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A Study on Dioxin Contamination in Herbicide Sprayed Area in Vietnam by GIS

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1. Introduction

During the Vietnam war (1961 – 1971), the United States military forces carried out the operation named Ranch Hand that sprayed over 19.5 million gallons of herbicide for defoliation over wide areas of forests and crops in Vietnam, Laos and Cambodia to deny their use by opposition force (Westing, 1984; Stellman et al., 2003a). Two thirds of the chemical herbicides used was Agent Orange, a 50:50 mixture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlophenoxyacetic acid (2,4-D) herbicides (IOM 2002). The defoliant 2,4,5-T was contaminated with an extremely toxic substance, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) known to have adverse effects on human health.

The Vietnam war ended over 35 years ago. There are still adverse effects of herbicide residues on people who lived in sprayed area and also its ecosystems (Schecter et al., 2001, 2003; Cau et al., 2003; Mai et al., 2007). In environmental health research, there is a recognized need to develop methodologies to carry out epidemiologic research. The geographic information system (GIS) is a technology which can improve the study of dioxin in Vietnam to estimate people's herbicide exposure. GIS can also combine both extensive and intensive databases on dispersal of herbicides, locations of US army military units and bases, locations of civilian population centers in Vietnam, then provide a unique basis to integrate and improve epidemiologic studies (Stellman et al., 2003b; Waring et al., 2005; Viel et al., 2008). GIS-based exposure assessment has been used in a small number of

epidemiologic environmental studies (Rushton, 2003). They can efficiently integrate records of where herbicides were used.

Results of investigations in a sprayed site, A Luoi Valley in southern Vietnam, demonstrate the apparent food chain transfer of TCDD from contaminated soil to cultured fish pond sediments to fish and duck tissues, then to humans as measured in whole blood and breast milk. A Luoi Valley is considered a microcosm of southern Vietnam, where the soils have been contaminated by numerous reservoirs of TCDD (Dwernychuk et al., 2002). Another study made clear that TCDD was increased in the blood of 19 of 20 persons taken from an Agent Orange contaminated site, Bien Hoa in southern Vietnam. TCDD levels among these persons, one of whom reached 271 ppt, was 135-times higher than among persons living in non-contaminated areas in northern Vietnam (Schecter et al., 2001). A project for searching dioxin hot spots was carried out in provinces in southern Vietnam. The results showed that dioxin contamination in soil and sediment are higher than standard, and indentified specific US bases as hot spots (797 pg/g TCDD in Bien Hoa, 227 pg/g TCDD in Da Nang, 194 pg/g TCDD in Phu Cat) (Dwenychuk et al., 2005, 2006).

In 2001, a Japanese medical research team initiated studies on human impacts of herbicide spraying. The study has shown significantly higher dioxins levels of serum, breast milk and adipose tissues in inhabitants of sprayed areas (Quang Tri province) than those in non-sprayed area (Ha Tinh province), while no significant difference was found on early indicators of adverse health effects such as liver or thyroid function and immunological activities. Dioxin levels in breast milk of people in the sprayed area are significantly higher than those in non-sprayed areas (Tawara et al., 2006). The study on mothers revealed high dioxin levels in breast milk. Kido et al.(2006) has shown visual acuities of both eyes of people in sprayed areas were also significantly lower than those in non-sprayed area at every condition with change of contrast from 100 to 2.5 % except for 2.5 % contrast of the left eye, the simple relationships between dioxin levels and visual acuity were shown as mild in both eyes.

In the world and Vietnam there have been numerous studies. They have shown the hazards of dioxin on the environmental and human health. However, some studies remain limited with respect to subject of study, sample size, method of study and difficulties in chemical analysis, etc. In addition, there were not many studies applying GIS to the study of dioxin levels in soil and breast milk in Vietnam.

The main objective of this study was to assess the correlation between dioxin concentration in soil, sediment and breast milk of females in Cam Chinh commune, Quang Tri province, Vietnam. Herbicides that contained high concentration of dioxin were sprayed during the Vietnam war.

2. Materials and methods

2.1 Study area

This study was implemented in two areas where herbicides were sprayed and not sprayed. A herbicide sprayed area in Cam Chinh commune in Quang Tri province was chosen as a case site. Quang Tri province received 150 herbicide missions with a quantity of 6602 l/hectare (10-80 committee, 1999; Vietnam Investment Review, 1999). Quang Tri borders the demilitarized zone along the 17th parallel that once divided the north from South Vietnam. The control site was Cam Phuc commune in Ha Tinh province, which did not experience herbicide operations during the war.

2.2 Estimation of spatial distribution of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)

If a mother's house or residential area was more polluted with PCDD/Fs, the mother will have higher level of dioxins in her body. With 16 soil samples and one sediment sample, spatial distribution of PCDD/Fs was estimated by Kriging interpolation method. There are some detailed methods in Kriging interpolation (Armstrong et al., 1988), however, Saito et al., (2000) referred "log-normal Kriging" consistently yields the best result for concentration of dioxin.

Kriging interpolator is a specialized interpolation method that assumes the distance or direction between sample points and shows spatial correlation that helps describe the surface (Largueche, 2006). The initial step, a list of mothers whose breast milk was collected from lactating females aged between 20-40 years old, was made at Quang Tri in 2002-2003; these breast milk samples were analyzed in the laboratory of Kanazawa Medical University, Japan. All mother's house locations were checked by GPS (Global Positioning System). GPS data have been coded as latitude (Y) and longitude (X) then located on the map in accordance with the position of their residence (Fig 2). Soil and sediment samples were taken in the same area (Fig1) and also analyzed in the Japanese laboratory. These data have been used to create a surface contour of dioxin of this area by using Geostatistical Analyst, one of the extension systems of ArcGIS.

2.3 Soil and sediment sample sites

The soil and sediment samples were taken randomly around Cam Chinh commune of Quang Tri province. Sixteen soil samples and one sediment sample were collected throughout Cam Chinh commune with a stainless steel core sampler. Cores were of the 0-10 cm depth fraction (Dwernychuk et al., 2002). At any give site, ten cores were collected, composited, and thoroughly mixed to represent a single sample for laboratory analysis. The location of each sample was geo-referenced using global positioning system (GPS). The locations of sampling sites are shown in Fig 1.

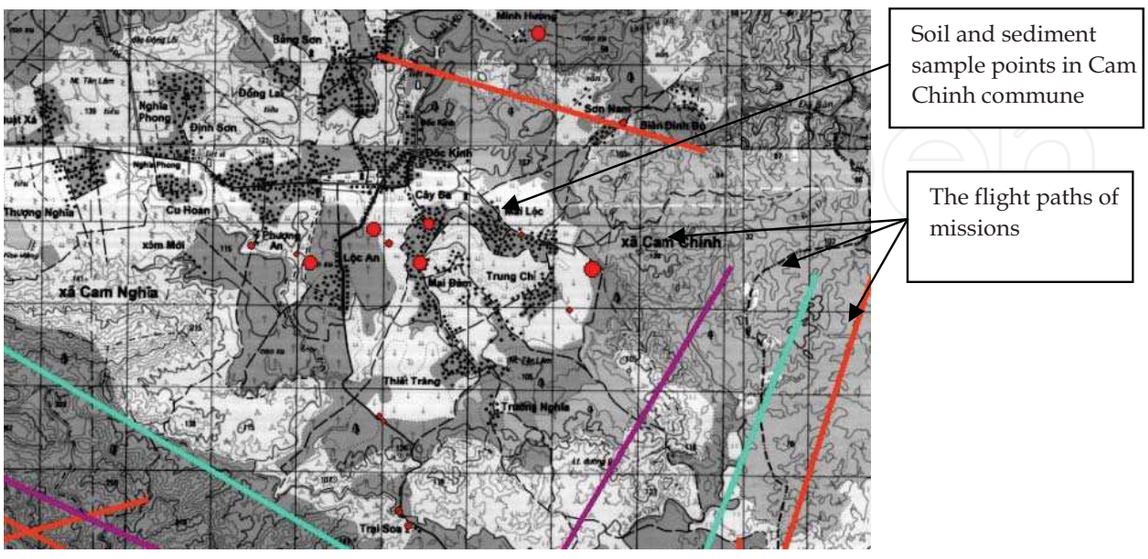

Fig. 1. Location of soil and sediment sample sites in Cam Chinh commune

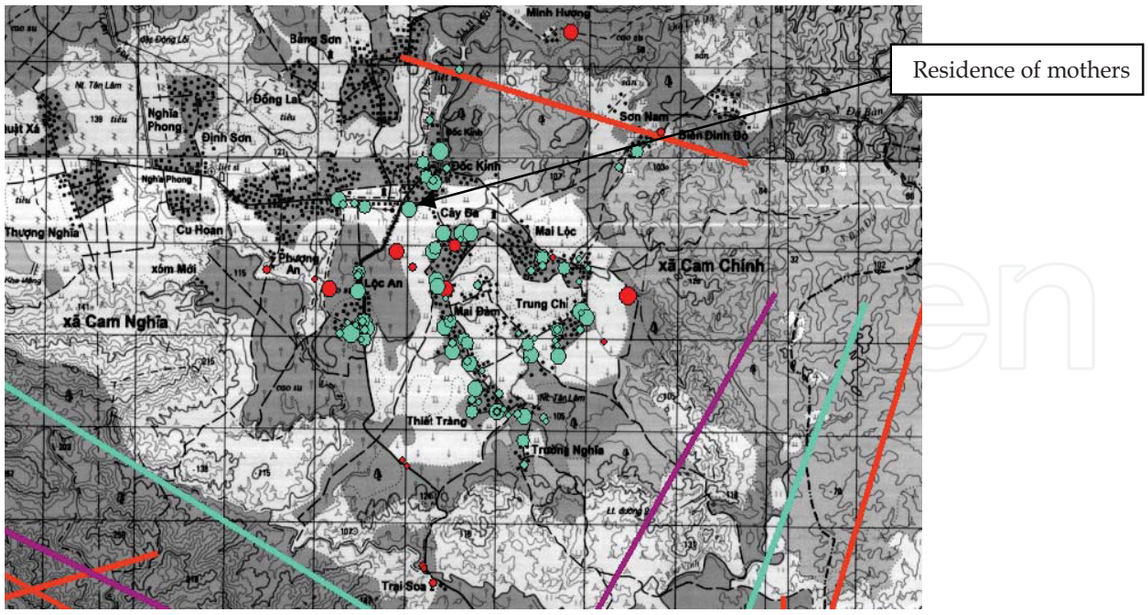

Fig. 2. Overlaid GPS data of breast milk samples in Cam Chinh map

2.4 Breast milk sampling site

In September 2002 and July 2003, breast milk samples were taken from lactating mothers aged between 20-40 years old in two communes. The study purpose was explained to the eighty-six lactating mothers in Cam Chinh commune and seventy-one lactating mothers in Cam Phuc commune by local authorities and medical staff. In sprayed area, 64 mothers lived in Cam Chinh commune, and 12 mothers were born in Cam Nghia commune but got married in Cam Chinh commune. Cam Nghia commune is near Cam Chinh commune; it is also one of the communes that was sprayed during the war. In the non-sprayed area, 71 mothers lived in Cam Phuc commune. All lactating mothers consented to cooperate to donate milk samples. The mothers were breast-feeding their infants aged from 20 days to one year. Donors provided 10-20 ml volume of milk. In each local clinic, samples were collected by the mothers themselves, and local medical staff. All samples were frozen immediately after collection. The residence of mothers was confirmed by GPS. The GPS data were coded as latitude (Y) and longitude (X) by an excel program and saved database. Dots were put on the map in accordance with position of their residence (Fig 2).

2.5 Survey method

A retrospective cohort survey, based on epidemiological interviewing was implemented. All lactating mothers in both communes who donated milk samples after participated in the survey participated. These mothers were interviewed directly by researchers of the 10-80 Division and Kanazawa University using a standard questionnaire to acquire information. Collected information during interview included personal habits like smoking, alcohol drinking, contraceptive drug use, history of pesticide contacting, disease history, number of pregnancies, age of each pregnancy and kinds of pregnant failures. Data were entered into computers using JMP® 6 software for analysis.

2.6 Analytic method

Statistic comparisons were made using chi-square and Wilcoxon signed rank test statistics for categorical variables and Turkey-Kramer HSD test for continuous variables (α=0.05 level). Odds ratios (OR) and 95% confidence intervals (95%) based on the chi-square and Wilcoxon signed rank test were calculated on epidemiology data.

All soil and sediment samples from Vietnam were frozen and brought to Ishikawa Prefectural Institute of Public Health and Environmental Science to analyze. Analysis methods of PCDD/Fs levels described in paper (Kakimoto et al., 2004; Oka et al., 2007). Breast milk samples were brought to the Center of High Technology of Kanazawa Medical University, Japan. All samples were spiked with ^{13}C-labelled surrogate standards prior to analysis. All extracts were subject to a series of chromatographic cleanup steps prior to analysis for PCDDs and PCDFs by a high resolution mass spectrometer (HRMS, HR-GC/MS; MA Station-JMS700, JOEL, Japan) equipped with a gas chromatograph (HP-6980), and measurements were performed by selected ion monitoring (SIM) method (Tawara et al., 2003; Nishijo et al., 2008). Concentration levels of dioxins were shown by actual measurement values and ones were converted to 2,3,7,8-TCDD toxic equivalents (TEQ), submitting the international Toxicity Equivalent Factor (TEF) of WHO-TEF in 1997 (Van den Berg et al., 1998), and WHO-TEF in 2005 (Van den Berg et al., 2006). For non-detectable (ND) and NDR (chromatographic peak was detected but did not meet quantification criteria) were not designated.

Maps representing summary findings of the study were generated using ArcGIS. The database was merged into the GIS software using a Kriging interpolation method to Geostatistical Analysis (Cattle et al., 2002; Wu et al., 2003). Distribution of dioxin contamination was estimated based on the soil samples. Dioxin levels in the soil of each mother's house were estimated and relation between the value of dioxin level in the soil of each mother's house and breast milk were analyzed by Kriging.

3. Results and discussions

The mean concentration of dioxin in soil of the sprayed area is 1.9 pg-TEQ/g; this value is significantly higher than 0.38 pg-TEQ/g in the non-sprayed area.

The reproductive cohort survey on mothers was carried out in two communes, one area sprayed with herbicide and another area not sprayed. The results on Table 1 show that mean dioxin level of breast milk in the sprayed area was significantly ($p<0.001$) higher than the non-sprayed area. A statistically significant difference was showed in mother's height and weight in non-sprayed area, this being higher than in sprayed areas, but BMI is not significant. The mothers have used pesticides in the non-sprayed area is significantly ($p<0.001$) higher than sprayed area, but other chemicals are not significant between the two areas. All mothers in the two areas do not use alcohol, and three mothers in the sprayed area smoke (3.6%). Cousins who have birth defects in the sprayed area were 15.2% and non-sprayed area 10.6%, and is not significant. Present disease of mothers in sprayed area (22.1%) was significantly ($p<0.05$) higher than non-sprayed area (8.6%). Reproductive failure history in sprayed area (22.1%) was significantly ($p<0.01$) higher than non-sprayed area (5.6%).

This study is to assess the correlation between dioxin contamination levels in soil and dioxin exposure levels in breast milk of females in Cam Chinh commune, Quang Tri province of Vietnam. One of the GIS technologies of Kriging interpolation method was used to check the

		Sprayed area		Non-sprayed area		p-value
		N	Mean ±SD or N (%)	N	Mean ±SD or N (%)	
Age	(years)	86	29.3 ± 5.7	71	26.1 ± 4.3	***[b]
Height	(cm)	79	152.4 ± 4.6	54	154.1 ± 5.5	*[b]
Weight	(kg)	85	43.6 ± 3.9	66	45.8 ± 4.0	**[b]
BMI	(kg/m^2)	78	18.8 ± 1.5	54	19.4 ± 1.8	n.s[b]
Contact with herbicides during the war	Yes	82	4(4.9%)	71	0 (%)	-
	No		71(86.6%)		71 (100%)	
	Don't know		7(8.5%)		0 (%)	
Using pesticides	Yes	82	7(8.5%)	68	31 (45.6%)	***[c]
Using other chemicals	Yes	84	2(2.4%)	65	0 (%)	-
Alcohol habit	Yes	83	83(100%)	69	69(100%)	-
Smoke status	Yes	83	3(3.6%)	71	0 (%)	-
Using contraceptive	Yes	80	3(3.8%)	65	1 (1.5%)	n.s[c]
Cousin who is birth defect people	Yes	79	12(15.2%)	66	7 (10.6%)	n.s[c]
Cousin have birth defect	(People)	12	1.3 ± 0.9	7	1 ± 0	n.s[b]
Present disease	Yes	86	19(22.1%)	70	6 (8.6%)	*[c]
Reproductive failure history	Yes	86	19(22.1%)	71	4 (5.6%)	**[c]
TEQ-PCDDs	[pgTEQ/gFat][a]	86	5.1 ± 3.2	71	2.1 ± 0.9	***[b]
TEQ-PCDFs	[pgTEQ/gFat][a]	86	6.0 ± 4.0	71	2.0 ± 0.8	***[b]
TEQ-TOTAL	[pgTEQ/gFat][a]	86	11.3 ± 7.0	71	4.2 ± 1.6	***[b]

[a]Data of dioxin levels were log transformed
[b] Wilcoson signed rank test, ***:p<0.001
[c] Chi-square
*:p<0.05; **: p<0.01; ***: p<0.001, n.s: not significant

Table 1. Comparison of characteristics and dioxin levels in breast milk of the mothers between herbicide sprayed and non-sprayed areas.

correlation; however, no significant correlation appeared (Fig 4, 6). We suspect the reason for the lack of correlation was the extended period since the end of the war (35 years passed). The half-life of dioxin elimination in the human body, which is estimated at 7 – 11 years (Mukerjee 1998; Kerger et al., 2007), and in the soil environment is 28.5 – 274 years (Sinkkonen et al., 2000). The second reason was the rather low number of the samples; 16 for soil, one for sediment and 86 for breast milk are highly imbalanced. In contrast, no significant relationship means present environment status represented by dioxin levels of soil is not consistent with human exposure that is represented by breast milk. There is one possibility that another exposure route such as exposure due to herbicides during Vietnam war might affect dioxin levels of breast milk.

In addition, we also found that the distributions of PCDDs and PCDFs in soils were quite different by the Kriging interpolation analysis. More details in differences are presented below. On the Kriging map of PCDDs, no correlation was shown between dioxin in soil and breast milk (fig 4). We can see red points (locations of soil samples) which are dioxin levels in soil, and blue points (locations where mothers are living) which are dioxin levels in breast milk. Around the red big points (high dioxin level) are dark colors and red small points (low dioxin level) are light. PCDDs were thought to come from Agent Orange, given Agent Orange in the Quang tri area was sprayed 1965 – 1970 (IOM 1998) (Fig 3).

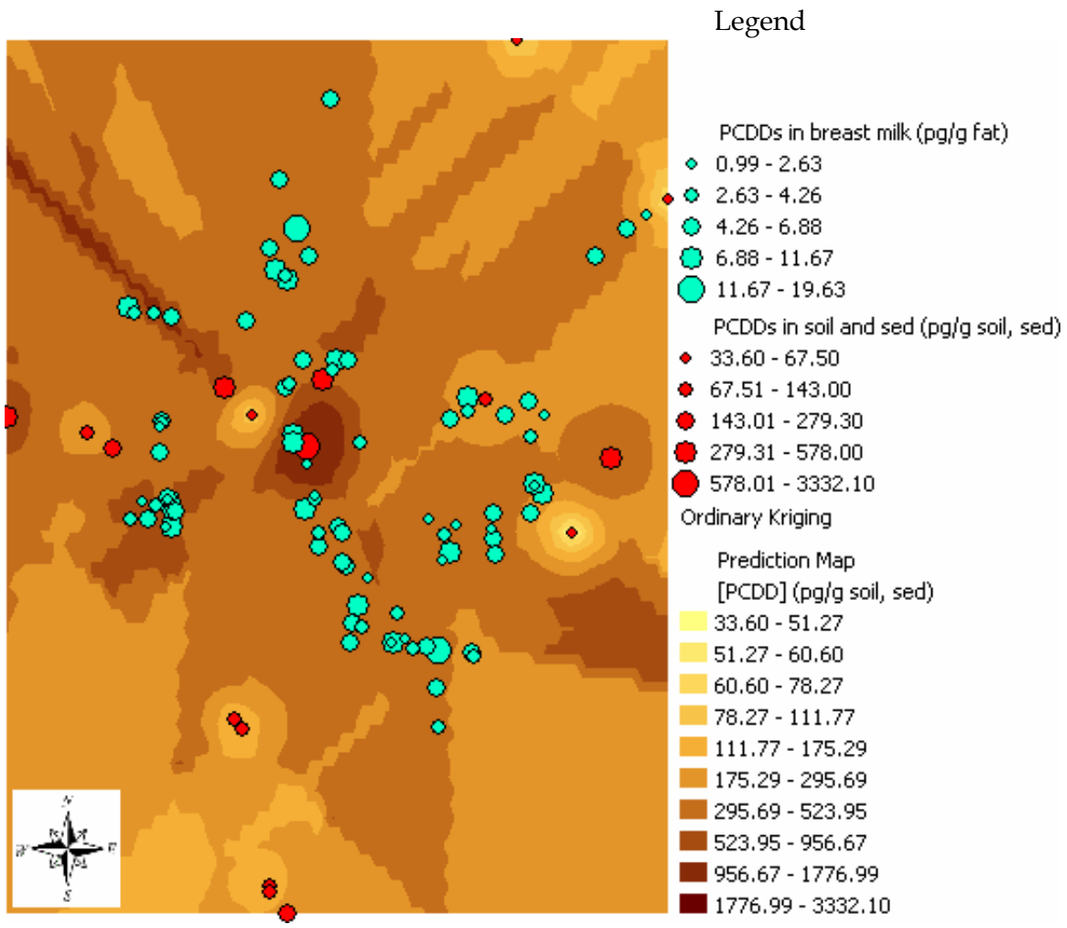

Fig. 3. Estimated distribution of PCDDs from soil samples by Kriging

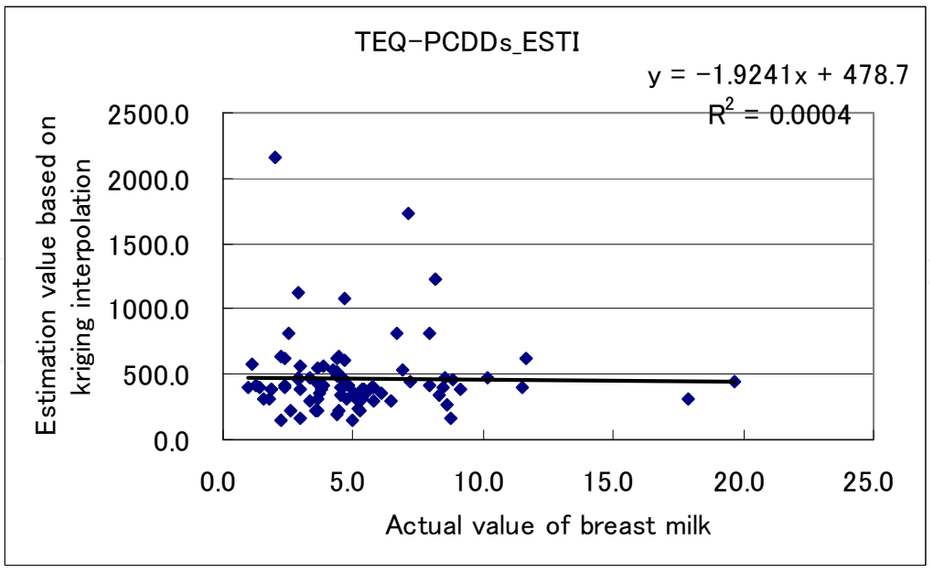

Fig. 4. Estimation value based on Kriging interpolation and actual value of breast milk

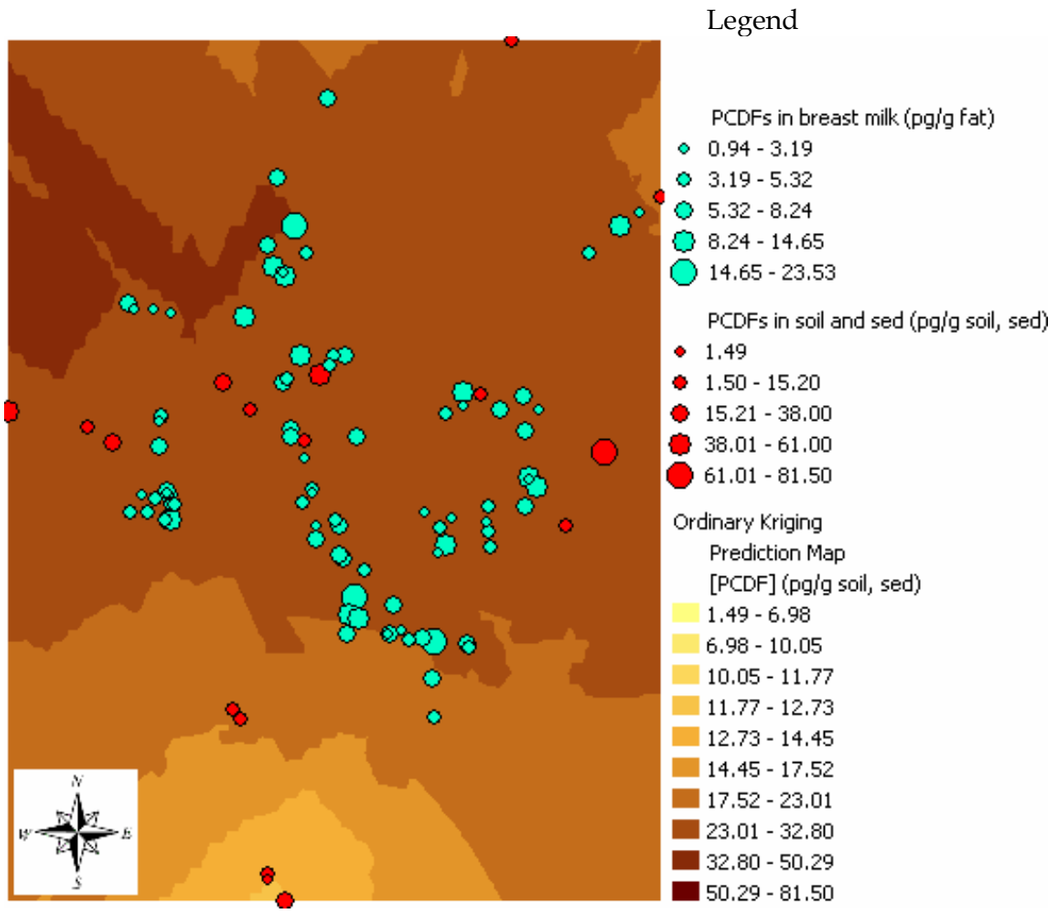

Fig. 5. Estimated distribution of PCDFs from soil samples by Kriging

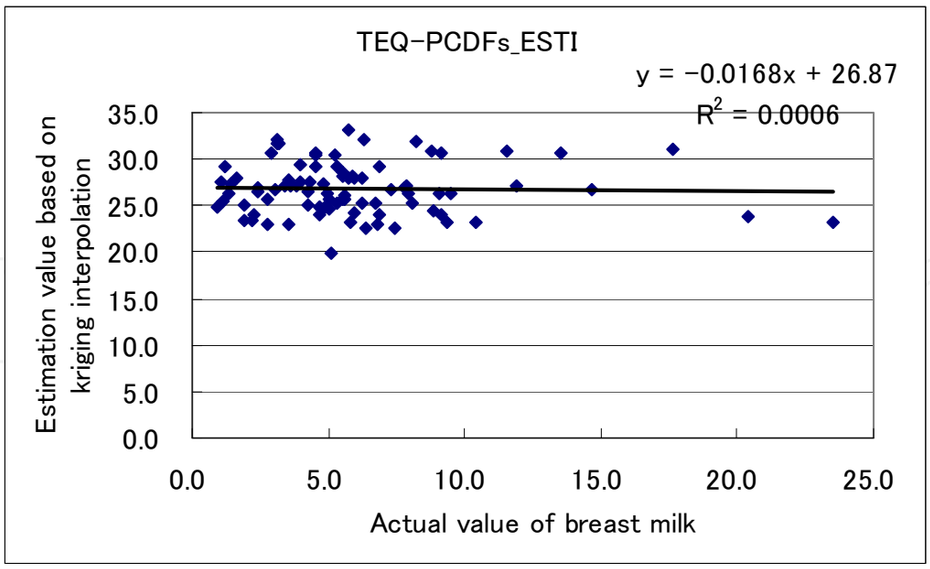

Fig. 6. Estimation value based on Kriging interpolation and actual value of breast milk

Results of the analysis show that PCDFs are not correlated with dioxin in soil and breast milk (Fig 6). On the Kriging map of PCDFs we can see red points which are dioxin levels in soil and blue points which are dioxin levels in breast milk (Fig 5). But location of red points is different with Kriging map of PCDDs and the dark color is also different, it is not around the high dioxin level in soil. PCDFs in this area are thought to come from herbicides other than Agent Orange sprayed during the war plus other chemical and pesticides applied after the war.
Reasons why we cannot detect clear correlation between estimated value of soil and breast milk follow: 1) duration after the war, 2) imbalanced alignment of soil samples, 3) larger area will be tested if people's immigration tendencies are taken into account. Then, more studies will be required to take a larger number of soil samples, which will be well-distributed in the study site including dioxin hot spots.

4. Conclusions

The concentrations of PCDDs, PCDFs in soil, sediment and breast milk samples collected in Cam Chinh commune, Quang Tri, Vietnam to assess correlations using Geostatistic algorithms of log-normal Kriging. Results showed that mean dioxin level of breast milk in sprayed areas was significantly higher than that in non-sprayed area. Significant correlations did not appear between estimated dioxin levels in soil by Kriging method and those in breast milk. There is one possibility that another exposure route such as exposure due to herbicides during Vietnam war might affect dioxin levels of breast milk. The distribution pattern of PCDDs and PCDFs is also quite different each other. More soil data should be needed to make more reliable geographical estimations.

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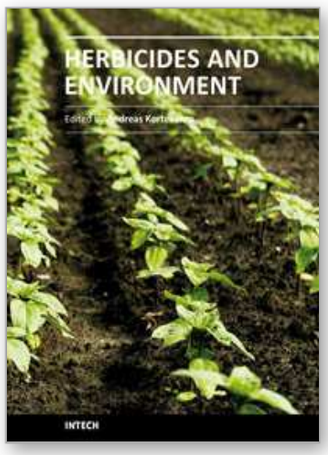

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Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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