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An integrative study to determine the Bioavailability of heavy metals in the soil

Raghad Shubbar Jaafar, Ahmed yousif, Zuhair A. Abdulnabi, Abdul Zahra Alhello and Hamid T. Al-Saad*

Marine Science Center, University of Basrah, Basrah, Iraq

ABSTRACT

Studying the distribution and availability of heavy metals in the soil has a great importance in assessing environmental quality. This study aims to a) Assess the level of Cadmium(Cd), lead (Pb) and Nickel(Ni) contamination in different soils from the province of Basra in southern Iraq; b) determine the available form of the studied metal using different extraction methods, namely (diethylene - triamine acid extraction method, and fumigation-extraction(chloroform-labile metals); and c) determined the potential dependence of the physiochemical properties of soil. The means of the total heavy metal content for Cd, and Pb were recorded as (0.381 mg.kg^{-1} and 28.933 mg.kg^{-1}), respectively, which were higher than the levels reported in the standard range, suggesting some degree of pollution with these metals. Meanwhile, the total heavy metal content of nickel (Ni) was within the range (4.227 mg·kg $^{-1}$). However, the available forms of heavy metals were extremely low, suggesting that the availability of heavy metals to the organism would be minimal under current conditions. Results also showed that the difference in the available form of metals according to the different extraction methods used, was less for the biological method (0.057, 3.007 and 0. 657 mg·kg^{-1}) compared with the chemical (0.060, 4.779 and 1.2724 mg·kg $^{-1}$) for Cd, Pb and Ni respectively. The results suggested that chemical and biological metals forms should be considered as a complementary technique rather than an alternative in evaluating the metal bioavailability from different pollution sources. The results indicated weak or lack of significant correlations between heavy metals and total organic carbon, electrical conductivity and pH, indicating that the activity of these metals may be affected by other factors.

Introduction

Pollution can be defined as the existence of pollutant in the environment.The term pollutant refers to any substance, living thing, a form of energy that leads to the destruction or threat of the ecosystem at present or in the future. Polluting the environment with heavy metal is now considered one of the most important environmental concerns(Jaafar et al., 2016).

Different pollutants that come from various anthropogenic activities, such as industry, agriculture, mining and transport can be accumulated in the soil. Therefore, soil is a critical environment. The pollution of soil by heavy metal has attracted considerable attention because they are nondegradable. The extent of pollution with heavy metals can be indicated by their total concentration,which cannot be considered an accurate indicator of the their toxicity (A. H. Parizanganeh, Bijnavand, Zamani, & Hajabolfath, 2012).Thus several recent studies have relied on bioavailability rather than the total concentration as a key to appreciating the environmental risks of metals, which is associated with as their

*Corresponding author's email : amialraghad@yahoo.com

negative impact associated only with their biologically available forms (Li & Naidu, 2005). So for site-specific hazard evaluation, bioavailability is considered useful tool because the knowledge of the available form of metals is important in deducting the concentration allows to exposure by human, in addition to its importance in selecting the appropriate type of bioremediation technique (Leita *et al.*, 2013). The bioavailable part also depends on the soil properties and the processes that occur to them that vary with age, hence studying the processes can be affected by bioavailability at a site,which is essential in determining the risk associated with pollution and selecting cleanup technologies. The passage of heavy metals from unavailable to available form is regulated by the chemical, physical , biological conditions and time, which determine the transfer of metals from the solid to the solution phase that are governed by properties such as pH, organic matter and electrical conductivity, cation exchange capacity, and redox potential (Liviana Leita *et al.,* 2009).

Successful method of bioavailability measurement

Choosing the suitable technique for heavy metals remediation, usually depends on bioavailability. Using complementary method in testing bioavailability form instead of depending on universal one, to address an obstacles related to the main difficulties in applying bioavailability in remediation procedures. Bioavailability cannot be defined by chemical and biological tests alone, but both must be considered as tools, to provide complete acquaintance on the bioavailability (Harmsen, 2008**).** Researcher commonly use serial extraction procedures to determine the available form of heavy metals, but this method give inadequate evidence about metal availability for all the metals exist in a multi-contaminated environment (Prokop *et al.*, 2003). The sequential extraction process faces a major problem, that is, the phase of nonselective extraction and the extraction of heavy metals during redistribution. To study the contaminated soil or sediments, partial, single extraction may not result in phase determination but will affect various stable and, relatively stable metal bonds are easier to use and suitable for many samples (Koukina, 2018). A disadvantages of the chemical method is, its incapability to produce an image of the transition of minerals from nonvital to the vital system. (Liao *et al.,* 2006). Consequently, biological methods which are

harry, sensitive and cheap way, and can integrate all parts of bioavailability, are considered a new trend in environment monitoring (Peijnenburg & Jager, 2003), A unique way to study the availability of contaminant in special organism. The organism in the ecosystem make the exact application of this type of approach difficult. Therefore, biological tests can be used to approximate some signs for the actual bioavailability in the form of test organisms. If the particular factor in the biological absorption of a chemical combination is acquired from a series of chemical procedures, then the bioavailability indicators can also come from chemical tests that define the kind of the bonds that connect a substance to chemical surfaces. On the basis, the present study aims to give information on the bioavailability derived from laboratory tests in which bioavailability is determined by biological and chemical approaches to avoid the drawback of each and complement the knowledge about heavy metals availability.

Materials and Methods

Study Area

The study area included six districts in Basra province in south of Iraq. The sample was collected in September 2017.
1. Oil fields in Northern Rumaila, are Station No. 1, 2 and 3
2. Urban area (Al-Sharash, Al-Salhia and Al-Ashar parking), are Stations No. 4, 5, 6 respectively

Soil sampling and handling

A total of 18 soil samples was collected in 0 - 20 cm depth from six different areas. Soil samples were labelled, stored in plastic bags and taken to the laboratory. In the laboratory, each sample was separately air-dried and milled using porcelain pestle and mortar, and then sieved with a 2 mm sieve. The fine soil fractions were collected in separate bags, and stored in a dry place for use in further analysis.

Soil analyses

Soil samples were analyzed for various physical and chemical characteristics such as, pH, EC, total organic carbon, and total concentration of heavy metals.

pH

pH was analysed according the method of Page,

Miller, and Keeney (1982),where 50 gm of soil was placed in glass beakers, and 100 mL of distilled water (1:2) was added. The mixed was shaken in a shaker, then beakers pliable to stop1h. A calibrated Lovibond pH 200 meter was used to measured soil pH.

(EC)

A total of 30g of each soil sample taken, in glass beakers, and saturated with distilled water to form paste. A vacuum pump was used to obtain soil extract. EC was recorded using a calibrated Lovibond con200 meter (Page *et al.,* 1982).

TOC (Organic C)

The method of (Page *et al.,* 1982) was followed to measure the total concentration of organic matter. The steps, weighted 2 gm of soil in an Erlenmeyer flask, and add 10 mL of 1 N $K_2Cr_2O_7$ to the flask while shaking. Then add 20 mL of concentrated H_2SO_4 shake again for 1min, and allowed to stand for 30 min. Add sulfuric acid to the soil suspension, stir the flask again, and leave for 1 min. Finally, add 200 mL distilled water and 10 mL of H_3PO_4 with 1 mL of diphenylamine indicator and adjust against 0.5 N $FeSO_4.7H_2O$ until the color changes from blue to red.

Total concentration of heavy metal

One gram of soil was digested using the acid mixture (1:1 $HCl : HNO_3$), and hot plate at 80 °C, allowing it to evaporate to near dry. Another digestion using the mixture of concentrated $HClO_4$ and HF acids was used to complete the digestion process. The remaining part from digestion was dissolved in 20 mL of (0.5 N) HCl and cooled for 10 min (Tüzen, 2003). The extractor was transferred into a 25 mL plastic container. This step was repeated twice, and all supernatants were combined. Finally, the volume was completed with deionised water to reach 25mL and sealed for heavy metal analysis. Metals concentration was determined from the calibration curve as follows:(μg metal/L sample (A*V)/w. Where A= mg metal in the processed sample from the calibration curve(mg/L),V is the final volume of the processed sample in(mL) and W denotes the dry weight equivalent to the sample(g).

Heavy metal bioavailability measurements

1. Chemicals methods
Diethylen-triamine pentaacetic acid (DTPA)-extrac-

tion method was used to determine the available portions of studied metals, where ten grams of air dried soil was placed in a flask for extraction using 20 mL of buffered (pH 7.3) of mixture (0.005 M DTPA+0.01 M TEA + 0.01 M CaCl2). The contents were shaken for 2 h. The mixture was then filtered through filter paper (Whatman No.42). The filtration was diluted with distilled water for 100 mL volume and stored in a clean plastic bottle for metal determination (Parizanganeh *et al.,* 2010). Heavy metal analysis was performed by a flame atomic absorption spectrophotometer (AAS 7000, Shimadzu, Japan).

Biological methods

The method of fumigation extraction as chloroform ($CHCL_3$-labile metals) was used, where all soil samples were subjected to water holding capacity(60%) for 7 days (Brookes, 1995); (Brookes *et al.,* 1995). Twelve subsamples of 10g moist soil were taken from each soil, and extracted with 25 mL of 1 M NH_4NO_3 (1:2.5 w:v), shacken up for 60 min at 200 rpm and then filtered through paper (Whatman No. 42). Twelve replicates were fumigated for 24h with ethanol free ($CHCl_3$) in vacuum sealed desiccators at room temperature. After fumigant removal, the samples were extracted as described for the nonfumigated replicates. After filtration, the soil extracts were acidified with 0.5mL 65% HNO_3 and stored at 4°C. Subsequently, Cadmium (Cd), Lead(Pb) and Nickel (Ni) were in measured in all extract by flame atomic absorption.CHCl3-labile Cd, Pb and Ni were calculated as the difference between those extracted from fumigated and nonfumigated soil (Khan *et al.,* 2009).

Statistical analysis

Statistical analysis was using one way analysis of variance to compare means, and significantly different means were separated using LSD; with posttest if P<0.05 using SPSS versio 10 software. The Persons correlation coefficient was used to determine the dependence of various parameters one another

Results and Discussion

Some physical and chemical properties of soil (pH), (EC) and (TOC)

Several factors can explain the concentration of heavy metal in environments. The current study fo-

cuses on important factors, such as pH, EC and TOC. Soil pH serves as a useful index of availability of heavy metal, and the physical properties of the soil. The present study recorded pH values ranging between 7.29 and 7.96 (Table 1), which indicated that the studied soils have neutral to subalkaline nature. Iraqi soil contains a high percentage of carbonate which leads to equalising the acidity of soil. These findings are consist those of (Al Obaidy *et al.,* 2016) who studied Baghdad soil, with the limits of pH (7- 8) because they have calcium carbonate (lime) and calcium sulphate in high quantities. Soil in urban areas with an alkaline reaction has been reported to be reletively common (Al-Ameri, 2011). The convergence of pH values recorded for soils can suggested that the effect of pH on metal availability is minimal and does not affect site characterisation (Al Obaidy *et al.,* 2016). EC expresses the material's ability to deliver electricity. The EC had the lowest value in the soil the Station, 2 (4.24 ms/cm), whereas the highest value was in the soil of Station, 6 (45.08 ms/cm). Variation in EC values showed that soluble salt concentration varied considerably. The level of TOC in the stations were in the following order: 6>4>1>3>2>5 (Table 1).

Table 1. pH, EC(ms/cm) and (TOC%)

Stations	pH	EC (ms/cm)	TOC(100%)
1	7.80	6.71	2.52
2	7.53	4.24	1.90
3	7.91	31.06	2.50
4	7.65	18.94	2.61
5	7.96	18.44	0.57
6	7.29	45.08	4.05

Total concentration of heavy metal

The total concentration of (Cd), (Pb) and Nickel (Ni) in Table 1 followed the order Pb> Ni> Cd. The concentrations of Cd and Pb were greater than that of the standard range. Whereas that of Ni was less. The metal concentrations in the studied soil showed wide ranges of 0.05125– 0.32193 mg·kg^{-1} for Cd, 6.56579–45.59435 mg·kg^{-1} for Pb, and 1.49223– 7.56223 mg·kg^{-1} for Ni. The concentrations revealed significant spatial variation (Fig. 1). The comparatively high levels of Cd and Pb occurred at Station6, where 'car exhausts' were the main cause of pollution of these two metals. The high concentration of Ni occurred near the oil drilling wells because the oil extraction operations contributed to raising the
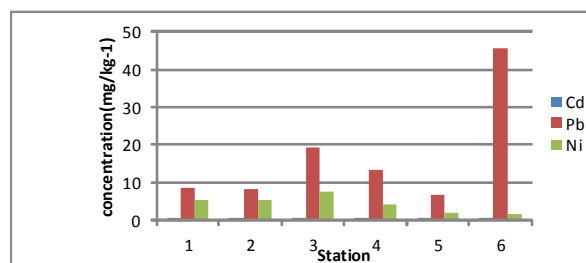

**Fig. 1.** Distribution of metals within the studied area

concentration of Ni compared with "the rest of the sources

Metal bioavailability study

Consciousness and anxiety about the adverse effects of high levels of heavy metal on ecosystems and organisms are growing. However, high levels of contaminants do not necessarily indicate negative effect, and the exact risk of heavy metal is due to the images available to them and not to the total concentration. Figs. 2, 3, 4 show the total and available concentration of Cd, Pb and Ni in the studied area using biological and chemical extraction methods. These figures show that the available concentration of metals was less than their total concentration because metals are frequently associated with differ-
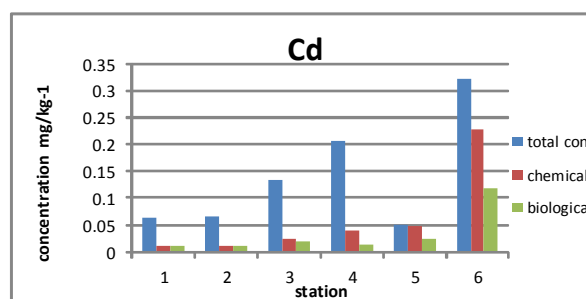
Fig. 2. Total concentration of Cd, and Comparison of chemical and biological Bioavailable in the Soils.
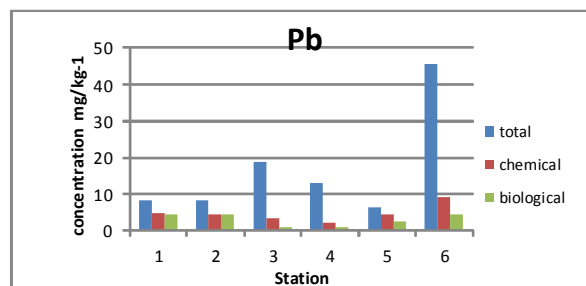
Fig. 3. Total concentration of Pb, and Comparison of chemical and biological Bioavailable in the Soils.
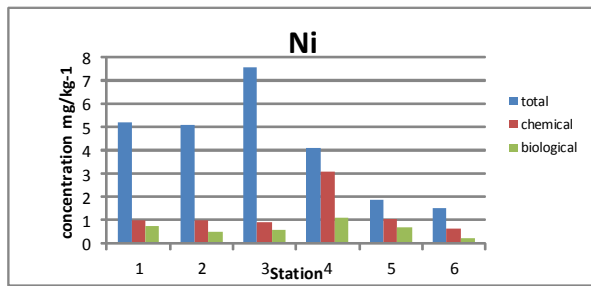
Fig. 4. Total concentration of Ni, and Comparison of chemical and biological Bioavailable in the Soils

ent soil constituents, making them nonfree, and the prominent role of soil characteristics, which affects their availability, this finding is consistent with other studies (Parizanganeh *et al.,* 2012), (Koukina S, 2018), and (Kiciñska *et al.,* 2019). The chemical method relied on the DTPA-extraction, which considered most efficient for extracting metals from soil regardless of their properties (Xiu-Zhen *et al.,* 2009), The results showed that the order of available concentration of metals using this method was Pb>Ni>Cd, and the recorded available concentration of Pb, and Ni was higher than that recorded in previous studies (Buccolieri *et al.,* 2010), (Nunes *et al.,* 2014), where for Cd it was perceptibly higher than those reported by (Massas *et al.,* 2010) and (Nunes *et al.,* 2014). The biological method used fumigation-extraction method, where in addition to their capability to store organic component as C, N, P, and S, soil bacteria can store macronutrients and heavy metals. Although a large quantity of metals is adsorbed by the cell-wall contents, a significant part is also transferred into bacterial cells (Gadd, 2004); (Khan *et al.,* 2009). So fumigation with $CHCL_3$, can extract these metals as CHCl3-labile metals of bacterial origin with extraction solutions. The results in Figure 3 showed that the order of the measured metals in this method followed the same order as the chemical method, although the concentrations

were lower, this finding agrees with previous works (Emili, 2012); (Baran and Tarnawski, 2015). The differences in the principle and the goal of these methods can cause differences in the mobility of metal or/and soil properties that affect the extraction, in addition long term heavy metal contamination of soil can decrease the total number and the microbial activity in the soil (Kumawat Yadav and Majumdar, 2017). The statistical analysis result showed a significant difference between the total concentration of metals and the available concentration measured in the chemical and biological methods and between the available concentration measured in both methods at a significant level ($P<0.05$). In view of the concentration that can be taken by living organisms, the main drawback of chemical methods is transferring the results obtained from nonbiological to biological systems (Liao *et al.,* 2006), that the current trend towards field monitoring has driven the development of biological methods such as new analytical tools capable of providing fast and sensitive measurements at a lower cost; many of them aimed at on-site analysis. These tools have also gained much attention since they integrate all aspects of bioavailability (Peijnenburg and Jager, 2003). Evidence of the validity of the foregoing is the results of the current study, which showed a clear difference between the total concentration of the studied metals and their available concentration measured in both the chemical and the biologic methods. This emphasizes the need to consider the study of the total concentration of elements as well as the available concentration measured in different ways complement each other to give a clear picture of the extent of heavy metals pollution in the studied area and to determine their environmental risk.

Pearson's correlation coefficient analysis

Correlation studies have been performed on the mean levels of the variables to find the relationship

Table 2. Total metal concentrations were compared with existing standard (mg·kg^{-1}) (A. H. Parizanganeh et al., 2012).

Standards	Cd	Pb	Ni
Mean in the studied area	0.38167	28.93377	4.227413
Maximum	3.21470	45.59435	7.60070
Minimum	.05125	16.56579	1.48870
USEPA[1]	-	10	40
WHO	-	20	20
GLC[2]	0.3	20	68

[1]United State Environmental Protection Agency; [2]Great London Council

among various studied variable. Table 3 shows the Pearson's correlation coefficient results with their significance levels (P<0.01 and P<0.05). The results indicated weak or lack of significant correlations between heavy metal and TOC, EC, and pH. The absence of correlations between heavy metal and TOC can imply that heavy metal are not controlled by the organic carbon content (Skordas *et al.,* 2015). This conclusion is also indicated by (Javed *et al.,* 2018). The EC did not show any significant correlation with the studied metals, except for a significant negative correlation (-0.742*)at (P<0.05) with the available concentration of Ni measured in the biological method; this finding is consisted with that of (Solgi, 2016), who specified the absence of a significant correlation between studied metals (Pb and Cd) and the EC of the soil. The results also showed no association between the concentration of metals and pH, indicating that the activity of these metals may be affected by other factors. This finding is consistent with the study of (Xuan *et al.,* 2018) regarding of Cd metal, whereas significant negative correlation was found for Pb with pH (P≤0.05).

In relation to the correlation among the studied metals, the results in Table 3 showed a significant correlation between the total concentration of Cd and Pb referring to a common origin for these metals in the soil; however the correlation was insignificant with Ni. (Shafie *et al.,* 2013) suggested that the associations between metals can differ considerably depending on their geochemical origin, including soil basis material and soil sort. (Covarrubias *et al.,* 2018) reported strong correlation between Ni and Cd, and no correlation with Pb. The results showed

that significant correlations are evident between the total metal form and the extractable form of DTPA for Cd and Pb, which confirms the lack of a strong relationship between the total form of metals with that for DTPA- extractable form for all studied metals (Topcuoglu, 2016). The results also showed the only significant correlation found between the total form of Cd and Pb with the biological extractable form of Ni, which can be referred to the same causes. In case of correlation with the method used to assess the available form of study metals, the results illustrated significant correlation between Pb and Ni, although the correlation was insignificant for Cd. The Severity ranges in DTPA-extractable and biological –extractable metal levels in Pb and Ni were determined, which may cause the difference in metal availability, in addition to the conditions of soil that can affect the result of the extraction process.

Conclusions

The results of the present study confirm the necessity of integrating all aspects related to the study of soil pollution with heavy metals. Relying on one side and neglecting the rest is impossible, as observed for the difference of the total concentration of the metals studied in the soil and between the available concentration measured depending on the chemical and biological methods. The results also show the importance of studying soil characteristics to determine their influence on pollution levels in the environment. The integration of metals pollution in the study can be considered important in se-

Table 3. Personal correlation coefficient among various studied variables.

	Cd/total	Pb/total	Ni/total	Cd/che	Pb/che	Ni/che	Cd/bio	Pb/bio	Ni/bio	TOC	EC	pH
Cd/total	1											
Pb/total	.709**	1										
Ni/total	-.413	-.371	1									
Cd/che	.715**	.936**	-.672-**	1								
Pb/che	.617*	.758**	-.586-*	.844**	1							
Ni/che	-.198	-.270	.034	-.264	-.667-**	1						
Cd/bio	.272	.273	-.596-*	.457	.492	-.318	1					
Pb/bio	.245	.183	-.288	.276	.696**	-.588-*	.202	1				
Ni/bio	-.499-*	-.694-**	.231	-.666-**	-.854-**	.833**	-.314	-.524-*	1			
TOC	-.026	-.041	-.019	-.024	.008	.050	-.209	.026	-.115	1		
EC	.586	.544	.549	.617	-.649	-.466	-.476	-.584	-.742-*	.595	1	
pH	-.188	-.195	-.222	-.169	.160	.221	-.343	.188	-.110	-.732-*	-.356	1

*. Correlation is significant at the 0.05 level (1-tailed). **. Correlation is significant at the 0.01 level (1- iled).

lecting appropriate strategies to eliminate or reduce pollution by heavy metal, which is one of the most important aspirations of the era to preserve the environment. Therefore, the integration of the results of the present study can be considered vital in selecting suitable bioremediation methods for treating the heavy metal pollution in soil.

Acknowledgments

Conflict of interests

The researcher asserts that there is no clash of interest

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