The Bioavailability and Evolution of Trace Metals in Environment: A Brief Review

Hui Zhang

School of Environmental Science and Engineering, Shanghai Jiaotong University, 800 Dongchuan Rd., Shanghai 200240, P.R. China

Abstract: As a kind of important pollutants, trace metals and the pollution have been become a concerned worldwide environmental problem. Despite the fact that the bioavailability of trace metals indicated by their speciation has been an indispensible parameter in the assessment and treatment of trace metal pollution, many studies suggest that the bioavailability of trace metals may change according to environmental conditions, and they can also transform between some speciation fractions. These transformations are related with factors such as the compositions, microorganism, time, and other physical-chemical conditions of the system. So, it is necessary to systematically understand and investigate for the factors to affect the transformation aside from analysis at certain time-place. The results of these understanding and investigations can be used for reasonably determining the allocation of financial and technical trace metals on environmental inspirations from the evolution of the speciation of the

Keywords: Trace metals, speciation, transformation.

1. INTRODUCTION

With the fast development of economy worldwide trace metals from large-scale industrial production and human life had been and are adding to ecosystems. The pollution of trace metals from rapid development has become one of the challenges to have to be faced by human today [1, 2]. In the study of trace metal pollution, either in natural or engineering systems, the speciation is a key parameter for assessment and treatment of the pollution [3-8].

Currently, the speciation data obtained at certain time-place conditions are used to assess the environmental impact for the trace metal pollution, especially used in soil and water sediment pollution research and engineering treatment. But the emphases of real effects of the transformation for the speciation of trace metals in environment seem relatively weak, and the significance of this transform in real environmental impacts are not well valued in practices. Although the research on the speciation of trace metals has been a basic work in the study of pollution problems, with understanding of this pollutant going deep and the analysis technique being advanced, the evolution that may affect metal's real bioavailability in inorganic systems and life entities should be taken into consideration to develop a counter plan for the pollution research and treatment [9-13].

speciation of trace metals as a significant topic in the research of trace metal pollution has become an indispensable subject in metal pollution problems. The speciation of trace metals from certain analysis procedure is considered as a key parameter in the assessment of trace metal bioavailability and, accordingly, the prevention and remediation plan for the pollution is generally formed based on the level of this speciation parameter from the analysis [14, 15]. This has been viewed as an irreplaceable measurement for pollution assessment by not only scholars but also public and policymakers. However, as more and more facts show that the speciation of the trace metals is in evolution in environment, except the significance of the data on the speciation at certain time-space condition, it is paying attention to that the speciation is in variation with the physical and chemical condition change in the system [15-19].

Because of its serious impact on the biotoxicity,

2. OVERVIEW ON CASES OF THE SPECIATION EVOLUTION

2.1. The Cases on Water Sediment

It has been demonstrated by a lot of work in recent years that the speciation of trace metals changes with the changes of conditions of environment. The work from Laing *et al.* on the sediment of the Scheldt River, Europe showed that the speciation of the metals varied with conditions such as hydrological regime, organic matter, salinity etc. [20, 21]. The same facts from aqueous systems were demonstrated too by recent work from UK etc. [22-25]. Furthermore, studies relative to aqueous systems by García-Delgado *et al.*

Address correspondence to this author at the School of Environmental Science and Engineering, Shanghai Jiaotong University, 800 Dongchuan Rd., Shanghai 200240, P.R. China; Tel: 86-21-54748942, Fax: 86-21-54746023, E-mail: huizhang@sjtu.edu.cn

on cadmium in sewage sludge from Salamanca province, Spanish in 2007 [26], Wang et al. on metals in the sediment from rivers in 2012 [27], Scheckel et al. on silver and zinc by aqueous environmental experiments in 2010 [28], and Prica et al. on lead, cadmium etc. on some aqueous environmental media in 2010 [29] all suggested that the speciation change with environmental conditions and it is not always constant. The work from Masson et al. on Arsenic from the Garonne and Dordogne rives, France in 2007, Verschoor et al. on experimental research for copper, nickel, and Zinc in 2011 and Duman Fatih et al. on metals in Sapanca lake, Turkey in 2006 suggested that temperature may be a factor to affect metal speciation change in the system [30-32]. Also, reported by Catalano et al. respectively in 2010 and 2012, conditions such as pH value, plant feature in the aqueous environment may be factors to affect the variation of the speciation of trace metals [33].

2.2. The Case on Soil

the А very interesting discussion about bioavailability of trace metals was from Dr. Alexander in a review for Environmental Science and Technology in 2000 on crisis assessment of soil system, and the bioavailability of metals in metal environmental problems, and its variation nature in environment media were raised in his presentation, based on a lot of important discussions together with organic pollutants. And, in this review, it was first pointed out that pollutant bioavailability may be varied under different conditions in environment and the general understanding of pollutant bioavailability from instant analysis seems to be exaggerated compared with its real impacts in the ecosystem, thus causing a huge waste in treatment of the pollution [34]. The facts of metal availability change in soil system had also been demonstrated by the work on repeated phytoextraction test recently completed by Zhu et al. and the work on coastal soil completed by Zheng et al. in 2016 [35, 36].

2.3. The Factors in the Speciation Evolution of Trace Metals

The speciation change of trace metals with the condition varyinged in environment has been revealed from work on water and sediments from Asia [37-40]. Components of the system, the concentration of the trace metals in the system, the time of the trace metal reacting with other matters in the system, and other physical-chemical conditions such as the value of pH, Eh etc. may all be factors to affect speciation change in

the system of soil, water, and other media such as fly ash of coal [41-43]. Some researchers have found that the sulfate can significantly affect the release of metals such as Ag, Cu etc. in the systems, especially under water-flooding conditions [44-47]. The speciation of the Zn in sediment may be very in different under different features of porewater and overlying water of the sediment [48]. The change of the speciation of trace metals (Cr, Cu, Pb, Zn, and Cd) can be induced to occur from the variation of the physical-chemical parameters of the system such as adding chlorinating agent or drying, revealed by some engineering treatment for sewage sludge and refuse landfill [49-51, 31]. It can be different in different kinds of soils for the speciation of the trace metals to change under same environmental conditions [52, 53].

Excepting these research findings, there are work to suggest to assess the bioavailability of trace metals using the feature of rare earth elements (REE) fractionation, the variation of soil enzyme activities (IR/reduced partition index), and delayed geochemical hazard model (DGH), based respectively on the differences in ionic radius, oxidation statuse and bonding of the rare earth elements (REE) drive fractionation of these elements in natural systems and the closed relationship between metal release and the soil enzyme activities or mechanism of chain reaction (Hg) [54-57].

Recently, the work on the sediment of the Dianshan lake, Shanghai showed the change and transform of the trace metal speciation within fractions of the speciation of the metals, and it was found by the Pollutant Behavior Chemistry Group, Shanghai Jiaotong University that the transformations of the speciation of metals defined and analyzed with BCR extraction procedure sequential might occur respectively between water and acid soluble fraction and reducible fraction, oxidisable fraction and residual fraction, and oxidisable fraction and reducible fraction etc. (Figure 1).

The studies mentioned above suggested that the variation of speciation of trace metals requires to be understood and be considered in not only researches but also treatment. Although it has been concerned by many scholars, much more work on this field should be done since it has become a current cutting-edge problem in trace metal pollution research and treatment. Because of the importance of the speciation and the speciation transformation of trace metals in metal environmental problems, based on the facts

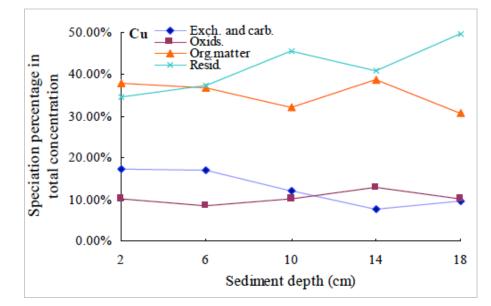


Figure 1: The relationship of the trace metal speciation in the sediment of the Dianshan Lake, Shanghai.

reviewed here if only the data of speciation of trace metals from instant sample were used to evaluate the pollution, it will cause unreasonable disposition for resources and funds, by ignoring the speciation transformation nature in systems.

3. SUMMARY

In general, the issue of speciation of trace metals, the understanding of speciation and its transformation is very important to offer significant references more objectively for comprehending and assessing the real impact of trace metal pollution in environment. The condition and effects of the speciation transformation of trace metals in environment should be emphasized to understand, assess, and treat impersonally and effectively as well as speciation data on the samples from certain time or site should be valued. Now, the issue on metal speciation and bioavailability is being paid more and more attention by scientists, and hope it will be made a point of doing this by policymakers too.

ACKNOWLEDGEMENTS

The study on the issue of bioavailability of trace metals discussed in this paper is funded by The National Science Foundation of China (grant No. 41572329) to Hui Zhang, and here I express my heartfelt thanks to this valuable financial assistance.

REFRENCES

 Bi X, Li Z, Sun G, et al. In vitro bioaccessibility of lead in surface dust and implications for human exposure: A comparative study between industrial area and urban district. Journal of hazardous Materials 2015; 297: 191-197. http://dx.doi.org/10.1016/j.jhazmat.2015.04.074

- [2] Lzquierdo M, De ME, Ortega MF, et al. Bioaccessibility of metals and human health risk assessment in community urban gardens. Chemosphere 2015; 135: 312-318. <u>http://dx.doi.org/10.1016/j.chemosphere.2015.04.079</u>
- [3] Rauret G, López-Sánchez JF, Sahuquillo A, et al. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. Journal of Environmental Monitoring 1999; 1: 57-61. http://dx.doi.org/10.1039/a807854h
- [4] Ure AM, Quevauviller P, Muntau H, et al. Speciation of heavy metals in soils and sediments - An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the commission of the European communities. International Journal of Environmental Analytical Chemistry 1993; 51(1-4): 1135-1511. http://dx.doi.org/10.1080/03067319308027619
- [5] Wang X, Wei D, Ma Y, et al. Derivation of Soil Ecological Criteria for Copper in Chinese Soils. PLOS ONE 2015; 10: 7. <u>http://dx.doi.org/10.1371/journal.pone.0133941</u>
- [6] Thanh PM, Ketheesan B, Yan Z, et al. Trace metal speciation and bioavailability in anaerobic digestion: A review. Biotechnology Advances 2016; 34(2): 122-136. <u>http://dx.doi.org/10.1016/j.biotechadv.2015.12.006</u>
- [7] Islam MN, Xuan PN and Jung HY. Chemical speciation and quantitative evaluation of heavy metal pollution hazards in two army shooting range backstop soils. Bulletin of Environmental Contamination and Toxicology 2016; 96(2): 179-185. http://dx.doi.org/10.1007/s00128-015-1689-z
- [8] Leonardo T, Farhi E and Pouget S. Silver Accumulation in the green microalga coccomyxa actinabiotis: Toxicity, *in situ* speciation, and localization investigated using synchrotron XAS, XRD, and TEM. Environmental Science and Technology 2016; 50(1): 359-367. <u>http://dx.doi.org/10.1021/acs.est.5b03306</u>
- [9] Ren J, Williams PN, Luo J, et al. Sediment metal bioavailability in Lake Taihu, China: evaluation of sequential extraction, DGT, and PBET techniques. Environmantal science and Pollution Research 2015; 22(17): 12919-12928. <u>http://dx.doi.org/10.1007/s11356-015-4565-9</u>

- [10] Zhang H. Behaviors of trace metals in environment--The pollution in regional and metropolis area (in Chinese). Shanghai Jiaotong University Press 2014; Shanghai.
- [11] Yoelvis BA, Carlos MA, Roberto M, et al. Mercury contamination of riverine sediments in the vicinity of a mercury cell chlor-alkali plant in Sagua River, Cuba. Chemosphere 2016; 152: 376-382. http://dx.doi.org/10.1016/j.chemosphere.2016.03.025
- [12] Uriel A, Matthias P and Sibylle M. Determination of moderately polar arsenolipids and mercury speciation in freshwater fish of the River Elbe (Saxony, Germany). Environmental pollution 2016; 208: 458-466. http://dx.doi.org/10.1016/j.envpol.2015.10.015
- [13] 016 Bolan S, Naidu R and Kunhikrishnan A. Speciation and bioavailability of lead in complementary medicines. Science of the Total Environment 2016; 539: 304-312. <u>http://dx.doi.org/10.1016/i.scitotenv.2015.08.124</u>
- [14] Stockdale A, Tipping E and Lofts S. Effect of Ocean Acidification on organic and inorganic speciation of trace metals. Environmental Science and Technology 2016; 50(4): 1906-1913. http://doi.org/10.1021/acc.oct.5b05524

http://dx.doi.org/10.1021/acs.est.5b05624

- [15] Ricevuto E, Lanzoni I and Fattorini D. Arsenic speciation and susceptibility to oxidative stress in the fanworm Sabella spallanzanii (Gmelin) (Annelida, Sabellidae) under naturally acidified conditions: An *in situ* transplant experiment in a Mediterranean CO2 vent system. Science of the Total Environment 2016; 544: 765-773. <u>http://dx.doi.org/10.1016/j.scitotenv.2015.11.154</u>
- [16] Pauget B, Faure O, Conord C, et al. In situ assessment of phyto and zooavailability of trace elements: A complementary approach to chemical extraction procedures. Science of the Total Environment 2015; 521: 400-410. <u>http://dx.doi.org/10.1016/j.scitotenv.2015.03.075</u>
- [17] Hayes SM, Root RA, Perdrial N, et al. Surficial weathering of iron sulfide mine tailings under semi-arid climate. Geochimica et Cosmochimica Acta 2014; 141: 240-257. http://dx.doi.org/10.1016/j.gca.2014.05.030
- [18] Duan D, Peng C, Xu C, et al. Lead phytoavailability change driven by its speciation transformation after the addition of tea polyphenols (TPs): Combined selective sequential extraction (SSE) and XANES analysis. Plant and Soil 2014; 382(1-2): 103-115. http://dx.doi.org/10.1007/s11104-014-2152-3
- [19] Matijevic L, Romic D, Romic M, et al. Soil organic matter and salinity affect copper bioavailability in root zone and uptake by Vicia faba L. plants. Environmenta Geochemistry and Health 2014; 36(5): 883-896. http://dx.doi.org/10.1007/s10653-014-9606-7
- [20] Laing DG, Vanthuyne D, Tack FMG and Verloo MG. Factors affecting metal mobility and bioavailability in the superficial intertidal sediment layer of the Scheldt estuary. Aquatic Ecosystem Health and Management 2007; 10(1): 33-40. <u>http://dx.doi.org/10.1080/14634980701212969</u>
- [21] Laing DG. Dynamics of heavy metals in reed beds along the banks of the river Scheldt. Thesis of PhD in Applied Biological Sciences. Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium 2006; pp.284, ISBNnumber: 90-5989-147-3.
- [22] Lv B, Xing M and Yang J. Speciation and transformation of heavy metals during vermicomposting of animal manure. Bioresource Technology 2016; 209: 397-401. http://dx.doi.org/10.1016/j.biortech.2016.03.015
- [23] Li WC, Deng H and Wong MH. Metal solubility and speciation under the influence of waterlogged condition and the presence of wetland plants. Geoderma 2016; 270: 98-108. <u>http://dx.doi.org/10.1016/i.geoderma.2015.10.012</u>
- [24] Dong Y, Rosenbaum RK and Hauschild MZ. Assessment of metal toxicity in marine ecosystems: comparative toxicity

potentials for nine cationic metals in coastal seawater. Environmental Science and Technology 2016; 50(1): 269-278.

http://dx.doi.org/10.1021/acs.est.5b01625

- [25] Lathouri M and Korre A. Temporal assessment of copper speciation, bioavailability and toxicity in UK freshwaters using chemical equilibrium and biotic ligand models: Implications for compliance with copper environmental quality standards. Science of the Total Environment. 2015; 538: 385-401. http://dx.doi.org/10.1016/j.scitotenv.2015.06.120
- [26] García-Delgado M, Rodríguez-Cruz MS., Lorenzo LF, Arienzo M, Sánchez-Martín MJ. Seasonal and time variability of heavy metal content and of its chemical forms in sewage sludges from different wastewater treatment plants. Science of the Total Environment. 2007; 382: 82-92. <u>http://dx.doi.org/10.1016/j.scitotenv.2007.04.009</u>
- [27] Wang X and Li Y. Distribution and fractionation of heavy metals in long-term and short-term contaminated sediments. Environmental Engineering Science. 2012; 29: 617-622. http://dx.doi.org/10.1089/ees.2011.0122
- [28] Scheckel K, Luxton T, Elbadawy A, Impellitteri CA, Tolaymat T. Synchrotron speciation of silver and zinc oxide nanoparticles aged in a kaolin suspension. Environmental Science and Technology 2010; 44: 1307-1312. <u>http://dx.doi.org/10.1021/es9032265</u>
- [29] Prica M, Dalmacija B, Dalmacija M, Agbaba J, Krcmar D, Trickovic J, et al. Changes in metal availability during sediment oxidation and the correlation with heim mobilization potential. Ecotoxicology and Environmental Safety 2010; 73: 1370-1377.

http://dx.doi.org/10.1016/j.ecoenv.2010.06.014

- [30] Duman F, Obali O and Demirezen D. Seasonal changes of metal accumulation and distribution in shining pondweed (Potamogeton lucens). Chemosphere 2006; 65: 2145-2151. http://dx.doi.org/10.1016/j.chemosphere.2006.06.036
- [31] Masson M, Schafer J, Blanc G and Pierre A. Seasonal variations and annual fluxes of arsenic in the Garonne, Dordogne and Isle Rivers, France. Science of the Total Environment. 2007; 373: 196-207. http://dx.doi.org/10.1016/j.scitotenv.2006.10.039
- [32] Verschoor AJ, Vink JPM, Snoo GR and Vijver MG. Spatial and temporal variation of watertype-specific no-effect concentrations and risks of Cu, Ni, and Zn. Environmental Science and Technology 2011; 45: 6049-6056. <u>http://dx.doi.org/10.1021/es2007963</u>
- [33] Nowack B, Schulin R and Luster J. Metal fractionation in a contaminated soil after reforestation: Temporal changes versus spatial variability. Environmental Pollution 2010; 158: 3272-3278. http://dx.doi.org/10.1016/j.envpol.2010.07.020
- [34] Alexander M. Aging, Bioavailability, and Overestimation of Risk from Environmental Pollutants. Environmental Science and Technology 2000; 34(20): 4259-4265. <u>http://dx.doi.org/10.1021/es001069+</u>
- [35] Li Z, Jia M and Wu L. Changes in metal availability, desorption kinetics and speciation in contaminated soils during repeated phytoextraction with the Zn/Cd hyperaccumulator Sedum plumbizincicola. Environmental pollution 2016; 209: 123-131. http://dx.doi.org/10.1016/ji.envpol.2015.11.015
- [36] Zheng R, Zhao J and Zhou X. Land use effects on the distribution and speciation of heavy metals and arsenic in coastal soils on Chongming Island in the Yangtze River estuary, China. Pedosphere 2016; 26(1): 74-78. http://dx.doi.org/10.1016/S1002-0160(15)60024-8
- [37] Liu H, Yi C and Tang Y. Distribution and speciation of heavy metals in sediments at a littoral zone of Meiliang Bay of Taihu lake. China Environmental Science (in Chinese with English abstract) 2010; 30(3): 389-394.

- [38] Ji F, Wang T, Hu X, He Q, et al. Movement and Trans formation of Heavy Metals in Water -Sediment in Water-Level-Fluctuating Zone of Three Gorges Reservoir Area. Environmental Science (in Chinese with English abstract) 2009; 30(12): 3481-3487.
- [39] Zhong L. The sedimentary records of heavy metals and its significance in the Dianshan Lake, Shanghai. Theses of MS in Environmental Science and engineering (in Chinese with English abstract), School of Environmental Science and Engineering, Shanghai Jiaotong University, Shanghai, China 2007; pp.72.
- [40] Thomas ALK, Mikutta C and Lohmayer R. Sulfidization of organic freshwater flocs from a Minerotrophic Peatland: Speciation changes of iron, sulfur, and arsenic. Environmental Science and Technology 2016; 50(7): 3607-3616. http://dx.doi.org/10.1021/acs.est.5b05791
- [41] Catalano JG, Huhmann BL, Luo Y, Mitnick EH, Slavney A and Giammar DE. Metal release and speciation changes during wet aging of coal fly ashes. Environmental Science and Technology 2012; 46: 11804-11812. http://dx.doi.org/10.1021/es302807b
- [42] Wang Y, Liu J, Wang G and Wang J. The influence of freeze/thaw cycles and water content on the form transformations of cadmium in black soils. China Environmental Science (in Chinese with English abstract) 2007; 27(5): 693-697.
- [43] Xiao Z, Yuan X and Li H. Chemical speciation, mobility and phyto-accessibility of heavy metals in fly ash and slag from combustion of pelletized municipal sewage sludge. Science of the Total Environment 2015; 536: 774-783. http://dx.doi.org/10.1016/j.scitotenv.2015.07.126
- [44] Donner E, Schecke K, Sekine R, et al. Non-labile silver species in biosolids remain stable throughout 50 years of weathering and ageing. Environmental Pollution 2015; 250: 78-86. http://dx.doi.org/10.1016/j.envpol.2015.05.017
- [45] Yang J, Zhu S, Zheng C, et al. Impact of S fertilizers on porewater Cu dynamics and transformation in a contaminated paddy soil with various flooding periods. Journal of Hazardous Materials 2015; 286: 432-439. http://dx.doi.org/10.1016/j.jhazmat.2015.01.035
- [46] Zheng SA, Zheng XQ and Chen C. Transformation of metal speciation in purple soil as affected by waterlogging. Internastional Journal of Environmental Science and Technology 2013; 10(2): 351-358. http://dx.doi.org/10.1007/s13762-012-0146-3
- [47] Kimball BE, Foster AL, Seal RR, et al. Copper speciation in variably toxic sediments at the Ely copper mine, Vermont, United States. Environmental Science and Technology 2016; 50(3): 1126-1136. http://dx.doi.org/10.1021/acs.est.5b04081

Received on 06-04-2016

[48] Xie M, Jarrett BA, Da SC, et al. Coupled Effects of Hydrodynamics and Biogeochemistry on Zn Mobility and Speciation in Highly Contaminated Sediments. Environmental Science and Technology 2015; 49(9): 5346-5353.

http://dx.doi.org/10.1021/acs.est.5b00416

- [49] Li R, Zhao W, Li Y, et al. Heavy metal removal and speciation transformation through the calcination treatment of phosphorus-enriched sewage sludge ash. Journal of Hazardous Materials 2015; 283: 423-431. http://dx.doi.org/10.1016/j.jhazmat.2014.09.052
- [50] Weng H, Ma X, Fu F, et al. Transformation of heavy metal speciation during sludge drying: Mechanistic insights. Journal of Hazardous Materials 2014; 265: 96-103. <u>http://dx.doi.org/10.1016/j.jhazmat.2013.11.051</u>
- [51] Lou Z, Zhu N and Li A. Evolution processes of trace metal speciation in leachates with different ages from Laogang refuse landfill, Shanghai. Desalination and Water treatment. 2016; 57: 8583-8590. http://dx.doi.org/10.1080/19443994.2015.1019364
- [52] Fan C, Zhang Y and Wang J. Application of Tessier-AAS to the Non-Biological Transformation Mechanism of ChemicalSpeciation of Lead in Red Soil in Agricultural Area of Central China. Spectroscopy and Spectral Analysis 2015; 35(2): 534-538.
- [53] Shangguan Y, Qin X, Zhao D, et al. Migration and Transformation of Heavy Metals in Soils by Lysimeter Study with Field Condition. Research of Environmental Sciences 2015; 28(7): 1015-1024.
- [54] Prudêncio MI, Valente T, Marques R, Sequeira Braga MA and Pamplona J. Geochemistry of rare earth elements in a passive treatment system built for acid mine drainage remediation. Chemosphere 2015; 138; 691-700. http://dx.doi.org/10.1016/j.chemosphere.2015.07.064
- [55] Valente TM, Antunes M, Sequeira Braga MA, Prudencio MI, Marques R and Pamplona J. Mineralogical attenuation for metallic remediation in a passive system for mine water treatment. Environmental Earth Sciences 2012; 66 (1): 39-54. http://dx.doi.org/10.1007/s12665-011-1205-7
- [56] Hu B, Liang D, Liu J, et al. Transformation of heavy metal fractions on soil urease and nitrate reductase activities in copper and selenium co-contaminated soil. Ecotoxicology and Environmental safety 2014; 110: 41-48. <u>http://dx.doi.org/10.1016/j.ecoenv.2014.08.007</u>
- [57] Zheng M, Feng L, He J, et al. Delayed geochemical hazard: A tool for risk assessment of heavy metal polluted sites and case study. Journal of Hazardous Materials 2015; 287: 197-206. http://dx.doi.org/10.1016/j.jhazmat.2015.01.060

Accepted on 18-05-2016

Published on 31-07-2016

DOI: http://dx.doi.org/10.15377/2410-3624.2016.03.01.1

© 2016 Hui Zhang; Avanti Publishers.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<u>http://creativecommons.org/licenses/by-nc/3.0/</u>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.