: pH: 7.55 ± 0.09 – 8.88 ± 0.67 ; EC: 409.78 ± 4.54 – 523.18 ± 11.77 $\mu\text{S}\cdot\text{cm}^{-1}$; DO: 5.00 ± 0.23 – 6.38 ± 0.21 $\text{mg}\cdot\text{L}^{-1}$. The metal, copper sulphate CuSO_4 (Sigma, Cas no. 7758-98-7), was examined on *L. minor*.

The preparation of the Saharan dust

Ten grams of desert soil imported from southern Tunisia was added to 1 liter of distilled water. The prepared mixture was allowed to stand in the sunlight for about 120 minutes. In this process, the muddy layer sank to the bottom and a clear layer of water remained at the top. This clear water was used in the experiment.

The calculation of the growth rates

All experiments were conducted in inert containers of a suitable size for *Lemna* growth. 100 mL of filtered culture water was used in chemically inert containers for each experiment (water samples taken from the culture were filtered with a 0.45 μm filtration unit prior to testing). Only specimens with 2 or 3 fronds were utilized for measurement, and a total of 21 fronds per container were selected for analysis at the end of the 7-day experimental period.

The growth rate of *L. minor* was calculated with the following formula, following OECD standards (OECD 2006).

$$\mu_{i-j} = \frac{\ln(N_j) - \ln(N_i)}{t_j - t_i}$$

Where:

$\mu_{i,j}$ represented the average specific growth rate from moment time i to j,

N_j represented the number of fronds observed in the test or control vessel at time j,

N_i represented the number of fronds observed in the test or control vessel at time i,

t_i represented the moment in time the period started,

t_j represented the moment in time the period ended.

The percentile biomass inhibition rates of *L. minor* were calculated with the following formula, following OECD standards.

$$\%I_b = \frac{b_c - b_T}{b_c} \cdot 100\%$$

Where:

I_b represented percent reduction in biomass,

b_c represented $\ln(\text{final biomass})$ minus $\ln(\text{starting biomass})$ for the control group,

b_T represented $\ln(\text{final biomass})$ minus $\ln(\text{starting biomass})$ in the treatment group.

The phytoremediation study

Two hundred and fifty millilitre beakers were used in the experiment; the tops of the test beakers were covered to prevent evaporation and possible accidental contamination (OECD 2006). In the study, 21 *L. minor* plants with 3 fronds were used for each experiment. The parameters (temperature, pH, electrical conductivity (EC), as well as the solved oxygen (O_2)) of all the test groups were measured. Two different design experiments were conducted, without the Saharan medium (WS) and with the Saharan medium (S), and 3 different concentrations (0.05, 0.05 and 5.00 ppm) of Cu were examined. The experiment continued for 7 days and was repeated 3 times. Water samples were taken every 24 hours. The ICP/MS (Thermo Scientific X-Series II) device at UNAM laboratory of Bilkent University (Ankara) was used for the laboratory study of Cu assignment. The control groups (0ppm) were also formed in the same conditions.

Instrumental analysis and quality control

In this study, the Thermo Scientific X-Series II ICP-MS device, Thermo Scientific ID 100 auto-diluter, and Cetac Asx-260 auto-sampler accessories were used for heavy metal measurements. In all the samples, a dilution of ultra-pure water with 2% nitric acid was used. In the preparation of the calibration curves, a High Purity Standards brand QCS-27 series calibration solution with 27 elements was used. The element concentration in the sample was taken into account and the correlation coefficient was considered to be more than 0.99 when preparing the calibration curves of all elements (Internal Standard of 10 ppb 209Bi). For the analysis of the parameters, the main run number was taken as 3, and sampling and washing durations were set to be 60 seconds considering tubing lengths. After every 40 readings, calibration and the blank solution readings were redone.

Statistical tests

All statistical calculations were carried out with the statistics program SPSS 21.0 (IBM, Portsmouth, UK).

Results and discussion

The parameters of temperature, pH, EC, DO of the Saharan medium (S) and the non-Saharan (WS) medium were measured at the beginning and at the end of the phytoremediation experiment (Table 1).

The removal potential of *L. minor*

Two different mediums including WS and S were exposed to 3 different concentrations (0.05, 0.50 and 5.00 ppm) for 7 days in this study. The removal efficiencies of *L. minor* were investigated in terms of concentration, time, and mediums, both separately and together, by using nonparametric tests and statistical comparison. According to the results, there were statistically significant differences between the different concentration groups in terms of removal. According to removal rates, the lowest absorption (16%) was observed at 0.50 ppm on the 1st day in the WS medium. The best removal values were observed at 5.00 ppm on the 4th day in the S medium (Figure 1).

The removal rates for the mediums were as follows:

WS: 24–58% at 0.05 ppm, 16–46% at 0.50 ppm,
26.8–61.6% at 5.00 ppm.
S: 48–76% at 0.05 ppm, 48–74% at 0.50 ppm,
43.8–79.6% at 5.00 ppm.

Some studies show that the removal rate increases as the concentration increases (Hanks et al. 2015, Mohammed 2016, Üçüncü et al. 2014). However, this inference was not observed in this study and some studies supporting our result are also available in the literature (John et al. 2008, Zhang et al. 2015).

Based on the results, Cu was effectively removed from WS and S mediums and thereafter the removal process efficiently

continued between the 1st and 4th day. These results showed that time is very important in the metal removing process, as well as the medium. When the removal amounts of Cu were analysed within the study, the optimum Cu removal was observed in the S medium. It was determined that *L. minor* continued to remove Cu from the mediums until the 4th day. Previous studies have indicated that metal removal is mostly completed at different times for *L. minor*. Axtell et al. (2003), Üçüncü et al. (2013b) and Aggoun and Benmaamar (2019) have stated that the metal removing process was mostly complete in the first 24 hours. Fikirdeşici-Ergen et al. (2017) observed that the continuous removing process was mostly completed on the 2nd day. In this study, the results observed that a total of 61.6% and 79.6% Cu was removed on the 4th day in the WS and S medium, respectively (Table 2). In remediation studies, there are many factors that influence the uptake of contaminants by the plant. Some of these include the resolution of the contaminant, exposure time, exposure concentration, the type of organism and absorbent (Fikirdeşici-Ergen and Üçüncü-Tunca 2018, Skjolding et al. 2014).

When the S medium and WS medium were compared, in all test groups, Cu was removed more successfully in the S medium than in the WS medium contaminated by 3 different concentrations of Cu (0.05 ppm, 0.50 ppm, 5.00 ppm) (Table 2). The removed Cu proportion in the S medium contaminated with 0.05 ppm of Cu showed no significant changes on the 1st, 2nd or 3rd day compared with the WS medium containing 0.05 ppm of Cu. A similar situation was also observed in the 5.00 ppm S and WS mediums on the 1st and 2nd day.

One of the important cases that affects the efficiency of uptake is that the available metal is functional cellularly for the absorbent in the medium. According to the metal uptake

Table 1. pH, EC ($\mu\text{S}\cdot\text{cm}^{-1}$), DO ($\text{mg}\cdot\text{L}^{-1}$) and T ($^{\circ}\text{C}$) values of mediums at the beginning and end of the experiment (7 days)

Medium		pH	EC ($\mu\text{S}\cdot\text{cm}^{-1}$)	DO ($\text{mg}\cdot\text{L}^{-1}$)	T ($^{\circ}\text{C}$)
WS	Initial	5.19–6.22	238.2–286.7	2.72–3.31	24
	Final	6.33–6.67	476.1–493.9	2.98–3.62	23
S	Initial	6.55–7.12	309.8–423.2	3.00–4.38	24
	Final	7.62–7.91	512.3–578.9	4.01–4.78	23

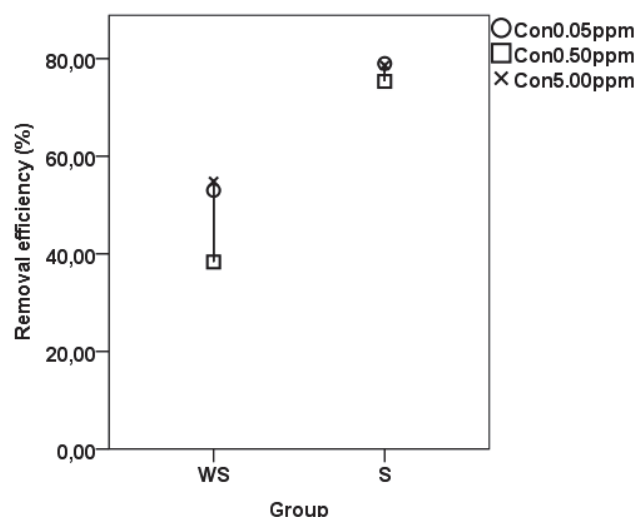


Fig. 1. Removal efficiencies of *Lemna minor* in the WS and S mediums

voluntary of the absorbent, transport pathways of the organism for the quantity of the transporters and the metal may cause alterations in removed metal rates.

Cu acts as a member of the regulatory proteins to participate in oxidative stress responses, mitochondrial respiration, photosynthetic electron transport system, cell wall metabolism, and hormone signals (Garcia-Molina et al. 2011, Ruiz et al. 2016, Yruela 2005). Cu acts as the cofactor of many enzymes such as Cu/Zn superoxide dismutase (SOD), cytochrome c oxidase, and amino oxidase, and it also has a fundamental role in oxidative phosphorylation and iron mobilization at the cellular level (Lee 2018, Yruela 2005). Although Cu is a basic micronutrient for plants, high levels of Cu exposure are highly toxic to plants. Symptoms such as chlorosis, necrosis, growth stunt, loss of color, and the inhibition of root growth are observed in plants exposed to high levels of Cu (Andresen et al. 2018, Fontanilla and Cuevas 2010).

At the cellular level, specific transporters are responsible for the uptake and secretion of metal ions. Accordingly, the demand for the accumulation of metal in living organisms, the transport pathways in vivo for the contaminant in the environment, and the low amount of or multiplicity of transporters can cause variations in the accumulation rates of metals. There are transporters involved in the transport of Cu in plants. The uptake of Cu from the cell membrane is the place where homeostasis is controlled. Different types of carrier proteins have been reported that mediate the uptake of Cu. COPT (Copper Transporter)/Ctr (Copper transporter) proteins are known as basic Cu transport groups belonging to various protein families in different organisms (Puig 2014, Yuan et al. 2011).

The other type of transporter that transports Cu into the organelles from the cytosol in plants is the P-type adenosine triphosphate pump (Williams and Mills 2005). It has also been reported that other metal transporters carry Cu to the cells. For example, YSL1 and YSL3 from OPT/YSL iron (Fe) transport family transported Cu seeds from the leaves of Arabidopsis (Grillet et al. 2013, Waters et al. 2006), and also ZIP2 and ZIP4 zinc (Zn) transported family transported Cu (Milner et

al. 2013, Puig et al. 2007). Cu is an important metal in terms of the structural and catalytic components of many enzymes and proteins (Hashimoto and Kambe 2015, Osredkar and Sustar 2011). For this reason, the rate of phytoremediation may increase due to the high number of Cu transporters in the plant.

Dust from the desert is not different on the ground or in the air. However, as dust is in contact with clouds during the transport process, a number of reaction mechanisms result in the reduction of iron, amino acids, and various other elements (Saydam 2014). Therefore, the atmospherically transported dust of the Sahara Desert allows the delivery of biologically important nutrients to surface waters and soils (Saydam 2014). Saharan dust particles include iron (Fe) and this nutrient is bioavailable. Dust, and therefore iron, is deposited into the ocean and sea, and phytoplankton can use iron in photosynthetic activity (Johnson and Meskhidze 2013, Schoffman et al. 2016). Fe is required for the synthesis of chlorophyll pigment, which is responsible for the photosynthesis process. Fe, which plays an active role in metabolic activities within the plant, is Fe(II). Fe(II), which is the ready-to-use iron element of nature, reaches the earth with the Saharan dust in rain (Rizzolo et al. 2017, Rout and Sahoo 2015). Fe is also a trace metal. Plants need Fe for its roles in redox chemistry. Fe composes the active sites in numerous enzymes involved in dissimilar processes, such as photosynthesis, mitochondrial respiration, oxidative stress protection, and various metabolic pathways (Gomez-Casati et al. 2018, Pilon et al. 2011).

In the experiments with 0.05 and 5.00 ppm concentrations, the amount of Fe in the water was higher than the amount of Fe in 0.50 ppm (Table 3). In other words, *L. minor* removed less of it. This may be due to the fact that the Cu remediation success at 0.05 and 5.00 ppm is better than at 0.50 ppm. Previous reports showed that it could be crosstalk between Fe and Cu. Waters and Armbrust (2013) explained that these 2 metals have to interact to increase or decrease Fe and/or Cu accumulation in leaves. They detected that Ferric reductase activity, an indicator of Fe requisition, was inhibited. This can also be explained by the fact that YSL1 and YSL3 from the OPT/YSL iron (Fe) transport family can transport Cu in plants (Grillet et al. 2013, Waters et al. 2006).

Table 2. After the remediation study, the amounts of Cu concentration in the water of S and WS mediums (7 days)

Medium	Initial	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
WS	0.05	0.038±0.006	0.030±0.006	0.026±0.003	0.021±0.006	0.023±0.004	0.026±0.006	0.027±0.004
S	0.05	0.026±0.005	0.021±0.005	0.015±0.005	0.012±0.001	0.012±0.002	0.013±0.003	0.014±0.004
WS	0.50	0.42±0.05	0.39±0.06	0.34±0.06	0.27±0.06	0.28±0.02	0.31±0.02	0.34±0.03
S	0.50	0.26±0.02	0.20±0.05	0.17±0.03	0.13±0.03	0.14±0.03	0.16±0.07	0.18±0.06
WS	5.00	3.66±0.70	3.08±0.62	2.49±0.37	1.92±0.24	2.34±0.32	2.50±0.38	2.56±0.33
S	5.00	2.81±0.32	2.06±0.41	1.42±0.21	1.02±0.26	1.18±0.16	1.28±0.16	1.65±0.12

Table 3. The initial amount of Fe detected in Saharan dust and the amount of the Fe in S medium during the experiment

Initial Cu	Initial Fe	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
0.05 ppm	0.6 ppm	0.52	0.48	0.36	0.33	0.28	0.31	0.26
0.50 ppm	0.6 ppm	0.42	0.38	0.17	0.12	0.09	0.16	0.21
5.00 ppm	0.6 ppm	0.56	0.51	0.44	0.35	0.46	0.48	0.50

Fe was not detected in WS medium

The growth rate

In the study, the toxic effect of Cu on *L. minor* was calculated based on the fronds. Growth and inhibition rates were calculated according to the control group using the obtained data. The estimation models of the time-dependent growth rates were established by a regression analysis. The R^2 values of the estimation models were found to be in the interval of 0.939–0.991 in the WS medium and 0.995–1.000 in the S medium (Fig. 2 and 3). 0.05 ppm > 0.50 ppm > 5.00 ppm was determined to be the average specific *L. minor* growth rate ($\mu_{i,j}$). The specific growth rate increased in the S medium at the 0.05 ppm concentration until the 4th day, while a decrease was observed on the 5th day in the same concentration. At 0.50 and 5.00 ppm concentrations, the growth rate increased until the 3rd day and decreased after the 3rd day. The results in the WS medium were also found to be similar. As the concentration increased, a decrease in the specific growth rate was detected. Based on the average specific growth rate of *L. minor*, it was found that *L. minor* in the S medium in the same concentration grows more than *L. minor* in the WS medium (66.6% at 0.05 ppm, 64.9% at 0.50 ppm, 65.7 at 5.00 ppm). When the percentile biomass inhibition rates (% Ib) of *L. minor* were calculated, no reduction in *L. minor* biomass in the S medium was observed while *L. minor* biomass reductions developed when the concentration was increased in the WS medium. This reduction was calculated as 24.6% (5 ppm) > 13.1% (0.50 ppm) > 9.8% (0.05 ppm), respectively, according to the concentration. This can be explained by the fact that various minerals, such as Fe, Mg, Ca and K contained in the dust have significant effects on the growth rate of *L. minor*. The iron

contained in the S medium is Fe^{2+} and is in a form that can be used directly by living organisms.

L. minor, which takes minerals in the S medium through its root systems and leaves, was enriched with nutrients and grew faster. In addition, the increased leaf average and specific growth rate of *L. minor* increased in the S medium relative to the WS medium. The effects of the soil, transported by the atmosphere, on the plant growth was investigated and it has been shown that plant development with desert origin soil is more efficient than with a fertilizer mixture (Bağcı and Şengün 2012). The development of various wheat species was investigated using Saharan dust and the results indicated that the effect of some of the wheat varieties on the growth parameters of the elemental composition of the Saharan dust is close to the chemical nutrient solution (Hewitt) (Yücekutlu et al. 2011).

Interestingly, the chlorosis rate was found to be higher at concentrations of 5.00 ppm and 0.05 ppm than at a concentration of 0.50 ppm. As a result of iron deficiency, chlorosis can occur in plants due to the inability to synthesize chlorophyll (Huang et al. 2012). The amount of Fe in water at 0.50 ppm and 0.05 ppm was higher than 0.50 ppm. That is, the Fe uptake was higher in the 0.50 ppm concentration experiment (Table 3).

Conclusion

At the end of the study, *Lemna minor* was found to be successful in removing copper. However it was found that the medium without Saharan dust (WS) was not as successful as the medium with Saharan dust (S) in the copper removing

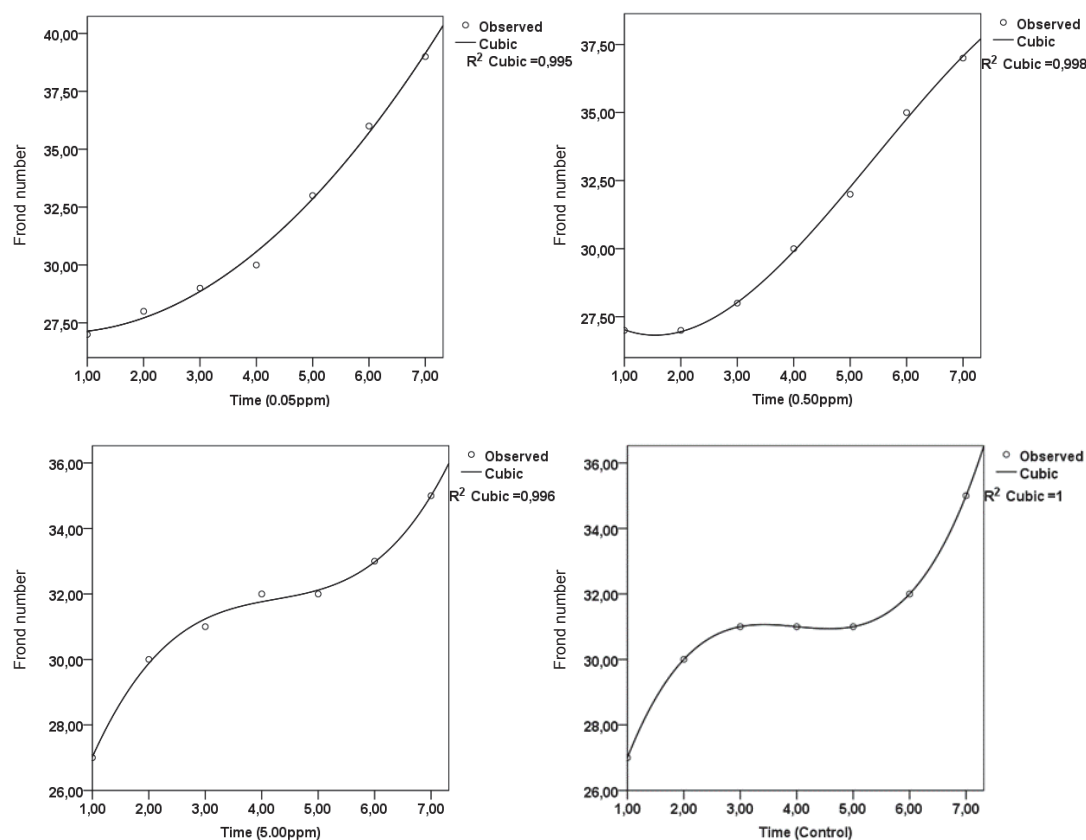


Fig. 2. Time dependent growth rate models for *L. minor* in the S medium

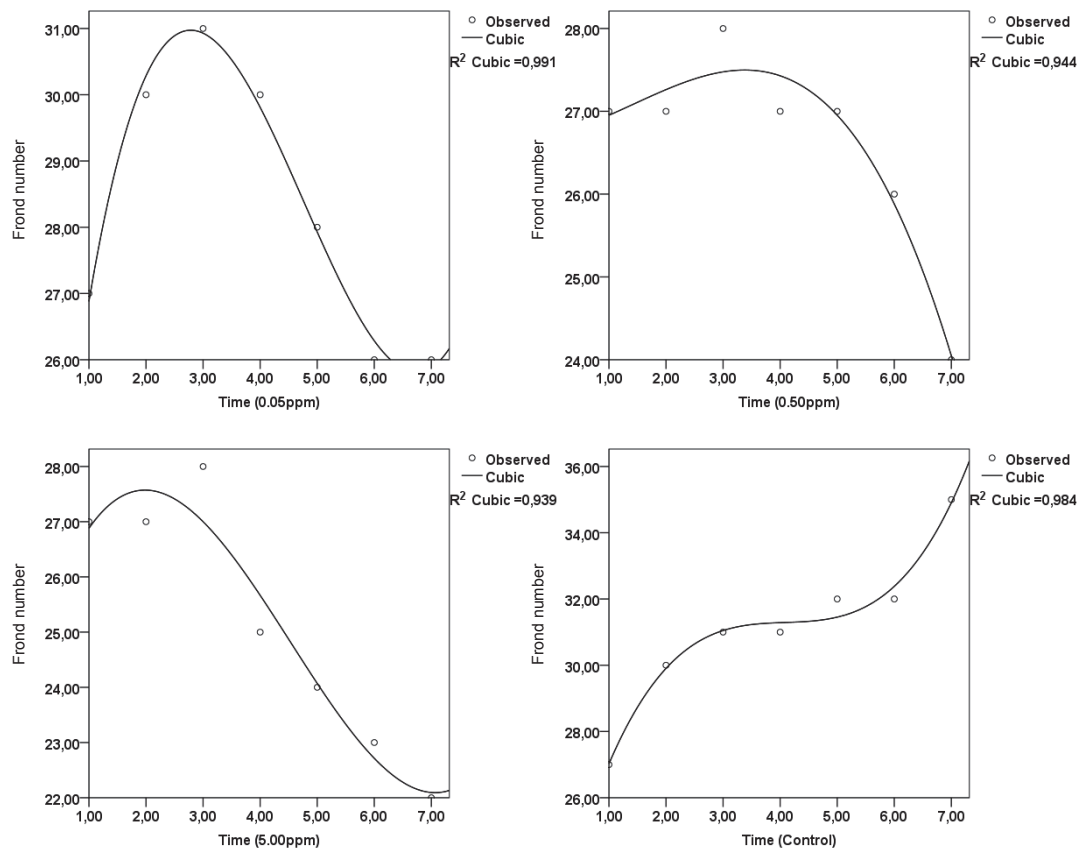


Fig. 3. Time dependent growth rate models for *L. minor* in the WS medium

process. The notable result of this study is that the removal efficiency on the 4th day was higher than on the 7th day. This result points to the importance of the time that the absorbent organism needs to be in exposure in the medium. The optimum removal value was observed to be at a maximum concentration in S medium (79.6%). The lowest removal value was found at 0.50 ppm on the 1st day in the WS medium (16%).

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