ANALYSIS OF FISH TISSUE CONCENTRATIONS OF DIOXINS AND FURANS USING PRINCIPAL COMPONENTS ANALYSIS (PCA)

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Introduction

Principal Components Analysis (PCA), sometimes called factor analysis, is a multivariate statistical technique that is used to reduce the dimensions of a data set so that trends in the data can be visualized more easily. In this paper we illustrate the application of PCA to polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) fish tissue data collected in Southern Mississippi. PCA is used to distinguish different potential sources of PCDD/Fs in fish, and to evaluate whether fish tissue samples tend to reflect any of 6 known industrial PCDD/F sources.

Materials and methods

Sample Collection

Sixty-one wild-caught and farm-raised catfish samples were collected from areas in southern Mississippi in March 2006. Sample collection is described in detail in Ferriby et al (2007)[1]. Briefly, 28 farm-raised catfish samples were purchased from farms (CF) or local grocery/seafood markets (SM) throughout southern Mississippi. Farm-raised catfish were either purchased as fillets, dressed samples (whole fish with no head or skin) or nuggets (small pieces of fish which may be cut from regular blocks or blocks of minced fish). Thirty-three wild catfish were collected from one location along the Mississippi River (MR), two locations along the Leaf River (LR), and two locations along the Pearl River (PR); one of which is referred to as the Ross Barnett Spillway (RBS). Although sampling focused on obtaining the edible portion of fish (i.e., fillets), the sampling team observed local residents catching and eating whole fish at one of the sampling locations (Leaf River). As such, whole fish were obtained from the Leaf River and were analyzed on a "whole fish" basis. All other wild-caught catfish were filleted prior to analysis. Each sample was measured for length and weighed, and final fillet weights were recorded for filleted fish.

Tissue Analysis

Fish tissue samples were analyzed by Vista Analytical Laboratory (El Dorado Hills, CA) for the 17 laterally-substituted PCDD/Fs using high resolution gas chromatography-mass spectrometry (HGC-MS) according to EPA Methods 1613. All non-detect concentrations were assumed to have a concentration equal to the limit of detection (LOD) divided by the square root of two. Lipid content was determined according to EPA Method 8290

Data analysis

Two PCAs were performed using the fish tissue PCDD/F data. The first PCA included only the fish tissue data collected across southern Mississippi. The purpose of this PCA was to determine if the levels measured in fish samples reflected similar or different potential sources of contamination. The second PCA includes all fish tissue data as well as data from 6 industrial known sources of PCDD/F: 2,4,5-T manufacture[2], 2,4-D manufacture[3], chlor-alkali process manufacturing[3,6], sewage sludge[2,4], pentachlorophenol manufacture[5], and pulp and paper mill processing[3]. The purpose of this PCA was to determine whether any of the industrial sources show similar PCDD/F profile characteristics to the fish tissue samples.

Wet weight PCDD/F concentrations were first converted to lipid concentrations. To decrease the impact of differences in measurement scale among the congeners for the PCA, sample concentrations were range transformed. First, the percent contribution to total concentration for each chemical was calculated for each sample (both fish tissue and industrial source samples) by dividing each PCDD/F congener by the sum of all PCDD/F concentrations for that sample, as appropriate. The effect of this calculation was to scale all concentrations for an individual between zero and one. The data were then range transformed for each chemical across all samples included in a PCA, using the transformation presented below:

$$r`_{ij} = \frac{(r_{ij} - r_{\min j})}{(r_{\max j} - r_{\min j})}$$

Where

r_{ij} = Relative concentration of the *j*th congener in the *i*th sample
$r_{min\,j}$ = Minimum relative concentration of the *j*th congener
$r_{max\,j}$ = Maximum relative concentration of the *j*th congener

The purpose of the range transformation was to minimize the effects of large concentrations on the percent contribution calculation performed previously[2]. After range transforming the data, PCA was performed using the factor analysis tool in SYSTAT for Windows, version 11.00.01 (2004). Because the data set was range transformed, the covariance matrix option was used. The minimum eigenvalue criterion was set at 0.0001. The focus of this study is on factors explaining more than 5% of variance. Rotation and resampling options were not used.

Results and discussion

PCA including fish tissue concentrations

Figure 1 illustrates the factor score plots for the PCA. Factors 1, 2, and 3 describe 52%, 14% and 12% percent of the variance associated with this data, respectively (a total of 78% of variance described). Figure 3 illustrates the fingerprints associated with each factor. Factor 1 shows a profile commonly seen with background samples of PCDD/F[3]. Factor 2 shows strong negative loading for HpCDD, 6HxCDD, 6HxCDF, and 6HpCDF, and strong positive loading for 2HxCDF, 9HxCDF, 9HpCDF, and OCDF. Factor 3 shows generally positive loading associated with the dioxins and negative loading associated with furans.

Based on the factor score plots in figure 1, several interesting trends are observed. First, the farm-raised (CF), seafood market (SM), and Leaf River (LR) samples tend to group together based on positive factor 2 scores; thus indicating that they are probably being fed similar kinds of food which has similar contaminants. Pearl River (PR), Ross Barnett Spillway (RBS) and Mississippi River (MR) samples tend to show negative factor 2 scores, and positive factor 1 scores. This indicates that wild fish tend to obtain their dioxin from a variety of different sources; which is not unexpected. Interestingly, the RBS samples show more in common with the MS river samples than the PR samples. Factor 3 shows positive scores for CF, SM, and PR samples and negative scores for MR, LR, and RBS. Based on the results of the PCA, PR, LR and RBS samples can all be distinguished, CF and SM samples can not. Factor 2 is useful for distinguishing the PR samples, factor 3 is useful for distinguishing LR samples, and factor 1 is useful for distinguishing RBS samples

PCA including fish tissue and industrial source concentrations

Figure 2 presents the factor score plots for the PCA. Factors 1, 2, and 3 describe 36%, 22% and 10% percent of the variance associated with this data, respectively (a total of 68% of variance described). Figure 4 presents the fingerprints associated with each factor. Factor 1 shows a profile commonly associated with background levels of PCDD/F: all congeners loading positive, with OCDD loading negative. Factor 2 shows generally positive loading associated with the dioxins and weak or negative loading associated with furans (similar to factor 3 in the above analysis). Factor 3 shows negative loading for HpCDD and 6HxCDF (similar to factor 2 above) and also negative loading for OCDF and TCDD. Factor 3 also demonstrates strong positive loading for 2HxCDF, 9HxCDF, 9HpCDF, and OCDF.

From the factor score plots in figure 3, the catfish samples do not tend to plot with any of the 6 industrial sources included in this analysis. That is, the catfish samples do not correspond with any of the industrial sources included in the PCA. However, samples that have been used to characterize a particular industrial source do not all plot together either. This indicates that there can be a range of congener profiles within a single source category. For example, factor 3 indicates slight overlap between chlor-alkali and fish tissue for certain chlor-alkali samples; but not others. This same trend can also be seen for pulp and paper sources. The factor score plots are probably demonstrating process design characteristics unique to the facility of origin for the samples included in the PCA. In general, it usually not adequate to reach conclusive views about relating fish concentrations to a particular industry or source based on a few samples from the literature. Instead, some local samples of sediment or particles from the specific outfall should be collected and analyzed.

ConclusionThis work indicates that PCA should be an effective tool for showing the differences in dioxin/furan congener patterns for fishes caught in different rivers. In the first PCA analysis presented: SM and CF samples tend to plot together for all factors, MR and RBS samples tend to plot together for all samples, LR samples can be

distinguished by factor 3 scores, PR samples can be distinguished by factor 2 scores. In the second PCA analysis, it was determined that none of 6 known industrial sources of PCDD/Fs showed a strong similarity to the fish tissue samples. It was also demonstrated that if a local comparison is to be made for fish tissue samples and a local industrial source, it is essential to get samples of the local source for comparison in PCA.

References:

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Figure 1: Factor score plots for PCA including fish tissue data.

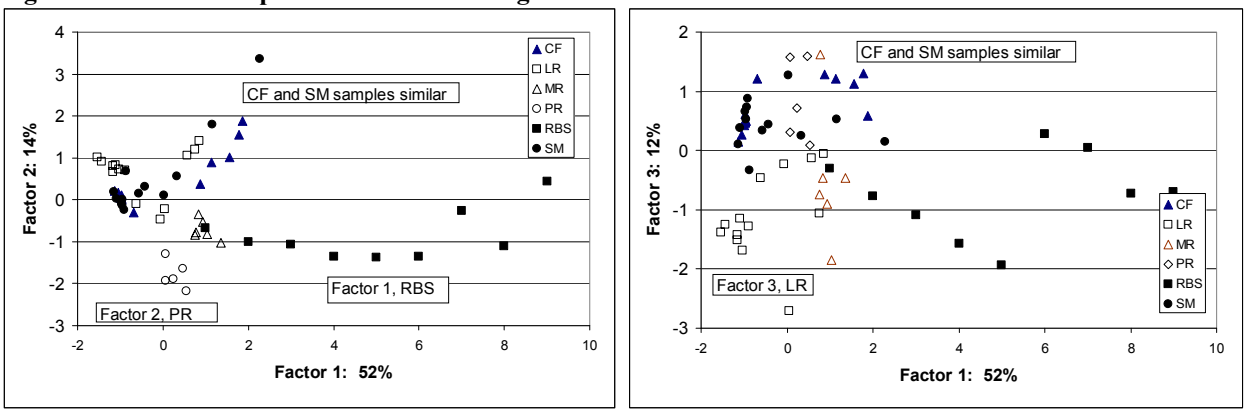
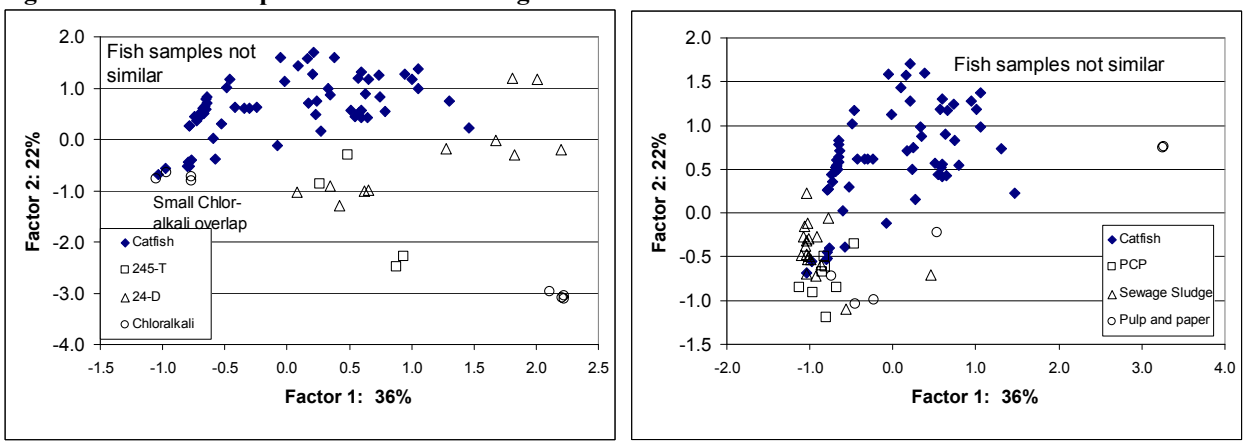


Figure 2: Factor score plots for PCA including fish tissue and industrial source data



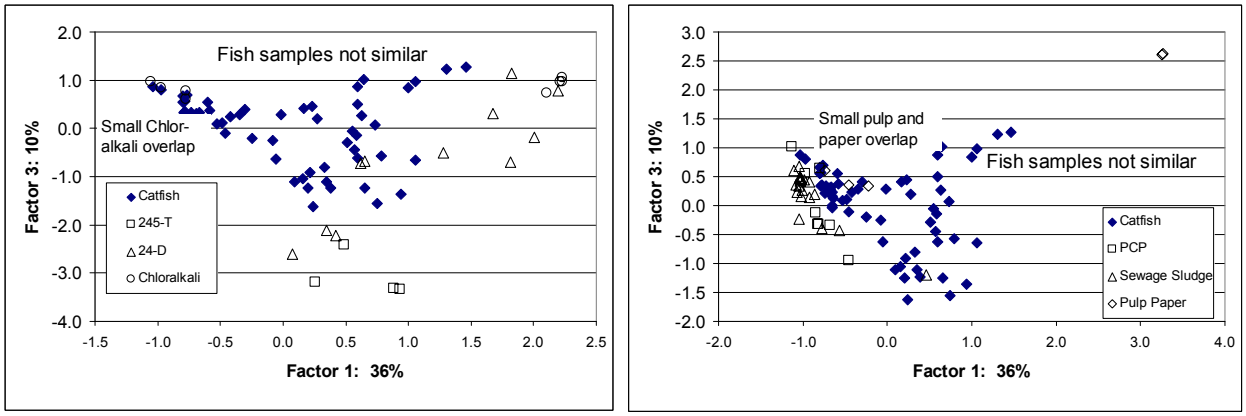


Figure 3: Factor loading plots for PCA including fish tissue data

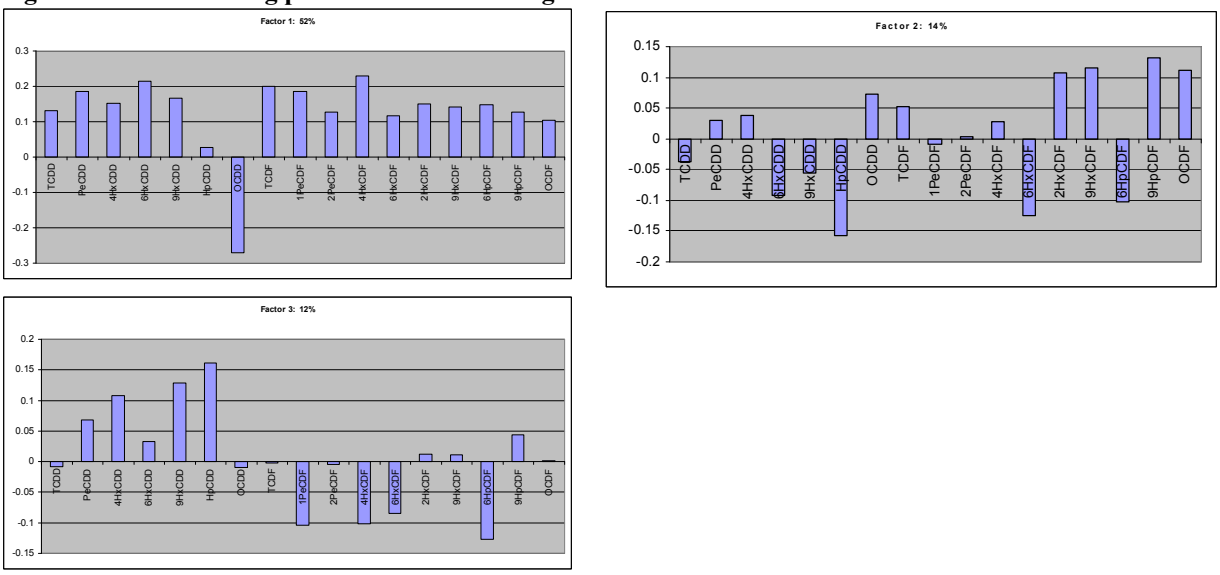


Figure 4: Factor loading plots for PCA including fish tissue and industrial source data