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The effect of incubation time and silk worm cocoon on mobility of zinc and copper in contaminated clay soil

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Original Article

Abstract

Of the problematic agents in the ecosystem, heavy metals have special importance because they are unabsorbable and have physiologic effects on living beings at low concentrations. This study has investigated the effect of silk worm cocoon on reducing mobility of zinc (Zn) and copper (Cu) for the first time. To this end, 5% cocoon adsorbent was added to the studied soil, which had been contaminated with Cu and Zn in separate containers at concentration of 600 mg/l. The experiment was performed in three repeats and two treatments (with and without adsorbent). Samples were incubated at 28°C at constant humidity for 3 hours, 1, 3, 7, 14, 21, and 28 days. Then both treatments were extracted using sequential extraction method and the concentration of Zn and Cu was processed using atomic absorption spectrophotometry. The results showed that there were changes in mobility of the Zn and Cu added to soil; adding silk worm cocoon to soil increased organic phase of Zn and Cu as compared to the soil without adsorbent. Data were analyzed by SPSS software. All comparisons of the means were performed at statistical level of 5% using Student's independent t-test. Student's independent t-test showed that the highest significant difference ($P < 0.05$) was observed in the organic fraction of the Cu-Zn contaminated soil with cocoon, as compared with the Cu-Zn contaminated soil without cocoon.

KEYWORDS: Soil, Zinc - Copper, Decreased mobility, Silk worm cocoon, Incubation

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Introduction

Today, environmental pollution has sharply increased along with industrial and technological growth in human societies. Different parts of the environment such as water, air, and soil are contaminated in different forms.[1] After water and air, soil is the third most important part of the environment for human beings.[2] Distribution of heavy metals in the environment, which accompanies industrial development and increasing population, is a major environmental problem in many countries.[3] Concentration of heavy metals in the soil can contaminate the food chain, and consequently endanger human health.[4] Due to their relative stability and non-biodegradability, heavy metals accumulate in different body organs like kidney, liver, and bones, and cause serious disorders.[5] The toxicity of heavy metals in plants ranges from reducing the product through their effect on the growth of the root and leaves to enzyme preventing activity.[6] Heavy metals are a group of elements that are considered pollutant at concentrations higher than their critical limit. A heavy metal is an element with an atomic mass higher than that of Fe (55.8 g/mol), and with a specific gravity of higher than 4.5 g/cc.[7] Copper (Cu) and zinc (Zn)

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are heavy metals that cause serious disorders in human and plant health at concentrations higher than threshold. As an essential element, Cu plays a role in the metabolism including development of bones, central nervous system, and ligaments. Overdose of Cu causes capillary disorder, liver and kidney complications, nervous system irritability, and depression. According to studies, Zn is more easily dissolved than other heavy metals and almost 60% of the soluble Zn in soil is in the form of soluble organic complex.[8]

These metals are naturally found at low concentrations in soil and rocks, and enter the environment through natural processes (geochemical and weathering), but human activities have increased their release and distribution in the environment.[9] The important human sources of contamination of soil with heavy metal contamination include mining, batteries, electric plating, electrolysis, mine survey, leather, manufacturing of electric appliances, polishing metal surfaces, pharmaceutical uses, atomic energy or aerospace utilities that dispose of their contaminated wastewater directly or indirectly in the environment.[10] Due to the environmental hazards of heavy metals, their removal has been attended to in recent decades. Surface adsorption is one of the most practical methods because of its easy application[11] In this method, heavy metals are adsorbed to the surface pores of adsorbents, which are water insoluble.[12] The use of natural materials as heavy metal adsorbent is more suitable for adsorbing heavy metals than other materials because they are not costly. Agricultural byproducts are suitable because they have hydroxyl, carboxyl, and phenol groups and have a high affinity with heavy metals.[13] These material are mainly the waste products of industrial and agricultural activities, and have cellulose origins.[14] Wastes like, tree bark, orange waste, wool, olive leave, pine needle leaves, cactus leaves, coconut skin, grapes waste, walnut shell, teakwood, apple waste, banana skin, and date stone, have been reported to be good adsorbents for surface metals.[12,15-19] Generally, this study aimed to determine the efficiency of silk worm cocoon in decreasing mobility of Cu and Zn through their effect on fractions of elements in clay soil at different time intervals.

Materials and Methods

Soil sampling was conducted at the depth of 0-30 cm from around the city of Hamedan. The soil was air dried and passed through a 2-mm sieve. Some of the physical and chemical properties of soil were determined in the following ways: texture was measured using hydrometer method and based on Stox law, pH was measured in a 1:5 water/soil solution using a pH meter, electric conductivity was measured in a 1:5 water/soil solution using an EC meter, sodium (Na^+) and potassium (k^+) were measured using film photometer, sulfate, nitrate and phosphorus were measured using spectrophotometer, bicarbonate was measured using neutralization with acid, and Cloride (Cl^-), Calcium (Ca^+), and Maguesium (Mg^{2+}) were measured by titration.[20-22] Some of the physical and chemical properties are shown in table 1.

The concentration of Zn and Cu was measured in 2 treat soils using nitric acid extraction by atomic absorption spectrophotometry.[23] The total concentration of Cu and Zn in silk worm cocoon was measured; silk worm cocoon was chopped into 1cm pieces and analyzed by inductively coupled plasma (ICP) (Verian710-Es).[24]

The studied soil was contaminated with Cu and Zn at the concentration of 600 mg/l in

Table 1. Some physical and chemical properties of the studied soils

Solution cations and anions (mg/l)								%			Soil texture	EC (ds.m$^-$)	pH
Ca^{+2}	K^+	Mg^{+2}	Na^+	PO_4^{3-}	Cl^-	SO_4^{2-}	NO^{3-}	Sand	Silt	Clay	Clay- loam	0.15	7.22
32	45	9.6	26.22	0.63	81.6	13.3	69.4	26	43	31			

EC: Electrical conductivity

separate containers, and mixed with 5% of the adsorbent (silk worm cocoon).[25,26]

Samples of soil with adsorbent and samples without adsorbent were incubated at 28°C.[27] At intervals of 3 hours, and 1, 3, 7, 14, 21, and 28 days, samples were taken from the soil in the incubator. The tests were performed in three repeats and two treatments (with and without adsorbent), and the concentration of Zn and Cu was measured at different fractions including exchangeable, carbonate, Fe-Mn oxide, organic, and residual fractions using sequential extraction and atomic absorption spectrophotometry (GBC-Avante).[23,28]

Heavy metal components of soil were fractionated using sequential extraction method of Salbu and the following components were recognized.[28]

Fraction 1. Exchangeable: extraction with 20 mL of 1 MNH_4OAc at pH 7 for 2 hours at room temperature.

Fraction 2. Specifically sorbed and carbonate-bound: extraction of the residue from F1 with 20 mL of 1 MNH_4OAc at pH 5 for 2 hours at room temperature.

Fraction 3. Fe–Mn oxides occluded: extraction of the residue from F2 with 20 mL of 0.04 MNH_2OH_HCl in 25% HOAc for 6 hours in a water bath at 60°C.

Fraction 4. Organically complexed HM: extraction of the residue from F3 with 15 mL of 30% H2O2 at pH 2 for 5.5 hours in a water bath at 80°C.

Fraction 5. Residual: after cooling, 5 mL of 3.2 MNH_4OAc in 20% HNO_3 was added to the residue of F4. Sample was shaken for 0.5 hours, and finally diluted to 20 mL with distilled water.[29]

The solutions obtained from each fraction were read by atomic absorption spectrophotometer.

Results and Discussion

Characterization of soil

Some physical and chemical properties of the studied soil are presented in table 1. Accordingly, pH was 7.22, EC was 0.15 ds/m, and the texture of the soil was loamy clay. On the basis of the results, the concentration of the whole Cu and Zn in non-contaminated clay and silk work cocoon was not detectable by atomic absorption spectrophotometer.

Changes of Cu in two treatment of soil

Accessible Cu in soils is mainly found as divalent cation on the surface of clay minerals or bound to organic matters of the soil. That is why preserving Cu is increased in clay and organic matters of the soil and its availability is also increased.[29] The results showed that Fe-Mn fraction had the highest adsorption of Cu (Figure 1, Table 2). Luo et al. reported the same results in contaminated paddy fields in China in that the highest concentration of Cu was found in the Fe-Mn fraction of the soil.[30] Bartlet and James found that the surface of iron oxide acts as active adsorbent and adsorbs most of the metal ions dissolved in soil.[31]

The order of increase in different fractions of soil is as follows:

Fe-Mn > exchange > carbonate > residual > organic

On the basis of the results, Cu in the organic fraction has significantly increased in the soil with cocoon compared with that of in the soil without cocoon at all times; it increased from 2.7% to 36.52% at 3 hours, from 0.005% to 37.69% on day 3, from 0.003% to 45.33% on day 7, from 0.003% to 23.14% on day 14, from 0.002% to 32.61% on day 21, and from 0.003% to 24.33% on day 28. Student’s independent t-test showed that the highest significant difference ($P < 0.05$, $P = 0.007$) was observed in Cu in the organic fraction of the Cu contaminated soil with cocoon as compared with the Cu-contaminated soil without cocoon.

As seen in figure 2 and table 3, the amount of organic matters increased relative to the blank soil. This shows that when silk worm cocoon is added, metals in the exchange fraction reduce and those in the organic phase increase, and their mobility reduces. Cu can bind with organic matters easily because it creates highly stable

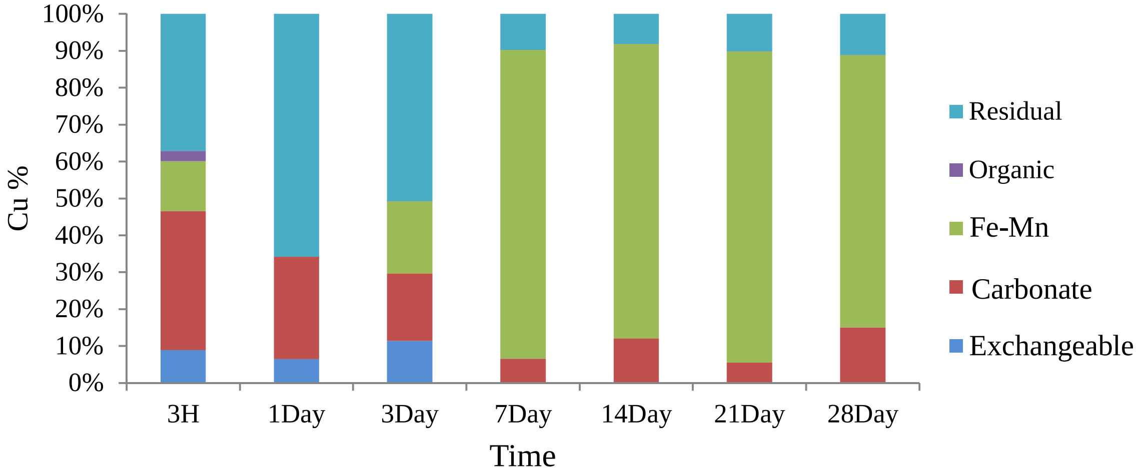

Figure 1. Different fractions of Cu in blank soil (%)

Table 2. Different fractions of Cu in blank soil (mg/kg^{-1})

Time	Exchangeable	Carbonate	Fe-Mn	Organic	Residual
3H	8.58	36.29	13.00	2.68875	35.73
1d	6.62	28.35	0.01	0.00750	67.38
3d	16.05	25.76	27.63	0.00750	71.47
7d	0.01	14.76	189.15	0.00750	22.00
14d	0.01	26.47	175.15	0.00750	17.83
21d	0.01	14.21	216.38	0.00750	25.96
28d	0.01	28.67	141.00	0.00750	21.35

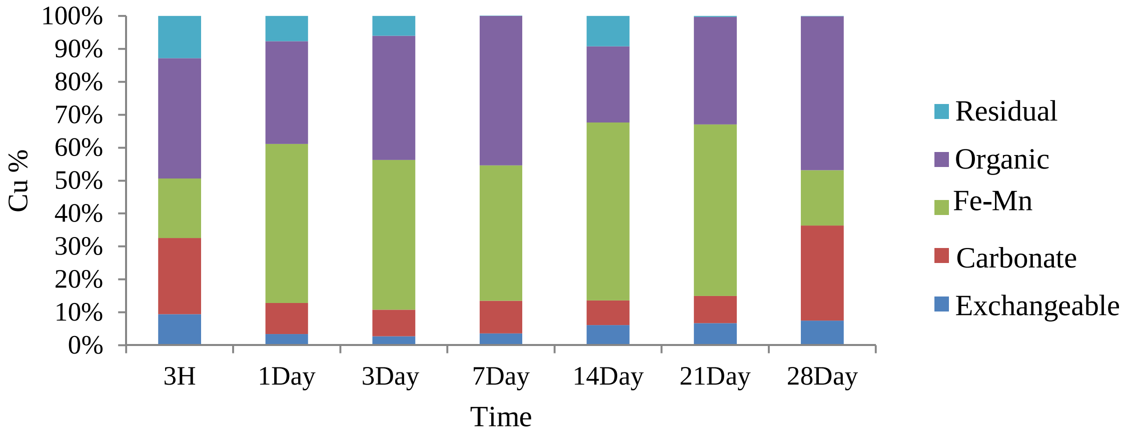

Figure 2. Different fractions of Cu in contaminated soil with silk worm cocoon (%)

Table 3. Different fractions of Cu in contaminated soil with silk worm cocoon (mg/kg^{-1})

Time	Exchangeable	Carbonate	Fe-Mn	Organic	Residual
3H	9.82	23.95	26.86	29.850	13.32
1d	12.00	33.14	243.43	37.250	26.84
3d	10.32	30.63	248.16	69.300	22.99
7d	9.54	26.39	156.92	73.840	0.01
14d	12.93	15.89	162.98	0.007	19.47
21d	13.81	17.12	153.55	21.180	0.54
28d	4.33	30.00	28.89	47.690	0.01

combinations in the soil. This binding stabilizes Cu, reduces Cu washing, and reduces availability of Cu for plants.[32] Furthermore, it was observed that on days 1 and 3, Cu in exchange fractions reduced and in organic fractions increased. On days 7, 14, 21, and 28, Cu in the Fe-Mn fraction reduced and in the organic fraction increased. At all times, except 3 hours, the highest fraction of Cu was reported bound with Fe-Mn. investigated soils of paddy fields in China, showed that the highest percentage of Cu was bound with organic matters.[33] The highest percentage of Cu reduction in residue was observed on day 1; it reached from 7.61% to 65%. Kim et al. reported that heavy metals were fixed using improved treatment of waste with oyster.[26] Lindsay found that organic residue decreased the mobility of Cu in soil, which results from the gradual formation of insoluble Cu complexes.[34] After the organic fraction, Fe-Mn fraction has the highest amount of Cu.

Furthermore, Jalali and khanlari reported that most of the added Cu was distributed in organic and Fe-Mn fractions.[27]

Changes of Zn in two treatment of soil

Solubility of Zn minerals during weathering especially in acidic and oxidative conditions mobilizes Zn. Zinc is found in many minerals in the soil, and because of the proximity of its ionic radius to that of Fe and Mg, it can substitute these ions in different mineral structures.[2] Based on our results, at all times except 3 hours, Fe-Mn fraction had the highest adsorption of Zn (Figure 3, Table 4).

The order of Zn increase in the fractions of soil is as follows:

Fe-Mn > carbonate > residual > exchange > organic

On the basis of the results, organic fraction increased in the soil with cocoon as compared with the soil without cocoon at all times; it

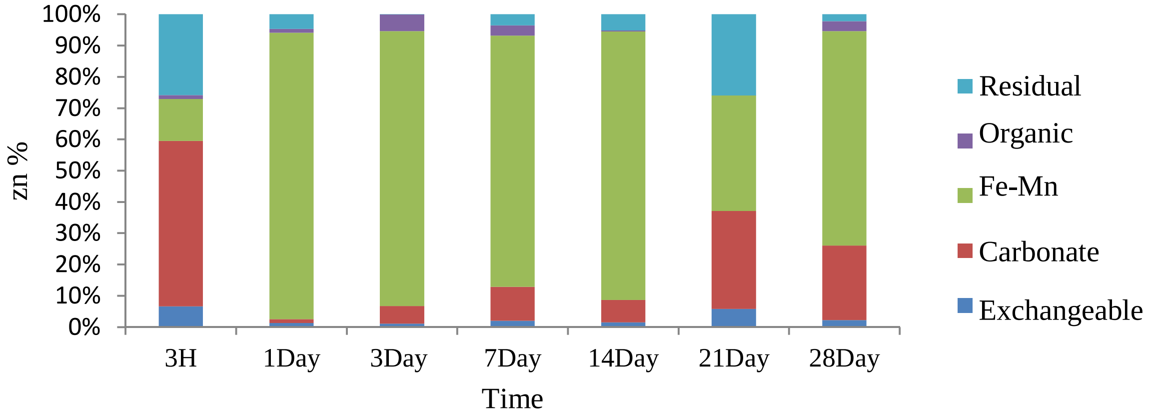

Figure 3. Different fractions of Zn in blank soil (%)

Table 4. Different fractions of Zn in blank soil (mg/kg^{-1})

Time	Exchangeable	Carbonate	Fe-Mn	Organic	Residual
3H	6.42	50.96	12.98	1.120	24.96
1d	5.82	5.98	422.28	5.350	21.58
3d	5.58	27.83	439.62	26.640	0.28
7d	7.24	39.10	289.20	11.940	12.74
14d	6.42	31.83	380.80	1.270	22.82
21d	5.95	32.18	37.87	0.007	26.61
28d	7.62	81.20	234.20	10.960	7.36

increased from 1.16% to 34.34% at 3 hours, from 1.16% to 29% on day 1, from 5.32% to 29.88% on day 3, from 3.31 to 32.15% on day 7, from 0.28% to 30.16% on day 14, from 0.007% to 31.04% on day 21, and from 0.31% to 3.21% on day 28. Student's independent t-test showed a significant difference ($P < 0.005$, $P < 0.001$) between organic fraction in the soil contaminated with Zn with silk worm cocoon, and that in the soil contaminated with Zn without silk worm cocoon. There was no significant difference observed in other fractions.

On the basis of our findings, at 3 hours, and on days 21 and 28, Zn in the carbonate fraction reduced and in the organic fraction increased. On days 1, 3, 7, and 14, Zn in the Fe-Mn fraction decreased and in the organic fraction increased (Figure 4, Table 5). On the basis of past researches, Zn dissolves more easily than other heavy metals, and about 60% of Zn is found as soluble organic complex in soil.[8] Kim et al., using egg shell to investigate the mobility of heavy metals, reported that the amount of heavy metal before using egg shell was 37% bound to organic fraction, but it increased to 52.2% after applying egg shell.[26] Clemente et al. found that using organic soil fixes Pb and Zn.[35] Luo reported similar results.[30]

Conclusion

In order to study the mobility of heavy metals in loamy clay soil, we used Salbu sequential extraction. On the basis of our results, the organic fraction had a significant increase in Zn and Cu at all times. Organic fraction in the soil with adsorbent of Cu increased more than that in the soil with adsorbent of Zn. Zn and Cu were reported to be higher in Fe-Mn fraction than other fractions in the blank soil. The residual fraction reduced in the soil with adsorbent of Zn and Cu. The exchange fraction of Zn had a significant decrease as compared to that of Zn.

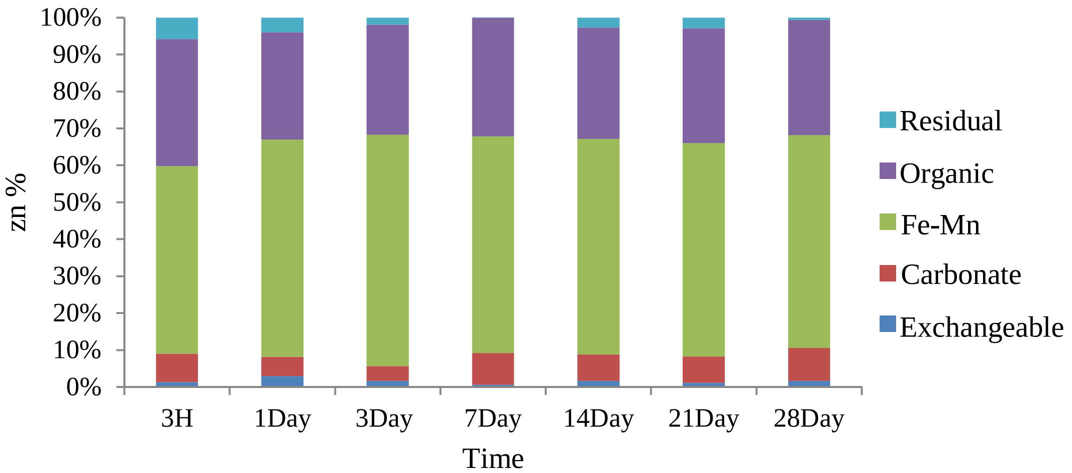

Figure 4. Different fractions of Zn in contaminated soil with silk worm cocoon (%)

Table 5. Different fractions of Zn in contaminated soil with silk worm cocoon (mg/kg)

Time	Exchangeable	Carbonate	Fe-Mn	Organic	Residual
3h	6.42	39.42	369.24	64.15	29.59
1d	12.87	22.62	367.71	16.57	17.42
3d	8.03	20.02	443.70	15.21	9.36
7d	2.67	34.93	343.97	28.76	0.01
14d	7.53	33.41	385.55	24.04	12.57
21d	5.35	32.54	378.18	28.76	13.31
28d	6.77	37.37	343.97	27.35	2.49

Student's independent t-test showed a significant difference ($P < 0.05$) between organic fractions in the Cu contaminated soil with silk worm cocoon and in the blank soil. Furthermore, a significant difference was seen in the organic fraction of Zn contaminated soil with silk worm cocoon as compared with that in the blank soil.

Conflict of Interests

Authors have no conflict of interests.

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