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Health Risk Assessment of Paraquat Contaminated in *Spirogyra* spp. (tao) in Chiang Mai, Thailand

Preechaya Tajai*, Assawin Daducale and Sarunya Chuanphongpanich

Faculty of Pharmacy, Payap University, Chiang Mai 50000, Thailand; tapreechaya@gmail.com

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Abstract

Spirogyra spp. (tao) is freshwater green algae which are consumed as an uncooked food especially in the north of Thailand. Aquatic plants especially algae are easily contaminated by pesticide residues from soil and water. Paraquat (1,1′-dimethyl, 4,4′-bipyridinium dichloride; PQ), a widely used herbicide among Thai farmers, is toxic, mutagenic, and carcinogenic to mammals through ingestion, inhalation and skin contact. This study aims to determine the concentrations of paraquat contaminated in *Spirogyra* spp. (tao) and health risk assessment on the consumption of *Spirogyra* spp. (tao) in Chiang Mai province, Thailand. The levels of paraquat were analyzed by using a simple, sensitive, and reliable method which was high performance liquid chromatography (HPLC). Seven samples were collected from the different districts. The levels of paraquat contamination were 5.07±0.00 to 12.69±0.10 mg/kg. The results showed that the concentrations of paraquat in all samples were lower than the standard level of the European Commission Regulation. Risk assessment of paraquat found that the hazard quotient was in the range of 0.18-0.46. It indicated that the consumption of *Spirogyra* spp. (tao) was expected to have no adverse health effects. However, the consumption of *Spirogyra* spp. (tao) should be a concern in terms of chronic exposure to toxic herbicide contaminated in the environment.

**Author for correspondence*

1. Introduction

In Thailand, the quantities of imported agricultural pesticides including herbicides have been increasing every year[1-3]. Paraquat was the second most commonly imported herbicide[2]. Pesticides have been reported to cause several chronic health problems in farmers, humans living in rural communities, and the exposed people[4]. The levels of pesticides were monitored in the rivers of Thailand. They found the insecticide and herbicide residues in levels above the advisable limits in most water samples including paraquat[4,5]. Moreover, herbicides including paraquat were found in maternal and umbilical cord serum in pregnant women who live near agricultural areas or work in the agricultural fields[2,6].

Spirogyra spp. (tao) is freshwater green algae which are consumed as an uncooked food especially in the north and northeast of Thailand. It is locally known as tao or thao which is in Zygnemaceae family[7]. Green algae is rich in protein, carbohydrate, fat, and dietary fiber. It also has biological activities such as antioxidant effects[7,8]. Aquatic plants especially algae are easily contaminated by heavy metals and pesticide residues such as herbicides from soil and water[9]. Moreover, the study of heavy metals and pesticides accumulation in water uses macroalgae because of their ability to concentrate and tolerate high level of heavy metals and pesticides[10]. Aquatic plants such as algae concentrate high amount of paraquat from water which was found in *Chara* sp. and *Spirogyra* sp. (2300 mg/kg and 1300 mg/kg, respectively)[11].

Para-Quat (PQ) is one of the most widely used herbicides especially in developing countries including Thailand[12-14]. There are strong evidences that paraquat causes genotoxicity. Moreover, paraquat is a weak mutagen supported by the *in vitro* evidence. The mutagenic effect of paraquat stems from its ability to generate oxidative stress and subsequently oxidative DNA damage[13,15,16]. More recent population-based evidence showed that skin cancer may associate with paraquat exposure[17,18]. Paraquat may contribute to cancer by mutagenic mechanism[13]. In most foodstuffs, the maximum residue level of paraquat is 0.05 mg/kg and the acceptable daily intake is 0.005 mg/kg[19]. Thus, the levels of paraquat were analyzed by using a simple, sensitive, and reliable method which was High Performance Liquid Chromatography (HPLC).

This study aims to determine the concentrations of paraquat contaminated in *Spirogyra* spp. (tao) and risk assessment on the consumption of *Spirogyra* spp. (tao) in Chiang Mai province, Thailand. Apart from the benefits of *Spirogyra* spp. (tao) as food and antioxidant, this study may raise the concern about toxic effects associated with the consumption of algae with high paraquat content.

2. Materials and Methods

2.1 Instrumentation and Chemicals

Analysis was performed on an Agilent 1200 HPLC system with photodiode array detector (DAD) (Agilent Technologies, Fremont, CA, USA). HPLC-grade paraquat, triethylamine, 1-hexane sulfonic acid, orthophosphoric acid and ethyl viologen were purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA) and handled with carefulness by wearing protective clothing and gloves. All reagents were analytical grade. Paraquat standard solutions were prepared by dissolving paraquat with ultrapure water and diluting to proper concentrations.

2.2 *Spirogyra spp*. Collection, Preparation, and Extraction

The samples (*Spirogyra* spp.) were collected from the local areas in Chiang Maiprovince, Thailand. The collecting sites were located in 7 different districts (Figure 1). Sample 1 and 4 were collected from the lake and rice field, respectively (Doi Saket District). Sample 2 was collected from the rice field (Mae on District). Sample 3 was collected from the lake (Mae Rim District). Sample 5 was collected from the river (San Sai District). Sample 6 was collected from the rice field (San Pa Tong District). Sample 7 was collected from the rice field (Mae Taeng District).

Seven samples of *Spirogyra* spp. were cleaned with distilled water and dried by hot air oven at 60°C for 48 hours. Two grams of each *Spirogyra* spp. (tao) sample was weighed and grounded. Then, 20 ml of water was added and sonicated at 60°C in water bath for 30 minutes. After the extraction, the extract solution was filtered with Whatman filter paper no. 1. After that, the extracts of each sample were kept at 4°C until use to analyze.

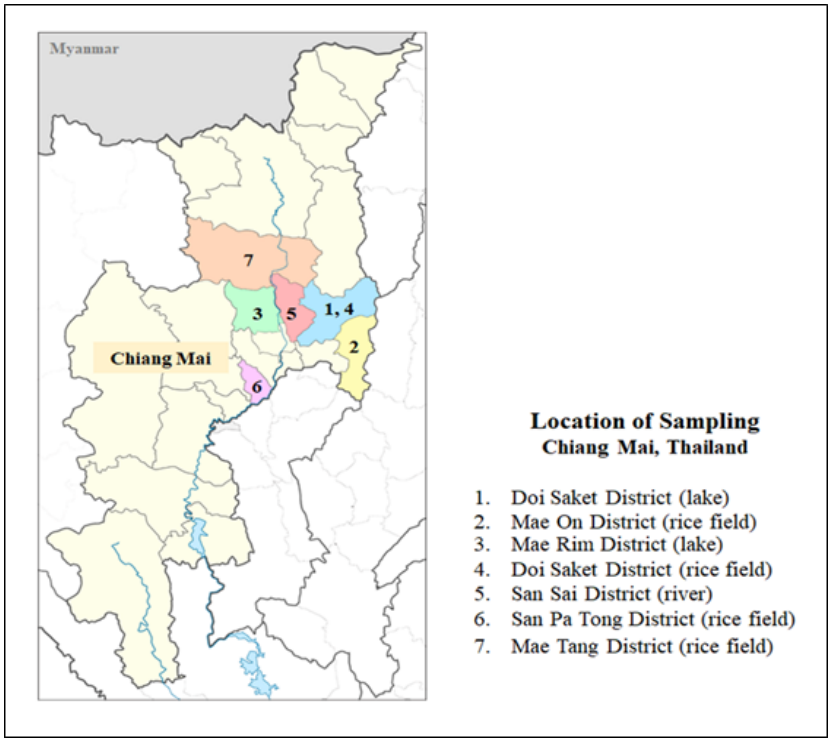


Figure 1. Location of 7 sampling sites located in 7 different districts.

2.3 Determination of Paraquat Contamination in *Spirogyra* spp. Extracts by HPLC

Chromatographic separation of paraquat was achieved on a ZORBAX SB-C18 column (4.6×150 mm 5 µm). The column temperature was set at 35.0 °C. The samples were eluted at a flow rate of 1 ml/min and the column eluent was monitored by photodiode array detector (DAD) with the UV absorption at 257 nm. Injection volume was set at 100 µl for each sample. Experiment procedure was with the installation of mobile phase buffer of 25 mM triethylamine, 5.3 mM sodium hexane sulfonate, and orthophosphoric acid (pH 2.5). Then, standard paraquat were prepared at 0, 20, 40, 50, and 60 ppm, respectively. Ethyl viologen was used as the internal standard. After that, each sample was diluted to proper concentration in order to be measured for the paraquat level in 3 replicates.

2.4 Human Health Risk Assessment of Paraquat Contamination

Equation 1[20,21] calculates the daily intake of paraquat by ingesting *Spirogyra* spp. per kilogram of body weight per day:

$$\text{Intake (mg/kg-day)} = \frac{\text{CF} \times \text{IR}}{\text{BW}}$$

In which, Intake: estimated daily intake (mg/kg/day), CF: the level of paraquat contaminated in *Spirogyra* spp. (mg/kg), IR: ingestion rate (kg/day), BW: body weight (kg).

Equation 2[20,21] calculates the Hazard Quotients (HQ) which is used to estimate the potential health risks associated with long term exposure to paraquat contaminated in *Spirogyra* spp. Equation 2 is used to calculate the HQ of non-cancerous diseases for adults and children that were developed by the Environmental Protection Agency (EPA) in the United States.

$$\text{HQ} = \frac{\text{Daily intake}}{\text{RfD}}$$

In which, Intake: estimated daily intake (mg/kg/day), RfD (mg/kg/day) is an oral reference dose for chronic

oral exposure which is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. The RfD is an estimate of a daily exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime[22]. The RfD value for paraquat is 4.5E-3 mg/kg/day[23].

3. Results

3.1 Validation of Analytical Method

Linearity was studied by using paraquat standard solutions which were prepared at 1,2,5,10 and 20 mg/L. The

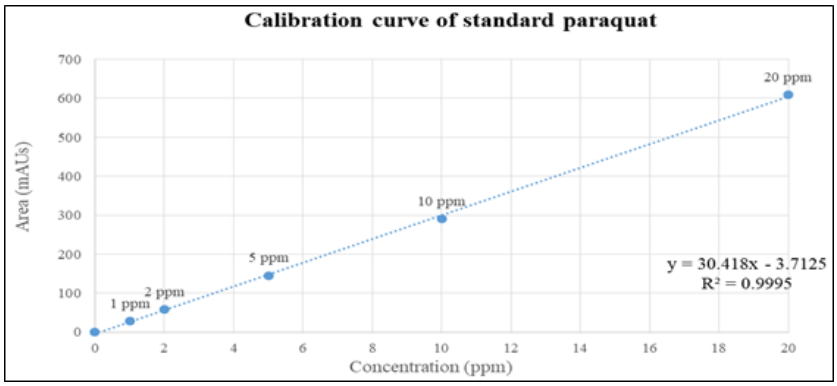


Figure 2. The calibration curve of standard paraquat.

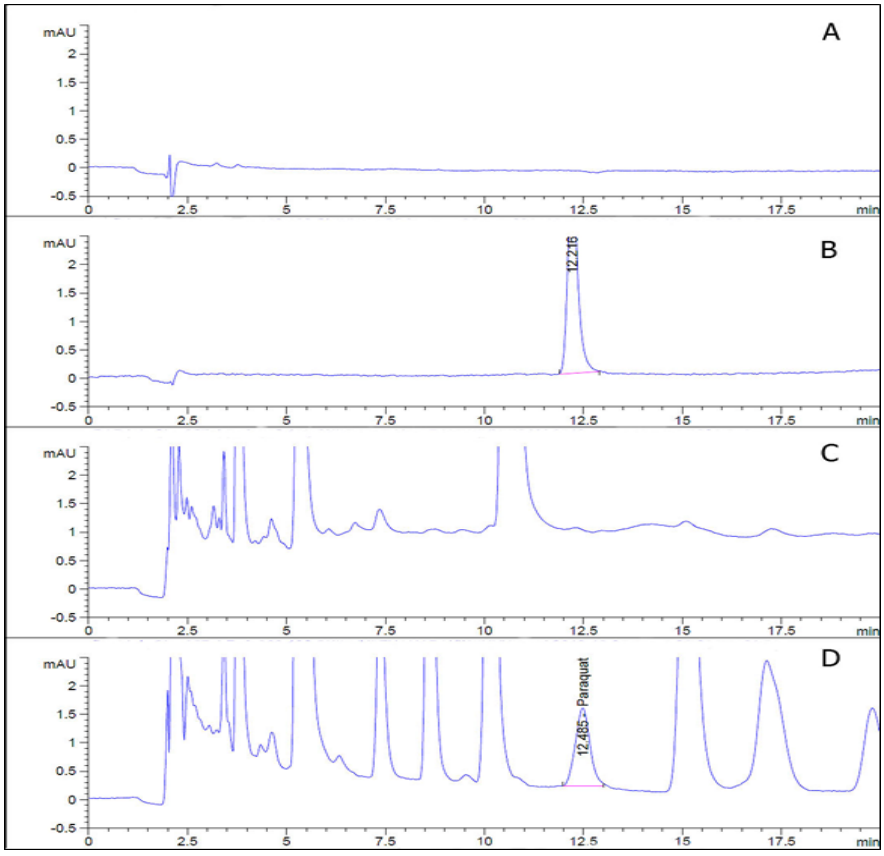


Figure 3. A chromatogram of blank (DI water), B chromatogram of standard paraquat 2 mg/L (tR = 12.216 min), C chromatogram of sample 1 which no paraquat found, D chromatogram of sample 4 which paraquat found (tR = 12.485).

relationship between peak area and amount of paraquat would be described by the equation: y = 30.418x – 3.7125. The regression equations for the calibration curves were used to calculate the concentration of paraquat in the samples. The correlation coefficient (R^2) was 0.9995 for paraquat.

The Limit of Detection (LOD) of this method can be determined from LOD = 3SN; (SN signal to noise ratio) and was calculated to be 0.1 mg/L (ppm), the Limit of Quantification (LOQ) can be determined from LOQ = 10SN; (SN = signal to noise ratio) and was calculated to be 0.5 mg/L (ppm).

Recovery analysis was obtained from plotting the calibration curves of 5 paraquat standard solutions at concentrations between 0-20 ppm and spiking paraquat into sample No. 2 (Figures 2, 3). Three replicates were tested for each concentration. The recovery was 97%.

3.2 Contamination Levels of Paraquat in *Spirogyra* spp.

The concentration of paraquat in sample 1-3 were lower than LOQ so there were reported as ND (non detectable). In sample 4-7, paraquat was found at 12.69276, 5.06868, 6.28994, and 8.161922 mg/kg, respectively (Table 1).

3.3 Human Health Risk Assessment of Paraquat Contamination

Human consumption of algal foods varies by nation, ranging from 9.6 (2014) to 11.0 (2010) g macroalgae/day (IR = 0.0103 kg/day)[24]. The overall mean body weight (BW) of Thai Adults is 63 kg[25]. The levels of paraquat contamination were 5.07±0.00 to 12.69±0.10 mg/kg (Table 1). Risk assessment of paraquat found that the hazard quotient was in the range of 0.18-0.46 (Table 2).

4. Discussion

The low concentrations of paraquat were found in sample 4-7 which were 12.69276, 5.06868, 6.28994, and 8.161922 mg/kg, respectively. The highest level was found in sample 4 (12.69276 mg/kg) which was collected from the rice field. However, the lowest level was found in sample 5 (5.06868 mg/kg) which was collected from the river. The excessive application of herbicides might be the major source of contamination in *Spirogyra* spp. (tao). Published study showed that the residues of pesticides can

Table 1. The levels of paraquat contamination in 7 samples of *Spirogyra* spp. (tao)

Sample	Concentration of PQ (ppm)			$\bar{X}$	SD	Weight (sample) gram	PQ levels in micro gram	PQ levels in mg/kg
	n1	n2	n3					
1.	0.122049	0.122049	0.122049	0.122049	0	2.0343	2.440989	ND
2.	0.122049	0.122049	0.122049	0.122049	0	2.0582	2.440989	ND
3.	0.365326	0.368614	0.365326	0.366422	0.002325	2.0565	7.328446	ND
4.	1.280306	1.300766	1.290536	1.290536	0.01023	2.0335	25.81072	12.69276
5.	0.509719	0.509593	0.509656	0.509656	6.28E-05	2.011	10.19312	5.06868
6.	0.635995	0.632183	0.634089	0.634089	0.001906	2.0162	12.68178	6.28994
7.	0.823546	0.853894	0.838718	0.838719	0.015174	2.0552	16.77438	8.161922

ND = Non Detectable, n = 3.

Table 2. The levels of paraquat contamination in *Spirogyra* spp. (tao), estimated daily intake, and Hazard Quotients for risk assessment of paraquat contamination

Sample	Daily intake (mg/kg/day)	PQ levels in mg/kg (CF)	SD (mg/kg)	Hazard Quotients (HQ)
1	ND	ND	ND	ND
2	ND	ND	ND	ND
3	ND	ND	ND	ND
4	0.002075	12.69276	0.100613	0.461148
5	0.000829	5.06868	0.000624	0.184153
6	0.001028	6.28994	0.01891	0.228523
7	0.001334	8.161922	0.147665	0.296535

ND = Non Detectable, n = 3.

accumulate in the environment from agricultural practice such as spraying[26]. In Thailand, insecticide and herbicide residues are found in the levels above the advisable limits in most water samples including paraquat[4,5]. Paraquat may be absorbed by aquatic plants especially algae and accumulate in the environment.

The previous study investigated the contamination of paraquat in some vegetables, crops and fruits in Nigeria using spectrophotometric method. Paraquat was found in all samples (150 samples) of crops, vegetables and fruits in the range of 0.04 to 0.27 ppm which was in the paraquat tolerance or maximum pesticide limits (MRLs: 0.5 mg/kg)[6,27]. The consumption of these products may not cause the acute (short term) serious health effects. However, the chronic (long term) effects of PQ in very low concentration may cause hazardous health effects such as Parkinson's disease and cancer. Our study showed that the concentrations of paraquat in all samples were lower than the standard level of the European Commission Regulation. Risk assessment of paraquat found that the hazard quotient was in the range of 0.18-0.46. It indicated that the consumption of *Spirogyra* spp. was expected to have no adverse health effects.

This study provides information about the levels of paraquat contaminated in *Spirogyra* spp. (tao) in the local areas in Chiang Mai province, Thailand. An alga is easily contaminated by paraquat due to their ability to concentrate high level of paraquat in water. Thus, the consumption of *Spirogyra* spp. (tao) should be a concern in terms of chronic exposure to very low concentration of toxic herbicide contaminated in the environment. Apart from the benefits of *Spirogyra* spp. as an antioxidant, this present study may raise the concern about toxic effects associated with the consumption of green algae (tao) with high paraquat content. There should be the caution with consumption of *Spirogyra* spp. (tao) if the contamination is higher than the Acceptable Daily Intake (ADI) of paraquat (0.005 mg/kg/day as PQ ion)[19].

Our results will be very useful for further study which aims to investigate the mutagenic effects of *Spirogyra* extracts contaminated with very low concentration of paraquat on the new cell model ($Ogg1^{-/-}Mutyh^{-/-}$ AS52 cells). This cell model is a sensitive tool to study toxic effects especially the mutagenic effects of a low dose exposure to environmental agents that can induce oxidative stress[13].

5. Conclusion

The levels of paraquat contamination in *Spirogyra* spp. (tao) were analyzed by using HPLC. Seven samples were collected from the different districts in Chiang Mai

province, Thailand. The levels of paraquat contamination were 5.07±0.00 to 12.69±0.10 mg/kg. The results showed that the concentrations of paraquat in *Spirogyra* spp. (tao) were lower than the standard level of the European Commission Regulation. Risk assessment of paraquat found that the hazard quotient was in the range of 0.18-0.46. It indicated that the consumption of *Spirogyra* spp. (tao) was expected to have no adverse health effects.

6. Credit Author Statement

PT: Conceptualization, Methodology, Investigation, Data curation, Writing- Original draft preparation, Writing-Reviewing and Editing, Visualization, Supervision; AD: Investigation, Resources, Data curation, Writing-Original draft preparation; SC: Resources, Data curation

7. Conflict of Interest

The authors declare that they have no potential conflicts of interest, financial or otherwise.

8. Acknowledgments

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9. References

1. Panuwet P, Siriwong W, Prapamontol T, *et al*. Agricultural pesticide management in Thailand: Situation and population health risk. Environ Sci Policy. 2012; 17:72-81. https://doi.org/10.1016/j.envsci.2011.12.005. PMid:22308095 PMCid:PMC3269779.
2. Kongtip P, Nankongnab N, Phupancharoensuk R, *et al*. Glyphosate and paraquat in maternal and fetal serums in Thai Women. J Agromedicine. 2017; 22(3):282-289. https://doi.org/10.1080/1059924X.2017.1319315. PMid:28422580.
3. Forté CA, Colacino J, Polemi K, *et al*. Pesticide exposure and adverse health effects associated with farmwork in Northern Thailand. J Occup Health. 2021; 63(1):e12222. https://doi.org/10.1002/1348-9585.12222. PMid:33973692 PMCid:PMC8112117.
4. Tirado R, Englande Jr A, Promakasikorn L, *et al*. Use of agrochemicals in Thailand and its consequences for the environment. 2008.
5. Pataranawat P, Kitkaew D, Suppaudom K. Paraquat contaminations in the Chanthaburi river and vicinity areas, Chanthaburi province, Thailand. Journal of Science Technology and Humanities. 2014; 10:17-24.
6. Konthonbut P, Kongtip P, Nankongnab N, *et al*. Paraquat exposure of pregnant women and neonates in agricultural areas in Thailand. Int J Environ Res Public Health. 2018; 15(6). https://doi.org/10.3390/ijerph15061163. PMid:29865285 PMCid:PMC6025106.
7. Wongsawad P, Peerapornpisal Y. Morphological and molecular profiling of Spirogyra from northeastern and northern Thailand using Inter Simple Sequence Repeat (ISSR) markers. Saudi J Biol Sci. 2015; 22(4):382-389. https://doi.org/10.1016/j.sjbs.2014.10.004. PMid:26150742 PMCid:PMC4486733.
8. Thumvijit T, Thuschana W, Amornlerdpison D, *et al*. Evaluation of hepatic antioxidant capacities of *Spirogyra neglecta* (Hassall) Kützing in rats. Interdiscip Toxicol. 2013; 6(3):152-156. https://doi.org/10.2478/intox-2013-0024. PMid:24678253 PMCid:PMC3967442.
9. Jaiswar S, Kazi MA, Mehta S. Bioaccumulation of heavy metals by freshwater algal species of Bhavnagar, Gujarat, India. J Environ Biol. 2015; 36(6):1361-1366.
10. Lupsor S, Stanciu G, Epure D, *et al*., editors. Heavy Metals and Pesticides Analysis from Black Sea Algae 2009, Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-90-481-2335-3_34.
11. Bauer Dial CA, Dial NA. Lethal effects of the consumption of field levels of paraquat-contaminated plants on frog tadpoles. Bull Environ Contam Toxicol. 1995; 55(6):870-877. https://doi.org/10.1007/BF00209467. PMid:8601067.
12. Blanco-Ayala T, Andérica-Romero AC, Pedraza-Chaverri J. New insights into antioxidant strategies against paraquat toxicity. Free Radic Res. 2014; 48(6):623-640. https://doi.org/10.3109/10715762.2014.899694. PMid:24593876.
13. Tajai P, Fedeles BI, Suriyo T, *et al*. An engineered cell line lacking OGG1 and MUTYH glycosylases implicates the accumulation of genomic 8-oxoguanine as the basis for paraquat mutagenicity. Free Radic Biol Med. 2018; 116:64-72. https://doi.org/10.1016/j.freeradbiomed.2017.12.035. PMid:29289706 PMCid:PMC5902796.

14. Tajai P, Suriyo T, Rangkadilok N, *et al*. Andrographolide, an antioxidant, counteracts paraquat- induced mutagenesis in mammalian cells. Asian Pac J Cancer Prev. 2021; 22(S1):3-8. https://doi.org/10.31557/APJCP.2021.22.S1.3. PMid:33576206.
15. Jovtchev G, Gateva S, Stergios M, *et al*. Cytotoxic and genotoxic effects of paraquat in Hordeum vulgare and human lymphocytes in vitro. Environ Toxicol. 2010; 25(3):294-303. https://doi.org/10.1002/tox.20503. PMid:19437450.
16. Zienolddiny S, Ryberg D, Haugen A. Induction of microsatellite mutations by oxidative agents in human lung cancer cell lines. Carcinogenesis. 2000; 21(8):1521-1526. https://doi.org/10.1093/carcin/21.8.1521. PMid:10910953.
17. Wesseling C, Antich D, Hogstedt C, *et al*. Geographical differences of cancer incidence in Costa Rica in relation to environmental and occupational pesticide exposure. Int J Epidemiol. 1999; 28(3):365-374. https://doi.org/10.1093/ije/28.3.365. PMid:10405835.
18. Wesseling C, van Wendel de Joode B, Ruepert C, *et al*. Paraquat in developing countries. Int J Occup Environ Health. 2001; 7(4):275-286. https://doi.org/10.1179/oeh.2001.7.4.275. PMid:11783857.
19. Zou T, He P, Cao J, *et al*. Determination of paraquat in vegetables using HPLC-MS-MS. J Chromatogr Sci. 2015; 53(2):204-209. https://doi.org/10.1093/chromsci/bmu041. PMid:24850699.
20. Bleam WF. Chapter 10 - Risk Assessment. In: Bleam WF, editor. Soil and Environmental Chemistry. Boston: Academic Press; 2012. p. 409-447. https://doi.org/10.1016/B978-0-12-415797-2.00010-8.
21. Liang Y, Yi X, Dang Z, *et al*. Heavy metal contamination and health risk assessment in the vicinity of a tailing pond in guangdong, China. International Journal of Environmental Research and Public Health. 2017; 14(12):1557. https://doi.org/10.3390/ijerph14121557. PMid:29231884 PMCid:PMC5750975.
22. Chattopadhyay S. Grain Incidents and Other Mercury Tragedies: Forms, Fate, and Effects. In: Wexler P, editor. Encyclopedia of Toxicology (Second Edition). New York: Elsevier; 2005. p. 464-469. https://doi.org/10.1016/B0-12-369400-0/01052-8.
23. Dourson ML, Knauf LA, Swartout JC. On Reference Dose (RfD) and its underlying toxicity data base. Toxicol Ind Health. 1992; 8(3):171-189. https://doi.org/10.1177/074823379200800304. PMid:1502696.
24. Wells ML, Potin P, Craigie JS, *et al*. Algae as nutritional and functional food sources: Revisiting our understanding. J Appl Phycol. 2017; 29(2):949-982. https://doi.org/10.1007/s10811-016-0974-5. PMid:28458464 PMCid:PMC5387034.
25. Wells JC, Treleaven P, Charoensiriwath S. Body shape by 3-D photonic scanning in Thai and UK adults: Comparison of national sizing surveys. Int J Obes (Lond). 2012; 36(1):148-154. https://doi.org/10.1038/ijo.2011.51. PMid:21386803.
26. Shaban NS, Abdou KA, Hassan NE-HY. Impact of toxic heavy metals and pesticide residues in herbal products. Beni-Suef University Journal of Basic and Applied Sciences. 2016; 5(1):102-106. https://doi.org/10.1016/j.bjbas.2015.10.001.
27. Akinloye, Adamson, Ademuyiwa, *et al*., Editors. Occurrence of Paraquat Residues in some Nigerian Crops, Vegetables and Fruits; 2011.