Mobility of zinc and copper in contaminated clay soil influenced by *Actinidia deliciosa* and incubation times

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Original Article

Abstract

As a low cost byproduct, *Actinidia deliciosa* shell can be made into sorbent materials which are used in heavy metals immobilization. It has been investigated as a replacement for currently expensive methods of heavy metal immobilization from soil. In this study, soil samples were contaminated with copper (Cu) and zinc (Zn) at the rate of 600 ppm in separate dishes. The 5% *Actinidia deliciosa* shell was added into the samples. The samples were incubated for 3 hours, and 1, 3, 7, 14, 21, and 28 days at 28° C with constant moisture. After incubation, metals in contaminated soil with *Actinidia deliciosa* shell and control soils were fractionated by the sequential extraction procedure. The results of this study indicated that addition of *Actinidia deliciosa* shell led to increased organic matter fraction and stabilized Cu and Zn in contaminated soil. In the control soils, the dominating chemical form for Zn and Cu were Fe-Mn oxides and residual, respectively. Sequential extraction also revealed that the addition of *Actinidia deliciosa* decreased the easily accessible fraction of Zn through the transformation into less accessible fractions. The experiment was performed in three replicates and two treatments.

KEYWORDS: *Actinidia Deliciou*s, Stabilization, Incubation, Sequential Extraction

Introduction

Contamination of heavy metals represents one of the most pressing threats to soil sources.[1] The pollution of the soil ecosystem with heavy metals is considered as a global environmental issue. Some of these elements such as copper (Cu) and zinc (Zn) are essential micronutrients for living organisms but at elevated concentrations, they can be toxic to higher plants and other organisms.[2] Cu and Zn have both natural resources such as weathering/erosion of parent rocks and ores deposits and anthropogenic resources such as mining,

smelting, energy, electroplating, fuel production, intensive agriculture, waste water irrigation, sludge dumping and dust.[3-7] In soil ecosystems, the toxicity of heavy metals depends upon various factors including total concentration of metals, specific chemical form, metal binding state and properties.[8] High levels of heavy metals may pose an important hazard to human health and the environment not only because of their direct toxic effects on organisms, but also due to their further potential for increasing exposure along the food chain through bioaccumulation.[2] As chemical hazards, heavy metals are non-biodegradable and remain almost indefinitely in the soil environment.[9]

To overcome the high costs and limits remediation technology sources, new technologies

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should be investigated which can be implemented at lower costs and without the possibility of adverse environmental consequences.[10,11]

The immobilization, which transforms heavy metals into less bio available forms, is considered as one of the most effective ways to remediate the heavy metals contaminated soils.[12,13]

Agricultural wastes or by-products have been investigated for their uses as sorbent materials for heavy metals due to the tendency of heavy metals to form stable complexes with organic ligands.[14,15] Many studies have been conducted to investigate the stabilization of heavy metals using conventional stabilizing agents such as Portland cement, quicklime, and fly ash.[16,17] Few studies have involved immobilizing heavy metals using natural wastes such as oyster shells.[18,19] The immobilization of heavy metal by calcined egg shell in contaminated soil performed by Kim et al.[20]

Actinidia deliciosa shell as a low cost byproduct can be made into sorbent materials which are used in heavy metals immobilization. It has been investigated as a replacement for expensive methods of heavy metals immobilization from soil currently.

Therefore, the present study aimed to evaluate the effect of Actinidia deliciosa and time on distribution and mobility of Zn and Cu in soil using the sequential method.

Materials and Methods

Soil sample was collected from 0-30 cm soil horizon, in Hamadan, Iran. Samples were air-dried and passed through a 2 mm sieve before analyzing. Particle size was determined by the hydrometer method. Sample was then analyzed for metals (Zn and Cu).The pH and electrical conductivity (EC) values (solid: distilled water = 1:5) of the soil sample was measured by a pH meter and an EC meter, respectively.

The soluble ions of the soil sample were analyzed such as sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), chloride (Cl^-), sulfate (SO_4^{2-}), nitrate (NO_3^-) and phosphate (PO_4^{3-}). Calcium, Mg^{2+}, and Cl^- were determined by titration. Sodium and K^+ were measured by flame photometry. Sulfate and PO_4^{3-} were determined by spectrophotometric turbidimetry. Nitrate was measured by colorimetry with an UV-visible spectrophotometer.[21]

Kiwi fruit with scientific name of Actinidia deliciosa, belongs to Actinidia class, Ericales order and Actinidiaceae family, and grows in temperate climate.

In this study, Actinidia deliciosa shell was washed with double-distilled water (DDW), oven dried at 70° C and powdered with electric grinder, then mixed with soil samples.

In addition, the total Cu and Zn concentrations were determined in the Actinidia deliciosa shell.[22]

Soil was placed in plastic cups and heavy metals were added at the rate of 600 ppm for Zn and Cu in separate dishes, and then each soil sample were mixed thoroughly. The soil was amended with Zn and Cu using $ZnCl_2$ and $CuCl_2$, respectively.[23]

The samples were incubated for 3 hours, and 1, 3, 7, 14, 21, and 28 days at 28° C. The appropriate amount of water was added to cause the soil to the estimated field capacity. The samples were kept moisture By adding distilled water as needed, all the incubation experiments were carried out in duplicate. Soil samples without Actinidia deliciosa shell and amended with heavy metals were prepared as the control soils.[23,24]

Prior to incubation, a sequential extraction of Salbu[25] method was used to extract metals in contaminated soil with Actinidia deliciosa shell and control soils (Table 1).[26] The metal concentrations for Zn and Cu were analyzed by ICP (inductively coupled plasma) and optical emission spectrometry (Varian 710-Es).

Graphs were plotted using Microsoft Excel 2010 and statistical analysis was performed using SPSS for Windows (version 18.0, SPSS Inc., Chicago, IL, USA).

Table 1. Sequential extraction procedure and the corresponding forms by the method of Salbu

Form/ association	Fraction	Operational definition
EXCH	F1	20 ml of 1 M NH4OAc[*] at pH 7 for 2 hours at room temperature.
CARB	F2	20 ml of 1 M NH4OAc at pH 5 for 2 hours at room temperature.
Fe–Mn	F3	20 ml of 0.04 M NH2OH-HCl[†] in 25% HOAc[‡] for 6 h in a water bath at 60°C.
OM	F4	15 ml of 30% H2O2[§] at pH 2 for 5.5 h in a water bath at 80°C.
RES	F5	5 ml of 3.2 M NH4OAc in 20% HNO3[‖] was added to the residue of F4. Sample was shaken for 0.5 hours, and finally diluted to 20 mL with distilled water.

[*]Ammonium acetate, [†]Hydroxylamine hydrochloride, [‡]Acetic acid, [§]Hydrogen peroxide, [‖]Nitric acid; EXCH: Exchangeable; CARB: Carbonate-bound; Fe–Mn: Fe-Mn oxides; OM: Organic matter; RES: Residual

Results and Discussion

Characterization of soils

Selected chemical and physical properties of the soils are given in table 2. Results showed that texture of soil was clay-loam. The pH and electrical conductivity (EC) values were 7.22 and 0.1215 (ds/m), respectively. Thus, the soil sample was neutralized and non-saline. Moreover, the results showed that no element was detected in the primary soil and Actinidia deliciosa shell.

Concentration of Cu in contaminated soil with Actinidia deliciosa shell and control soil

Tables 3 and 4 represent the Cu concentration in the samples in each fraction, and figure 1 shows the change of Cu (%) in different fractions as well.

Furthermore, tables 3 and 4 illustrate the concentration of Cu in five fractions in the control soil and contaminated soil with Actinidia deliciosa shell, incubated for 3 hours, 1, 3, 7, 14, 21, and 28 days. There were some differences between the control soil and the contaminated soil with Actinidia deliciosa shell in Cu distributions.

Table 2. 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^-	$SO4^{2-}$	NO_3^-	Sand	Silt	Clay	Clay-loam	0.1512	7.22
32	45.2	9.6	26.2	0.6	81.6	13.3	69.4	26	43	31			

EC: Electrical conductivity

Table 3. Concentration (mg/kg) of Cu in each fraction in the contaminated soil with Actinidia deliciosa shell

Time	Exchangeable	Carbonate	Fe-Mn oxides occluded	Organic matter	Residual
3 hours	17.5	48.4	71.5	30.6	70.6
1 day	16.2	42.1	334.9	143.3	55.0
3 days	414.1	31.5	629.7	269.8	47.0
7 days	34.6	61.2	87.2	37.3	2.3
14 days	36.7	70.7	88.9	38.1	2.3
21 days	38.1	48.5	102.2	43.8	32.3
28 days	34.2	40.0	84.6	37.1	5.0

Table 4. Concentration (mg/kg) of the Cu in each fraction in the control soil

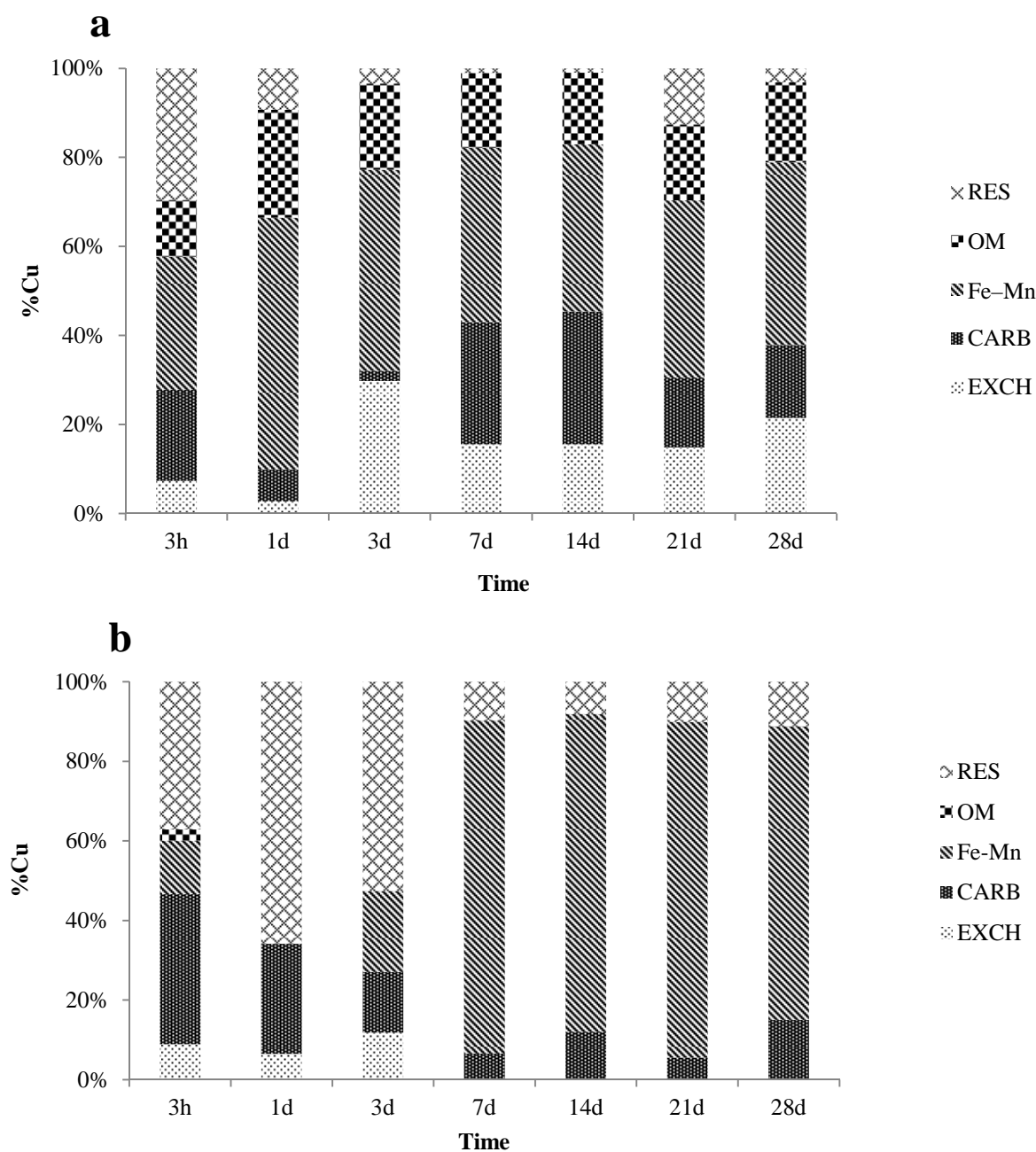| Time	Exchangeable	Carbonate	Fe-Mn oxides occluded	Organic matter	Residual
3 hours	8.5	36.2	13.0	2.6000	35.7
1 day	6.6	28.3	0.01	0.0075	67.3
3 days	16.0	20.7	27.6	0.0075	71.4
7 days	0.01	14.7	189.1	0.0075	22.0
14 days	0.01	26.4	175.1	0.0075	17.8
21 days	0.01	14.2	216.3	0.0075	25.9
28 days	0.01	28.6	141.0	0.0075	21.3

Figure 1. The change of Cu (%) in different fractions with time in contaminated soil with (a) Actinidia deliciosa shell (b) in the control soil

RES: Residual; OM: Organic matter; Fe–Mn: Fe-Mn oxides; CARB: Carbonate-bound; EXCH: Exchangeable

According to the statistical comparisons, performed by the 95% level using the independent t-test, there was a statistically significant difference between the amount of absorbed Cu in the organic matter (OM) fraction in the contaminated soil with Actinidia delicious and the control soil, about the carbonate fraction; there was also a statistically significant difference between the two cases. Regarding the other soil fractions, there was not a statistically significant difference between the contaminated soil with Actinidia deliciosa shell and the control soil (Table 5).

Table 5. Statistical test results for Cu in each fraction between the contaminated soil with Actinidia deliciosa and the control soil

Fractions	Sig	df	t
Exchangeable	0.160	6.02	1.57
Carbonate-bound	0.002	9.89	4.21
Fe-Mn oxides	0.310	8.21	1.04
Organic matter	0.040	6.00	2.50
Residual	0.630	11.48	-0.04

Sig: Significant; Df: Degree of freedom

The addition of Actinidia deliciosa shell had resulted in increased Cu concentrations in OM fraction and thus Cu stabilized. Cu has high affinity for organic matter.[23] Ok et al. indicated that waste oyster shells (WOS) amendments improved the soil quality and stabilized heavy metal in contaminated soil.[11] In the contaminated soil with Actinidia deliciosa shell, the highest contents of Cu are associated with Fe-Mn fraction, with the following order:

Fe-Mn > OM > CABN > EXCH > RES

The results also showed that in the contaminated soil with Actinidia deliciosa shell, the highest contents of Zn in OM fraction are associated at 1 day (24.2%) (Figure 1). On the other hand, although Cu has absorbed in the organic phase, the exchangeable amount has increased. A major part of the residual fraction in the contaminated soil with Actinidia deliciosa shell decreased as compared to the control soil, which showed that part of the Cu from the residual fraction has moved to the organic and exchangeable fractions. Moreover, at 7-14-21-28 days Fe-Mn fractions has decreased and at 1-3 days and 3 hours carbonate the fraction has decreased, which represented the Cu movement from these fractions to the organic and exchangeable fractions. Results of the Cu classification in the control soil showed that Fe and Mn fraction had maximum percentage of Cu absorption at 7, 14, 21, and 28 days, and the remaining fraction had the maximum percentage of the Cu absorption at 3 hours and 1-3 days.

In the control sample, the dominating chemical form for Cu was the RES, with the Fe-Mn of the secondary importance, with the following order:

RES > Fe-Mn > CABN > EXCH > OM

Cu absorption in the OM fraction at all times was lower than the other soil fractions, so that, in 3 hours, it was 2.79% and in the other times i.e. 1, 3, 7, 14, 21, and 28 days, it was negligible (Figure 1).

Besides, after the OM fraction, the RES fraction had the lowest amount of the Cu absorption in all the time periods.

Concentration of Zn in contaminated soil with Actinidia deliciosa shell and the control soil

Tables 6 and 7 illustrate the Zn concentration in the samples in each fraction and figure 2 shows the change of Zn (%) in different fractions.

Moreover, the tables 6 and 7 suggest the concentration of Zn in five fractions in the control and contaminated soil with Actinidia deliciosa shell, incubated for 3 hours, 1, 3, 7, 14, 21, and 28 days. There were some major differences between the control and contaminated soil in the Cu distributions.

According to the statistical comparisons, using the independent t-test, at 95% level, there was a statistically significant difference in the organic and exchangeable fractions in the Zn absorption in the contaminated soil with Actinidia deliciosa shell and the control soil. There was no statistically significant difference in comparison to the other soil fractions (Table 8).

The addition of Actinidia deliciosa shell had resulted in increased Zn concentrations in OM fraction and stabilized Zn. Yoon et al. indicated that treatment with WOS was effective for the adsorption and stabilization of the heavy metals in soil.[27]

Figure 2 shows that OM fraction increased markedly following Actinidia deliciosa shell addition in soil in all the times periods.

The results also showed that in the contaminated soil with Actinidia deliciosa shell, the highest contents of Zn in OM fraction are associated at 3 hours (28.7%) (Figure 2). Besides, there was no high difference between the 3
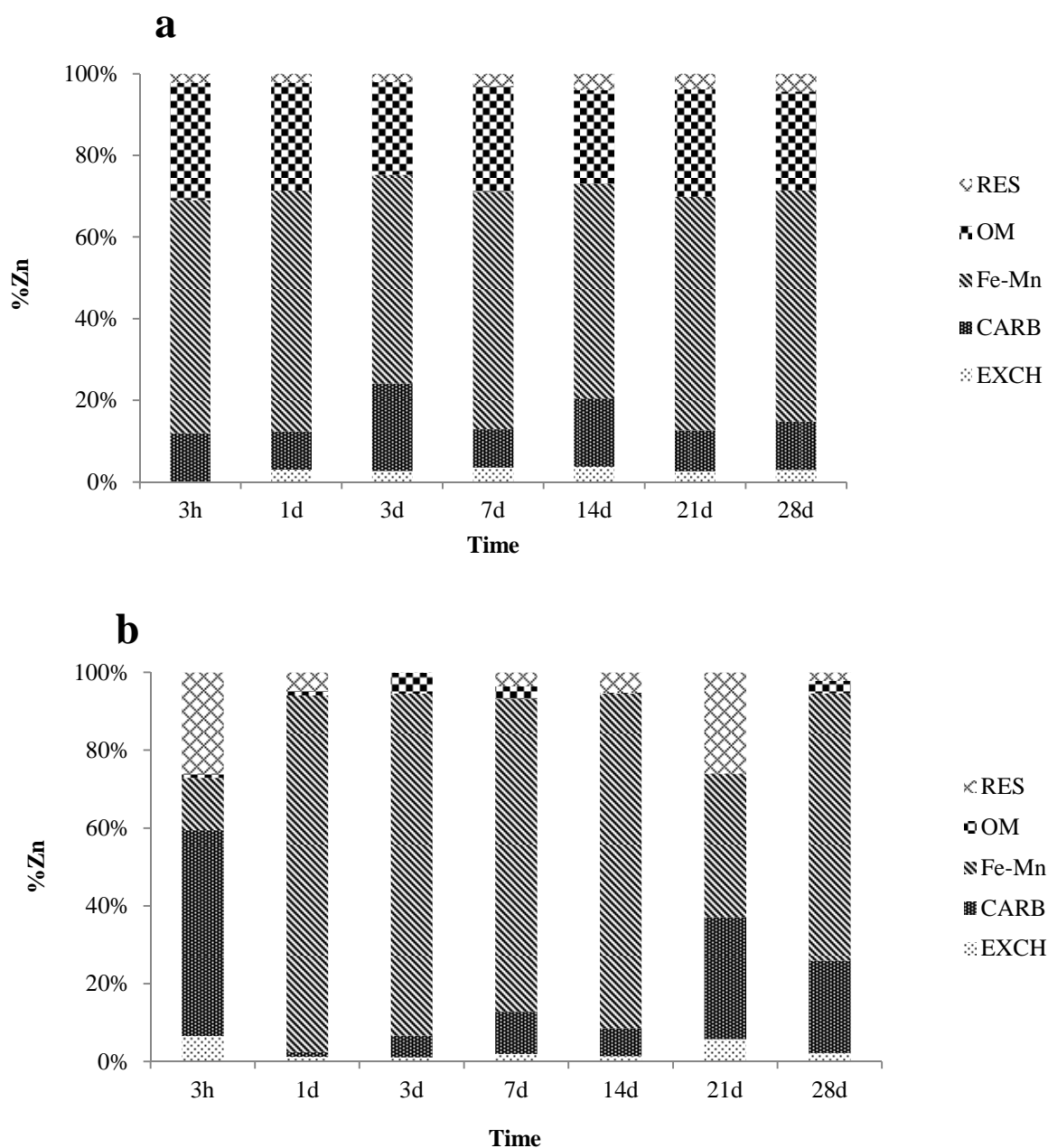
a

b

Figure 2. The change of Zn (%) in different fractions with time in contaminated soil with (a) Actinidia deliciosa shell (b) in control soil
RES: Residual; OM: Organic matter; Fe–Mn: Fe-Mn oxides; CARB: Carbonate-bound; EXCH: Exchangeable

Table 6. Concentration (mg/ kg) of Zn in each fraction in the contaminated soil with Actinidia deliciosa shell

Time	Exchangeable	Carbonate	Fe-Mn oxides occluded	Organic matter	Residual
3 hours	0.51	48.1	232.0	116.8	9.1
1 day	12.4	38.2	242.4	108.6	9.1
3 days	15.2	124.5	296.6	131.4	11.9
7 days	15.7	41.7	263.9	117.6	13.3
14 days	15.3	68.5	220.5	95.8	16.5
21 days	10.4	41.0	234.5	107.7	15.5
28 days	10.6	42.0	203.2	87.1	15.3

Table 7. Concentration (mg/ kg) of Zn in each fraction in the control soil

Time	Exchangeable	Carbonate	Fe-Mn oxides occluded	Organic matter	Residual
3 hours	6.4	50.9	12.9	1.1	24.9
1 day	5.2	5.9	422.2	5.3	21.5
3 days	5.5	27.8	439.6	26.6	0.2
7 days	7.2	39.1	289.2	11.9	12.7
14 days	6.4	31.8	380.8	1.2	22.8
21 days	5.9	32.1	37.8	0.0075	26.6
28 days	7.6	81.2	234.2	10.9	7.3

Table 8. Statistical test results for Zn in each fraction between the contaminated soil with Actinidia deliciosa and the control soil

Fractions	sig	df	t
Exchangeable	0.04	6.24	2.47
Carbonate-bound	0.21	11.09	1.31
Fe-Mn oxides	0.8	6.36	-0.2
Organic matter	0.000	10.24	15.33
Residual	0.4	7.12	-0.9

Sig: Significant; Df: Degree of freedom

hours and 28 days after the 28 days. These results demonstrated that the main effect of Actinidia deliciosa shell as adsorbent has occurred in 3 hours.

Therefore, the Zn amount in the exchangeable fraction -which is the available fraction for the plant-, has decreased from 6.65% in the control soil to 0.12% to the contaminated soil with Actinidia deliciosa shell (Figure 2). Almas et al. found that the heavy metal in the mobile fractions decreased significantly in the soil treated with organic matter.[28]

In the contaminated soil with Actinidia deliciosa shell at all the times except from 3 hours and 21 days, the Fe and Mn fractions decreased as compared to the control soil, which showed a small amount of Zn from this fraction has moved to the organic fraction. Furthermore, after 3 hours and 21 days, carbonate and exchangeable fractions in the contaminated soil with Actinidia deliciosa shell have decreased and transferred to the organic fraction. Kim et al. found that the application of calcined eggshell particularly decreased the mobile fraction of the heavy metals.[20] Ok et al. reported that sequential extraction revealed that the addition of rapeseed residue decreased the easily accessible fraction

of cadmium by 5-14% and lead (Pb) by 30-39% through the transformation into less accessible fraction.[29]

In the contaminated soil with Actinidia deliciosa shell, the highest contents of Zn were associated with Fe-Mn fraction, with the following order:

Fe-Mn > OM > CABN > RES > EXCH

In the control soil, the dominating chemical form for Zn was Fe-Mn oxides, suggesting that Zn had a preference for oxides at the expense of other fractions.

After 28 incubation days, the Fe-Mn oxides fraction remained the most dominant fraction in all the times, except 3 hours, with the following order:

Fe-Mn > CABN > RES > EXCH > OM

Conclusion

In this study, Actinidia deliciosa shell was used to immobilize Cu and Zn in contaminated soil. The addition of Actinidia deliciosa shell led to increased OM fraction. Besides, according to the statistical comparisons, performed to the 95% level using the independent t-test, there was a statistical significant difference between the amount of absorbed Cu and Zn in the OM fraction in the contaminated soils with Actinidia delicious and the control soils. In the contaminated soils with Actinidia deliciosa shell, most of the added Cu and Zn were found in the Fe-Mn fraction. In the control samples, most of the Cu and Zn were found in the residual and Fe-Mn oxides fractions.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

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