Safety Assessment of Heavy Metals in Rice, Cultivated Habitats (Soil and Water, etc.) and Cooked Rice that may Arise from Environment

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Abstract— Heavy metals were analyzed in approximately 30,000 samples from agricultural, fisheries and livestock food sources in order to assess food safety in Korea from 2012–2014 as part of a project with Korean Ministry of Food and Drug Safety (KMFDS). This study reports the Pb, Cd, As (analyzed by ICP-MS and ICP-OES) and Hg (using gold amalgamation analysis) contents found in rice during these studies. Rice was the most widely studied food as it is a staple of the Korean diet.

Rice, rice seed, rice straw, rice root, the fertilizer used for cultivation and related environmental samples (soil and water) were sampled from cultivation sites for analysis. Analysis was also performed to determine changes in heavy metal contents during the cooking process. Water was analyzed after rinsing rice once, twice, three times, and soaking rice for 30 minutes. Rice that had been boiled for 30 minutes was also analyzed.

Sampling and analytical methods used were carried out in accordance with the Republic of Korea Food Code and Standards Act. The environmental samples were referenced to USEPA. Validation was carried out using QA / QC and the analyses passed a FAPAS test for external quality assurance.

We analyzed the correlation between rice heavy metal concentrations, agricultural products and the environmental surroundings. As and Cd concentrations in the rice root were found to be several times larger than in the surrounding soil and fertilizer used, while Pb concentrations were lower. However, these concentrations were lower in the final rice grain. We also found that rinsing removed As, but there was little added benefit after 2 or 3 rinses.

Index Terms— rice, soil, heavy metals, environment contamination, cooked rice, analysis

I. INTRODUCTION

Persistent heavy metals such as Lead, cadmium, and inorganic arsenic are known to be carcinogenic and pose many other health risks, for example with the respiratory and nervous systems[1]. Contaminants in the environment are concentrated as they move up the food chain through bio-magnification. Therefore, it is important to assess environmental contamination in order to ensure food safety and can therefore pose an even greater risk to human health [2].

The study also showed that plants grown in a heavy metal rich environment accumulated significant amounts of heavy metals through biochemical processes and were damaged [3].

Heavy metals also disturb plant metabolism, affecting respiration, phytosynthesis, stomate opening and plant growth [4]. Movement of heavy metals from soil to plant represents the main source of human exposure to heavy metals is Korea.

Arsenic has a higher transfer rate to rice than fruits and vegetables, and it is known that increases in soil heavy metal content result in higher heavy metal concentrations in crops. Therefore, it is important to monitor environmental concentrations of heavy metals in order to assess health risks [5].
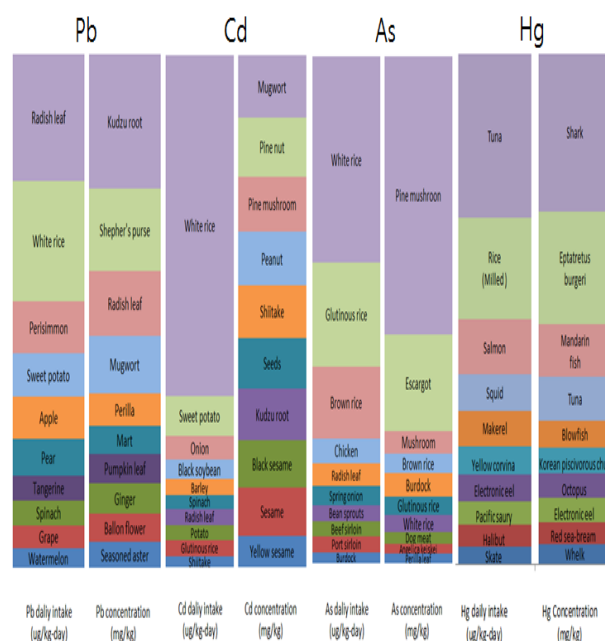

Fig.1. Top 10 Foods Consumed in Korea Ordered Based on Concentrations and Daily Intake of Pb, Cd, As and Hg (2012~2014, KMFDS)

During 2012-2014, Korean Ministry of Food and Drug Safety (KMFDS) conducted a heavy metal safety assessment using approximately 30,000 samples of raw food materials in Korea from the agricultural, fisheries and livestock sectors. This study concerns the Pb, Cd, As, and Hg contents of rice, which is a major staple of the Korean diet (Fig 1). Rice, rice seed, rice straw, rice root, the fertilizer used for cultivation and related environmental samples (soil and water) were sampled from cultivation sites in Korea for analysis. Analysis was also performed to determine changes in heavy metal

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contents during the cooking process. Water was analyzed after rinsing rice once, twice, three times, and soaking rice for 30 minutes. Rice that had been boiled for 30 minutes was also analyzed.

II. MATERIALS AND METHODS

A. Materials

We chose three sites in Gangneung (north eastern region of South Korea, Fig. 2) where local rice plantations were formerly mining areas. This rice was cultivated in 2014.
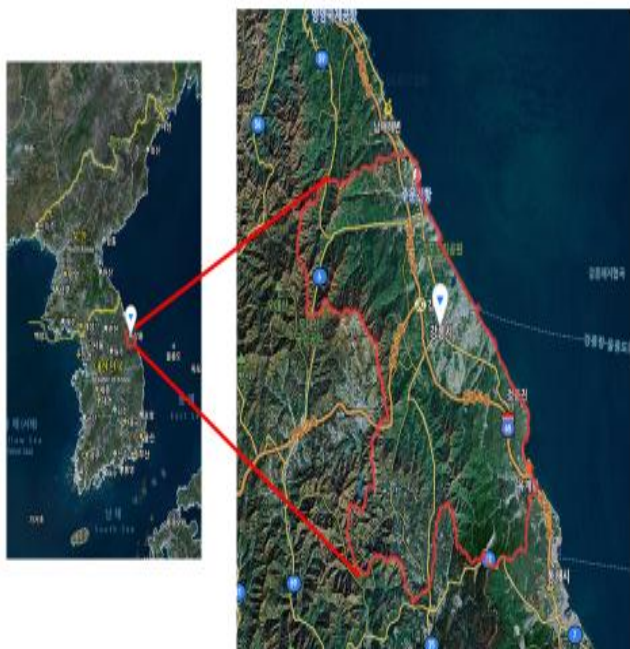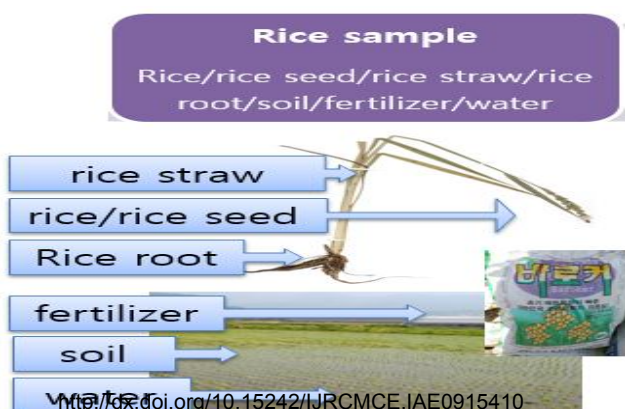

Fig.2. Map of Gangneung Sampling Sites, South Korea

Two kilograms each of rice, rice seed, rice straw, rice root, the fertilizer used for cultivation and related environmental samples (soil and water) were collected for monitoring (Fig 3).

Depending on the cooking method, rinsed once with water, rinsed twice with water, rinsed three times with water, 30 minutes referred to water, heating the rice for 30 minutes were monitored.

Before analysis, samples were homogenized finely in a blender and sieved with a 2 mm sieve, placed in polyethylene bags and stored in a freezer (-18 ℃ below). Soil was sampled at a depth of 15cm, air-dried, sieved (<2 mm) and stored in sealed polyethylene bags for storage before analysis.

Sampling and analytical methods used were carried out in accordance with the Republic of Korea Food Code and Standards Act. The environmental samples were referenced to USEPA.

Fig.3. Rice and Environmental Samples Used

B. Reagents and standards

For all analyses, special grade reagents and "Millipore water" (purified to 18 MΩ , Milli Q Genesis) were used. Lead, cadmium, arsenic and mercury reference standards were purchased from Merck (Merck KGaA, Damstadt, Germany). Pretreatment was conducted with 62% nitric acid (Dong Woo Fine Chem, Co. Ltd, lkan, Korea) , 37% hydrochloric acid (Dong Woo Fine Chem, Co. Ltd, lkan, Korea) and 30% hydrogen peroxide (Electronic grade), and all glassware was PTFE material.

For the validation of the analysis method, standard reference materials Bovine Liver (NIST 1577c), Oyster tissue (NIST 1566b) and Tomato leave (NIST 1573a) were used.

All glassware was immersed for 24 hours in 10% nitric acid and then washed with distilled water before use.

C. Heavy metals analysis of environmental samples

Soil samples were dried in air, crushed by hand or a rubber mallet, and sieved to 2 mm. Reducing the soil particle size increases the acid decomposition efficiency.

Approximately 0.5~1g of homogenized sample was placed into a Teflon vessel with 7 ml 62% nitric acid and 1 ml hydrogen peroxide, and digested in a microwave digestion system (ETHOS, Milestone, Italy; Table Ⅰ, Fig 4).

TABLE Ⅰ
MICROWAVE CONDITIONS

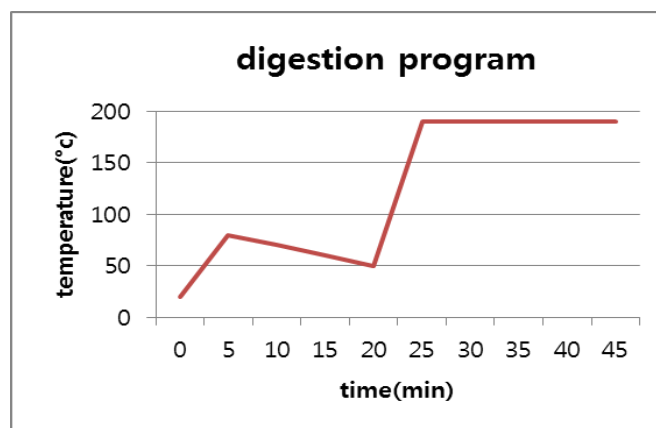| Step	Conditions
Preparation	• 0.5~1g sample material + 62 % HNO$_3$ (7 ml) + 30 % H$_2$O$_2$ (1 ml) • Heated to 60℃ for 10 min then 80℃ for 25 min, then 2 ml Milli Q water was added • Vessel was sealed
Digestion	• step.1 5 min. 80℃, 1000 watt • step.2 5 min. 50℃, 1000 watt • step.3 15 min. 190℃, 1000 watt • step.4 20 min. 190℃, 1000 watt
Completion	• Cool down • Top up to 25 ml using Milli Q water

Fig.4. Microwave Digestion Program

Pb, Cd and As were analyzed by ICP-MS (ELAN DRC-II, Perkin-Elmer, Waltham, MA, USA) and by ICP-OES (Varian 720ES, Agilent, US) using masses 208, 114, and 75

respectively. Settings are listed in Table Ⅱ.

TABLE Ⅱ
ICP-OES & ICP-MS CONDITIONS

METHOD		ICP-OES	ICP-MS
Plasma (W)		1150	1500
Coolant flow (L/min)		12	17
Nebulized flow (L/min)		0.70	1.05
Auxiliary flow (L/min)		0.5	2.00
Sample Introduction System / torches	Sample uptake flow (mL/min)	1.00	1.00
	Spray chamber	Cychronic type	Cychronic type
	Nebulizer	Concentric type	Concentric type
	Torch	EMT Duo	Demountable
Analyte	Pb Mass	-	206,208
	Cd Mass	-	112,114
	As Mass	-	75

Mercury was analyzed using gold amalgamation with a mercury analyzer (DMA80, Milestone, Vergamo, Italy). Samples of 0.1 g were analyzed under the conditions listed in Table Ⅲ.

TABLE Ⅲ
DMA CONDITIONS

Description	Conditions
Working range	0.1~100 ng
Wavelength	253.7 nm
Dry time	70 seconds
Drying temperature	200 ℃
Decomposition time	3 minutes
Decomposition temperature	650 ℃
Purge time	60 seconds
Amalgamator time	12 seconds
Signal recoding time	30 seconds

D. Cooking process

Heavy metal leachates in Milli Q water were monitored after rinsing rice once, twice, three times, and soaking for 30 min. Rice was also analyzed after boiling in Milli Q water for 30 min. Analytical methods were the same as those described above.

III. RESULTS AND DISCUSSION

A. Validation

Internal quality assurance (NIST standards listed in section II B) and external quality control (performed on a periodic basis by FAPAS) were assessed by QA / QC. Table IV lists the measurement parameters for limit of detection (LOD) and limit of quantification (LOQ) considered for reference with Toxic Substance Monitoring Guidelines 2012.

The calibration curves were built on 8 different concentrations, so that concentrations of all analytes in the samples were within linear range of calibration curves. Measurements were carried out using the full quantitative analysis mode.

TABLE Ⅳ
DETECTION LIMIT

	Toxic Substance Monitoring Guidelines	British Standard	Kaiser's equation
LOD / LOQ	$LOD = 3\sigma$, $LOQ = 10\sigma$, σ = standard deviation	$LOD = 3\sigma$, $LOQ = 6\sigma$, σ = standard deviation	$Kaiser's\ eq = \dfrac{k \times RSD\ of\ blk \times Conc.\ of\ STD}{\frac{intensity\ of\ STD - intensity\ of\ blk}{intensity\ of\ blk}} \times 100$ k = constant RSD = relative standard deviation SBR = signal to background ratio
Ref.	(KMFDS, 2012. 09)	BS EN 15763:2009	R.H. Kaiser, Anal.chem, 42, 53A(1987)

Internal quality control results are listed in Tables V, VI and VII. The external quality control United Kingdom FAPAS test received a satisfactory grade. Results are shown in Table VIII.

TABLE Ⅴ.
NIST CRM OYSTER TISSUE(1566B)

Analyte	Reference (mg/kg)	Estimate (mg/kg)	RSD (%)	Recovery (%)
Pb	0.308	0.314	1.08	101.4
Cd	2.48	2.419	1.35	103.7
As	7.65	7.337	2.18	100.7
Hg	0.037	0.034	2.01	94.6

TABLE Ⅵ
NIST CRM TOMATO LEAVE(1573A)

Analyte	Reference (mg/kg)	Estimate (mg/kg)	RSD (%)	Recovery (%)
Cd	1.52	1.430	1.00	94.1
As	0.112	0.115	0.69	102.4
Hg	0.034	31.77	1.55	93.4

TABLE Ⅶ
NIST CRM BOVINE LIVER(1577C)

Analyte	Reference (mg/kg)	Estimate (mg/kg)	RSD (%)	Recovery (%)
Pb	62.8	59.87	1.55	95.34
Cd	97	98.77	1.42	101.8
As	19.6	20.37	2.52	103.81
Hg	5.36	5.19	0.27	96.83

TABLE VIII
FAPAS RESULTS

Analyte	Milk Powder (2014.06)	Metallic Contaminants in Offal (Liver) (2014.10)
Pb	0.4	1.2
Cd	-0.4	-1.1
As	-0.1	1.4
Hg	0.1	0.4

*The satisfactory range, i.e. lZl<2

B. Results

Measurement uncertainties presented in the figures were within ±3%. Measurements are summarized in Table IX. Figure 6 shows the heavy metal contents of the raw materials studied. The water showed very low levels of all heavy metals and therefore cannot be considered as a source for the rice. While both the fertilizer and soil samples had very elevated levels of Pb, Cd and As compared to the water, they were within national average levels for soil in Korea [5]. Of interest is that both As and Cd concentrations in the rice root were larger than in both the soil and fertilizer, while the Pb concentration was slightly lower. Furthermore, the heavy metal concentrations continued to decrease from root to straw to seed to rice.

As rice is rinsed further, the As continues to be leached out. The data suggests that, after 2 or 3 rinses, there is little added benefit to further washing before boiling.

Figure 5 shows the concentration trends during rice preparation.

TABLE IX
ANALYSIS RESULTS

| Samples (n=3)	Pb(ppb)	Cd(ppb)	As(ppb)	Hg(ppb)
cooked rice	2.20	1.78	24.55	0.0509
soaked for 30 min.	1.73	0.67	28.32	0.101
rinsed three times with water	2.18	1.12	23.65	0.102
twice rinsed with water	1.56	1.31	24.31	0.102
once rinsed with water	1.73	0.70	28.73	0.102
rice	7.48	7.17	128.9	0.0501
Rice seed	97.07	12.52	218.62	0.0500
rice straw	31.66	43.66	813.5	0.0500
rice root	5287.24	52.36	2065.77	0.0203
Fertilizer	5612.63	10.89	1215.53	0.0509
Soil	5931.54	9.95	544.1	0.0516
water	0	0.003	0.34	0.104
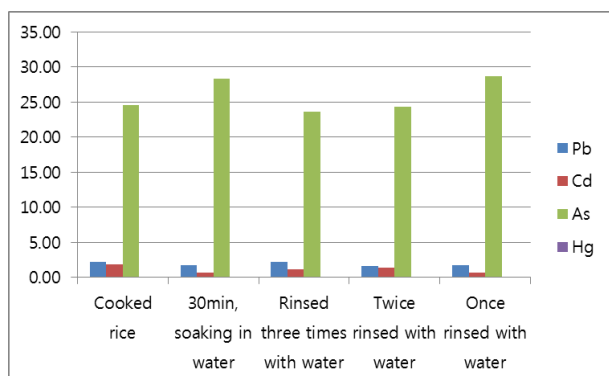
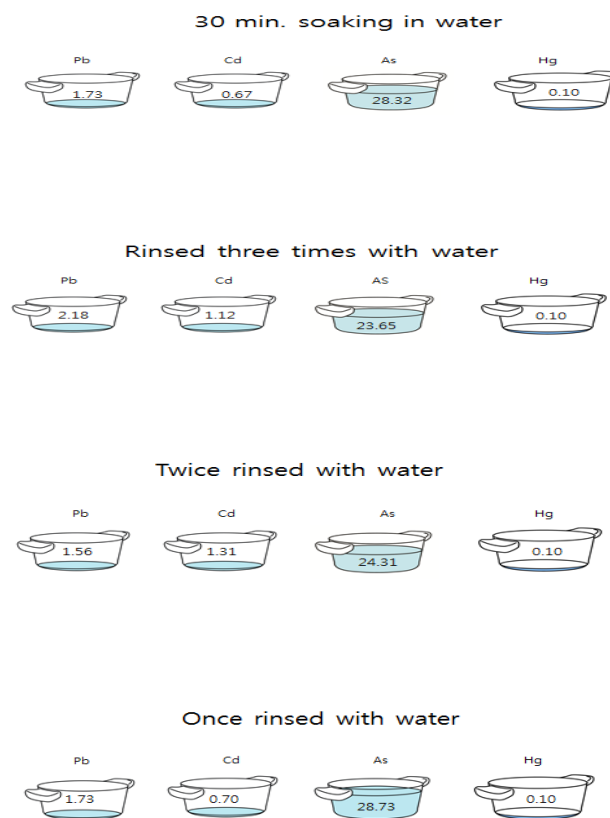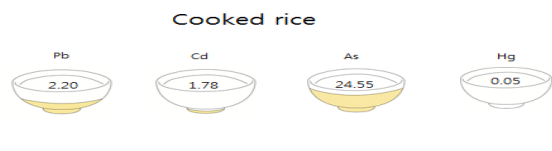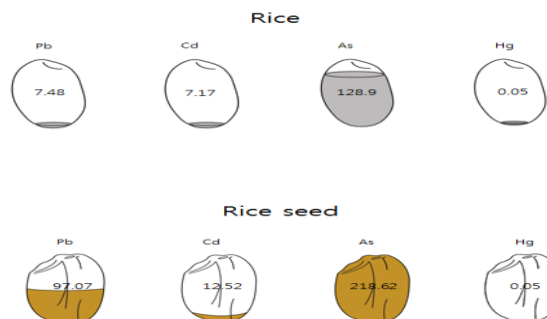Fig. 5. Heavy Metal Contents [ppb] of Cooking Process
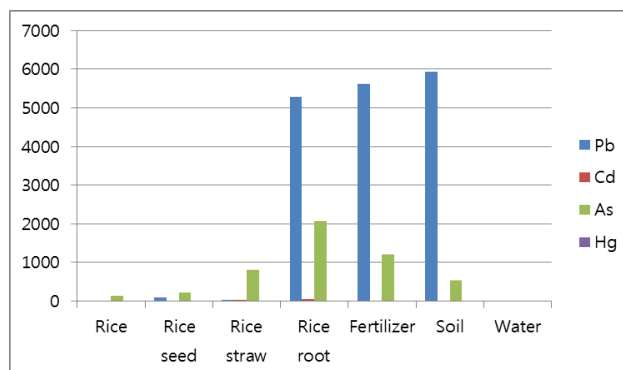
Fig. 6. Heavy Metal Contents [ppb] of The Raw Materials Studied

Rice straw

Pb 31.66 Cd 43.66 As 813.5 Hg 0.05

Rice root

Pb 5287.24 Cd 52.36 As 2065.77 Hg 0.02

Fertilizer

Pb 5612.63 Cd 10.89 As 1215.53 Hg 0.05

Soil

Pb 5931.54 Cd 9.95 As 544.1 Hg 0.05

Water

Pb 0 Cd 0.003 As 0.34 Hg 0.1
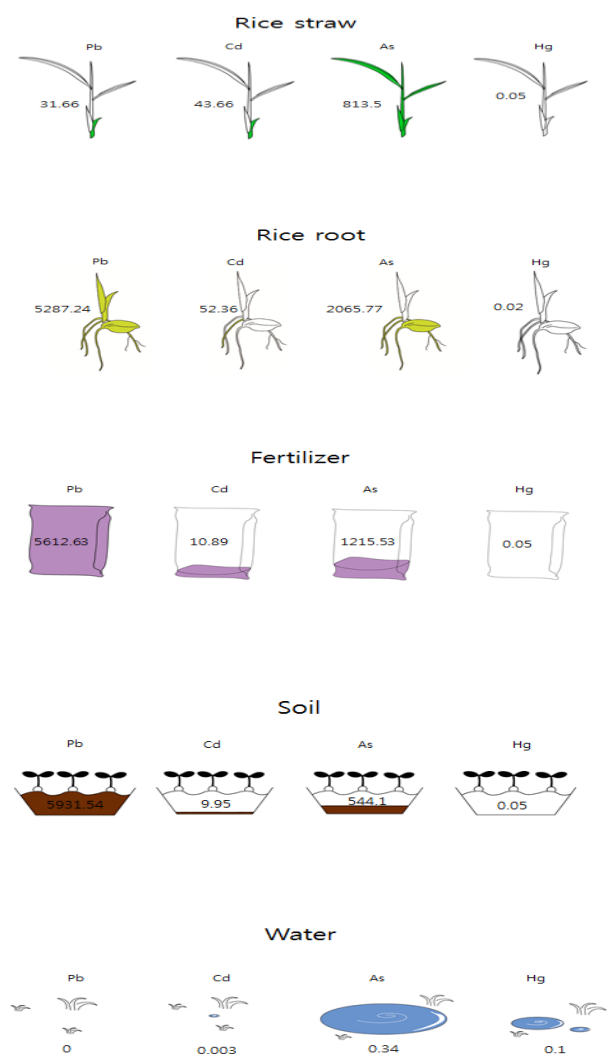
C. Conclusions

We analyzed the correlation between rice heavy metal concentrations, agricultural products and the environmental surroundings. As and Cd concentrations in the rice root were found to be several times larger than in the surrounding soil and fertilizer used, while Pb concentrations were lower. However, these concentrations were lower in the final rice grain. We also found that rinsing removed As, but there was little added benefit after 2 or 3 rinses.

REFERENCES

[1] WHO(World Health Organization). Arsenic and Arsenic Compounds. Environmental Health Criteria. 2001.224. Geneva, Switzerland.

[2] S.M.Han, S.W.Kang, H.J.Kim, D.H.Kim, *The Annual Report of Busan Metropolitan city Iinstitute of Health & Environment*, 2012. 22(1), 201-215

[3] Atanasiu L., (1984), Plants Ecophysiology, (in Romanian), Scientific ans Encyclopedic Press, Bucharest, Romania

[4] Samical, A.I., Hotea, V., Oros, V., Juhasz, J., Pop, E., 2008. Studies on transfer and bioaccumulation of heavy metals from soil into lettuce, Environ. Eng. Manage. J. 7 (5), 609-.615.

[5] Ji-Young Kim, Ji-Ho Lee, Anitha Kunhikrishnan, Dae-Won Kang, Min-Ji Kim, Ji-Hyock Yoo, Doo Ho Kim, Young-Ja Lee, Won Il Kim, 2012. Transfer Factor of Heavy Metals from Agricultural Soil to Agricultural Products. 31. NO.4, 300-307. Korean J Environ Agric.

[6] Brun, L.A., Maillet, J., Richarte, J., Herrmann, P., Remy, J.C., 1998. Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils, Environmental Pollution. 102, 151-161.

http://dx.doi.org/10.1016/S0269-7491(98)00120-1

[7] Cao, H., Chen, J., Zhang, J., Zhang, H., Qiao, L., Men, Y. 2010. Heavy metals in rice and garden vegetables and their potential health risks to inhabitants in the vicinity of an industrial zone in Jiangsu, China, J. Environmental Sciences. 22(11), 1792-1799.

http://dx.doi.org/10.1016/S1001-0742(09)60321-1

[8] Cui, Y.J., Zhu, Y.G., Zhai, R.H., Chen, D.Y., Hung, Y.Z., Qui, Y., 2004. Transfer of metals from soil to vegetables in an areas near a smelter in Nanning, China Environ. Int. 30 (6), 785-791.

http://dx.doi.org/10.1016/j.envint.2004.01.003

[9] Huang, R., Gao, S., Wang, W., Staunton, S., Wang, G, 2006. Soil arsenic availability and the transfer of soil arsenic to crops in suburban areas in Fujian Province, southeast China, Sci Total Environ. 368, 531--541.

http://dx.doi.org/10.1016/j.scitotenv.2006.03.013

[10] Jung, G.B., Kim, W.I., Lee, J.S., Shin, J.D., Kim, J.H., Yun, S.G., 2005. Availability of heavy metals in soils with different characteristics and controversial points for analytical methods of soil contamination in Korea, Korean J. Environ. Agric. 19(2), 128-133.

[11] Kim, J,Y., Choi, N.G., Yoo, J.H., Lee, J.H., Lee, Y.G., Jo, K.K., Lee, C.H., Hong, S.M., Im, G.J., Hong, M.K., Kim, W.I., 2011. Monitoring and Risk Assessment of Cadmium and Lead in Agricultural Products, Korean J Environ Agric. 30(3), 330-338.

http://dx.doi.org/10.5338/KJEA.2011.30.3.330

[12] K.S.Chung, B.-I.Choi, S.Y.Kim, D.Y.Song, K.H.Kim, H.J.Seong, 1999. Dissolution Properties for the Inorganic Pollutants in Soils, Korea. Analytical science & technology, 12(1).

[13] S.W.Park, M.Y.Yoon, J.K.Kim, B.J.Park, W.I.Kim, J.D. Shin, O.K.Kwon, and D.H.Chung, Rice Safety and Heavy Metal Contents in the Soil on "Top-Rice" Cultivation Area, South Korea, J.Fd Hyg. Safety, 2007, 23(3), 239-247.

[14] Y.H Lee, S.T Lee, J.Y Heo, M.G Kim, K.P Hong, E.S Kim, W.D Song, C.W Rho, J.H Lee, W.T Jeon, B.G Ko, K.A Roh, S.K Ha, 2010, Monitoring of Heavy Metal Contents from Paddy Soil in Geongnam Province, South Korea, Korean J. Soil Sci. Fert. 43(3), 289-295.

[15] S.Y.Kim, M.Sidharthan, Y.H.Yoo, C.Y.Lim, J.H.Joo, J.S.Yoo, H.W.Shin, *Algae*, 2003.18(4), 349-354.

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