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Geospatial Visualisation of Food Contaminant Distributions: Polychlorinated Naphthalenes (PCNs), Potentially Toxic Elements (PTEs) and Aflatoxins

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Abstract: Large volume of multidimensional data can be summarised, both in terms of tabulated statistics, and as graphic geospatial visualisations. The latter approach allows rapid interpretation and communication of complex information to stake-holders such as regulators, risk assessors and policy makers. In the main study on polychlorinated naphthalene (PCN), individual samples representing different edible fish species were analysed from around the UK. PCNs were observed in all samples with nearly all of the twelve measured congeners being detected. Summed congener concentrations ranged from 0.7 ng/kg ww (turbot) to 265 ng/kg ww (sprats). The highest contamination levels were recorded for sprats and mackerel with mean summed concentrations of 67 ng/kg ww and 68 ng/kg ww respectively. Two ancillary studies, on potentially toxic elements (PTEs) in crabs from China and aflatoxin in children's blood from Tanzania, demonstrate the wide applicability of this approach. The PTE contents in crab showed strong dependence on the tested tissues and elements, and crabs from Tai and Yangcheng Lakes showed obviously higher PTE levels than the other lakes. Geospatial distribution of the aflatoxin biomarker AF-alb in children's serum from 3 locations showed how individual anthropometric or socio-economic data reveals the relationship between family size, socio-economic score and magnitude of serum aflatoxin levels. In addition to facilitating the flow of interpreted data to stakeholders, these techniques can direct the formulation of risk mitigation activities and help with the identification of data gaps. When combined with hierarchical cluster analyses, correlations within the data can also be predicted.

Keywords: PCN-TEQ, fish, spatial analysis, visualization, risk mitigation, hierarchical clustering

1. Introduction

The visualisation of the spatial distribution of contaminant concentrations in foods sampled across large areas allows the rapid and efficient communication of information for regulators, risk assessors and managers. In particular, this mode of presentation of geospatial intelligence is especially relevant when multidimensional data is involved, where the sheer volume of generated data makes interpretation time-consuming and difficult. When combined with the outputs from the geospatial analysis of contaminant distributions in regions or countries, visualisation provides a powerful tool for the study of risk identification and establishment. It can also provide rapid and effective representation of affected or at-risk areas which aids the development of mitigation activities. Additionally, the technique can be complemented with hierarchical cluster analyses to allow the prediction of correlations within data sets.

Some web-based mapping resources such as Google Maps (Google Maps 2016) provide a powerful platform for the visualisation of geographical data. These platforms can be developed to efficiently represent and explore the complex inter-relationships between food contaminant occurrences, e.g. in different edible fish species or in different crop producing regions, as demonstrated in this paper. Using an interactive webpage, specific aspects derived from occurrence data can be represented in location and magnitude by different coloured circles or radiation patterns corresponding to their GPS position and to represent different species for example. The extent of contamination or the magnitude of the occurrence can be superimposed on this data by varying the radii of circles or the offset distances for radiation patterns.

Polychlorinated naphthalenes (PCNs) are legacy industrial chemicals widely used as dielectrics and flame retardants in the 20th century and are now recognised as persistent organic pollutants (POPs), as evidenced by their listing in Annex A and C of the Stockholm Convention. Environmentally, they occur ubiquitously with reports of contamination of biota, sediments and air, both in temperate as well as Polar Regions (Biddleman et al., 2010; Braune and Muir 2017). Biochemically, they demonstrate properties of persistence and high bioaccumulation potential, coupled with a similarity in structural configuration to polychlorinated dibenzodioxins and furans (PCDD/Fs). This latter property is related to human health concerns as many PCN congeners have been reported to contribute to dioxin-like toxicity (Villeneuve et al., 2000; Falandysz et al., 2014; Fernandes et al., 2017) and elicit different effects such as mortality, embryotoxicity, hepatotoxicity, dermal lesions, teratogenicity and carcinogenicity (Behnisch et al., 2003; Falandysz et al., 2014). A recent review of their occurrence in human tissue and foods (Fernandes et al., 2017) show higher levels in fish relative to other foods.

A number of trace elements, particularly heavy metals, have long been recognised as potentially toxic elements (PTEs). Nutritionally, some trace elements, such as selenium, zinc, copper, etc. are essential to health but may be toxic at high levels of dietary intake. Environmental sources are the main contributors to contamination of food which is the major source of the overall exposure of consumers to PTEs, although other routes may also be significant (e.g. oral exposure via drinking water, occupational exposure by inhalation). Certain food groups naturally accumulate some elements (e.g. fish and shellfish are known to accumulate arsenic and mercury and cereals can accumulate cadmium) and consequently contain high concentrations of these elements compared to other foods. PTEs may enter marine and aquatic environments and bio-accumulate in species at any point during growth and harvesting. Chinese mitten crab (*Eriocheir sinensis*) is widely consumed in China because of its unique aroma and umami taste, but has also been reported (Xu et al., 2016; Shou et al., 2012) to be at high risk of PTE accumulation with the tissues of crabs exhibiting diverse PTE (e.g. lead and cadmium) bioaccumulation due to feeding behaviour and environmental factors.

Aflatoxins are contaminants that are produced by the moulds, *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B1 is recognised as a genotoxic carcinogen and is the most potent of the fourteen aflatoxins that are natural products of these fungi. Within crops such as maize and groundnuts, the growth of these moulds is promoted by poor storage conditions combined with ambient humidity and temperature. Although hepatocellular carcinogenesis is generally recognised as the most lethal biological effect, the stunting of growth in childhood (affecting 165 m. children world-wide) that occurs in some countries in the African continent, may show an association with dietary aflatoxin occurrence (Gong et al., 2002; 2004). High levels of childhood aflatoxin exposure were observed during a study on three regions of Tanzania in 2010 (Shirima et al., 2013; 2015)

This paper focusses on the occurrence and geospatial mapping of these contaminants to provide effective visualisation of their distribution within geographical regions. The main focus of this work is on the distribution of PCN contamination in fish, but the applicability of this approach is also demonstrated for PTEs and aflatoxins, in different geographical locations. For PCNs, occurrence is mapped for different edible marine fish species from locations in the North Atlantic Ocean. PTE contents of three types of tissues (muscle tissue, hepatopancreas and sexual organs) of Chinese mitten crabs were determined, and mapped for samples taken from 5 lakes in Jiangsu province, China. For Aflatoxin B1, visualisation of human exposure as measured in blood AF-alb biomarkers in 3 rural locations in Tanzania was superimposed with related anthropometric data to examine correlations between these variables and exposure.

2. Experimental

2.1 Polychlorinated Naphthalene study

In the main study on PCNs, 75 samples of Sea Bass, Herring, Mackerel, Mullet and Sardine were identified, as part of a larger study investigating other contaminants such as brominated flame retardants, PCDD/Fs, etc. Samples were collected in major fishing areas, mainly but not exclusively, from waters around the UK, Ireland and Northern France. In general, selected fish sizes approximated to those that are commercially sold for consumption. The following PCN congeners were included in this study - PCN-52/60, 53, 66/67, 68, 69, 71/72, 73, 74, & 75.

The analytical methodology for the determination of PCN concentrations in fish species has been described elsewhere (Fernandes et al., 2010). In brief, sample aliquots were fortified with ^{13}C-labelled PCN analogues of target compounds and extracted using mixed organic solvents. PCNs were chromatographically fractionated from potential interferants such as PCBs, on an activated carbon column and further purified using adsorption chromatography on alumina. Analytical measurement was carried out using high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS). Additional control was provided by the inclusion of procedural blanks and a reference material.

The quality control criteria used for evaluating data, and method performance parameters have been reported before (Fernandes et al., 2010). There are no available reference materials (RMs) specific to PCNs, but the same reference material that is used for PCDD/F and PCB analysis (cod liver oil), was analysed during the course of this work with results showing good consistency and agreement with established values. The reporting limits (LOQ quoted as "<") for all analytes incorporate the relevant procedural blank and were derived using the current guidance on LOQ estimation (European Commission, 2017).

2.2. Potentially Toxic Elements in Crabs

The sampling sites included 5 major lakes in Yangtze River Delta, China, i.e., Yangcheng Lake, Tai Lake, Changdang Lake, Gucheng Lake and Hongze Lake. 8 crabs were collected from each lake, rinsed with tap water, and dissected quickly to obtain the sexual gland, hepatopancreas and muscle. The sample tissues were digested using a microwave-assisted method (López et al., 2003), then Cadmium (Cd), Lead (Pb), Mercury (Hg) and Copper (Cu) in the digested solutions were measured by inductively coupled plasma mass spectrometry (F.J.Sanchez L., 2003) (ICP-MS, XSeries II, Thermo Fisher Scientific, USA). Blanks and QC samples included and the LOQs ranged from 3-5 μg/kg.

2.3 Aflatoxins in Serum

The aflatoxin data is derived from a study (Shirima et al., 2015) that investigated 3 regions in Tanzania (Iringa, Tabora and Kilimanjaro). Dietary intake data and anthropometric indices-body weight, recumbent length, etc. were recorded. Validated methodology was used to measure aflatoxin–albumin adducts (AF-alb), as biomarkers in blood plasma taken from 166 children. A description of this methodology and quality parameters has already been given before (Chapot., 1991) but in brief, the tests included extraction of albumin, digestion of protein, purification, and ELISA quantification of AF-alb adducts. Each batch of plasma was analysed with three positive controls and one negative control. Samples were measured in ELISA in quadruplicate on at least two occasions on separate days. The detection limit was 3 pg of aflatoxin-lysine equivalents per milligram albumin.

2.4 Geo-spatial mapping

For the geo-spatial mapping, an interactive webpage based on Google Maps was used (Li et al., 2018) to integrate sample location (GPS), with contaminant concentration data. The webpage primarily consisted of user selective control fields such as individual or summed contaminant, species, occurrence scaling, colour legend of selected species/contaminants and a help field. Each sample was presented with a circle centred at its GPS coordinate location. The magnitude of occurrence was indicated by the radius of each circle (ng/kg for PCNs; µg/kg for PTEs; pg/mg for AF-alb), which can be simultaneously colour coded to variables such as socio-economic status. The design allows multiple species or congeners to be selected, differentiated by colour coding and line type (solid, dashed, dotted) as applied to relevant circle representations. The aflatoxin study used plasma AF-alb biomarker data and/or contaminant levels within maize/other foods at the designated sampling region together with hazard indicators such as child weight z-scores. This approach offers efficient data exploration without full statistical analyses.

With the volumes of data generated in this study, hierarchical clustering (HC) analysis can complement the above approach, a feature which would allow the user to investigate if certain locations or species/individuals are susceptible to concentration levels of concern. HC is a typical algorithm to analyse the similarities (or dissimilarities) of objects in variable space (Smoliński et al., 2002). In order to have a better understanding of the geographical distribution, or independence of occurrence of the congeners, HC was employed in the PCN study e.g. by dividing the fish samples into 3 clusters with the PCN congener concentration as input variables to investigate if correlations existed between the species and spatial locations. HC analysis was performed using R language with the Ward method as the amalgamation rule and Euclidean distance as metric (Smoliński et al., 2002).

3. Results and Discussion

The raw data from the PCN study is very large and has been presented in a sponsor report (Fernandes et al., 2015). Raw data from the mitten crab study is provided in the supplementary information (SI). Descriptive statistics are summarised in Tables 3.1 and 3.2, by analyte type.

3.1 PCN

PCN concentrations are reported in ng/kg wet weight (ww) as per convention. Congeners were selected for measurement based on toxicological significance as described in earlier studies (Fernandes et al 2010; Falandysz et al., 2014). From the point of view of food safety, and given the known AhR receptor active responses (Falandysz et al., 2014; Fernandes et al., 2017), these concentrations have also been converted to toxic equivalents (TEQs) using a similar approach to PCDD/Fs and dioxin-like PCBs (Van den berg et al., 2006). The TEQ values were calculated using relative potencies (REPs) as used in other studies (Fernandes et al., 2010; 2011; 2017; Jongchu et al., 2018; Lili et al., 2018).

PCNs were detected in all of the samples analysed with variations in patterns of occurrence and levels of concentrations depending on the species and location. Higher concentration levels were recorded for penta- and hexa-chlorinated PCNs (e.g. PCNs 52, 53 and 66/67) with lowest levels occurring mainly for the octa-chlorinated PCN-75. The extent of positive detection was high, at 86% of all measurements. The patterns of occurrence are dominated by PCN-52 but the occurrence of other congeners varies widely depending on the species as shown in Figure 3.1A (average pattern for each species, except for halibut which was a single sample), whereas the absolute concentrations appeared to be more dependent on the location of the sample. Summed concentrations (sum of twelve measured congeners) showed a range extending from 0.7 ng/kg ww (turbot) to 265 ng/kg ww (sprats). Highest concentrations were recorded for sprats and mackerel with mean concentrations of 67 ng/kg ww and 68 ng/kg ww respectively. Given the large geographical range of the sampling region, these observations are indicative, although other studies (Fernandes et al., 2009) appear to show similar findings. However, other parameters such as size and the age of the fish, and seasonal physiological changes (e.g. spawning periods, etc.) will undoubtedly influence the findings.

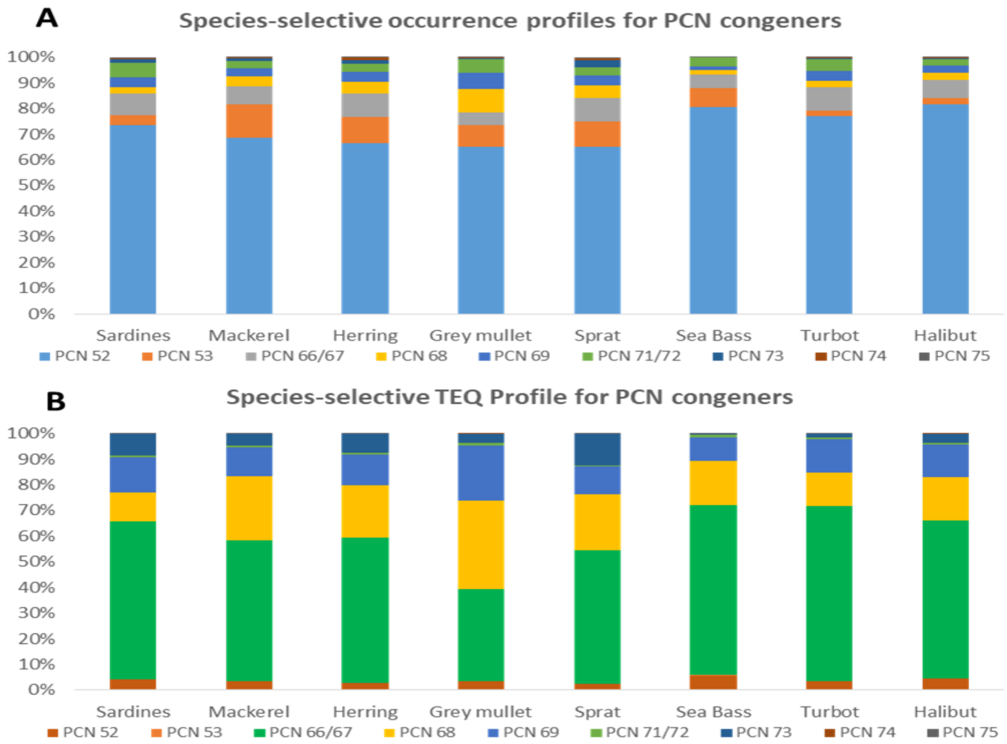


Figure 3.1 Average pattern (A), of PCN congener occurrence and (B), TEQ, based on species

Table 3.1. Statistical summary of PCN occurrence in various species of edible marine fish

Species (N) & average fat		Sum PCNs Lipid basis	Sum PCNs whole weight	*PCN TEQ whole weight	Species (N) & average fat		Sum PCNs Lipid basis	Sum PCNs whole weight	*PCN TEQ whole weight
		ng/kg fat	ng/kg w.w.				ng/kg fat	ng/kg w.w.	
	MIN	22	5.4	0.004		MIN	138	29.4	0.014
Sardines	MEDIAN	172	16.6	0.007	Sprat	MEDIAN	335	46	0.027
(12)	MEAN	291	19.8	0.009	(15)	MEAN	680	66.5	0.044
12.9	MAX	1215	63.1	0.031	13.8	MAX	2390	264.8	0.204
	MIN	112	10.1	0.002		MIN	302	14.2	0.004
Mackerel	MEDIAN	451	50.5	0.024	Sea Bass	MEDIAN	848	29.2	0.008
(14)	MEAN	648	68	0.035	(13)	MEAN	999	29.4	0.01
11.7	MAX	1654	243	0.17	4.3	MAX	3084	48.5	0.026
	MIN	141	18.3	0.009		MIN	132	0.7	<0.001
Herring	MEDIAN	231	29.7	0.016	Turbot	MEDIAN	246	3.5	0.002
(6)	MEAN	431	38.7	0.024	(6)	MEAN	343	5.3	0.003
11.9	MAX	1342	89.5	0.069	1.4	MAX	828	15.5	0.009
	MIN	125	4.2	0.001					
Grey mullet	MEDIAN	554	12.4	0.006	Halibut		253	5.85	0.003
(9)	MEAN	1293	14.7	0.007	(1)				
4.0	MAX	7572	33.5	0.014	2.3				

*PCN TEQ calculated using REP values from Fernandes et al. 2010 Env. Sci. Technol., 44, 3533–3538. Sum PCNs = sum of 12 congeners

The variation in congener occurrence is reflected in the TEQ distribution (Figure 3.1B), although here, the higher REPs of congeners PCN-66/67, PCN-68 and PCN-69 (Falandysz et al., 2014) make them the largest contributors to the cumulative TEQ. The TEQ values for these fish samples (Table 3.1), range from <0.001 to 0.2 ng/kg ww and correlate well with the summed concentrations, with highest average TEQ concentrations for mackerel and sprats at 0.035 and 0.044ng/kg ww. This range of values is substantially lower than the corresponding TEQ arising from PCDD/Fs and PCBs in fish, but it does contribute to the overall burden of dioxin-like toxicity.

Preliminary geospatial mapping analysis showed that the variables that would provide the most useful interpretation of the results were likely to be location, species and PCN congener (including sum of congeners). The visualisation plots of the concentrations (Figure 3.2A) revealed that although locations across the southern/eastern UK coasts and northern France showed a majority of concentrations above the average for sum PCNs (39 ng/kg ww), the highest PCN concentrations were recorded in samples of mackerel and sprats from the Irish Sea. This distribution is confirmed when plots of the indicative PCN congeners are examined e.g. PCN-52 as the most abundant congener measured in this study (Figure 3.3A), and PCN-66/67 as the largest contributor to the PCN TEQ (Figure 3.3B). In terms of species, Figure 3.2A also reveals that of the measured species, mackerel, sprats and sea-bass are the most susceptible to PCN contamination. Considering the significant difference between species, analysis of individual species was necessary to examine the spatial distribution of PCNs exposure. Using grey mullet as an example, HC analysis was performed to determine the correlations between grey mullet samples with PCN congener concentrations used as input variables. Figure 3.2B clearly shows that cluster 2 and cluster 3, present higher level of PCNs and appeared along the north coast of France.

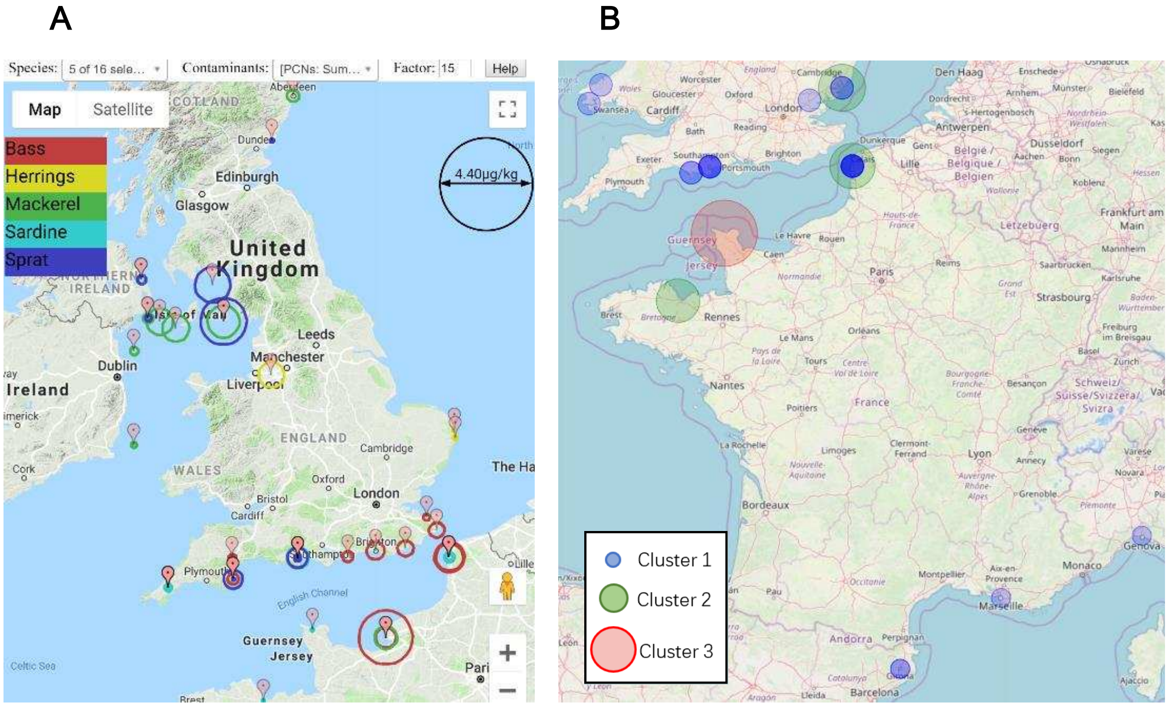


Figure 3.2. (A) Spatial distribution of sum PCN congeners for locations around the UK; (B) HC analysis for PCN levels of Grey mullet

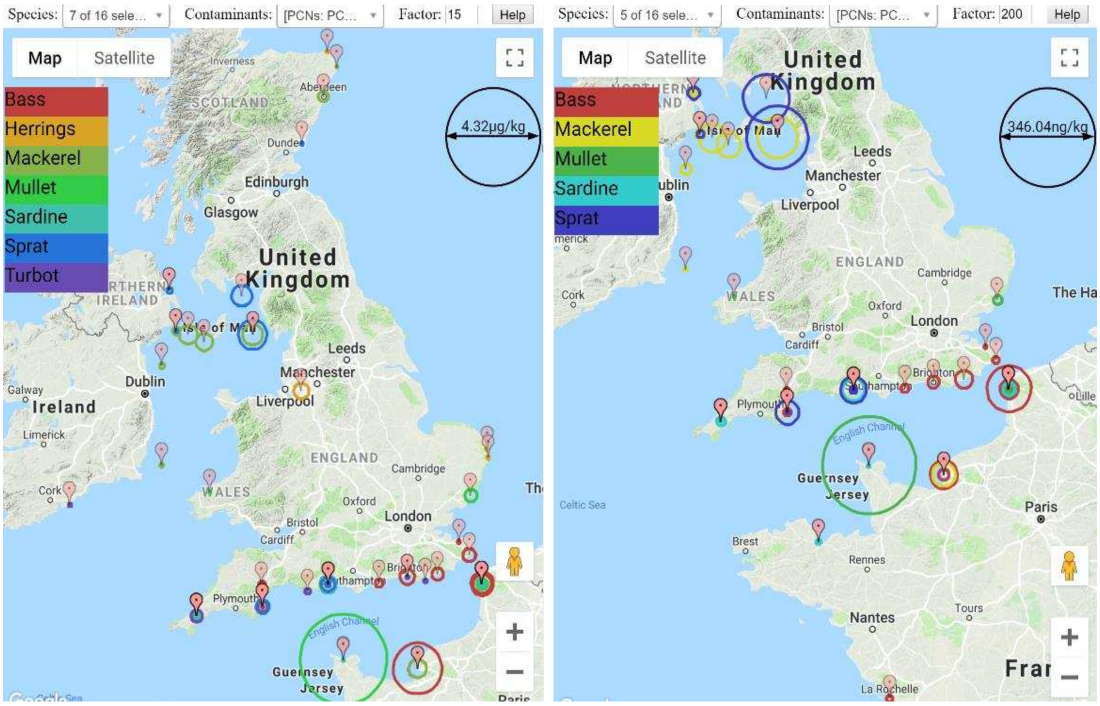


Figure 3.3. Spatial distribution of A. PCN-52 and B. PCN-66/67

3.2 Analysis and visualization of PTEs in Crab

A statistical summary of the measured PTE concentrations in different crab tissues is presented in Table 3.2. The relatively high occurrence of Cu in all samples follows a normal distribution for fish and shellfish (De Mora et al., 2004) species but concentrations for some of the other PTEs showed a strong dependency on the type of tissue. The geometric mean concentration for Cd in the hepatopancreas was significantly higher than in the sexual gland or muscle. Conversely, the mean concentration of Hg in muscle was approximately double the concentration in the hepatopancreas and about 50-fold higher than in the sexual gland. There was no significant difference observed for Pb in different tissue in terms of the geometric mean. The maximum concentrations generally reflect the means apart from Hg in the sexual gland which shows a large difference. Such characteristics of PTE occurrence in crab tissues can be conveniently superimposed on the geographical locations of the samples using geospatial mapping. In Figure 3.4A the distribution of Cd with location as a variable, immediately displays the higher occurrence levels in the Tai and Yangcheng lakes relative to the other lakes. In this plot, only Cd in the hepatopancreas is immediately apparent, mainly due to the higher concentrations in this tissue (131 to 2382 μg/kg with an average value of 579 μg/kg, compared to e.g. <5.0 to 21 μg/kg with average value of 2.4 μg/kg in the sexual gland). Although the immediate effect is to obscure the detail for the muscle and the sexual gland, this can easily be overcome by using the scaling factor to expand the scale of the plot as shown in Figure 3.4B which shows the distribution for Hg plotted at a much higher magnification. Here the highest concentrations occur in the muscle tissue. The relatively high accumulation of Cd in hepatopancreas was also observed by Ma et al., 2008 and Wang et al., 2008, who suggested that this could be due to the hepatopancreas playing an important role in Cd detoxification in this species (Hopkin and Nott, 1980).

Besides single-factor comparison, multiple factors including tissue, location and element, could be compared at the same time with a radiation pattern. As shown in Figure 3.5, each vertex of the radiation pattern presents an element, the colour presents tissue part. Figure 3.5 clearly shows that PTE occurrence in the crabs strongly depended on tissue type and individual PTEs, which is consistent with Table 3.2 and Figure 3.4. Spatially, crabs from Tai Lake and Yangcheng Lake showed higher level of PTE bio-accumulation than the other 3 lakes.

Table 3.2. Statistical summary of the measured PTE contents in different crab tissues

Tissue parts	Statistics	PTEs (μg/kg)			
		Cd	Pb	Hg	Cu
Hepatopancreas	Min.	131	14.6	<3.0	3687
	Median	461	40.9	11.5	25123
	Mean	579.	52.3	11.4	30424
	Max.	2382	210.2	25.2	79060
Sexual Gland	Min.	<5.0	12.9	<3.0	3996
	Median	<5.0	42.4	<3.0	19469
	Mean	2.4	59.0	0.4	24278
	Max.	21.0	277.1	9.2	59659
Muscle	Min.	<5.0	17.2	<3.0	7763
	Median	<5.0	32.1	23.6	15485
	Mean	3.9	50.2	21.7	16040
	Max.	42	349	47	31425

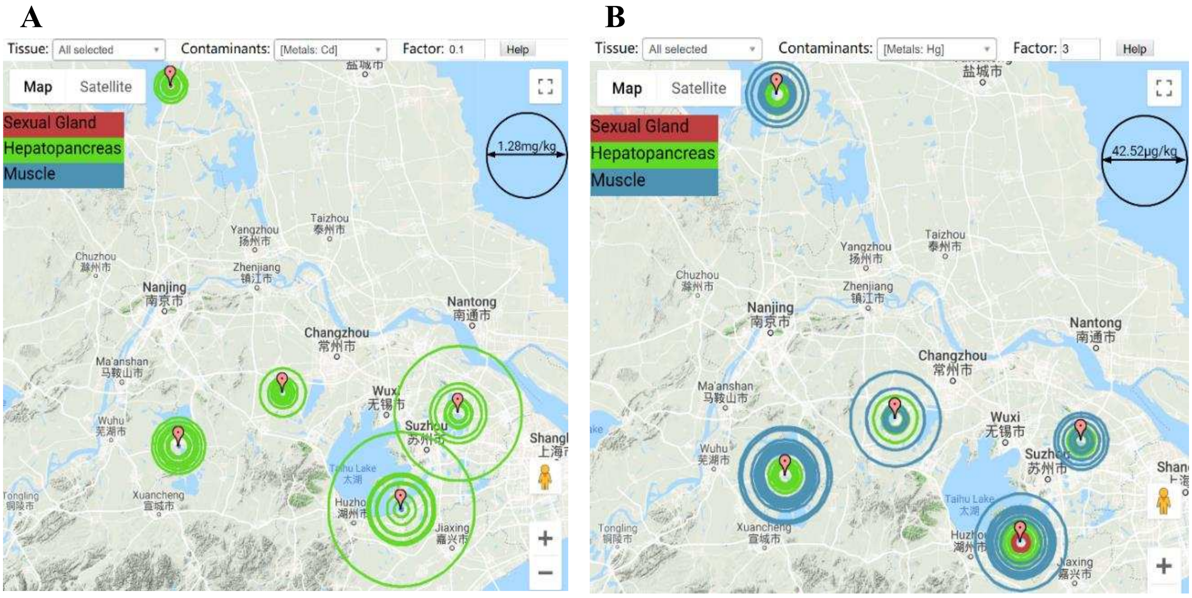


Figure 3.4. Spatial distribution of A. Cd and B. Hg for different tissues of crab

Tissue: All selected

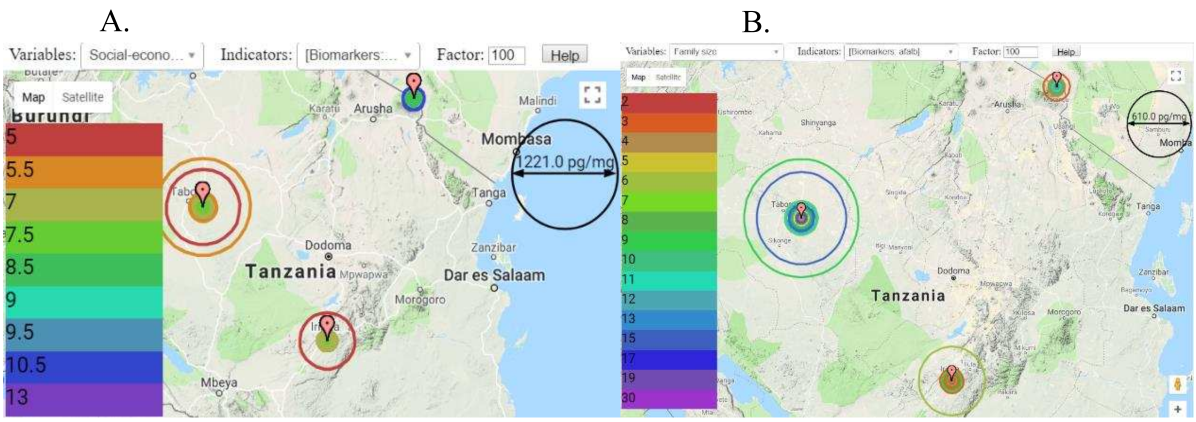
Contaminants: [Metals: Cd, ...

Factor: 0.4

Figure 3.5. Radiation pattern of Cd, Hg and Pb for different tissues

3.3 Aflatoxin

These preliminary results of aflatoxin analysis demonstrate the initial exploratory potential of the application. Geospatial analysis was applied to the distribution of the aflatoxin biomarker AF-alb in children's blood from 3 locations, to individual anthropometric or socio-economic data. The examples in Figures 3.6A and 3.6B show the magnitude of AF-alb content overlaid with socio-economic status and family size respectively and shows how potential associations for indicators may be explored. The Tabora region (located centre of Figures 3.6A & B) shows relatively stronger associations. Generally, the characteristic of poorer social economic state (lower score) show higher level of aflatoxin biomarker, which means more risk for aflatoxin exposure. Similarly, correlation to child hazard data (e.g. stunting) can be performed to evaluate biomarker to hazard indicators.



A. Variables: Social-econo... Indicators: [Biomarkers:... Factor: 100

B. Variables: Family size Indicators: [Biomarkers: afalb] Factor: 100

Figure 3.6. Selected spatial distribution for the aflatoxin biomarker AF-alb in Tanzania with A. associated socio-economic status colour coding and B. family size colour-coding, respectively.

3.4 General Discussion with focus on advantages of geo-spatial visualisation

Figures 3.2-3.6 demonstrate rapid and effective geo-spatial visualisation of the large amount of data associated with these studies. Although spatially distributed data can also be summarised in tabular format, navigating listed numerical data can be time consuming and tedious when compared to the amount of information that is quickly apparent from the visual plots. Areas of higher contamination that may relate to relatively higher risk, or locations requiring attention, are immediately apparent from the plots and enable rapid qualitative assessment of potential associations within data. So for example, in the case of the PCN contamination of edible fish species, it is immediately apparent from the plots that fish from the northern reaches of the Irish Sea between Northern Ireland and South-West England, and the English Channel show a higher levels of PCNs. This also allows for possible geographical associations with sources to be made e.g. Commercial PCN sold as Seekay waxes were manufactured at Runcorn (Crookes and Howe, 1993) on the southern bank of the river Mersey which flows into the Irish Sea. In the case of the AF-alb data from Tanzania, the introduction of socio-economic data relating to the families of the children who provided blood samples, as a variable within the geospatial analysis, allowed potential associations with poorer socio-economic scores and larger family sizes to be correlated with higher AF-alb levels in the blood

(Figure 3.6). The plot also shows that the relatively affluent area around Kilimanjaro in the north of the country was associated with smaller family sizes and lower AF-alb blood levels.

If sufficient data are available, more detailed analyses, e.g. HC, can be conducted or alternatively, where data are lacking, this promotes more informed planning, e.g. where additional research/data acquisition is required, and enables greater transparency across stakeholders. These techniques and the resulting visualised data also allow interpreted information to become more amenable to non-specialist stakeholders, e.g. risk assessors and managers who are then empowered to direct the formulation of risk mitigation activities and also help with the identification of data gaps. Another aspect of the techniques is the ability to retrospectively add new types of data that may become available e.g. rates of crop failure or any changes due to temporal or climatic conditions or spontaneous events.

4. Conclusions

The geo-spatial visualisation approach applied to the data presented here allows the rapid and effective evaluation of complex and multidimensional data sets. Three diverse datasets on PCNs, PTEs and aflatoxins were analysed demonstrating the versatility of the system. In the main study on PCNs, correlations between location and species with PCN congeners was demonstrated, showing that mackerel and sea-bass were the edible species that were most susceptible to PCN contamination, and the Northern reaches of the Irish Sea and the English Channel showed relatively higher levels of contamination than the other marine areas studied. In two ancillary studies, data on potentially toxic elements (PTEs) in crabs from 5 lakes in China and aflatoxin levels in maize from three regions of Tanzania, demonstrate the wide applicability of this visualisation approach. The PTE contents in crab showed strong dependence on the tested tissues and elements, and crabs from Tai Lake and Yangcheng Lake showed obviously higher level of PTE accumulation than the other lakes. Geospatial distribution of the aflatoxin biomarker AF-alb in children's blood from 3 locations in Kenya showed how individual anthropometric or socio-economic data reveals the relationship between family size, socio-economic score and magnitude of serum aflatoxin levels. In addition to facilitating the flow of interpreted data to stakeholders, these techniques can help direct the formulation of risk mitigation activities and also help identify gaps in data and knowledge.

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Declarations of interest: None

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