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**Biological and ecological factors related to trace element levels in harbour porpoises
(*Phocoena phocoena*) from European waters**

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Abstract

Selected trace elements (Cd, Cu, Hg, Se, Zn) were measured in the kidneys and the liver of 104 harbour porpoises (*Phocoena phocoena*) stranded along the coasts of France, Galicia (Spain), Ireland, Scotland (UK), and the Netherlands. Generally, relatively low concentrations of toxic elements were encountered in the tissues of European porpoises, except for two individuals, which displayed high hepatic Hg concentrations. Also, elevated Cd levels obtained in Scottish porpoises could be related to their feeding preferences and this result suggests an increase of the proportion of cephalopods in their diet with latitude. Moreover, significant geographical differences were seen in hepatic Zn concentrations; the elevated Zn concentrations displayed by porpoises from the Netherlands may relate their poor health status. Variation in metal concentrations within porpoises from the North Sea is likely to reflect a long-term segregation between animals from northern (Scotland) and southern areas (the Netherlands), making trace elements powerful ecological tracers.

Keywords: heavy metals, mercury, cadmium, zinc, marine mammals, age, diet, multivariate analysis, tracers

1. Introduction

Harbour porpoises (*Phocoena phocoena*) are one of the most common cetaceans in European waters (Hammond et al., 2002). Through the last century, declines in harbour porpoise populations have occurred in some northern European areas, particularly in the Baltic Sea (Benke et al., 1998; Koschinski, 2002), the southern North Sea and the English Channel (Klinowska, 1991; DOE, 1995). Several potential threats such as fishery by-catch, depletion of food resources, marine traffic and exposure to contaminants may have been responsible for the decline of these stocks (Evans, 1991; IWC, 1994; Koschinski, 2002). Consequently, the harbour porpoise has been categorised as vulnerable by the IUCN (The World Conservation Union) and it also constitutes a prioritised species in conservation and management for the UK (UK Biodiversity Plan Action; DOE, 1995), North Sea and adjacent areas (ASCOBANS, Agreement on the Conservation of Small Cetaceans of the Baltic and North Seas).

Harbour porpoises are relatively long-lived upper-level predators, which mainly inhabit coastal temperate areas (Read, 1999). Although these areas constitute productive systems in terms of providing nursery areas and habitats, they are also the repository for toxic agents and hazardous materials from industrial, agricultural and urban sources. These numerous sources contribute to sewage wastes, organic compound and heavy metal environmental enrichment. The question of whether environmental pollution is changing the dynamics of marine mammal populations remains yet unresolved. Indeed, bioaccumulation and long-term exposure to contaminants could be expected to pose a threat to the health and viability of these upper-level predators, especially for those living in industrial coastal areas, as it is the case for harbour porpoises in the north-eastern Atlantic (Aguilar & Borrell, 1995).

To date, experimental studies have shown that exposure to persistent organic contaminants could cause reproductive failure (e.g. Reijnders, 1986), immunosuppression (e.g. Ross et al., 1996), and diseases (e.g. De Guise et al., 1995) in some marine mammal species. As well, some studies have correlated high levels of mercury (Hg) and zinc (Zn) with the health status in free-living harbour porpoises (Siebert et al., 1999; Bennett et al., 2001; Das et al., 2004a; Strand et al., 2005; Kannan et al., 2006). However, causal connections to outbreak of diseases and increased mortality are likely to be complex, which make it difficult to evaluate the extent to which pollutants may contribute to adverse health effects *in situ* (Busbee et al., 1999). In particular, one additional aspect to consider regarding exposure to metals is that marine mammals have developed efficient detoxification capacities to support high levels of some toxic metals like Hg and cadmium (Cd) (see reviews by Cuvin-Aralar & Furness, 1991 and Das et al., 2000).

Concerning Hg, the demethylation of organic Hg in the liver leads to its co-precipitation with selenium (Se). Resulting non-toxic granules of tiemannite (HgSe) are stored in the liver, making it the ultimate organ of retention of Hg (Koeman et al., 1973; Martoja & Berry, 1980; Nigro & Leonzio, 1996). In the kidneys, Hg would rather be linked to proteins rich in sulphur (Chen et al., 1974; Neve & Therond, 1991), which could constitute a second route for Hg detoxification. Like Hg in the liver, Cd levels can also be particularly elevated in the kidneys of marine mammals (Wagemann & Muir, 1984; Dietz et al., 1998). In fact, potential toxic effects of Cd are mitigated by the binding to metallothioneins (MTs) in the liver and the kidneys (Klaassen & Liu, 1997; Teigen et al., 1999). MT is a low molecular weight protein, which also plays a key role in the homeostasis of Zn and copper (Cu) (Webb & Cain, 1982).

Although trace element levels in harbour porpoises are now well-documented for some northern European areas, i.e. the North Sea, the Baltic Sea and the Celtic shelf (Law et al., 1991, 1992; Siebert et al., 1999; Bennett et al., 2001; Das et al., 2004ab; Strand et al., 2005; Ciesielski et al., 2006), an overview of metal accumulation in porpoises is still required over a larger geographical scale in order to determine whether metallic pollution could constitute a particular threat for the populations that are generally considered as “at risk” (i.e. from northern areas) compared to more “healthy” ones (i.e. from southern areas). Therefore, by providing trace element data for some non-documented southern areas (i.e. the Atlantic coasts of France and Spain), as well as contemporaneous data for parts of northern Europe, this study aims at identifying geographical variations of trace element bioaccumulation (Cd, Cu, Hg, Se, Zn) in the liver and the kidneys of harbour porpoises stranded along European coasts, and at identifying the main factors influencing concentrations (including diet and persistent organic pollutant levels - POPs). The influence of diet on metal concentrations was investigated through fatty acid measurements since they constitute efficient integrated indicators of diet (e.g. Iverson et al. 1997, 2004; Bradshaw et al., 2003). Detoxification efficiency in these populations is also considered through the measurements of the essential elements that are linked to the neutralisation of Hg (i.e. Se) and linked to Cd detoxification (i.e. Cu and Zn), through MT induction. In addition, we investigated health status of some animals but these data were used at the interpretation stage only since infectious disease mortality is generally regarded as a consequence rather than a cause of high contaminant burdens (see Jepson et al., 2005).

2. Material and methods

Sampling and data collection

Between 1997 and 2003, 104 stranded harbour porpoises were collected along the coasts of Scotland (UK, $n = 36$), France ($n = 24$), Ireland ($n = 22$), Netherlands ($n = 19$), and Galicia (Spain, $n = 3$) (see Fig. 1). All carcasses were in good post-mortem condition, varying from a very fresh state to slightly decomposed. During each necropsy, individual characteristics of each animal were recorded and some tissues were sampled for further analyses, following the protocol from Kuiken & Hartmann (1991). Field information included date and location of stranding, gender and blubber thickness. Teeth were sampled in order to determine the age, blubber was used for fatty acid analyses and POPs measurements, and lastly, the kidneys and liver were dedicated for trace element analyses.

Temporal variation in trace element levels were investigated both at the year and at the quarter levels. Quarters were defined as Q1, January to March, Q2, April to June, Q3, July to September, and Q4, October to December. In addition, an index of nutritive condition was determined following blubber thickness, i.e. B1, < 10 mm, B2, 10-15 mm, B3, 15-20 mm, B4, 20-25 mm, and B5, > 25 mm, and 5) > 25 mm. Note that blubber thickness may vary seasonally in cetaceans (Elsner, 1999; Lockyer et al., 2003; Learmonth, 2006), but since we include season as an explanatory variable, any marginal effect of blubber thickness is arguably be related to condition. Where possible, necropsy included assessment of disease status and cause of death, with porpoises that died from trauma (i.e. by-catch, predation) being separated from those that presented significant pathologies (mainly pneumonia and lung parasitism).

Age was determined following the recommendations of Perrin & Myrick (1980). Briefly, this procedure consists of counting Growth Layer Groups (GLGs) from teeth sections, assuming that one GLG equals one year. Moreover, after lipid extraction of the inner blubber layer from a complete blubber core, 31 fatty acids were analysed by gas chromatography with flame ionisation detection (GC-FID). Concerning POPs analyses, organochlorines (18 PCB congeners, p,p'-DDE and HCB) were determined by gas chromatography with electron capture detection (GC-ECD) whereas brominated flame retardants (5 PBDE congeners) were determined using gas chromatography mass spectrometry with negative chemical ionisation (GC-NCI/MS). More details about these methods and detailed results are presented in Pierce et al. (2004), Zegers et al. (2005) and Learmonth 2006.

Trace element analyses

Tissues (kidneys, liver) dedicated to trace element analyses were stored in plastic bag at -20°C until being processed in the laboratory. Then, all equipment used in the sample processing was cleaned, and subsequently decontaminated for 24 h in a solution composed of 35 ml HNO_3 (65%) and 50 ml HCl (36%) for 1 L of Milli-Ro quality water. Fresh samples were freeze-dried and ground to powder. The mean ratio between dry weight (d.wt.) and wet weight (w.wt.) was 0.29 for liver and 0.23 for kidney. Each sample was then treated in duplicate.

For total Hg measurements, aliquots ranging from 0.5 to 2 mg of dried material were analysed in an Advanced Mercury Analyser spectrophotometer (Altec AMA 254). Hg determination in the AMA 254 involved evaporation of Hg by progressive heating to 800°C under oxygen atmosphere for 3 min, and subsequent amalgamation on a gold net. The net was then heated to

liberate the collected Hg, which was measured by Atomic Absorption Spectrophotometry (AAS). For other trace elements analyses (i.e. Cd, Cu, Se and Zn), 2 aliquots of approximately 200 mg of each homogenised dry sample were digested with 3.5 ml of 65% HNO₃ at 60°C for 3 days. The digested contents were then diluted to 10 ml in milli-Q quality water. Then Cd, Cu and Zn contents were assayed using a flame (Varian 250 Plus) AAS with deuterium background correction whereas Se and some low Cd contents were analysed with graphite furnace AAS (Hitachi Z-5000) with Zeeman background correction.

Quality controls were made using standard reference materials from National Research Council of Canada, i.e. dogfish liver (DOLT-2 and DOLT-3), dogfish muscle (DORM-2), and lobster hepatopancreas (TORT-2). These reference materials were treated and analysed under the same conditions as the samples. Results were in good agreement with the certified values (see Table 1). In addition, the laboratory participates in intercalibration exercises organised by the International Atomic Energy Agency (cf. Coquery et al., 1999). During the last exercise, our laboratory was classified in group 1, indicating the good quality of results for all analysed elements (Azemard et al., 2006). Detection limits ($\mu\text{g}\cdot\text{g}^{-1}$ d.wt.) were 0.004 for Cd, 0.5 for Cu, 3 for Zn, 0.8 for Se, and 0.005 for Hg. Metal concentrations in porpoise tissues were expressed as $\mu\text{g}\cdot\text{g}^{-1}$ w.wt.

As in previous studies on marine mammals (e.g. Falconer et al., 1983; Wagemann & Muir, 1984; Mackey et al. 1995), we found that concentrations of Cd were relatively low in the liver of some harbour porpoises. We report only renal Cd concentrations in this paper because far more porpoises were analysed for Cd in this tissue than in the liver. As well, Se was measured only in the liver since this tissue constitutes the ultimate site of Hg detoxification (Koeman et al., 1973; Martoja & Berry, 1980).

Data treatment

All concentrations below the detection limit were replaced with “dummy values” that were half of the detection limit in order to allow further statistical comparisons (Gibbons & Coleman, 2001). The sampled porpoises from Ireland included two mother-foetus pairs, allowing us to make some preliminary observations about relationships between metal concentrations in mother and foetus tissues.

Because trace element concentrations may depend on numerous biological and ecological factors (e.g. Aguilar et al., 1999), redundancy analysis (RDA) was used to relate trace element data to putative explanatory factors. This analysis was carried out on normalised data, excluding data for the two foetuses, using the software package Brodgar (www.brodgar.com). The explanatory variables selected were location and date (year, quarter) of stranding, gender, blubber thickness classes, age, fatty acid profiles and POPs concentrations. Because RDA is based on regression, the number of explanatory variables had to be smaller than the number of samples. A selection of variables was also required for the fatty acids and POPs explanatory variables. Hence, principal component analysis (PCA) was applied to the POPs data and to the fatty acid data and, in each case, the scores of the first two PC axes were used as explanatory variables in the RDA. Nominal data were coded as a set of dummy variables, each taking the value 0 or 1. Finally, Spearman's rank correlations were calculated in order to determine relationships between trace elements. In addition, we focussed on the Hg detoxification process through the calculation of Hg:Se molar ratio. This ratio was calculated as: $\text{Hg:Se} = (\text{Hg } (\mu\text{g}\cdot\text{g}^{-1} \text{ w.wt.}) / \text{Se } (\mu\text{g}\cdot\text{g}^{-1} \text{ w.wt.})) \times (78.96 \text{ (g}\cdot\text{mol}^{-1}) / 200.59 \text{ (g}\cdot\text{mol}^{-1}))$, where $200.59 \text{ g}\cdot\text{mol}^{-1}$ and $78.96 \text{ g}\cdot\text{mol}^{-1}$ are the atomic mass of Hg and Se, respectively.

3. Results

Trace element levels

Two foetus/mother pairs were collected along the Irish coast. As trace element concentrations in foetuses were excluded in the further statistical analyses, Table 2 gives the concentrations exhibited by the two foetus/mother pairs, as well as mean concentrations of the overall data set (without foetuses, $n = 102$).

Concentrations of Cd, Hg, and Se were particularly low in foetuses ($< 1 \mu\text{g}\cdot\text{g}^{-1}$ w.wt.) compared to mothers, and especially in the case of hepatic Hg and Se. Indeed, a 6 year-old female collected along the Irish Sea coast exhibited 140 and 65 fold higher concentrations of hepatic Hg and Se, respectively, than its foetus. Conversely, hepatic Cu concentrations were about 5 times higher in foetuses than their mothers. No such differences between foetuses and mothers were detected for Cu in the kidneys or for Zn in either liver or kidneys.

Concerning overall data (excluding foetuses), the highest coefficients of variation were encountered for Cu, Hg and Se in liver, and Cd in kidneys ($\text{CV}\% > 100$). For these elements, maximal concentrations were obtained in animals aged from 4 to 6 years-old, except in the case of hepatic Cu, for which the highest concentrations was encountered in a 0.5 year-old individual. Maximal renal Cd and hepatic Hg concentrations were obtained in porpoises from the northern North Sea ($11.9 \mu\text{g}\cdot\text{g}^{-1}$ w.wt.) and Irish Sea ($165 \mu\text{g}\cdot\text{g}^{-1}$ w.wt.) respectively. In addition, several maxima occurred in porpoises from Southern North Sea, i.e. for renal Hg, and hepatic Cu, Se and Zn.

Factors influencing trace element concentrations

Table 3 summarises results from RDA, which related trace element data to location (countries) and date (years, quarters) of stranding, gender, blubber thickness classes, age, blubber fatty acid profiles and blubber POPs concentrations. All investigated explanatory variables together explained 36.0 % of the variation of trace element data, among which the first two axes respectively explained 49.5% and 15.8% of (explained) metal variability (Table 3). We observed close relationships between axis 1, Hg and Se, as well as between axis 2 and Cd (Fig. 2a).

Hence, the most important explanatory variables were age ($p = 0.005$), and the Netherlands (i.e. the difference between samples from the Netherlands and those from elsewhere, $p = 0.005$). The second PCA axis score summarising fatty acid profiles had a weakly significant effect ($p = 0.04$), as did season (quarter 1, $p = 0.04$, quarter 3, $p = 0.045$). Age was well-correlated to axis 1 (i.e. to Hg and Se) and to a lesser extent to axis 2 (i.e. to Cd) (Table 3, Fig. 2a). Renal Cd concentrations were also clearly linked to the fatty acid profile (PCA axis 2) (Table 3, Fig. 2ac). Individual plots showed that Scottish animals were those with the highest Cd levels (cf Fig. 2a and 3). Note that the “Netherlands” variable was equally correlated to both axes 1, 2, and 5 (Table 3). Examination of biplots highlighted that porpoises from the Netherlands displayed higher Zn concentrations in their liver than animals from other countries (Fig. 2ab).

The seasonal variation in hepatic Cu and renal Hg concentrations may be of minor importance in relation to the overall variability of trace element data, since effects of the dummy variables representing quarters 1 and 3 were not correlated to the first two axes of the RDA (Table 3).

Note also that POPs concentrations in blubber were not related to trace element levels in the kidneys and the liver (Table 3).

Relationships between trace elements

Table 4 presents Spearman correlation values (R_S). Hepatic Se was closely and positively correlated to hepatic and renal Hg ($R_S = 0.909$ and 0.889 , respectively). Figure 4 illustrates geographical variation in the relationship between Hg and Se in the liver. Generally, Hg:Se molar ratios ranged from 0.16 to 1.27, with only seven animals from four areas (northern and southern North Sea, north-western Scotland, Irish Sea) displaying a molar excess of Hg. Most animals (i.e. 73%) exhibited a Hg:Se molar ratio lower than 0.8.

4. Discussion

Cadmium

Relatively low Cd concentrations were encountered in the kidneys of harbour porpoises from the western coasts of Europe. Indeed, mean renal Cd level of the overall sample ($n = 102$) was only $1.32 \pm 1.81 \mu\text{g.g}^{-1}$ w.wt. (Table 2). Aguilar & Borrell (1995) firstly hypothesized that such low levels could be due to the physiological peculiarity of the harbour porpoise, or reflect stable low levels of Cd in their environment. In fact, Cd concentrations in the tissues of marine mammals are generally considered to be diet-related (Aguilar et al., 1999). Feeding on cephalopods is probably a major source of Cd for small cetaceans both because of the high Cd levels in these prey and because Cd is present mainly in bioavailable forms in their tissues (Bustamante et al., 1998; 2002).

As a result, close relationships were previously found to occur between renal Cd concentrations and muscular stable isotope ratio of nitrogen in porpoises from European waters (Das et al., 2004a). Here, RDA showed a correlation between renal Cd concentrations and one of the synthetic variables summarising fatty acid profiles (Fig. 2ac, Table 3), which would be expected to reflect diet. Note that Scottish porpoises would be those with the highest Cd levels (Figs. 2ac, 3) and that the maximal Cd concentration obtained during this study ($11.9 \mu\text{g}\cdot\text{g}^{-1}$ w.wt.) was found in a 4 year-old individual from this area (Table 2). Such a high Cd concentration nevertheless remains far below those displayed by harbour porpoises from higher latitudes (i.e. Greenland and Iceland; Table 5), in which renal Cd levels often reach the $50 \mu\text{g}\cdot\text{g}^{-1}$ w.wt. threshold suspected to produce toxic effects in humans (Elinder & Järup, 1996). The much higher Cd levels commonly encountered in harbour porpoises from arctic areas occur despite the fact that the concentration of Cd in seawater is about the same in temperate and arctic waters (Møhlenberg & Jensen, 1980; Elinder, 1985; Asmund, pers. comm. *vide* Palludan-Müller et al., 1993). In fact, Cd levels are well-known to be elevated in arctic sediments and organisms (e.g; MacDonald & Sprague 1988; AMAP, 1998; Dietz et al., 1998; Zauke et al. 1999), and especially in cephalopods (Bustamante et al., 1998). The squid biomass occurring in stomach contents is much higher in porpoises from Greenland (up to 37%; Heide-Jørgensen & Lockyer, 1999) than in European ones (up to 7.3%; Pierce et al., 2004), which could explain the higher renal Cd levels obtained in Greenland animals compared to European animals. Within Europe, Scottish porpoises consume a larger proportion of cephalopods than porpoises from the other studied countries (Pierce et al., 2004; Santos et al., 2004; Spitz et al., 2006). Such a differential consumption of cephalopods might also induce higher Cd levels in the tissues of Scottish porpoises as demonstrated elsewhere for common dolphins (Lahaye et al., 2005). Consequently, the overall south to north increase of Cd concentrations in the kidneys of harbour porpoises from north-eastern Atlantic (see Table

5) strongly suggests a diet modification at the population scale, which would be characterised by an increase of cephalopod consumption with latitude, maybe related to feeding over deeper waters.

Regarding relationships between metals, the essential elements Cu and Zn are commonly related to Cd (Das et al., 2000), which would suggest induction of MTs and possible competition or increase in metal binding-sites (Klaassen & Liu, 1997; Wagemann et al., 1988; Teigen et al., 1999). Here, neither the Cd-Cu nor Cd-Zn relationships were significant (Table 6). Hence, the low Cd concentrations to which porpoises may be exposed in European waters are probably not sufficient to induce Cu or/and Zn ion displacement from MTs, and consequently leading to co-accumulation with Cd. Such results could easily be confirmed through MT measurements in the kidneys of porpoises.

Hepatic Cu concentrations were higher in foetuses than their mothers (Table 2), presumably due to an important transplacental transfer of this metal during pregnancy (e.g. Underwood, 1977; Law, 1996; Wagemann et al., 1988; Yang et al., 2004). Elevated Cu concentrations in offspring could be due to either bioaccumulation of Cu during pregnancy coupled with low excretion rates in foetus or a specific biochemical requirement for development (Wagemann et al., 1988). In other words, foetuses could have only limited Cu excretion *via* the bile or elevated MTs in the liver to store essential elements for growth. Conversely, the Cd levels obtained in the kidneys of foetuses were extremely low (Table 1), which is common in marine mammal foetus tissues (Honda & Tatsukawa, 1981, 1983; Fujise et al., 1988; Wagemann et al., 1988; Law et al., 1992; Caurant, 1994; Yang et al., 2004; Lahaye et al., submitted). This clearly indicates a limited transfer of Cd during pregnancy (e.g. Bell, 1984).

Mercury

Mercury in porpoise's prey species occurs mainly in the organic form (e.g. Bloom, 1992; Bustamante et al., 2006), and methyl-Hg could be transferred from the mother, through the placenta, and accumulated in the foetus (Law et al., 1992). Here, Hg concentrations found in the tissues of foetuses were particularly low ($< 1 \mu\text{g}\cdot\text{g}^{-1}$ w.wt.), in comparison to concentrations found in their mothers (up to 140 fold higher in the mother's liver, Table 2). Although organic Hg was not measured during this study, the low Hg levels encountered in foetuses suggest that transplacental transfer from mother to foetus is not a major accumulation route for the offspring. Such a limited maternal transfer of Hg during pregnancy has been several times been pointed out for other cetacean species (Itano & Kawai, 1981; Honda & Tatsukawa, 1981, 1983; Fujise et al., 1988; Law et al., 1992; Caurant et al., 1993; Yang et al., 2004; Lahaye et al., submitted).

Excluding the two foetuses, the range of Hg concentrations found in this study was wide, especially in the liver, where concentrations varied from 0.28 to $165 \mu\text{g}\cdot\text{g}^{-1}$ w.wt., against only 0.11 to $5.8 \mu\text{g}\cdot\text{g}^{-1}$ w.wt. in the kidneys. Although some porpoises from the North Sea have been reported to exhibit renal Hg concentrations reaching $30 \mu\text{g}\cdot\text{g}^{-1}$ w.wt. (Siebert et al., 1999), the low renal Hg levels obtained during this study are within the range of values reported in most studies carried out on this species over a large spatial scale (Gaskin et al., 1979; Falconer et al., 1983; Palludan-Müller et al., 1993; Teigen et al., 1993; Joiris et al., 2001; Das et al., 2003; 2004a).

Furthermore, and contrary to Cd, a strong increase of Hg concentrations with age occurred in both the kidneys and the liver (Fig. 2a and Table 3), as reported in several studies on harbour porpoises and also on other marine mammals (see review by Aguilar et al., 1999). Such a

strong relationships with age may partly explain the important variability of Hg concentrations in the liver (Table 2). The two highest concentrations (i.e. 165 and 139 $\mu\text{g}\cdot\text{g}^{-1}$ w.wt., Table 5) were recorded in animals that were only 5 and 6 years-old, from Irish Sea and the Netherlands respectively. These two porpoises displayed Hg concentrations in liver that were at the bottom of the range likely to induce toxic effects in marine mammals (i.e. 100-400 $\mu\text{g}\cdot\text{g}^{-1}$ w.wt.; Wagemann & Muir, 1984). Such extreme concentrations have previously been reported in the liver of harbour porpoises and other marine mammals from the Irish Sea and Dutch waters and they were attributed to anthropogenic inputs (Reijnders, 1986; Law et al., 1992; Simmonds et al., 1993; Das et al., 2004a). However, when looking at the population scale, no significant differences in Hg accumulation were detected among areas (Table 3, Figs. 2a and 3). Hence, excluding these two individuals from the Irish Sea and the Netherlands, Hg levels would be equivalent throughout the study area, indicating that the northern populations generally considered as “at risk” are not particularly more threatened by exposure to Hg than the more “safe” populations from southern areas.

Numerous studies have discussed the protective effect of Se on Hg toxicity and a molar ratio of 1:1 for Hg:Se has been reported to reflect efficient Hg detoxification in many studies involving marine mammals (e.g. Koeman et al., 1973; Palmisano et al., 1995; Caurant et al., 1996; Dietz et al., 2000). Selenium is also an essential element that is incorporated into anti-oxidant enzyme systems, and especially glutathione peroxidase (Bondy, 1996). As pointed out by Dehn et al. (2005), a 1:1 Hg:Se molar ratio would indicate that almost all available Se is bound to Hg. Owing to the oxy-radical scavenging involvement of Se, tissue ratios close to 1:1 could be also a possible indicator for compromised health (Dietz et al., 2000). Most of European porpoises from this study exhibited a Hg:Se ratio lower than 1:1 (Fig. 4), indicating an excess of Se compare to Hg, and thus also its bioavailability for its other functions (e.g. with glutathione peroxidase).

Finally, among the 102 studied porpoises, only 7 displayed a molar excess of Hg and these individuals were all collected along the coasts of the Netherlands and Irish Sea (Fig. 4). Based upon the relationships between Hg and Se in the liver, formation of tiemannite would occur in the liver of porpoises from European waters, except in the case of a limited number of individuals, which originated from two areas that are well-known for their anthropogenic background of chemical inputs.

Zinc

Zinc is essential to the integrity of the immune system of mammals. Law et al. (1991) hypothesized a range of liver concentrations within which Zn could be regulated as 20-100 $\mu\text{g}\cdot\text{g}^{-1}$ w.wt. Thirteen of the 102 porpoises sampled exhibited concentrations outside this range. Respectively, 5 and 3 individuals originated from the Netherlands and the Irish Sea, and a particularly elevated concentration (288 $\mu\text{g}\cdot\text{g}^{-1}$ w.wt) was encountered in a 9 year-old porpoise (Table 2). Among these 13 porpoises with high Zn hepatic levels, 11 individuals had a deteriorated health status, i.e. high parasitic burden and pulmonia (Pierce et al. 2004). Such particular high Zn levels were previously reported in the liver of harbour porpoises from the North Sea that exhibited deteriorating health condition (emaciation and bronchopneumonia) (Das et al., 2004a). This increase of Zn concentrations in the liver could be due to a general redistribution of this metal within the organs (muscle and blubber to liver) as a result of protein and lipid catabolism. Although emaciation was not directly evaluated in our samples, trace element concentrations were not related to blubber thickness (Table 3), suggesting that only the presence of a pathology may be associated with the observed high Zn hepatic levels. In humans, it is well-established that infection is associated with Zn redistribution, and in particular, that concentrations in liver rise as a result of acute-phase protein synthesis (Scott,

1985; Hambridge et al., 1986; Amdur et al., 1991). As Zn does not accumulate with age (Fig. 2a), the high Zn concentrations encountered in porpoises that exhibited pathological features are probably a response to infections rather than a direct cause. In addition, given that half of the Netherlands sampling was composed of animals with pathology, the higher Zn levels obtained for this area (Fig. 2ac, Table 3) could also be related to their poor health status. Note also that particularly high POPs levels have been encountered in the blubber of Netherlands porpoises, suggesting that this compromise health status could be induced by organic pollution in this area (Pierce et al. 2004).

5. Conclusions

The present study confirms results from various studies on the Cd and Hg in marine mammals from Arctic and temperate regions, which indicates that animals from lower latitude display far lower Cd levels in their tissues and that the Hg levels are about the same in both areas, despite a higher anthropogenic influence in temperate waters (Palludan-Müller et al., 1993; Szefer et al., 2002; Bustamante et al., 2004; Das et al., 2004a). This is likely to reflect lower rates of Cd exposure through temperate marine food webs compare to polar and subpolar areas, for which Cd natural enrichment of food webs is well-established (MacDonald & Sprague 1988; AMAP, 1998; Dietz et al., 1998; Zauke et al., 1999).

In general, stock structure (genetic and ecological) should be considered when considering conservation and management needs for a given species. Within European waters, both mitochondrial DNA (mtDNA) and microsatellite data suggest that harbour porpoises would constitute a single continuous population, which would be characterised by a fine scale partitioning according to latitude (Fontaine et al. submitted). Trace element concentrations are now well-known as interesting tracers to provide to provide further ecological information

about a given species (e.g. Das et al., 2003, 2004a; Lahaye et al., 2005; Caurant et al., 2006). Here, some renal Cd level differences occurred among northern and southern areas of the North Sea. In addition, animals from the southern area also displayed higher Zn levels than those stranded northern (probably because of the poor health status displayed by Netherlands individuals). Such results indicate that a long-term ecological segregation would occur in porpoises from northern and southern areas of the North Sea. Furthermore, it has to be pointed that some Netherlands porpoises were displaying the highest values of both hepatic Hg and Zn, plus Hg:Se in the liver, but also PCBs in blubber (Pierce et al., 2004). Despite no correlations between POPs concentrations in blubber and trace elements have presently been detected, the simultaneous occurrence of particular elevated levels of some metallic and organochlorine contaminants in Netherlands porpoises could also make their health status compromised.

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Tables

Table 1. Trace element levels (mean \pm SD; $\mu\text{g}\cdot\text{g}^{-1}$ d.wt.) in standard reference materials.

Metals	Standard reference materials (NRCC)							
	TORT-2		DORM-2		DOLT-2		DOLT-3	
	Certified value	Observed value	Certified value	Observed value	Certified value	Observed value	Certified value	Observed value
Cd	26.7 \pm 0.6	26.2 \pm 0.2	0.046 \pm 0.008	0.046 \pm 0.001	20.8 \pm 0.5	20.6 \pm 0.4	19.4 \pm 0.6	19.2 \pm 0.4
Cu	-	-	-	-	25.8 \pm 1.1	26.2 \pm 1.0	31.2 \pm 1.0	30.1 \pm 0.8
Hg	0.27 \pm 0.06	0.28 \pm 0.02	4.64 \pm 0.26	4.46 \pm 0.20	2.14 \pm 0.28	2.08 \pm 0.11	3.37 \pm 0.14	3.36 \pm 0.02
Se	-	-	-	-	6.06 \pm 0.49	5.82 \pm 0.40	7.06 \pm 0.48	6.70 \pm 0.56
Zn	-	-	-	-	85.8 \pm 2.5	86.5 \pm 2.3	86.6 \pm 2.4	85.1 \pm 4.3

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Table 2. Trace element concentrations ($\mu\text{g.g}^{-1}$ w.wt.) in 2 foetus/mother pairs of harbour porpoises, relative to mean levels (\pm SD) of the overall population data (n = 102). CV% is the percentage of variation, na refers to not analysed samples and y.o. to years old.

Trace element concentrations and sample characteristics	Pair 1 (Irish Sea)		Pair 2 (Southern Ireland)		Overall population data			For highest concentrations:
	foetus	mother	foetus	mother	mean \pm sd	range	CV %	area; age
Hg in liver	0.52	69.6	0.65	36.0	17.3 \pm 27.0	0.28 - 165	156	Ireland; 5 y.o.
Hg in kidney	na	2.7	0.17	1.96	1.57 \pm 1.28	0.11 - 5.8	82	Netherlands; 6 y.o.
Cd in kidney	na	0.56	0.05	1.62	1.32 \pm 1.81	0.002 - 11.9	137	Scotland; 4 y.o.
Se in liver	0.57	36.8	0.94	23.8	11.0 \pm 17.1	0.31 - 105	156	Netherlands; 6 y.o.
Cu in liver	87.2	19.3	58.9	11.1	12.7 \pm 21.5	2.1 - 194	170	Netherlands; 0.5 y.o.
Cu in kidney	na	2.7	1.79	2.9	3.80 \pm 1.32	2.1 - 8.9	35	France; 0 y.o.
Zn in liver	25.6	39.3	46.9	26.0	62.2 \pm 45.3	15.5 - 288	73	Netherlands; 9 y.o.
Zn in kidney	na	18.8	24.7	20.8	22.7 \pm 6.3	14.8 - 62.4	28	Ireland; 0 y.o.
Body length (cm)	40.0	151	63.5	171	135 \pm 24.5	81.5 - 187	18	
Age (y.o.)	na	6	na	6.5	4.1 \pm 4.1	0 - 24	102	

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Table 3. Results of redundancy analysis (RDA). For each axis, the table shows correlations with explanatory variables, the eigenvalue as percentage of inertia (E%) and as percentage of sum of all canonical eigenvalues (E/ SE%). Note: total inertia = 1.00; sum of all canonical eigenvalues = 0.36. F statistics of conditional effects refer to the increase in explained variation due to adding extra explanatory variable.

Variable	F statistic	P-value	Correlation with axes					Involved metals	Figures
			1	2	3	4	5		
Age	13.054	0.005	0.78	0.36	0.06	0.06	0.07	HgL, HgK, SeL, CdK	2a
Netherlands	4.914	0.005	0.51	0.41	0.08	0.02	0.42	ZnL	2ab
Quarter 3	2.499	0.045	0.03	0.14	0.51	0.41	0.21	CuL	not shown
POPs axis 2	2.458	0.050	0.04	0.09	0.14	0.32	0.14		
Fatty acids axis 2	2.245	0.040	0.19	0.63	0.23	0.05	0.01	CdK	2ac
Quarter 2	1.839	0.105	0.24	0.18	0.04	0.16	0.22		
Quarter 1	2.053	0.040	0.01	0.02	0.14	0.33	0.30	HgK	not shown
France	1.594	0.160	0.05	0.08	0.21	0.48	0.02		
Blubber 4	1.546	0.180	0.07	0.17	0.33	0.31	0.15		
Irlande	1.446	0.215	0.12	0.13	0.05	0.00	0.71		
Female	1.433	0.200	0.34	0.15	0.07	0.09	0.08		
Fatty acids axis 1	1.095	0.350	0.06	0.23	0.31	0.06	0.22		
Blubber 3	1.080	0.385	0.17	0.14	0.08	0.29	0.01		
Scotland	1.074	0.340	0.29	0.36	0.03	0.30	0.19		
POPs axis 1	0.833	0.465	0.12	0.11	0.19	0.15	0.12		
2002	0.739	0.600	0.16	0.06	0.08	0.37	0.09		
2001	0.841	0.540	0.14	0.02	0.24	0.05	0.31		
Blubber 2	0.535	0.765	0.08	0.01	0.18	0.02	0.13		
Blubber 1	1.419	0.200	0.35	0.14	0.01	0.13	0.37		
2003	0.498	0.800	0.14	0.01	0.10	0.21	0.05		
		E%	17.77	5.66	4.64	3.06	2.62	Cumulative E% = 33.7	
		E/SE%	49.46	15.75	12.90	8.51	7.30	Cumulative E/SE% = 93.9	

Table 4. Spearman rank correlation matrix between trace elements in the liver and the kidneys of harbour porpoises stranded along the European coast. Bold characters refer to significant correlations ($\alpha = 0.05$).

	Liver				Kidney			
	Hg	Se	Cu	Zn	Hg	Cd	Cu	Zn
Liver								
Hg	+1							
Se	+0.909	+1						
Cu	+0.003	+0.036	1					
Zn	+0.068	-0.002	+0.004	+1				
Kidney								
Hg	+0.889	+0.855	-0.055	+0.019	+1			
Cd	+0.529	+0.544	-0.020	+0.086	+0.350	+1		
Cu	-0.219	-0.299	+0.290	+0.160	-0.252	+0.030	+1	
Zn	-0.062	-0.115	+0.065	+0.399	-0.112	+0.177	+0.498	+1

Table 5. Hepatic Hg and renal Cd levels ($\mu\text{g}\cdot\text{g}^{-1}$ w.wt.) in harbour porpoises (literature data).

Key: * refers to geometric mean; ** d.wt. converted in w.wt. on the basis of the d.wt. : w.wt.

ratio obtained during this study. LD is limit of detection.

Geographical location	Reference	Hepatic Hg			Renal Cd		
		n	Mean \pm sd	Range	n	Mean \pm sd	Range
Greenland							
West*	Palludan-Müller et al., 1993	44	4,2	0.48 - 20.7	26	13,2	0.11 - 72.5
West	Strand et al., 2005	2	6,6	6.9 - 6.3		na	na
South West	Szefer et al., 2002		na	na	42	55,3	0.32 - 210
Northwestern Atlantic							
Canada (Bay of Fundy)	Gaskin et al., 1979	68	12	0.5 - 112		na	na
US	Mackey et al., 1995	6	9.9 \pm 15.2	0.56 - 38.6		na	na
North Sea							
Iceland**	Das et al., 2004a	11	4.6 \pm 4.1	0.41 - 12.8	11	4.4 \pm 3.9	< LD - 54.7
Norway	Teigen et al. 1993	92	2.9 \pm 3.2	0.26 - 9.9		na	na
Norway**	Das et al., 2004a	21	4.1 \pm 2.9	0.29 - 9.3	20	1.38 \pm 1.04	< LD - 3.7
Scotland (East)	Falconer et al., 1983	23	na	0.28 - 15.9	23	na	0.17 - 7.42
Scotland (East)	This study	24	8.7 \pm 10.1	0.38 - 31	19	2.30 \pm 2.56	0.002 - 11.9
Denmark	Strand et al., 2005	15	8.5 \pm 10.2	0.42 - 32.8		na	na
Denmark**	Das et al., 2004a	17	6.4 \pm 10.4	0.29 - 42.6	15	0.25 +/- - 0.23	0.023 - 0.81
Denmark (Inner waters)	Strand et al., 2005	20	6.4 \pm 20	0.22 - 92		na	na
Germany**	Das et al., 2004a	14	4.1 \pm 5.2	0.29 - 16.2	12	0.92 \pm 2.1	< LD - 7.59
Belgium and France	Das et al., 2004a	27	6.7 \pm 19.1	0.17 - 99.8	48	0.71 \pm 0.71	< LD - 2.8
Netherlands and France	This study	22	25.8 \pm 33.6	0.88 - 139.4	22	0.84 \pm 0.85	0.04 - 3.52
Baltic Sea							
Poland	Szefer et al., 1994		na	na	4	1.41 \pm 1.05	0.21 - 2.7
Poland	Ciesielski et al., 2006	14	6.6 \pm 16.9	0.46 - 65.1		na	na
Germany**	Das et al., 2004a	9	1.31 \pm 1.04	0.26 - 3.4	9	0.25 \pm 0.35	< LD - 1.15
Celtic shelf							
Scotland (North West)	This study	5	12.4 \pm 11.8	1.2 - 26.0	2	6,4	2.3 - 10.4
Ireland**	Das et al., 2003	5	7.0 \pm 12.2	1.19 - 28.7	7	0.90 \pm 0.78	0.09 - 2.3
Ireland (South)	This study	12	16.1 \pm 20.8	0.28 - 54.9	14	1.03 - 0.84	0.04 - 3.19
Ireland and Scotland (Irish Sea)	This study	12	30.0 \pm 48.8	0.81 - 165.0	11	0.60 \pm 0.56	0.002 - 1.66
Ireland and Wales (Irish Sea)	Law et al., 1992	29	21.8 \pm 44.1	0.6 - 190		na	na
England and Wales	Bennett et al., 2001						
	(infectious disease)	37	20.0 \pm 5.5	na		na	na
	(physical trauma)	49	12.3 \pm 3.4	na		na	na
France (Channel)**	Das et al., 2003	4	2.6 \pm 3.2	0.87 - 7.5	4	0.35 \pm 0.62	< LD - 1.29
France (Channel)	This study	8	10.7 \pm 14.3	0.80 - 40.0	8	0.60 \pm 0.71	0.01 - 1.64
Bay of Biscay							
France	This study	13	17.9 \pm 21.9	0.99 - 65.5	13	1.45 \pm 1.37	0.12 - 5.12
Spain (Galicia)	This study	3	1.20 \pm 0.30	0.96 - 1.58	2	0,09	0.03 - 0.14
Black Sea							
Black Sea	Joiris et al., 2001	57	1,9	0.14 - 9.9		na	na
Black Sea**	Das et al., 2004b	41	2.4 \pm 2.8	0.17 - 8.8	42	1.33 \pm 1.08	< LD - 3.5

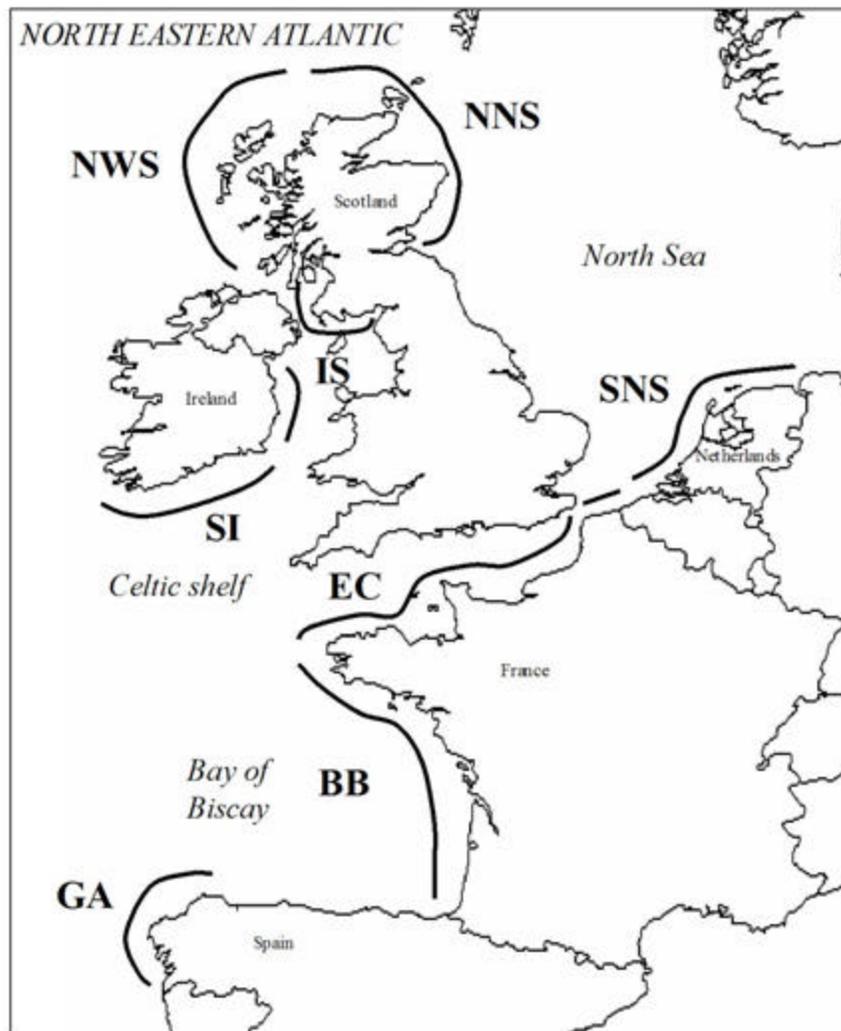
Figure captions

Figure 1. Map of the sampling area from which stranded harbour porpoises were collected: NNS = Northern North Sea (Scottish east coast); SNS = Southern North Sea (Dutch/French coast, north of Calais); NWS = Northwest coast of Scotland; IS = Irish Sea (Scottish west coast south of Campbeltown and Irish east coast); SI = Southern Ireland (Celtic Sea); EC = Channel (French north coast, south of Calais); BB = Bay of Biscay (French west coast); GA = Galician north and west coasts.

Figure 2. RDA biplot on trace element data: a) axes 1-2, b) axes 1-5, c) axes 2-3. With: Cd, Cu, Hg, Se, Zn “L or K” referring to “in Liver or in Kidneys”; “B1 to B5”, to Blubber classes; “Q1 to Q4”, to Quarters; “FA 1 and 2”, the first two axes of the PCA carried out on Fatty Acids; “POP1 and 2”, the first two axes of the PCA carried out on POPs; “Neth”, Netherlands; “Scot”, Scotland; “Irl”, Ireland; and “Fr”, France.

Figure 3. Molar Se and molar Hg concentrations in liver tissue of harbour porpoises stranded along the European coast: NNS = Northern North Sea; SNS = Southern North Sea; NWS = Northwest coast of Scotland; IS = Irish Sea; SI = Southern Ireland; CH = Channel; BB = Bay of Biscay; GA = Galicia.

Fig 1:



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Fig 2a:

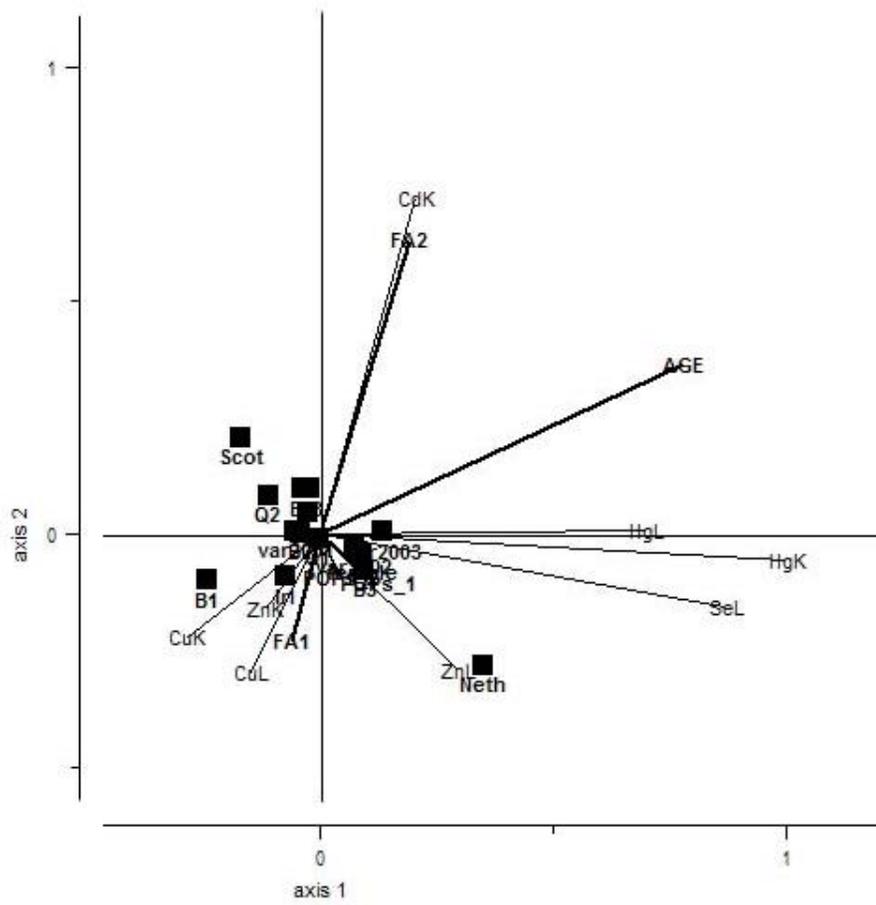


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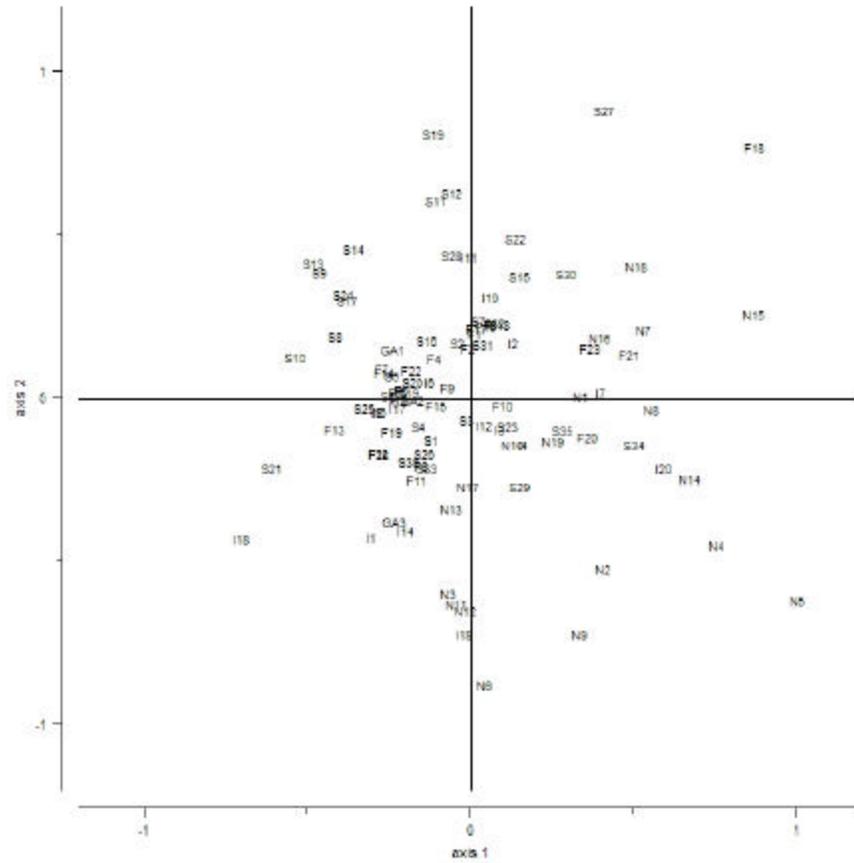


Fig 4:

