



**Journal of  
Toxicology and  
Environmental Health  
Sciences**

**Volume 9 Number 5 May 2017  
ISSN 2006 - 9820**



*Academic  
Journals*

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## Full Length Research Paper

## Biosorption of heavy metals onto different eco-friendly substrates

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Received 26 February, 2017; Accepted 28 March, 2017

Fungi play an important role in biosorption of heavy metals in heavily contaminated soil. Five metals-tolerant fungal species were isolated from two different contaminated soil (soils 1 and 2). The number of fungal colonies isolated from the contaminated soil 2 was higher than that of soil 1. The most resistant fungal species for the toxic studied metals (Pb, Cd, Cu and Zn) was *Rhizopus stolonifera* followed by *Macrophomina phaseolina*. It was established that the metal toxicity was related to the contamination levels, the physicochemical properties including pH, conductivity, organic matter and carbonate contents of the soils. This study confirmed the good ability of different chemicals (CaCO<sub>3</sub>, MO, Zeolite and phosphate) and biological fungal substrates (*M. phaseolina* and *R. stolonifera*) in bioremediation of polluted soils and reducing different heavy metals levels as compared to the control, especially for fungi. *M. phaseolina* amendment was superior in reducing the chemically available heavy metals in the studied soils.

**Key words:** Heavy metals, fungal adsorption, soil remediation, amendments, sequential extraction technique.

### INTRODUCTION

The basic environmental elements constituting ecosystem is the soil, which is the important material basis of humans to survive and develop. The soil contaminated by heavy metals manifests as concealment, accumulation, reversibility and protraction. The major ways of heavy metal contamination are surface water and agricultural soil polluted by industrial effluent, chemical fertilizers and pesticides (Hezbollah et al., 2016), while sewage is the main source of water pollution used for irrigation (Salawu et al., 2015).

To prevent the heavy metal contamination, sources of

contamination should be controlled and remediation of contaminated soil should be enhanced (Zhou et al., 2004). Recently, reducing the number of pollutants and improving the quality of the treated soils have been studied for the development of cheaper and more effective remediation technique. One of the most alternative treatments is adsorption. The adsorbents may be of mineral, organic or biological origin, zeolites, industrial byproducts, agricultural wastes, biomass and polymeric materials (Kurniawan et al., 2005). Microorganisms (bacteria, fungi and algae) are effective

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heavy metal sequestration for physicochemical methods (Brierley, 1990; Gadd, 1993; Niu et al., 1993; Wong and So, 1993; Wong et al., 1993). Removal of potentially toxic metals from polluted industrial and domestic effluents have already been used in large scale using certain microorganisms. These microorganisms have been shown to possess ability to survive by adapting or mutating at high concentrations of toxic heavy metals. To increase the tolerance of fungi for the bioleaching process, the adaptation of these fungi exposed to heavy metal ions has been examined and developed (Yang et al., 2009).

Generally, two mechanisms have been proposed for heavy metal tolerance in fungi. The first one is an extracellular (chelation and cell-wall binding) sequestration and the second is intracellular physical sequestration of metal by binding to proteins or other ligands to prevent it from damaging the metal sensitive cellular targets. Thus, extracellular mechanisms are mainly implied in the avoidance of metal entry, whereas intracellular systems aim to reduce the metal burden in the cytosol. In the first mechanism, different organic molecules that do not belong to the matrix of the cell wall are excreted by the fungal cell to chelate metal ions. Binding to the cell wall is called biosorption. The presence of various anionic structures, such as glucan and chitin gives negative charge to the cell surface of microorganisms (Maghsoodi et al., 2007; Bellion et al., 2006), which gives microorganisms the ability to bind metal cations. In the intracellular mechanism, metal transport proteins may be involved in metal tolerance, either by extruding toxic metal ions from the cytosol out of the cell or by allowing metal sequestration into the vacuolar compartment (Khan and Maqsood, 2007; Le et al., 2006).

In the present study, fungal-tolerant heavy metals will be screened in the two polluted soils. Moreover, although the separation of various chemical forms of heavy metals is very difficult, the use of sequential extraction methods provides an important approach and relevant environmental information on polluted samples. Therefore, the present study used sequential extraction scheme: (1) To predict the metal distribution among different fractions in two contaminated soils, determined periodically by a four-steps chemical fractionation procedure. (2) To compare biosorption capacity of chemical and biological treatments to degrade available metals contaminated soils. (3) To evaluate the changes in the speciation of studied metals in amended soils after treatments.

## MATERIALS AND METHODS

### Instrumentation

The metal determination in the extract was carried out by means of atomic absorption spectrophotometry (Model Solaar 969, ATI Unicam Comp.) equipped with a digital direct concentration read

out and an air-acetylene burner using single element hollow cathode lamps (ATI Unicam Comp.). When the concentrations were under the detection limit of flame, the AAS external standards in diluted acid were used to calibrate the accuracy of atomic absorption.

### Reagents and glassware

All glassware and plastic materials used were previously treated for 24 h in 2 M nitric acid and rinsed with double distilled water and then with ultra-pure water. 50 ml of acid - washed polyethylene centrifuge tubes were used for extraction, while 50 ml polyethylene vessels were used in the extracting solutions.

### Samples collection

Two contaminated soils were selected for this study, one influenced by urban and wastewater (soil 1), and the other was influenced by industrial wastes (soil 2). The surface soils (0 to 20 cm) of the contaminated sites (ca 20 samples from each site using sterile polyethylene bags) were sampled, air-dried, and then hand-crushed using the mortar and sieved through 2 mm stainless steel. Samples were finally homogenized and stored until the analyses.

### Analytical procedure

The pH-values and electrical conductivity (E.C.) of soil samples were measured in 1: 2.5 suspension of sample : bidistilled water using a pH-meter (Orion Research, Model SA520, U.S.A.) and conductivity meter (HANNA Instruments, HI 8033 Italy). Carbonates and organic matter were determined using standard procedures (APHA, 1992); (Nelson and Sommers, 1982).

### Choice of reagents and leaching conditions

In the choice of extracting reagents, particular emphasis was placed on the selectivity, suitability and extracting efficiency of each leaching solution. The extraction methods, which are the most informative for environmental purposes are the total element, moderately (reducible and oxidizable) and easily extractable element extraction techniques. The former defines and includes both elements from the rock matrix and the non-residual elements (those adsorbed from the aqueous medium). The three other extraction techniques show no association with the type of rock forming the soil and give results only for the moderately and weakly hold elements, which include those originating from polluted waters.

### Sequential extraction scheme

The Tessire et al., (1979) sequential extraction method was applied in triplicate to 2 g of soil samples (< 2 mm). The reagents and operating conditions for this method is summarized in Table 1. The procedure was conducted in five steps, assuming the forms of Cd, Pb, Zn and Cu extracted were (1) exchangeable (2) associated with Fe-Mn oxides (or reducible) (3) associated with organic matter (or oxidizable) (4) structurally bound in residual fraction.

### Isolation of micro-organisms from polluted sites

The soil samples (10 g) were first suspended in 100 ml of sterilized water; the mixture was agitated for 30 min at room temperature and then diluted (10- to 10 000-fold). Aliquots of 100  $\mu$ l of different

**Table 1.** Chemical extraction scheme for metal speciation in soil samples.

Reagent	Shaking time and temperature	Fraction
40 ml of 0.11 mol/l acetic acid	16 h at room temperature	Water-soluble (available)
40 ml of 0.1mol/l hydroxylamine oxides hydrochloride (pH 2)	16 h at room temperature	Occluded in Fe or Mn reducible)
10 ml 30% H <sub>2</sub> O <sub>2</sub> (pH 2)	1 h at room temperature and 1 h at 85°C	Organically bound and sulphides (oxidizable)
then 10 ml 30% H <sub>2</sub> O <sub>2</sub> (pH 2)	1 h at 85°C	
cool, add 50 ml mol/l ammonium acetate (pH 2)	16 h at ambient temperature	
Concentrated acid mixture (HCl: HNO <sub>3</sub> : HF)		Structurally bound (residual fractions)

**Table 2.** The physico-chemical characteristic of the two studied contaminated soils, soils 1 (Influenced by urban and wastewater) and 2 (influenced by industrial wastes).

Location	pH	Conductivity (µS/cm)	Organic matter (mg/g)	CaCO <sub>3</sub> (Mg/g)	Total content (µg/gm dry soil)			
					Pb	Cd	Cu	Zn
Soil1	7.12	78	19	21	16.35	5.66	225.96	176.07
Soil2	6.75	912	25	2.1	41.47	2.63	124.09	198.49

dilutions were placed on 2% malt extract agar (MEA) plates (three replicates) to make sure the growth of micro-organisms is present in samples. After at least 3 days of incubation at 25°C, developed colonies were randomly picked and isolated. Pure cultures of isolated micro-organisms were identified using the keys of Gilman (1957) and Domsch et al. (1980).

### Screening and choice of heavy metal-resistant microorganisms

The purified isolates were screened on the basis of their tolerance to Cu, Pb, Zn and Cd. A disk of mycelium was inoculated aseptically onto MEA plates supplemented individually with 1, 10, 30, 50, 100, 300, 600 and 1000 ppm of heavy metals. The inoculated plates were incubated at 25°C for 14 days. The effect of the heavy metals on the growth of the isolates tested was estimated by measuring the radius of the colony extension (cm) when compared with the control (medium without metals). The minimum inhibitory concentration (MIC) was calculated which is defined as the lowest concentration of metals that inhibit visible growth of the isolate. The isolates which showed resistance to the studied heavy metals were selected for the following experiments.

### Different treatments for contaminated soil

Six chemical and biological treatments were used to compare and evaluate the effectiveness of chemical remediation techniques. Seven different slurries of soil (1 g soil : 25 ml H<sub>2</sub>O) were put in polyethylene bottles and treated according the following methods: (1) 1 g calcium carbonate (CaCO<sub>3</sub>) added to increase soil pH to 7.0; (2) a high quantity of calcium phosphate (10 mg P); (3) 1% manganese oxide (5 g); (4) 1 g. of *Macrophomina phaseolina* (5) 1 g *Rhizopus stolonifer*; (6) 1% synthetic zeolite (5 g, Sigma Chemical Company, USA); & (7) kept as a control. Each treatment was performed in triplicate and incubated for two weeks at room temperature (25°C).

### Quality control and analysis

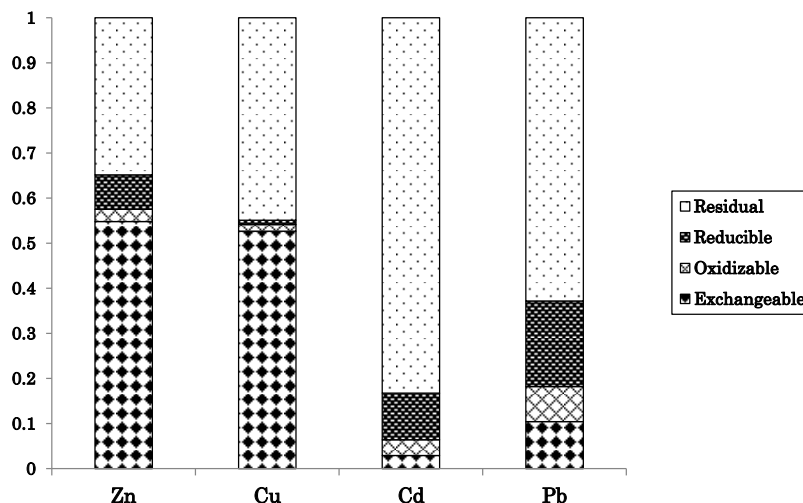
The analyses of the sequential extractions procedure were replicated three times. A blank was also run at the same time. All glassware and plastic containers were previously soaked in supra pure nitric acid (Merck) overnight, and rinsed with de-ionized water.

## RESULTS AND DISCUSSION

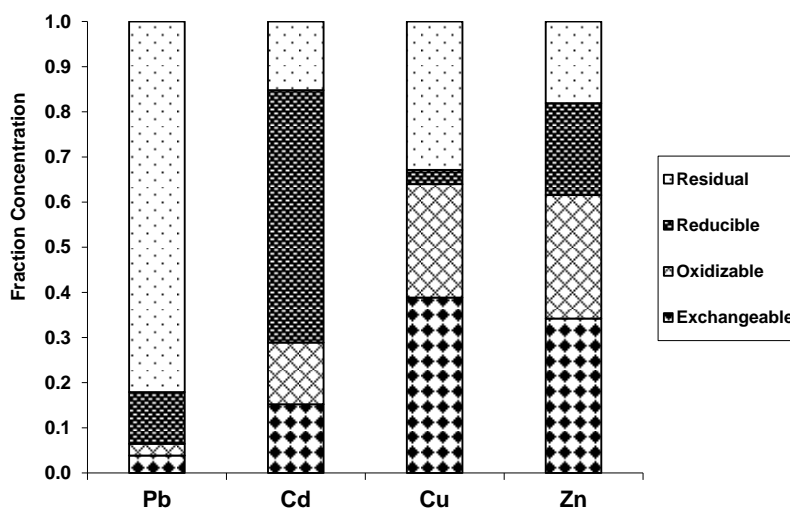
The monitoring of physicochemical properties of the studied contaminated soils showed that, a significantly higher organic matter content (25 mg/g) was observed in soil 2 (Table 2) caused by industrial wastes. This organic matter acts as a scavenger for metals and may provide sites for cations due to ligand or groups that form chelates and/or complexes with the metals (Fawzy, 2000). These findings tend to support the hypothesis that all geochemical processes leading to recycling and accumulation of trace metals in soils are associated with and influenced by organic matter.

The electrical conductivity values of the soil samples are 78 and 912 µS/cm for soils 1 and 2, respectively (Table 2). The highest conductivity value was observed at soil 2 (912 µS/cm), as a result of the effluent wastes from the factory. These effluents enriched with highly conducting materials, which can be adsorbed on the surface of the suspended matter and are deposited on the bottom and tend to increase the electrical conductance of the soil (Fawzy, 2000). On the other hand, there was pronounced decrease in the conductivity value obtained at soil 1 (78 µS/cm).

The pH values of the soil samples were 7.12 and 6.75



**Figure 1.** Metal fraction concentrations ( $\mu\text{g/g}$  dry soil) of lead, cadmium, copper, zinc in the contaminated soil 1.



**Figure 2.** Metal fraction concentrations ( $\mu\text{g/g}$  dry soil) of lead, cadmium, copper, zinc in the contaminated soil 2.

(Table 2). A minimum pH value recorded at soil 2, may be ascribed to the increase of organic matter decomposition, which leads to release of  $\text{CO}_2$  causing a drop of pH value. The hydrogen ion concentration (pH) is probably a single important factor influencing metal adsorption onto both inorganic and organic surface (Fawzy, 2008). The other major difference in soil properties of those contaminated soils was carbonates content (21 and 2.1 mg/g, for soils 1 and 2, respectively).

The total metal concentration provides little indication of metal specification bioavailability, mobility and reactivity in soil samples (Sánchez-Martin et al., 2007; Babel and Dacera, 2006). The total content is the predominant fraction for most of the studied metals (Table 2). Results

in Table 2 reflect highest total Cd and Cu concentrations in soil 1 (5.66 and 225.96  $\mu\text{g/g}$ , respectively), while soil 2 recorded the highest total Pb (41.47  $\mu\text{g/g}$ ) and Zn (198.49  $\mu\text{g/g}$ )

### Fractionation of heavy metals before treatment

In the sequential extraction scheme used in this study, the mobility and hence the possible bioavailability of metals decrease from readily exchangeable to residual. Figures 1 and 2 compare the mobility potential of heavy metals in different forms. It was noticed that Cu and Zn have the highest ability and susceptibility to be released



**Table 3.** Mean values sequential fractionation ( $\mu\text{g/g}$  dry soil) of Pb, Cd, Cu, Zn and recovery of summation percentage in the two contaminated soils (Soils 1 and 2).

Soil	Metal fractionation by different reagent (dry soil) ( $\mu\text{g/g}$ )			Sum of fractions $\mu\text{g/g}$ (dry soil)	Residual metal concentration (dry soil) ( $\mu\text{g/g}$ )	Recovery of a summation percentage
	HOAC	$\text{NH}_2\text{OH.HCl}$	$\text{H}_2\text{O}_2+\text{NH}_4\text{OAC}$			
Lead						
Soil 1	1.7	1.3	3.1	6.1	10.25	17.9
Soil 2	1.6	1.08	4.75	7.43	34.04	37.3
Cadmium						
Soil 1	0.16	0.19	0.57	1.08	4.58	19.1
Soil 2	0.4	0.36	1.47	2.23	0.4	84.8
Copper						
Soil1	1.44	2.11	76.74	80.29	65.38	0.44
Soil 2	3.9	31.17	48.23	83.3	40.79	67.13
Zinc						
Soil 1	13.49	4.76	96.49	114.74	61.33	65.17
Soil 2	40.44	54.26	67.92	162.62	35.87	81.93

from the soil samples, while Pb and Cd have the lowest mobility.

The presence of an acid-soluble portion of Pb indicates its sensitivity to the acidic condition and tendency to leach easily. Metal accumulation in the residual fraction prevailed with Pb (10.25 and 34.04  $\mu\text{g/g}$ ) mostly present as a major chemical form in soils 1 and 2, respectively (Table 3). But the other three fractions (which are soluble, reducible and oxidisable forms) of Pb are almost equally important (1.7 to 1.6, 1.3 to 1.08, 3.1 to 4.75  $\mu\text{g/g}$  of the total content for each fraction) in both contaminated soils 1 and 2, respectively (Table 3). This finding agreed with results reported by Chen et al. (2000).

Moreover, Cd showed a higher residual form concentration (4.58  $\mu\text{g/g}$ ) in the untreated soil 1. The same metal in soil 2 (Table 3) was found in highest concentration in oxidizable fraction (1.47  $\mu\text{g/g}$ ). Beside this, a remarkable reduction of the residual fraction observed in Cd content in soil 2 could be described (ascribed) as the dissolution and decomposition of inorganic and organic compounds, respectively of soil in this fraction leading to an increase in the concentration levels of the other fractions (Simth 1996). Jackson and Allowy (1992) reported that Cd and Pb may cause serious problems through food chains.

In both control soils, Cu and Zn displayed the highest potential mobility (Table, 3) with values of (0.44 to 67.13% for Cu) and (65.17 to 81.93% for Zn) for soils 1 and 2, respectively, with respect to total metal concentrations (Table 3). Higher concentration of heavy metals soil may increase uptake of these elements by crops and potentially affect human health via food chains (Chen et al., 2000)

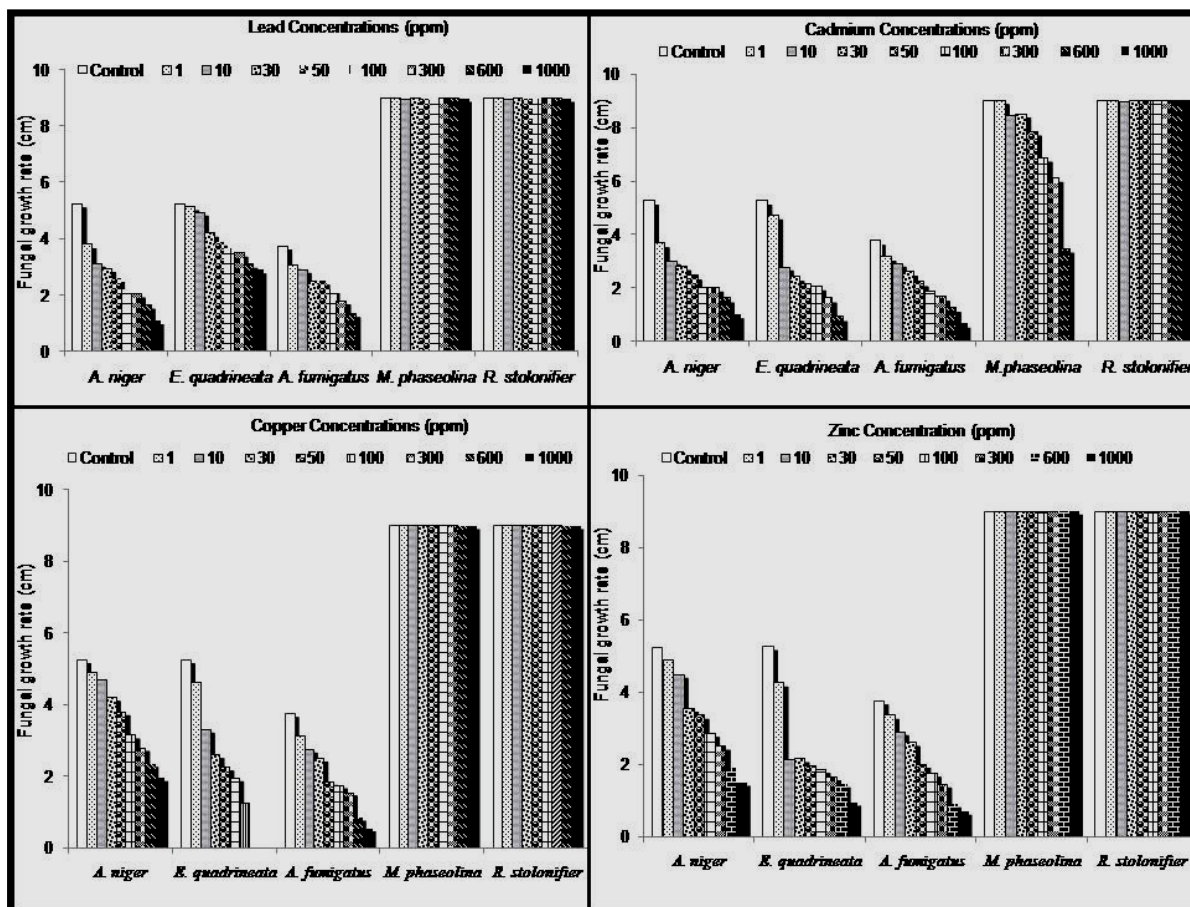
Overall, Pb and Cd would not be expected to have a high toxicity potential. But the presence of these toxic metals in the environment can be harmful to humans and

living species even in low concentration. Since toxic metals do not degrade into harmless end-products, they can accumulate in living bodies and get concentrated through the food chain (Singh et al., 2011). However, Zn and Cu although not usually with high toxicity potential (compared to Pb and Cd toxicity) they should be carefully monitored if these soils are to be reused for agricultural purposes.

#### Fungal tolerant to Pb, Cd, Cu and Zn isolated from the two studied soils

In this study, five fungal species were isolated from both soils 1 and 2. *Emmericilla quadrilineata* (100 colonies/g), *Aspergillus niger* (650 colonies/g) and *Macrophomina phaseolina* (50 colonies/g) were isolated from soil 1, while *Aspergillus niger* (2650 colonies/g), *Rhizopus stolonifera* (200 colonies/g) and *Aspergillus fumigatus* (1350 colonies/g) were isolated from soil 2. The number of fungal colonies isolated from soil 2 contaminated sediment soils by industrial waste was higher than that from the soil 1 contaminated sediments urban and waste water. This variation would be referring to the high sugar content in the soil 2 which refers to sugar cane factory waste. This variation was clear with the number of *A. niger* colonies (650 and 2650 colonies/g) isolated from soils and soil 2, respectively.

Screening of the resistance of these fungal species to Pb, Cd, Cu and Zn has been studied. Generally, the most resistant fungal species to these elements up to 1000 ppm was *R. stolonifera* followed by *M. phaseolina* which showed resistance with all studied elements even with high concentrations expects cadmium at 1000 ppm recorded MIC<sub>100</sub>. While the remaining studied fungal species (*A. niger*, *A. fumigatus* and *E. quadrilineata*) were



**Figure 3.** Fungal growth rate exposed to different concentrations of toxic metals when compared with that of the control (without metals).

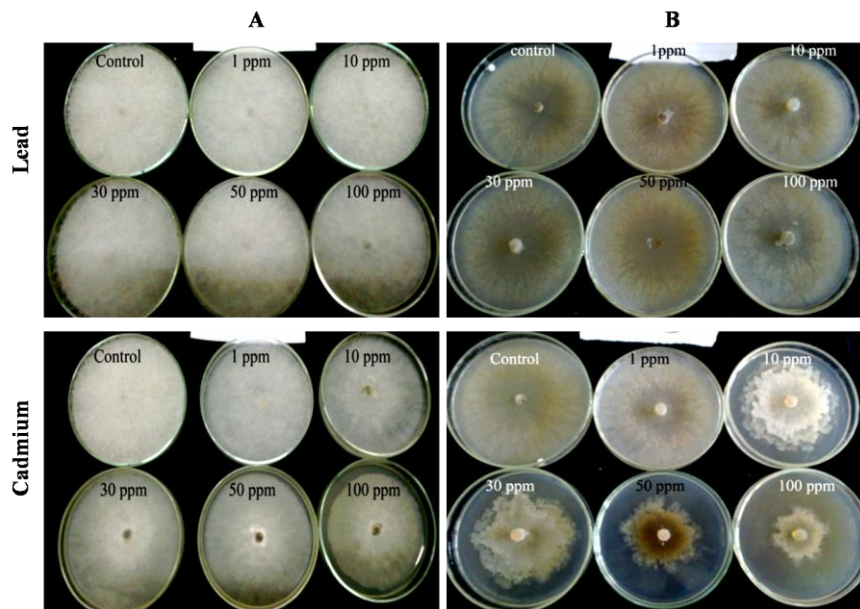
slightly sensitive to these heavy metals (Figures 3 and 4). *E. quadricincta* recorded MIC<sub>100</sub> with copper and cadmium at 600 and 1000 ppm, respectively, and MIC<sub>50</sub> with zinc and lead at 10 and 1000 ppm respectively. Cadmium showed a similar effect on both *Aspergillus* species, whereas MIC<sub>90</sub> was observed at 1000 ppm, and MIC<sub>50</sub> of *A. fumigatus* and *A. niger* with zinc 100 and 300 ppm, respectively. *A. fumigatus* showed more sensitivity to copper than *A. niger* (MIC<sub>90</sub> and MIC<sub>60</sub> at 1000 ppm), respectively. In the case of lead, *A. fumigatus* completely inhibited with 1000 ppm. Despite that, only 50 ppm was enough to inhibit 50% of *A. niger*, the fungus showed stable growth rate up to 1000 ppm (Meizhen and Junfeng, 2014).

A similar observation on the toxic effect of higher concentration of heavy metals on the growth of fungi has been reported (Rao et al., 1997; Malik, 2004; Joshi et al., 2011). Ahmad et al. (2005) reported that the biomass of *Rhizopus* sp. had a higher adsorption capacity as compared to *Aspergillus* sp. biomass. The differences may be ascribed to the intrinsic ability of the organism, its chemical composition of cell wall leading to various types

of interaction of metals with fungi (Gadd, 1993). According to the authors' knowledge, this is the first study on the resistance of *E. quadricincta* for heavy metals.

### Fractionation of heavy metals after treatment

Some chemical techniques for immobilizing metals in soils are application in polluted soils to reduce the soluble concentration of heavy metals in soils by precipitation, adsorption, or complexation (Chen et al., 2000). Recently, many industrial, agricultural and forestry sources are used as sorbents such as, red mud (Nadaroglu et al., 2010), sunflower stalks (Hussein, 2010), spent grain (Li et al., 2010), wheat bran (Yao et al., 2012), *Aspergillus niger* (George et al., 2012), *Scolymus hispanicus* L. (Barka et al., 2010), eggshell and coral (Chakravarty et al., 2012), maize bran (Tofan et al., 2011), saw dust and neem bark (Naiya et al., 2009), citrus peels (Njikam and Schiewer, 2012), Rosa gruss and teplitz (Bhattia et al., 2005), *Echornia speciosa* (Abdel-Halim et al., 2003), *Cupressus sempervirens*,



**Figure 4.** *R. stolonifera* (A) and *M. phaseolina* (B) resistance to different concentrations of lead and cadmium for 14 days when compared with the control (without lead or cadmium).

*Eucalyptus longifolia* and *Pinus halepensis* (Al-Subu, 2002) and *Pleurotus cornucopiae* (Danis, 2010).

Fawzy (2008) used seven substrates to investigate their efficiency to reduce the extraction of heavy metals concentration in a heavily contaminated soil, and deduced that the most effective treatments in decreasing available metal concentrations were calcium carbonate, zeolite, manganese or iron oxide and phosphate. The present study was conducted to compare the efficiency of different substrates: calcium carbonate, zeolite, phosphate, manganese oxide with fungi isolated from the contaminated soils in immobilizing metals in polluted soils.

Table 4 shows the sequential extraction concentration of the measured metals (Pb, Cd, Cu and Zn) treated with working substrates in different fractions. It is noteworthy that the mobility of the studied metals in treated soils generally decreases. A significant decrease recorded in exchangeable Pb and Cd fractions of soil 1 treated with *M. phaseolina*, *R. stolonifera*, carbonate, zeolite, phosphate or manganese oxide (Table 4). The effective role of fungus in reducing the mobility of heavy metals is due to their ability to accumulate significant amount of metals (Iskandar et al., 2011). Say et al. (2001) studied the biosorption of Cd (II), Pb (II) and Cu (II) with the filamentous fungus *Phanerochaete chrysosporium* and reported that the fungal cell walls have a negative charge due to the arrangement of the carboxyl and phosphate groups of the cell walls.

The more significant function of *M. phaseolina* and *R. stolonifera* was observed to reduce Pb and Cd in both

contaminated soils and Zn in soil 1 (Tables 4 and 5) as compared to the calcium carbonate which may be attributed to high capacities of metals binding to cell walls and may exhibit high values of intracellular accumulation (Blanquezet et al., 2004). Kapoor and Viraraghavan (1997) revealed that biosorption of heavy metal by fungi occurs as a result of ionic interaction and complex formation between metal ions and the functional group present on the fungal cell surface. These functional groups which may be involved in the biosorption of heavy metals include phosphate, carboxyl, amine and amide groups (Akhtar et al., 1996).

Table 5 showed a remarkable role of phosphate, manganese oxide, *Macrophomina phaseolina* and *Rhizopus stolonifera* in reducing Pb and Cd concentration in available fraction in soil 2. Application of manganese oxide, carbonate and zeolite reduced the mobility of Cd, Cu and Zn in two studied soils (Tables 4 and 5), this is in agreement with other studies reported by Mench et al. (1994) and Lee (1996). Liu et al. (1998) studied the effects of composts and calcium carbonate on the uptake of cadmium and lead by vegetables grown in polluted soils; they reported that application of calcium carbonate materials significantly reduces the solubility of heavy metals in contaminated soils. Many studies also indicated that application of manganese oxides mixed in contaminated soils could reduce the concentration of soluble Cd or Pb in soils (Mench et al., 1994). The mobile phase of Cu content into the contaminated soils (Tables 4 and 5) can be transformed to unavailable form after amendment with manganese oxide, zeolite and carbonates

**Table 4.** Distribution fractions of each studied metal ( $\mu\text{g/g}$ ) in Soil 1 (influenced by urban and wastewater).

Metals	Control	<i>M. phaseolina</i>	<i>R. stolonifera</i>	$\text{CaCO}_3$	MO	Zeolite	Phosphate
<b>Pb</b>							
F1	1.7	0.87	1.05	1.1	1.3	1.1	1.2
F2	1.3	7.62	2.04	2.8	2.14	1.81	1.16
F3	3.1	1.62	2.69	7.2	2.03	7.4	8.3
F4	10.25	11.22	10.63	6.85	0.56	1.17	0.11
<b>Cd</b>							
F1	0.16	0.01	0.02	0.02	0.02	0.03	0.03
F2	0.19	0.08	0.11	0.34	0.22	0.26	1.58
F3	0.57	0.17	0.20	11.73	8.7	10.43	4.34
F4	4.58	14.3	16.98	9.00	1.43	2.00	5.00
<b>Cu</b>							
F1	76.74	44.88	55.49	52.64	30.03	42.68	52.00
F2	2.11	2.12	1.41	7.76	20.08	5.1	10.66
F3	1.44	6.03	8.51	4.05	6.17	23.26	22.2
F4	65.38	13.39	37.98	34.05	14.64	62.64	14.02
<b>Zn</b>							
F1	96.49	73.72	86.67	78.9	94.02	94.51	83.41
F2	4.76	30.18	19.20	23.13	37.34	146.46	38.76
F3	13.49	10.19	7.88	37.11	36.86	71.99	1.46
F4	61.33	24.86	18.27	42.81	70.91	49.28	50.67

Soil1: Influenced by urban and wastewater. F1: Water soluble and exchangeable; F2: Bound to Fe and Mn oxides; F3: Bound to organic matter; F4: Residual metal concentration.

**Table 5.** Distribution fractions of each studied metal ( $\mu\text{g/gm}$ ) in Soil2 (influenced by industrial wastes).

Metals	Control	<i>M. phaseolina</i>	<i>R. stolonifera</i>	$\text{CaCO}_3$	MO	Zeolite	Phosphate
<b>Pb</b>							
F1	1.6	0.9	1.1	1.4	1.16	1.09	0.53
F2	1.08	8.59	7.59	7.85	2.14	7.71	0.95
F3	4.75	2.28	4.52	3.7	5.14	15.11	6.23
F4	34.04	12.17	12.48	15.74	15.09	18.81	15.31
<b>Cd</b>							
F1	0.4	0.23	0.23	0.34	0.19	0.8	0.37
F2	0.36	0.04	0.004	1.03	1.03	1.64	1.13
F3	1.47	2.02	3.02	0.04	1.03	0.49	1.12
F4	0.4	1.03	1.03	1.63	0.043	0.18	0.23
<b>Cu</b>							
F1	48.23	45.7	57.1	39.7	43.7	32.7	39.9
F2	31.17	42.06	51.13	39.63	36.35	33.05	38.01
F3	3.9	1.62	4.01	3.1	3.78	18.00	6.8
F4	40.79	47.15	31.53	42.08	61.36	22.30	25.11
<b>Zn</b>							
F1	67.92	35.2	49.8	31.34	46.01	38.92	46.65
F2	54.26	32.72	26.92	54.05	2.47	52.28	45.73
F3	40.44	24.3	28.25	3.87	46.35	28.13	13.64
F4	135.87	143.98	138.01	156.57	175.52	128.78	130.1

Soil1: Influenced by urban and wastewater. F1: Water soluble and exchangeable; F2: Bound to Fe and Mn oxides; F3: Bound to organic matter; F4: Residual metal concentration.

as substrates.

## Conclusion

The application of sequential extraction method to study samples provides relevant information on possible toxicity of heavy metals in contaminated soils and gives valuable information on the mobility of these metals, helping in predicting their behavior to the ecosystem. *M. phaseolina* and *R. stolonifera* recorded significant roles as good biosorbent agents for Pb, Cd, Cu and Zn and showed better uptake capacity for Pb, Zn and Cd as compared to Cu. This uptake capacity increased in *M. phaseolina* as compared to *R. stolonifera*. The chemical remediation techniques, using calcium carbonate, manganese oxide, zeolite and phosphates can significantly reduce the availability of studied metals and then reducing their toxicity potential. However, these techniques certainly require intensive further improvements and studies in details to optimize the conditions for maximum bioadsorption of heavy metals of contaminated soils.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Evaluation of occupational exposure to metal dust on the liver of road construction workers in Abuja metropolis

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Received 28 February, 2017; Accepted 11 April, 2017

Increasing air pollution levels due to rapid urbanization and growth in infrastructural facilities coupled with low level of standard safety procedures have been observed in Nigeria, and become a major source of concern. The study evaluated the effects of occupational exposure to metal dust on the liver of road construction workers in Abuja metropolis. Blood samples were used in the measurement of Lead, cadmium, magnesium and iron as indicators for metal toxicity. Bilirubin, liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP)) and proteins as biomarkers for liver function. Samples were collected from different road construction sites. One hundred (n = 100) subjects were recruited and served as the exposed group and another one hundred (n = 100) control as unexposed (control) group. Samples were analyzed using Atomic Absorption Spectrometry for the toxic metals (Lead and Cadmium) and trace elements (Magnesium and Iron); and Selectra ProS Clinical Chemistry Auto Analyzer for the liver function tests respectively. This study showed a statistically significant increase in Lead, Magnesium and Iron ( $p < 0.001$ ) when exposed subjects were compared with unexposed subjects. Test for liver function showed a significant reduction in total bilirubin ( $p < 0.01$ ) and direct bilirubin ( $p < 0.05$ ) respectively. Albumin also show statistically significant increase ( $p < 0.05$ ) on comparison of exposed and unexposed subjects. This study indicates the presence of metal dust in road construction workers occupationally exposed, and suggestive of liver impairment following continued exposure to these toxic metals and trace elements.

**Key words:** Occupational exposure, metal dust, liver.

## INTRODUCTION

Pollution of the ecosystem by heavy metals is a global problem because these metals are not easily destructible and most of them have harmful effects on living organisms (Shinggu et al., 2010). About 4 to 8% of deaths occurring annually in the world are associated to

air pollution related with anthropogenic activities (Lopez et al., 2005). Environmental source of air pollutants can be inorganic, organic or both. Among the inorganic pollutants originating from construction site activities, heavy metals such as lead (Pb), cadmium (Cd) are of a

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major concern due to their harmful and potential carcinogenic characteristics. In dusty environment, it has been established that an adult could inhale about 100 mg dust per day (Leung et al., 2008). Exposure to high levels of heavy metals can result to acute and chronic toxicity, such as damage to the central and peripheral nervous system, immune system, lungs, kidneys, blood composition, liver and even death.

Respirable fraction of heavy metal dust is composed of very fine dust which is able to reach the lower bronchioles and alveolar region of the lung. It has been reported that heavy metals absorbed in respirable dust suspended particulates produces tissue damage of the lungs (Pope et al., 1995). The health effects of toxic metals in the air and from road deposited dust on humans is better appreciated if one considers the fact that an active person typically inhales 10,000 to 20,000 L of air per day (Gbadebo and Bankole, 2007). During inhalation, these heavy metals may enter alveolar sacs deep inside the lungs and also blood stream thereby affecting vital organs such as the liver, kidney, etc (Gbadebo and Bankole, 2007). Air is very essential for human existence. Abuja the federal capital territory of the federal government of Nigeria like any other fast growing city in the world is witnessing yearly influx of residents who are coming to seek greener pasture, and because most of the new residents have no formal education and good technical training, coupled with the alarming unemployment rate, they end up doing any job which include road construction. Although urbanization can offer opportunities for improvements in human health (e.g., better access to health care and some amenities such as good roads, clean city), it can also result in environmental degradation (Gong et al., 2012). Roads play a vital role in inducing social and economic processes (Bai et al., 2009). Not much data is available on adverse effects of toxic metal dust in construction sites in Abuja. This study will attempt to generate and evaluate the level of some of these metals (pb, cd, fe and mg) in the blood and also ascertain if they have any effect on the liver and subsequently use the data to advise appropriate management on how best to protect workers and residents living around road construction sites.

## MATERIALS AND METHODS

### Study area

This study was carried out in the metropolitan city of Abuja, Federal Capital Territory (FCT), located in the North central geopolitical zone of Nigeria. Abuja which was founded in 1828 has a coordinate of 9°4'N 7°29'E and a land mass of 713 km<sup>2</sup>. It has a population of 979,876 according to the 2006 National population census (Anang, 2011) (Figure 1).

### Study population/subjects

A total of two hundred (200) apparently healthy men comprising of

100 occupationally exposed whom have worked for a minimum of five years, above 18 years of age and with no serious health challenge in recent years and willing to participate in the study and 100 non exposed men who are above 18 years of age, and not have been exposed to metal dust and also apparently healthy without any serious health challenge in recent years, were recruited for this study after obtaining informed consent and ethical approval from the Health Research Ethics Committee of the FCT Administration, Abuja.

### Biochemical measurements

The blood level of the metals were analyzed using Atomic Absorption Spectrometry method (Jackson and Chen, 1996) while the liver profile was analyzed using Selectra ProS, a Clinical Chemistry Auto Analyzer made in Holland that operates Beer-Lambert Principle (Harris, 1995).

### Statistical analysis

Statistical analysis including descriptive statistics was carried out using the Statistical Package for Social Sciences (SPSS). Values were expressed as mean  $\pm$  standard error of the mean. The paired t-test was used to determine significant differences and the p-value was set at  $p < 0.001$ .

## RESULTS AND DISCUSSION

The results obtained from this study are indicated in Tables 1 and 2. Occupational exposure to metal dust mostly Lead is one of the most prevalent overexposures in road construction sites. Industries with high potential exposure apart from road construction include other forms of construction, smelter operations, radiator repairs and firing ranges. Table 1 shows magnesium level of the exposed group to be significantly ( $p < 0.001$ ) increased when compared with unexposed subjects, also exceeding the upper limit of the reference range (0.66 to 1.07 mmol/L). Magnesium is a major intracellular cation for normal neuromuscular activity. Intracellular magnesium is an important cofactor for various enzymes, transporters and nucleic acids necessary for normal cellular function, replication and energy metabolism (Bringhurst et al., 2012). Fifty percent of total body magnesium resides in bone, while almost all extra skeletal magnesium is located inside the cells. Although serum magnesium levels have been argue not represent total body magnesium levels because on 1% of body magnesium in found in the extracellular fluid (Bringhurst et al., 2012), the levels exceeding 2 mmol/L are reported to cause vasodilation and neuromuscular blockage (Bringhurst et al., 2012). Mean magnesium levels in this study exceeded 2 mmol/L indicates occupational exposed subject could be prone to neuromuscular blockage.

Lead serves no useful purpose in the human body, with its presence leading to toxic effects, affecting every organ. Lead has the ability to mimic the action of calcium, affecting calcium dependant or related processes; and

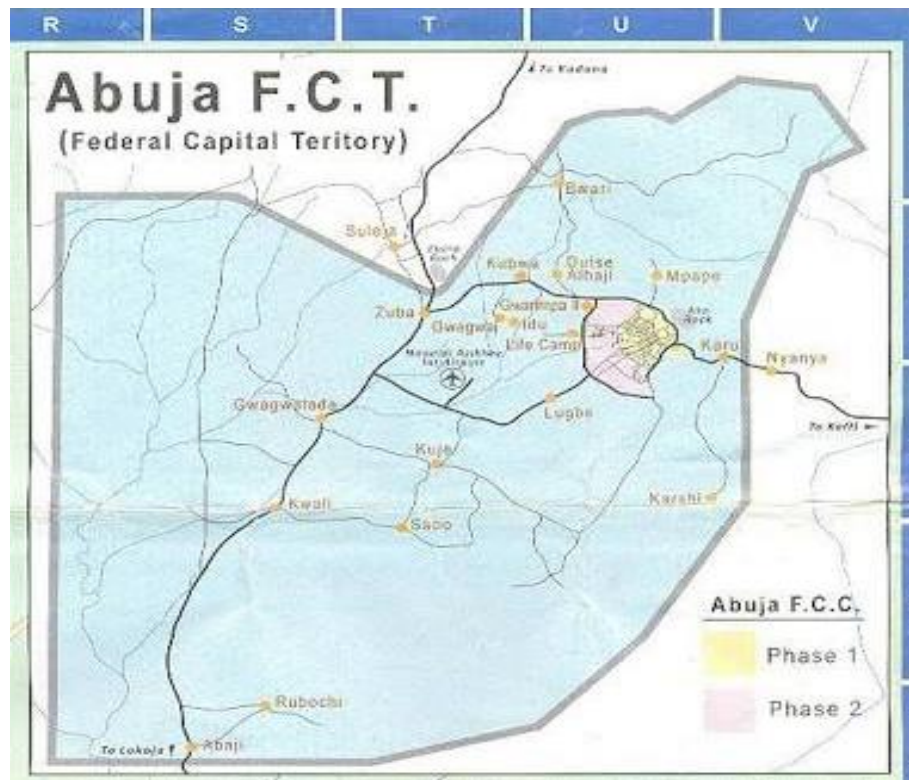


**Table 1.** Mean  $\pm$  SEM of metals in exposed and unexposed subjects.

Variable	Mean $\pm$ SEM		P-value	Level of significance
	Exposed	Unexposed		
Mg (mmol/L)	3.25 $\pm$ 0.13	0.56 $\pm$ 0.02	0.001	Highly significant
Fe ( $\mu$ g/L)	126.99 $\pm$ 2.78	113.27 $\pm$ 2.87	0.001	Highly significant
Lead ( $\mu$ g/L)	17.16 $\pm$ 0.49	12.28 $\pm$ 0.31	0.001	Highly significant
Cd ( $\mu$ g/L)	0.78 $\pm$ 0.04	0.85 $\pm$ 0.02	0.206	Not significant

**Table 2.** Mean  $\pm$  SEM of LFT results in exposed and unexposed subjects.

Variable	Mean $\pm$ SEM		P-value	Level of significance
	Exposed	Unexposed		
TB ( $\mu$ mol/L)	10.26 $\pm$ 0.57	17.43 $\pm$ 1.63	0.001	Highly significant
DB ( $\mu$ mol/L)	4.65 $\pm$ 0.28	7.26 $\pm$ 0.98	0.01	Highly significant
AST (U/L)	22.91 $\pm$ 1.63	28.88 $\pm$ 4.44	0.218	Not significant
ALT (U/L)	22.61 $\pm$ 1.15	26.73 $\pm$ 3.54	0.267	Not significant
ALP (U/L)	151.95 $\pm$ 5.81	192.26 $\pm$ 12.79	0.006	Highly significant
TP (g/L)	81.53 $\pm$ 0.70	80.86 $\pm$ 0.65	0.493	Not Significant
ALB (g/L)	42.67 $\pm$ 0.55	41.11 $\pm$ 0.59	0.048	Significant

**Figure 1.** Map of Abuja (FCT, 2015).

interact with proteins (including sulfhydryl, amine, phosphate and carboxyl groups) (ATSDR, 2005). Significant ( $p < 0.001$ ) increased lead levels in the

exposed subjects than unexposed subjects was observed which could have resulted from constant exposure to the exhaust fumes of motor vehicles powered by tetraethyl

lead and from the dust raised by tilling the surface of earth (Barbosa et al., 2005) as inhalation is a major source of lead absorption. Neurological and behavioural effects have been documented in lead-exposed workers (ATSDR, 2005), such effects includes; decreased libido, depression, mood changes, and headache, diminished hand dexterity, diminished cognitive performance, increased nervousness etc (ATSDR, 2005).

Cadmium levels in exposed subjects have no significant ( $p > 0.05$ ) difference with the unexposed subject suggesting that cadmium toxicity is mostly associated with exposure to paint and paint derived products. Iron is an essential metal for the body. While excess iron accumulation causes organ dysfunction through the production of reactive oxygen species, there is a sophisticated balance in body iron metabolism of storage and transport, which is regulated by several factors including the newly identified peptide hepcidin. As there is no passive excretory mechanism of iron, iron is easily accumulated when exogenous iron is loaded by hereditary factors, repeated transfusions, and other diseased conditions. This study reveals a significantly ( $p < 0.001$ ) increased iron level in exposed subjects when compared with unexposed subjects, though, this increase did not exceed physiological limits of serum iron (65 to 176  $\mu\text{g/dL}$ ).

Bilirubin is not only a waste end-product but also an antioxidant that may protect against diseases associated with oxidative stress (Schwertner and Vitek, 2008). Bilirubin is known to be associated with decrease in cardiovascular risk in men (Ollinger et al., 2005). Total and direct bilirubin reduced significantly  $p < 0.001$  and  $p < 0.01$  respectively in exposed subjects when compared with unexposed subject and could be suggestive of the high amount of lead in the exposed group which interferes with the production of bilirubin (Balistri et al., 1980). In several prospective studies, an inverse relationship has been reported between bilirubin cardiovascular disease (CVD) (Ganotakis et al., 2007) coronary heart disease, myocardial infarction, ischemic heart disease, and all-cause and cancer mortality in men (Heejin et al., 2009). In a recent cross-sectional study, it was shown that total bilirubin and stroke prevalence have an inverse association in the representative national data in which higher bilirubin level was not only associated with reduced stroke prevalence but also associated with favorable stroke outcomes (Heejin et al., 2009). Bilirubin level in this study was however within physiological range. Alkaline phosphatase which has a direct relationship with bilirubin also is significantly decreased ( $p < 0.006$ ) and this could be attributed to the same effect that Lead has on bilirubin.

Albumin constitutes more than half of the total protein in the body and plays a significant role in regulating plasma oncotic pressure. Albumin was significantly increased ( $p < 0.048$ ) in the exposed group and this could be as a result of the reduced cadmium levels in the

group.

## Conclusion

This study has shown that there is actually a negative effect of occupational exposure to metal dust on the liver of road construction workers in Abuja metropolis. And the positive effect magnesium and iron has on the liver of the exposed group which served as a compensatory mechanism for the liver enzymes (the amino transferases). The negative effect of lead on the liver is as a result of persistent exposure to metal dust and lack of safety rules adherence. It should be noted that occupational exposure to metal dust is not avoidable but it could be reduced if workers are given adequate training on how to minimize exposure by providing personal protective equipment and ensuring compliance by the supervisors, because a healthy workforce is the greatest asset of any organization. Furthermore, occupationally exposed workers to metal dust should be provided with regular health check to enable early impairment of the liver and other organ related illness to be detected in time and initiate therapy. As a matter of great concern, the agency of government responsible for supervision and enforcement should hold construction companies without good safety policies to account, and formulate procedures where none exist.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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