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Metals and organic contaminants including pesticides and musk substances in earthworms from three localities in Sweden.

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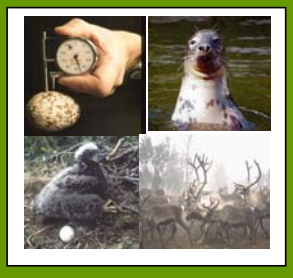


Content

Sammanfattning

Denna rapport är utförd på uppdrag av Naturvårdsverket (Överenskommelse 221-1031). Syftet var att kartlägga aktuella halter av ett antal miljögifter i daggmask från tre olika områden i Sverige. Syftet var också att jämföra halter i daggmask med övriga matriser, i första hand stare (*Sturnus vulgaris*) som har använts i den terrestra miljöövervakningen av jordbrukslandskapet sedan tidigt 1980-tal och att utvärdera hur väl daggmask lämpar sig som matris i den terrestra miljöövervakningen av miljögifter.

Daggmask utgör en viktig födokomponent för många fåglar och mindre däggdjur som i sin tur är viktiga födokomponenter för rovfåglar och andra terrestra predatorer. Daggmask kan därför vara en viktig länk mellan halter i jord och halter i levande organismer högre upp i näringskedjan. I Sverige förekommer ett tjugotal arter av daggmask tillhörande fem släkten[1], alla av familjen Lumbricidae. Dessa förekommer i olika typer av biotoper och har lite olika preferenser på jordens beskaffenhet. De arter som i första hand har varit av intresse i det här sammanhanget är de relativt storvuxna arterna av släktet Lumbricus, Allolobophora/Aporrectodea och i viss mån även Denrobaena. Det har legat utanför den här studien att gå närmare in på artantal och artfördelning på de olika insamlingslokalerna, inte heller har hänsyn tagits till ålder/grad av könsmognad hos de insamlade individerna. Daggmaskarna insamlades under sista dagarna av augusti och först halvan av september och inget urval gjordes vid insamlingstillfället, annat än att märkbart skadade individer valdes bort. I samband med frysningen av maskarna, c:a tre dygn efter insamlingen gjordes ett nytt urval där enbart friska och aktiva maskar valdes ut.

När det gäller val av substanser har vi utgått från de substanser som tidigare har analyserats, framför allt i stare sedan starten av övervakningsprogrammet. Dessa är ett antal metaller (Ca, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, Pb, V, Zn), klorerade substanser såsom PCB (CB-28, CB-52, CB-101, CB-118, CB-153, CB-138, CB-180), ΣDDT (DDT, DDE, DDD), HCB, α-, β-, och γ-HCH (lindan). Vidare har bromerade flamskyddsmedel, BFRs (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-209) samt HBCD och ett antal perfluorerade substanser, PFCs (PFHxA, PFHpA, PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFTriA, PFTeA, PFBS, PFHxS, PFOS, PFDcS, PFOSA) analyserats.

Även ett antal fenolära substanser (pentaklorfenol, n-OP, t-OP, nonylfenol, triclosan), ett femtiotal olika pesticider och femton mysksubstanser inkluderades i analysprogrammet. Samtliga analyserade metaller låg över detektiongränsen i alla prov från de tre olika lokalerna.

[1] Antalet arter och artindelning är för närvarande under revidering.

Högst kadmiumhalter (1,52-5,25 µg/g våtvikt) hittades i mask från Fleringe och i mask från en av lokalerna i Grimsö (3,01 µg/g våtvikt). Högst halt av bly (3,22 µg/våtvikt) hittades i ett av proven från Grimsö (inte samma prov med högst kadmiumhalt). I samtliga övriga prov låg halterna av bly klart under 1 µg/g våtvikt. Kvicksilver förekom i låga men kvantifierbara halter i samtliga prov. Högst halt (0,104 och 0,125 µg/g våtvikt) hittades i två av proven från Grimsö. Halterna av kobolt, vanadin och krom var något högre i Tyrestaproven jämfört med prov från de övriga lokalerna. Mask från Fleringe hade betydligt högre halt av kalcium jämfört med mask från de andra lokalerna.

Av de analyserade PCB kongenerna förekom CB-153 och CB-138 i kvantifierbara halter i sex respektive fem prov. Högst halt förekom i ett av proven från Fleringe. I detta prov förekom även CB-180.

DDE förekom i kvantifierbara halter i samtliga analyserade prover. Högst halt (4672 ng/g fettvikt) hittades i ett av Grimsöproven (Morskoga) och i ett av Fleringe proven (1399 ng/g fettvikt). Det mest anmärkningsvärde var den höga DDT-halten (5383 ng/g fettvikt) som också förekom i Morskogaprovet. I detta prov förekom även en hög DDD halt (1321 ng/g fettvikt). DDT hittades inte i något av de övriga proven.

Bromerade substanser förekom låga men kvantifierbara halter i samtliga prov utom i ett från Grimsö (Bergshyttan) där inga BFRs hittades. BDE-47 förekom i alla Tyresta proven, i ett av Grimsöproven och i två av Fleringe proven. Den högsta halten hittades i ett av Fleringeproven (Utoje). I detta prov hittades förutom BDE-47 (2,87 ng/g fettvikt) även BDE-99 (2,23 ng/g fettvikt), BDE-100 (0,82 ng/g fettvikt) och BDE-153 (0,39 ng/g fettvikt). BDE-209 hittades i ett av proven från Grimsö (20,3 ng/g fettvikt). Detta prov innehöll inte någon av de andra analyserade BFRs. HBCD hittades inte i något prov.

Av de fenolära substanserna förekom pentaklorfenol i samtliga analyserade prov (2,6-18 ng/g våtvikt). Högst halt hittades i ett av Tyrestaproven. Oktylfenol och triklosan hittades inte i något av proven medan nonylfenol förekom i tre prov, två från Tyresta och ett från Grimsö. Ett stort problem när det gällde analyserna av dessa substanser var de höga detektions och kvantifieringsgränserna vilket gör att det är svårt att dra några egentliga slutsatser angående halterna av dessa ämnen i daggmaskproverna.

Perfluorerade substanser förekom i mycket låga halter i samtliga prov. De tre vanligast förekommande PFCs var PFTriA som förekom över kvantifieringsgränsen i samtliga prov. PFOS förekom över kvantifieringsgränsen i alla prov utom i ett (Tyresta) och PFNA förekom i kvantifierbara halter i sex prov. PFHxA, PFDcA, PFBS, PFHxS, PFDcS och PFOSA hittades inte i något prov.

Av analyserade pesticider hittades klorpyrifos, metribuzin och propikonazol i proven från Fleringe. Halterna var mycket låga, i nivå med detektionsgränsen. I ett av proven från Grimsö hittades endosulfansulfat (70 ng/g). Inget av proven innehöll mer än en substans.
Ingen av de analyserade mysksubstanserna hittade i något av proven.
Fetthalten var mycket låg, runt 0,5 % i samtliga maskprov.
Generellt sett var halterna av de analyserade ämnena låga till mycket låga. Undantaget är provet från Morskoga som innehöll höga halter av DDT, DDE och DDD. Framförallt är det DDT halten i dessa prov som är förvånande. Förhöjda DDE halter har tidigare observerats i unga starar från Grimsölokalen Morskoga. Halterna i unga starar från Morskoga har dels varierat kraftigt mellan åren under perioden 1984-1995, dels var medelvärdet för hela perioden betydligt högre i starar från Morskoga jämfört med starar från övriga Grimsölokaler (Odsjö 2000). Även ett av Fleringe proven innehöll förhållandevis hög halt av DDE. Högre DDE halter har tidigare kunnat noteras i unga starar från Fleringe under perioden 1983-1995 jämfört med starar från övriga lokaler (Odsjö 2000). Kadmiumhalten var högst i maskarna från Fleringe. Att kadmiumhalten har varit högre i biota från Fleringe jämfört med Tyresta och Grimsö har tidigare setts i unga starar (Odsjö 2000; Odsjö et al. 2008).
Det är svårt att se några tydliga geografiska tendenser i det begränsade materialet i den här studien. Materialet är för litet för att analyseras statistiskt men generellt kan man säga att daggmask från Fleringe och i viss mån Grimsö verkar vara något högre belastade av miljögifter. Men det måste påpekas att variationen är stor mellan provpunkterna vilket indikerar relativt lokal belastning. Provtagningsområdena både i Fleringe och Grimsö är belägna i områden där ett aktivt jordbruk har bedrivits i många år vilket sannolikt påverkar resultatet, speciellt när det gäller halter av ΣDDT och i viss mån kadmium.

Summary

The present study was carried out on mandate of and in cooperation with the Swedish Environmental Protection Agency (SEPA) according to Agreement 221-1031. The aim was to obtain knowledge of the levels of certain environmental contaminants in a matrix, earthworm (different species) that has not previously been examined in Sweden. The aim was also to compare the levels of contaminants in earthworm with levels previously found in young starlings (*Sturnus vulgaris*), that has been used in environmental monitoring of contaminants

in terrestrial biota since the early 1980s. A purpose was also to evaluate the possibility of using earthworms in monitoring of contaminants in terrestrial biota.

Earthworms are important food components for many birds and small mammals that further on are important food components to raptors and other predators in terrestrial food chains. Earthworms are thus an important link between levels of bioavailable contaminants in soil and detritus and levels in biota further up in food chains. In Sweden there are about twenty different species in five different genera, all belonging to the Lumbricidae family[2]. Different species have somewhat different preferences on soil quality and also are more or less abundant in different biotopes. In this study, the larger species in the Lumbricus, Allolobophora/Aporrectodea and, to a certain extent also Dendrobaena genus have been of the largest interest. It has been beyond the scope of this study to further analyse the number of species at each sampling spot. Neither has the age or the maturity of individuals been taken into account.

Earthworms were collected during the last days of August and the first half of September. No selection of individuals was made at the sampling occasion other than that damaged individuals were removed. Three days after sampling the worms were frozen in liquid nitrogen. At that occasion only healthy looking individuals were sampled.

The substances that were analysed were the same as earlier has been analysed in the monitoring programmes on contaminants in biota. These are metals and some essential elements (Ca, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, Pb, V, Zn), the chlorinated substances PCB (CB-28, CB-52, CB-101, CB-118, CB-153, CB-138, CB-180), ΣDDT (DDT, DDE, DDD), HCB, α-, β-, γ-HCH (lindan). Furthermore some brominated flame retardants, BFRs (BDE-47, BDE-99, BDE-153, BDE-154, BDE-209, HBCD), perfluorinated compounds PFCs (PFHxA, PFHpA, PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFTriA, PFTeA, PFBS, PFHxS, PFOS, PFDcS, PFOSA), and phenolic compounds (pentachlorophenol, n-OP, t-OP, nonylphenol, triclosan) were analysed. Moreover, about fifty different pesticides and fifteen different musk substances were included.

All of the analysed metals and elements were above LOQ in all of the analysed samples. The highest cadmium levels were found in earthworms from Fleringe (1,52-5,25 µg/g ww) and in one of the samples from Grimsö (3,01 µg/g ww). One of the samples from Grimsö contained 3,22 µg/g ww of lead (not the same with the highest Cd levels). In all of the other samples the lead levels was well below 1 µg/g ww. Mercury was found in low but

[2] The number of species is under revision.

quantifiable levels in all of the samples. Highest level (0,104 and 0,125 µg/g ww) was found in two of the Grimsö samples. Levels of cobolt, vanadium and chromium were somewhat higher in the Tyresta samples. Earthworms from Fleringe had higher calcium levels compared to earthworms from the other sampling locations.

CB-153 and CB-138 was found in quantifiable levels in six and five samples respectively. Highest level was found in one of the Fleringe samples. This sample also contained CB-180.

DDE was found in all of the analysed samples. The highest level (4672 ng/g lw) was found in one of the Grimsö samples (Morskoga) and one of the Fleringe samples (1399 ng/g lw). The most conspicuous was the high level of DDT (5383 ng/g lw) that also was found in the Morskoga sample. This sample also contained high levels of DDD (1321 ng/g lw). DDT was not found in any of the other samples analysed.

BDE-47 was found in all of the Tyresta samples, in one of the Grimsö samples and in two of the Fleringe samples. The highest level was found in one of the Fleringe samples. This sample also contained BDE-99, BDE-100, and BDE-153. BDE-209 was found in one of the Grimsö samples (20,3 ng/g lw). This sample contained none of the other analysed BFRs. HBCD was not found in any of the analysed samples.

Pentachlorophenol was found in all of the analysed samples (2,6-18 ng/g ww). The highest level was found in one of the Tyresta samples. Nonylphenol (48 ng/g ww =LOQ) was found in two samples from Tyresta and one sample from Grimsö. Due to analytical problems, high LOQ, and large risks of contamination in the sampling and analytical procedure for these substances it is difficult to draw conclusions concerning the actual levels in biota.

Octylphenol and triclosan was not found in any of the analysed samples.

Perfluorinated substances were found in very low levels in all of the analysed samples. PFTriA was found above LOQ in all samples. PFOS was found in all samples but in one sample (Tyresta), the level was semi quantitative (<LOQ). PFNA was found in six of the samples. PFHxA, PFDcA, PFBS, PFHxS, PFDcS och PFOSA was not found above LOD in any of the analysed samples.

Of analysed pesticides, klorpyrifos, metribuzin and propikonazol were found at, or just above LOD in the samples from Fleringe. None of the samples contained more than one of these pesticides. Endosulfansulfat (70 ng/g) was found in one of the samples from Grimsö.

None of the analysed samples contained any musk substances.

The fat content was very low, about 0,5% in the earthworms.

Generally most of the analysed substances were found in low or very low levels in all of the analysed samples. An exception is the sample from Morskoga (Grimsö) that contained

surprisingly high levels of DDT, DDE and DDD and especially the high level of DDT in this sample is remarkable. It has earlier been observed that DDE levels in young starlings from Morskoga were higher compared to DDE levels in starlings from the other Grimsö localities. The between year variation in DDE levels starlings from Morskoga has also been large in 1983-1995 (Odsjö 2000). In addition, one of the Fleringe samples contained relatively high levels of DDE but no DDT was found in these samples. In earlier reports it has been found that starlings from Fleringe had the highest levels of DDE in 1983-1995 compared to starlings from the other sampling localities (Odsjö 2000).

These results indicate that both these sampling areas still have elevated levels of ΣDDT. Earthworms from Fleringe had the highest levels of cadmium although there was a large variation between the sampling spots. Higher cadmium levels have earlier been detected in young starlings from Fleringe compared to starlings from the other sampling locations (Odsjö 2000; Odsjö et al. 2008).

From the present study it is difficult to se any obvious geographical patterns due to the rather small material. Generally the sampling areas at Grimsö and Fleringe appear to have somewhat higher loadings of pollutants, especially of ΣDDT and cadmium.

Aim

This work was carried out on request of and in cooperation with the Swedish Environmental Protection Agency (SEPA).

The aim was to investigate the possibility of using earthworms in the terrestrial environmental monitoring of contaminants in the agricultural landscape. Earthworms are included in the diet of many birds and small mammals such as moles. Because of this they can be seen as a link between contaminant levels in soils and living organisms further up in food webs.

Organisation

The earthworms were collected and prepared by staff from the Department of Contaminant Research at the Swedish Museum of Natural History (SMNH).

Chemical analyses were performed by Lars Petersson and Vera Galgan, Department of Chemistry, National Veterinary Institute (metals), Ulla Eriksson ITM Department of Applied Environmental Science, Stockholm University (chlorinated and brominated compounds), Margaretha Adolfsson–Erici, ITM Department of Applied Environmental Science, Stockholm University (phenolic substances), Urs Berger ITM Department of Applied Environmental

Science, Stockholm University (perfluorinated compounds), Jenny Kreuger Department of Environmental Assessment, Swedish University of Agricultural Sciences (pesticides) and ALS Scandinavia AB (musk substances).
Results have been evaluated and the report has been prepared by Ylva Lind at the Department of Contaminant Research, Swedish Museum of Natural History.
The study has been carried out in cooperation with Britta Hedlund and Axel Hullberg at SEPA who also gave the financial support.

Introduction

The Swedish Environmental Monitoring Programme was initiated by SEPA in 1980 in order to monitor of environmental contaminants in biota. The monitoring programme on contaminants in terrestrial environment have been subdivided into three different parts, one concerning mountain environments of northern Sweden, one concerning forest environments and one concerning the rural agricultural landscape. These different ecosystems are represented by different species, chosen to be representative for each ecosystem (Odsjö and Olsson 1979; Odsjö and Olsson 1979; Odsjö and Olsson 1989). Starling (*Sturnus vulgaris*), a migratory bird have been chosen to represent the agricultural landscape. Fledglings of starlings have been collected yearly from 8-9 localities since the early 1980s and from one of the localities, Krankesjön in southern Sweden, even since 1967. The reason for using fledglings instead of adults is that the fledglings are raised on food collected within 500 m from the nest and thus represent the area from where they are collected (Ref). Nestlings of starlings are entirely raised on food of animal origin and the diet consists of a variety of invertebrates where earthworms can constitute a considerable part. Earthworms have not earlier been included in the Swedish monitoring programmes but are included in the German monitoring programme on contaminants in the environment since the 1990s (Quack et al. 2003). Earthworms are an important food for many bird species and also for small mammals such as moles, hedgehogs and badgers. There are also certain insect and spider species that feed on earthworms. Earthworms can thus be considered an important link between contaminant levels in soil and contaminant levels further up in food webs. The present work was done to investigate the levels of a number of both traditional environmental contaminants as well as more recent discovered contaminants in earthworms collected from three of the localities where starling have been collected since the 1980s.

In Sweden about 20 different species of earthworms are known although the number of species as well as the taxonomy of earthworms are being revised due to modern techniques (Gustavsson et al. 2009). In the present study, no thorough determination of species was made but a superficial calculation of the relative abundance of species on each locality was performed. As the speciation of earthworms is not yet performed this is not included in the present report.

Beside analyzes of known contaminants such as metals and chlorinated compound there are also regular studies of new and/or poorly investigated substances in different matrices in order to evaluate the need to include these in a more regular monitoring. In the present study, analyses of certain musk substances were included for this purpose. Musk substances are lipid soluble and chemically stable substances that are present in many household products. They are potentially distributed in the environment through air and sewage treatment plants. In 2002-2003, a screening study was made on the levels of some musk substances in fish and sediment from a number of places in Sweden. Musk substances has also been analysed in blue mussel from the Swedish west coast. Musk substances were frequently found in sludge from sewage treatment plants.(Naturvårdsverket 2005).

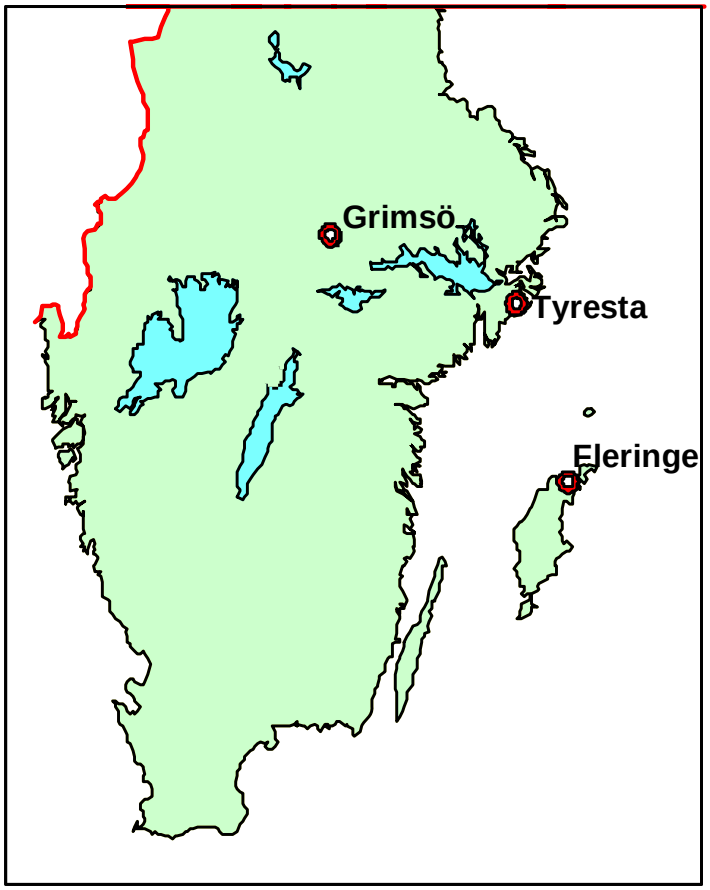
Material and methods

Earthworms

All of the earthworm species in Sweden belong to the Lumbricidae family. All earthworms live on dead organic matter but the species and the number of species differ between different biotopes. The highest number of both species and individuals are usually found in garden soils while there are few species that live in raw humus soils (Julin 1949). There is also a vertical differentiation with the larger species such as *Lumbricus terrestris* and *Allobophora longa* living deeper while smaller species such as *Dendrobaena* sp and *Lumbricus castaneus* living closer to the surface. Younger individuals, being smaller also usually lives closer to the surface compared to older ones (Julin 1949; Buch 1987). For this study, there was no separation on species or age/maturity but all living and healthy looking earthworms found were collected.

Localities

Three of the localities where starlings have been collected since the 1980s were chosen for earthworm collection (Figure 1). Earthworms were collected close to Tyresta village, a rural area about 20 km south of Stockholm were starlings have been collected since 1983. The Tyresta village is situated just outside Tyresta National Park and Nature Reserve and is considered as a comparatively unaffected region in the vicinity of a large city, Stockholm. Earthworms were collected from three different spots in pastures and meadows with and without grazing horses. These spots were located between 200 and 400 m apart. The second locality was Grimsö in the county of Västmanland in central Sweden where starlings have been collected since 1981. At Grimsö, earthworms were also collected at three different spots, just outside the garden of Grimsö manor at Grimsö village, at a meadow at Morskoga, and at a meadow in Bergshyttan. These collection spots were situated 4-6 km apart. The third locality was Fleringe at the northern part of Gotland were starlings have been collected since 1983. At Fleringe earthworms were collected from a grove, close to a pasture with clover and grazing sheep (Nors), from a pasture and ditch-bank close to a field with remains of a carrot growing (Utoje) and from a pasture with grazing sheep (Skymnings). These collection spots were situated 0,5-2,5 km apart. The collections at Grimsö and Fleringe were made in an area with an active and ongoing agriculture while the collection area at Tyresta was dominated by recreation activities.



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Sampling and preparation

Earthworms were collected in late August and early September 2010 by digging with a pitchfork. All worms that were not visibly injured were collected. When enough worms (150-200 g) were found at each collection spot, they were rinsed from soil and plant material and put in clean glass jars on filter paper. After three days in a dark environment at a temperature of approximately 15° C, the worms were rinsed in water and frozen in liquid nitrogen in lumps of 15-20 g. Only live and healthy looking worms were frozen but no separation was made on mature and immature individuals. Whole, frozen earthworms were cut in pieces and aliquots considered representative for each sampling spot were prepared. From each sampling spot, some adult individuals were preserved in alcohol for later determination of species[3].

[3] No list of species is yet available.

Chemical analyses

Aliquots of frozen earthworms representing the three different sampling spots, at each of the sampling localities, Tyresta, Grimsö, and Fleringe were prepared.

Analysis of metals and elements

Fourteen metals and elements (Ca, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, Pb, V, Zn) were analysed in homogenates of whole earthworms. Results are given in µg/g wet weight. Combustion of organs (5 g tissue for multi-element determination using HNO_3, $HClO_4$ and H_2SO_4; about 3 g tissue for analysis of Hg using HNO_3 and $HClO_4$) was performed by automatic wet digestion according to a standard program (Frank 1976; Frank and Petersson 1983; Frank 1988; Frank et al. 1992). An electrically heated block of aluminium was used (Foss Tecator Digestion System, Model 40, Foss Tecator AB, Höganäs, Sweden).

Analysis

Analysis of 13 elements (Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, V and Zn) was performed using an inductively coupled plasma atomic emission spectrometer (ICP-AES,,Jobin Yvon-Horiba SA, 91165 Longjumeau, France).

The determination of Hg was performed by using cold vapour (CV)- ICP-AES. (The methods are accredited according to SS-EN-ISO/IEC 17025).

Quality control was performed using appropriate reference materials (NCS ZC 71001 Beef Liver and DORM-3). As all the analysed metals and elements were above LOD in all samples, no LOD for the analyses is given.

The chemical analyses on metals were carried out by the Department of Chemistry, National Veterinary Institute, Uppsala.

Analysis of brominated flame retardants

Brominated flame retardants were analysed in homogenates of whole earthworms (Table 1). Results are given in ng/g lipid weight.

The samples of 10 g tissue were extracted with a mixture of acetone/*n*-hexane and *n*-hexane/diethyl ether. The organic phase was liquid/liquid partitioned with a solution of sodium chloride/phosphoric acid. The aqueous phase was reextracted with *n*-hexane and the combined organic phases were evaporated to dryness. The lipid content was determined gravimetrically. After treatment of the dissolved lipid extract with concentrated sulphuric acid (Jensen *et al*., 1983), the samples were analysed by gas chromatograph/mass

spectrometry (GC-MS) in electron capture ionization (ECNI) mode. A 30 m DB-5 MS fused silica column (0.25 mm i.d., 0.25 μm film thickness) was used for the lower brominated analytes while a 15 m DB-5 MS fused silica column (0.25 mm i.d., 0.10 μm film thickness) was used for BDE 209. Ammonia was used as the reaction gas. The mass fragments monitored were *m/z* 79 and 81 for all brominated compounds and *m/z* 237 and 239 for dechlorane, used as internal standard(Sellström et al. 2003).

Table 1. Brominated compounds and LOQ (ng/g lipid weight) for each compound analysed in homogenates of whole of earthworms from three different localities in Sweden.

		LOQ
BDE-47	2,2´, 4,4´-tetrabromodiphenyl ether (TeBDE)	1,0
BDE-99	2,2´, 4,4´, 5-pentabromodiphenyl ether (Pe2BDE)	1,0
BDE-100	2,2´, 4,4´, 6-pentabromodiphenyl ether (Pe1BDE)	0,3
BDE-153	2,2´, 4,4´, 5,5´-hexabromodiphenyl ether	0,3
BDE-154	2,2´, 4,4´, 5,6´-hexabromodiphenyl ether	0,3
BDE209	2,2´,3,3´,4,4´,5,5´,6,6´-decabromdiphenyl ether	5
HBCD	hexabromcyclododecane	2

Analysis of chlorinated substances

A number of chlorinated compounds were analysed in earthworms (Table 2)

The samples for the analysis of the chlorinated substances were extracted and cleaned-up in the same way as the brominated substances but analysed by a gas chromatograph equipped with an EC-detector. Two fused capillary columns of 60 m (0.25 mm i.d, 0.25 μm film thickness) were used in parallell, one DB-5 and one DB-1701. Argon/Methane was used as make-up gas and CB 53 as internal standard (Eriksson et al. 1997). Results are given in ng/g lipid weight. The analyses of chlorinated compounds were carried out by the Department of Applied Environmental Science (ITM), Stockholm University.

Table 2. Chlorinated compounds and LOQ (ng/g lipid weight) for each compound analyzed in homogenates of whole earthworms from three different localities in Sweden.

		LOQ
HCB	Hexachlorobenzene	4
α-HCH		4
β-HCH		5
γ-HCH	LINDAN	5
DDE	p,p'-DDE	3
DDD	p,p´-DDD	4
DDT	p,p´-DDT	6
CB-28	2,4,4'–trichlorobiphenyl (1-orto)	4
CB-52	2,5,2',5' –tetrachlorobiphenyl (2-orto)	4
CB-101	2,4,5,2',5' –pentachlorobiphenyl (2-orto)	4
CB-118	2,4,5,3',4' –Pentachlorobiphenyl (1-orto)	4
CB-153	2,4,5,2',4',5' –hexachlorobiphenyl (2-orto)	4
CB-138	summan av CB-138 (2,3,4,2',4',5' –hexachlorobiphenyl l (2-orto)) och CB-163 (2,3,3',4',5,6-hexachlorobiphenyl)	4
CB-180	2,3,4,5,2',4',5' –Heptachlorobiphenyl (2-orto)	4

Analysis of phenolic compounds

Five phenolic compounds were analysed in earthworms (Table 3)

The sample, 3 g of tissue, was homogenized with hexane/acetone twice, and the organic phases were treated with sodium chloride / phosphoric acid. The aqueous phase was reextracted with hexane and the combined organic phases were evaporated to dryness. The lipid content was determined, and the residue was redissolved in hexane/MTBE. The phenols were extracted into KOH/ethanol, and neutral compounds were removed by extracting the aqueous phase with hexane. After acidification of the aqueous phase, the phenolic compounds were extracted into hexane, and converted into their pentafluorobenzoyl esters followed by determination by GC/ECNI/MS (Allmyr et al 2006). Following surrogate standards were added to the muscle homogenate: ^{16}d-Bisphenol A, ^{13}C-6 pentachlorophenol, ^{13}C-12 triclosan and 4-n-nonylphenol.

As the lipid content of the earthworms was very low (0,5%) the results are reported on wet weight basis.

The analyses of phenolic compounds were carried out by the Department of Applied Environmental Science (ITM), Stockholm Univerity.

Table 3. Phenolic compounds and LOQ (ng/g wet weight) for each compound analysed in homogenates of whole earthworms from three localities in Sweden.

	LOQ
Pentachorophenol (PCP)	1,1
n-oktylphenol (4-n-oktylphenol, n-OP)	8,9
t-oktylphenol (4-t-oktylphenol, t-OP)	3,3
Nonylphenol (NP)	48
Triclosan	1,6

Analysis of perfluorinated compounds

Fifteen different perfluorinated substances were analysed in earthworms (Table 4). Sample extraction and clean-up was based on the method by (Powley et al. 2005) with modifications for biota samples described by (Verreault et al. 2007). In short, 1 g of the homogenized liver was spiked with the mass-labeled internal standards. Extraction was performed twice with 5 mL acetonitrile in an ultrasonic bath. The combined extracts were concentrated to 1 mL and subjected to dispersive clean-up on graphitized carbon. The cleaned-up extract was added to aqueous ammonium acetate. Precipitation occurred and the extract was centrifuged before instrumental analysis of the clear supernatant. Aliquots of the final extracts were injected automatically on a high performance liquid chromatography system coupled to a tandem mass spectrometer Compound separation was achieved on an C18 reversed phase column with a binary gradient of buffered (ammonium acetate) methanol and water. The mass spectrometer was operated in negative electrospray ionization mode. Quantification was performed in selected reaction monitoring chromatograms using the internal standard method. The analyses on perfluorinated compounds were carried out by the Department of Applied Environmental Science (ITM), Stockholm University.

Table 4. Perfluorinated compounds and LOD (ng/g wet weight) for each compound analysed in homogenates of whole earthworms from three different localities in Sweden. PFTriA and PFOS were above LOD in all the analysed samples.

		LOD (ng/g ww)
PFHxA	perfluorohexanoate	0,45
PFHpA	perfluoroheptanoate	0,25
PFOA	perfluorooctanate	0,35
PFNA	perfluorononanonate	0,15
PFDcA	perfluorodecanoate	0,25
PFUnA	perfluoroundecanoate	0,2
PFDoA	perfluorododecanoate	0,2
PFTriA	perfluorotridecanoiate	
PFTeA	perfluorotetradecanoate	0,2
PFPeDA	perfluoropentadecanoate	0,2
PFBS	perfluorobutane sulfonate	0,5
PFHxS	perfluorohexane sulfonate	0,4
PFOS	perfluorooctane sulfonate	
PFDcS	perfluorodecane sulfonate	0,2
PFOSA	perfluorooctane sulphonamide	0,15

Analysis of musk substances.

Fifteen different musk substances were analysed in earthworms (Table 5).

The worms were homogenized before preparation and 2,51-2,75 ml of homogenate was used for the extraction. To each sample 50 µl of IS (AHTN-D3 + MuskXyl-D15) were added. The samples got extracted 3 times 1 hour in an ultra sonic bath using Acetone and Hexane. For the clean up nonane was added as a keeper and the extracts got eluted in two steps first with Heptane and a mix of Heptane and dichloromethane and second with dichloromethane and a mix of dichloromethane and ethylacetate over a column with silica gel and AgNO3.

This extract got concentrated to a volume of 0,5 ml. 1 µl of this extract was used for the analysis with GC-MSD and the compounds are quantified with the intern standard. For the calibration extern standards, one for each compound reported was used.

Musk substances were analysed by ALS Scandinavia AB.

Table 5. Musk substances and LOD (µg/kg wet weight) for each compound analyzed in homogenates of whole earthworms from three localities in Sweden.

	LOD (µg/kg wet weight)
musk ambrette	<1,0
musk xylene	<1,0
musk moskene	<1,0
musk tibetene	<1,0
musk ketone	<1,0
cashmeran	<1,0
celestolide	<1,0
phantolide	<1,0
traseolide	<1,0
galaxolide	<2,0
tonalide	<2,0
4-amino-musk xylene	<1,0
2-amino-musk xylene	<1,0
2-amino-musk ketone	<1,0
galaxolide lactone	<1,0

Analysis of pesticides

A number of pesticides potentially used in industry, agriculture, horticulture, forestry, and house-holds were analysed (Table 6).

Sample preparation was performed by mincing 3 g of tissue with a scalpel followed by adding surrogate standards before extraction. The sample was extracted three times with *i*) acetone, *ii*) dichloromethane and *iii*) a mixture of acetone and dichloromethane, using both ultrasonic bath (20-30 min) and shaking (30-40 min). Extraction clean-up was carried out using size exclusion chromatography (SEC) packed with Bio-Beads S-X3. The final extracts (3 g tissue/mL) were analysed on a gas chromatography system coupled to a mass spectrometer (GC-MS). The mass spectrometer was operated in either electron ionization (EI) or negative chemical ionization (NCI) mode using the standard procedure applied when analysing semi-polar and non-polar pesticides in water (accredited method OMK 51). Quantification was performed against an external standard curve (relative the internal standard) with results corrected for recovery efficiency.

Pesticides were analyzed according to the standard procedure applied when analyzing semi-polar and non-polar pesticides in water (accredited method OMK 51). This method has earlier been applied for analysing pesticides in biological samples (muscle tissue of starling) (Odsjö et al. 2008). However, it was obvious in the final quantification step that the

earthworm extractions would have required a more specified clean-up procedure as there was more background noise in the earthworm samples compared to the starling samples (personal communication with Märit Peterson, SLU). Concequently, it was not possible to detect some of the substances that was analysed in starling muscles. This was also the reason for the somewhat higher detection limit for some of the substances in some samples.

The analyses of pesticides were carried out by the Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences.

Table 6. Pesticides analyzed and LOD (µg/kg wet weight) for each compound in homogenates of whole earthworms from three localities in Sweden. *The LOD differed between the samples (for explanations see Analyses of pesticides)

	LOD (µg/kg wet weight)	
alaklor	4	
alfacypermetrin	0,2	
bitertanol	2	
cyflutrin	0,3	
betacyflutrin	0,6	
cypermetrin	0,6	
deltametrin	1	
dilufenikan	1	
diklorbenil	1	
diuron	2	
alfa endosulfan	0,1	
beta endosulfan	0,1	
endosulfansulfat	0,1	
esfenvalerat	0,1	
etofumesat	5	
fenitrotion	5	
fenmedifam	30	
fenpropimorf	4	
flurtamon*	2	10
fluberidazol	2	
iprodion	10	
isoproturon	2	
karbofuran	10	
klorfenvinfos	0,3	
klorpyrifos*	0,07	0,1
lambda-cyhalotrin	0,05	
lindan (gamma-HCH)	0,1	
alfa-HCH	0,1	
beta-HCH	0,3	
delta-HCH	0,2	
metalaxyl	6	
metamitron	20	
metribuzin	2	
permetrin	3	
prokloraz	10	
propikonazol	6	
propyzamid*	3	10
prosulfokarb*	20	6
simazin	2	
terbutylazin	3	
desetylterbutylazin (DETA)	0,7	
toklofosmetyl	1	
trifluralin	0,7	
tau-fluvalinat	0,5	
vinklozolin	0,05	

Limit of detection (LOD) and limit of quantification (LOQ)

For the analyses on chlorinated, brominated, and phenolic compounds, the limits of quantification (LOQ) have been reported. The LOQ denotes the lowest level of a substance where a reasonably accurate quantification is possible. For lipid soluble substances that are determined on lipid weight basis, LOQ is dependent on the lipid content of the sample. Thus, the LOQ for the analysis differs between samples. Values below LOQ have been assigned with a minus in the lab reports. The expression "below LOQ" is used in the text to denote these cases but no actual numbers are given. Values below LOQ have not been included in the figures but are included in the tables in the Appendix. For metals and elements, and perfluorinated compounds the limit of detection (LOD) for the analysis has been given in the lab reports and these values have either been assigned with a minus (metals and phenolic compounds) or with < (perfluorinated compounds). In the text, these cases are referred to as "below LOD". In the report on analysis of perfluorinated compounds values that are below LOQ but above LOD are shown in *italics* in table 5 in the Appendix. These values are considered to be semi quantitative but are included in the figures. The reason that these values are included although they are below LOQ is that these results gives an indication on the levels of these substances in earthworms from background and reference area in Sweden.

Results

No statistical treatment has been performed, as the number of samples available from each locality was too small.

Lipid content

The lipid content was very low, around 0,5 % in the earthworms in this study.
The lipid content of earthworms from the different sampling localities is shown in Table 7.

Table 7. Lipid content (%) in earthworms from the different sampling sites.

sampling site	lipid %
Tyresta 2	0,47
Tyresta 3	0,44
Tyresta 4	0,43
Grimsö (village)	0,52
Grimsö (Morskoga)	0,51
Grimsö (Bergshyttan)	0,55
Fleringe (Nors)	0,50
Fleringe (Utoje)	0,59
Fleringe (Skymnings)	0,64

Metals and elements

All of the fourteen metals and elements analysed in homogenates of whole earthworms were above LOQ in all samples. The levels of metals and elements found in homogenates of whole earthworms at the different localities are shown in Appendix, Table 1.

Cadmium and lead

The levels of cadmium and lead in homogenates of whole earthworms are shown in Figure 2 Cadmium level was considerably higher than lead level at all sampling locations except at Grimsö (Bergshyttan). Earthworms collected at Fleringe (Nors) and Fleringe (Utoje) had the highest cadmium levels 5,25 and 4,51 µg/g ww respectively. There was a rather large variation in cadmium levels between the different sampling spots at both Grimsö and Fleringe while the Tyresta samples varied less.

Mercury

The mercury level in homogenates of whole earthworms is shown in Figure 3. The highest level of mercury was found at Grimsö (village) and Grimsö (Morskoga), 0,104 and 0,125 µg/g ww respectively. One of the Tyresta samples contained 0,092 µg/g ww. Mercury levels varied considerably between the sampling spots at both Tyresta and Grimsö while the variation was less in the Fleringe samples.

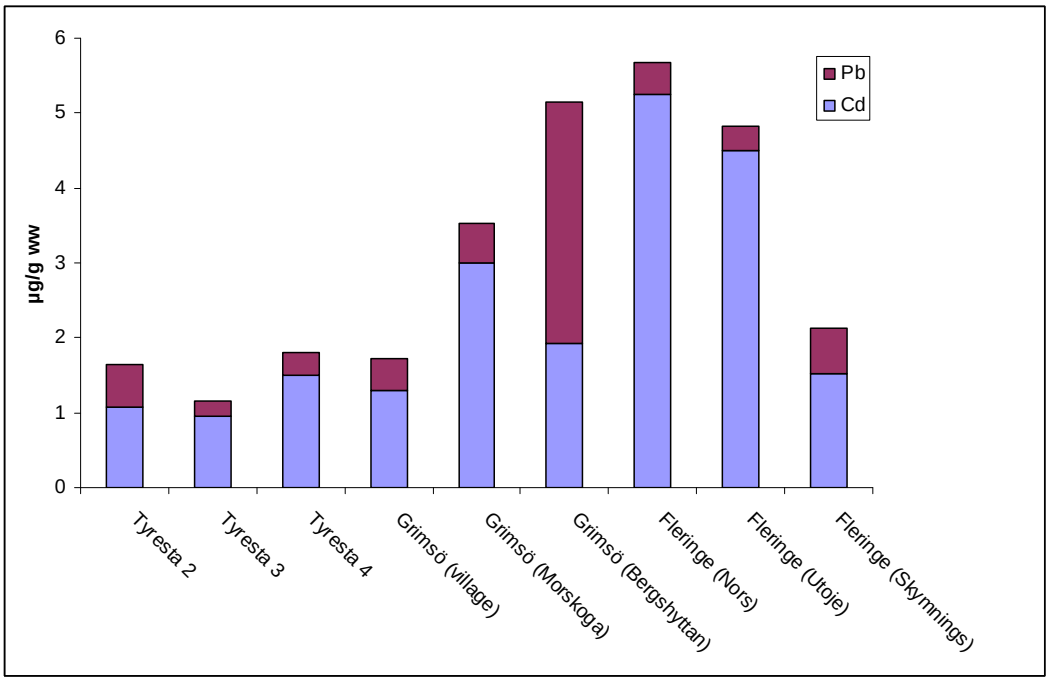

Figure 2. Cadmium and lead (µg/g ww) in homogenates of whole earthworms from three localities in Sweden.

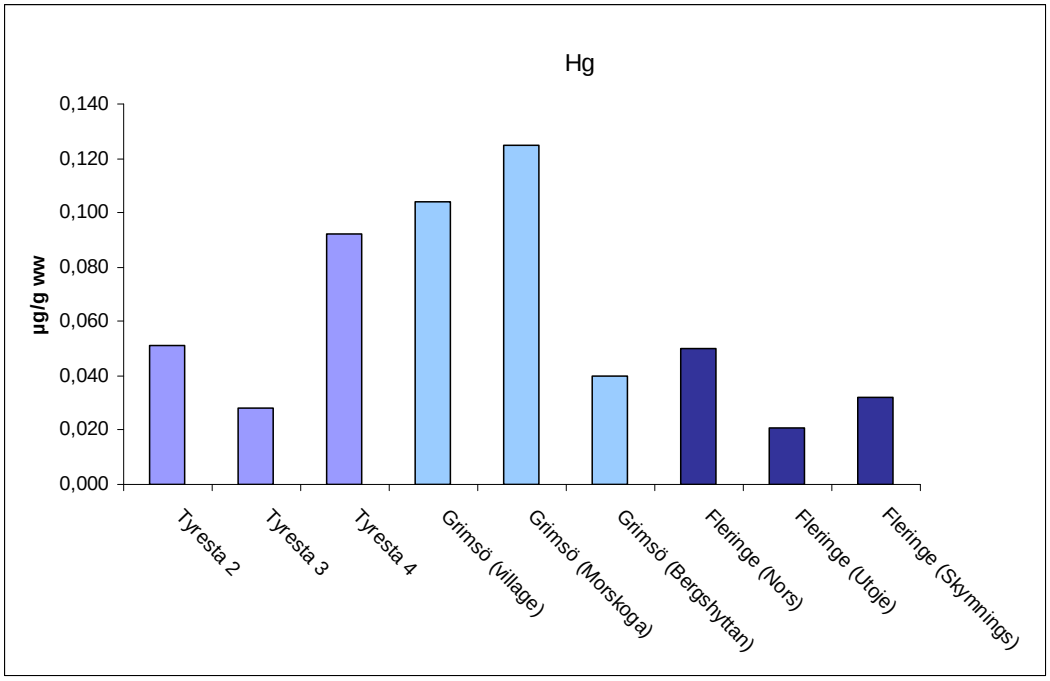

Figure 3. Mercury (µg/g ww) in homogenates of whole earthworms from three localities in Sweden.

Chromium, nickel, and vanadium

The toxic metals chromium and nickel are shown together with vanadium in Figure 4. Vanadium level was somewhat higher in earthworms collected at Tyresta.

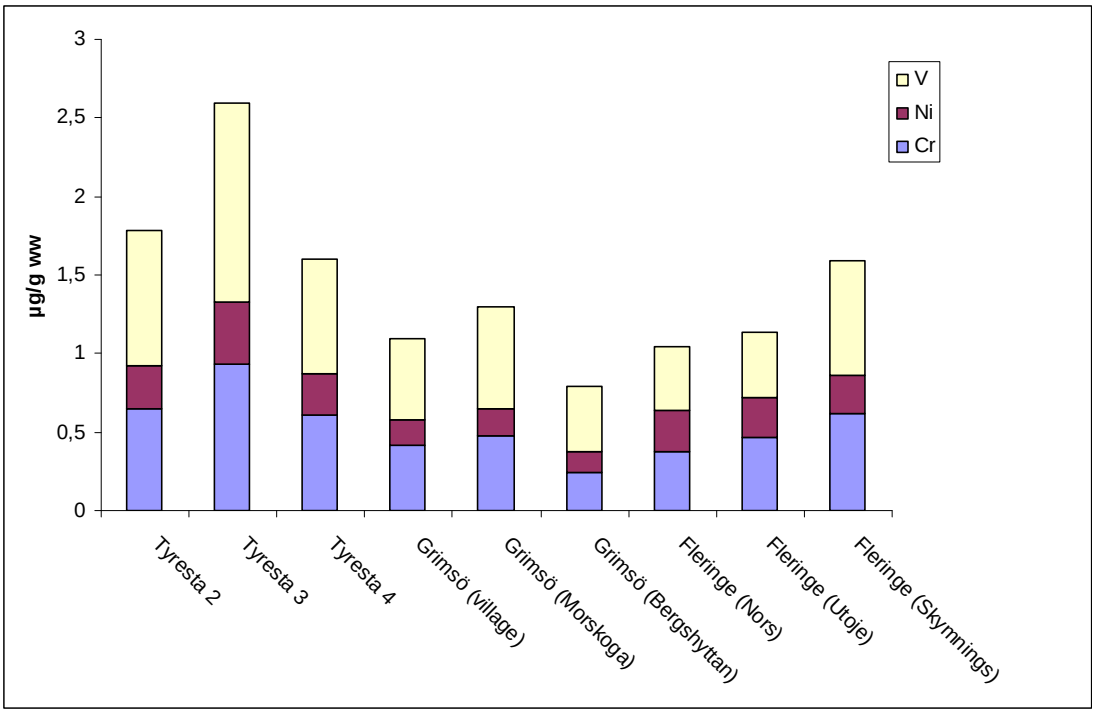

Figure 4. Chromium, nickel, and vanadium levels (µg/g wet weight) in homogenates of whole earthworms from three localities in Sweden.

Essential metals and elements

Levels of metals and elements considered to be essential are shown in Figure 6. Calcium and zinc levels are highest in earthworms collected at Fleringe. Cobolt and iron levels are somewhat higher in the Tyresta samples.

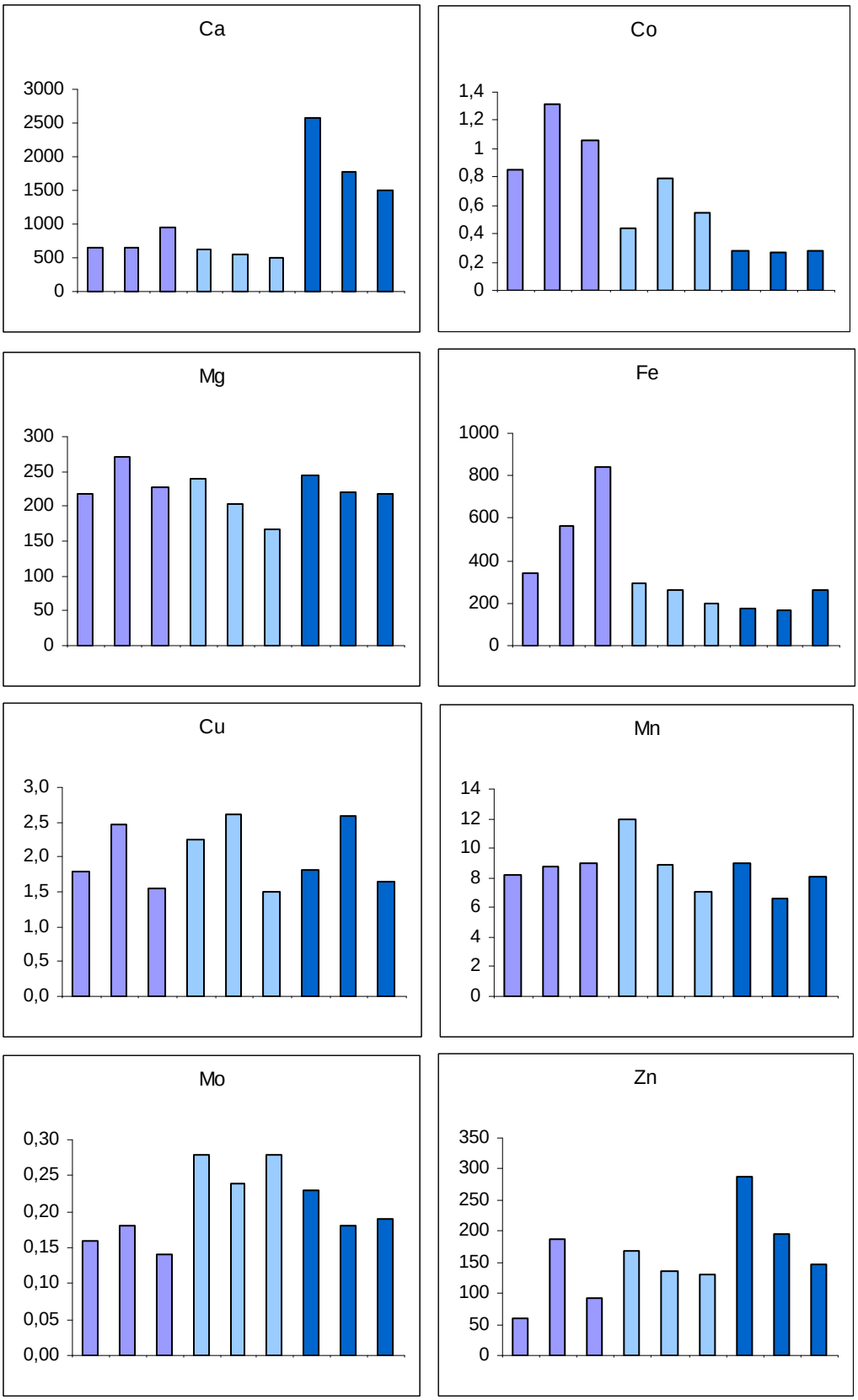

Figure 6. Essential metals and elements (µg/g ww) in homogenates of whole earthworms from three localities in Sweden. The staples from left to right are: Tyresta 2, 3, 4, Grimsö (village), Grimsö (Morskoga), Grimsö (Bergshyttan), Fleringe (Nors), Fleringe (Utoje), and Fleringe (Skymnings).

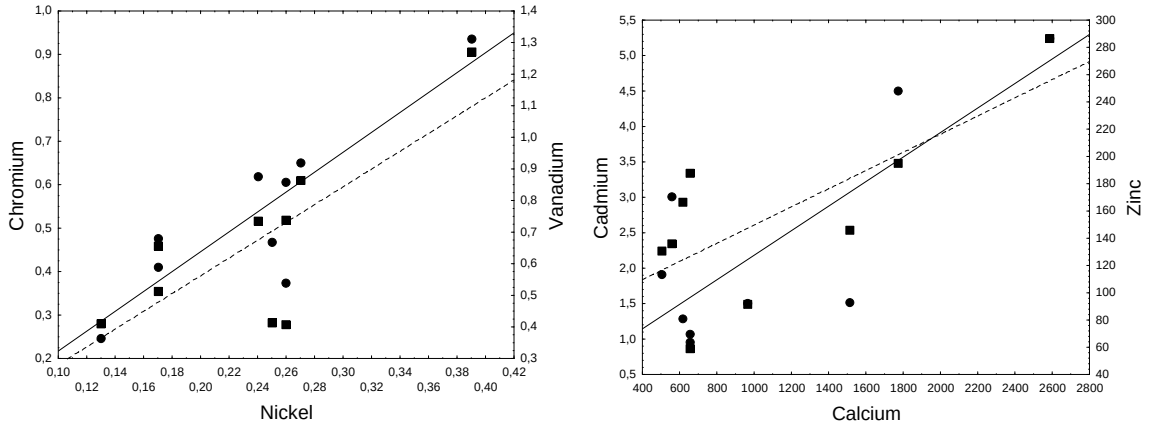

Figure 5. Correlation between Ni, Cr and V (left) and Ca, Cd and Zn (right) in earthworms.

Summary of metals and elements.

All of the metals and elements analysed in homogenates of whole earthworms were above the limit of quantification (LOQ). Cadmium level was highest in two of the samples collected at Fleringe. Higher cadmium levels has earlier been detected in kidney of young starlings collected at Fleringe and analysed yearly in 1983-1999 (Odsjö 2000). The higher cadmium levels found in biota at this sampling locality is probably due to the fact that the sampling is conducted in an area where artificial phosphate fertilizers based on minerals containing high cadmium levels has been used.

The calcium content in earthworms from Fleringe was considerably higher compared to the calcium content of earthworms from the other sampling areas. This is a reflection of the higher calcium content in soil from Gotland. This difference could not be detected in liver of starlings when samples from eight different localities (including Fleringe) was analysed (Odsjö et al. 2008). Cobolt and iron levels were slightly higher in earthworms from Tyresta. Vanadium, chromium and nickel were also slightly higher in the Tyresta samples. Some of the metals were highly correlated in the earthworm samples. The strongest correlation was between cadmium, calcium, and zinc and between nickel, chromium, and vanadium (Fig 5). Similar correlations are not found in analysed organs of starlings and bank voles. Metal levels with the exceptions of the essential elements Cu, Mg, Mo, and Fe were generally higher in earthworms compared to the levels in liver of young starlings (Odsjö et al. 2008). This is probably due to a greater ability of starlings to regulate the uptake and body levels of essential elements. The levels of all the analysed metals and elements in earthworms are probably a good reflection of the water soluble/bioavailable levels in soil and water at the sampling spot.

Chlorinated compounds (CLCs)

Fourteen chlorinated compounds were analysed. Results from all the analyses of chlorinated compounds are shown in Appendix Table 2.

α-HCH, β-HCH and lindan (γ-HCH), CB-28, CB-52, CB-101 and CB-118 were not found above LOQ in any of the samples analyzed.

DDE was found in quantifiable levels in all samples (Figure 7). DDT was below LOD in all the analysed samples expect in the sample from Grimsö (Morskoga). This sample on the other hand contained an unexpectedly high level, 5383 ng/g lw of DDT[4]. DDE and DDD were also found in comparatively high levels in the Morskoga sample, 4672 respectively 1321 ng/g lw (Figure 7). In this sample, HCB was also found in low levels, but none of the analysed CB congeners. One of the samples from Fleringe (Nors) also contained comparatively high levels of DDE (3599 ng/g lw) and detectable levels of DDD (54,4 ng/g lw) but no DDT above LOD. None of the Fleringe samples contained any HCB.

HCB was found in one of the samples from Tyresta and in two of the samples from Grimsö (Morskoga and Bergshyttan) (Figure 8). CB-153 and CB-138 was found in all of the samples from Fleringe. Detectable levels of CB-180 were also found in two of the samples from Fleringe. CB-153 was present in two of the samples from Tyresta and in one of the samples from Grimsö (Grimsö village). The lowest level of total analysed CLCs were found in the Grimsö (Bergshyttan) sample while the highest total level was found in the Grimsö (Morskoga) sample (Figure 7 and 8). The Fleringe (Nors) sample contained the highest level (85, 3 ng/g lw) of ΣCB138, 153, 180 (Figure 8).

[4] As this level of DDT was surprisingly high, an extra quality control was performed with GC-MS (ECNI) in order to confirm that the sample really contained DDT. The DDT level has been confirmed as far as possible.

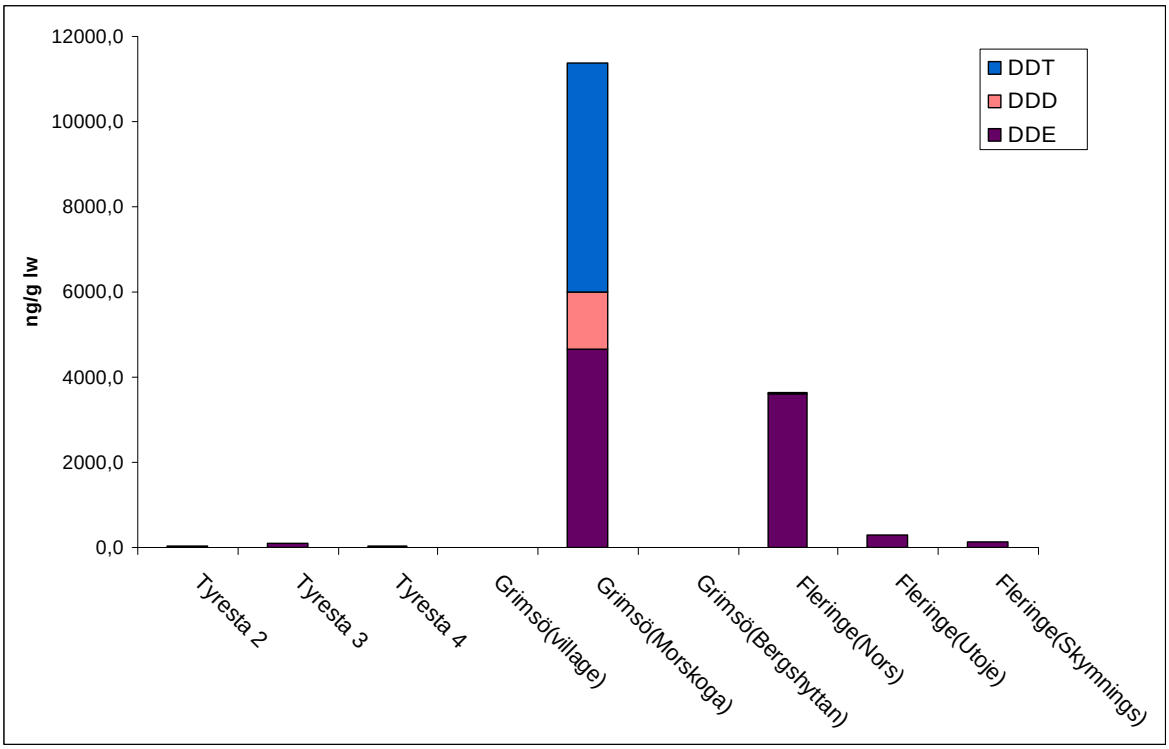

Figure 7. DDT, DDE and DDD (ng/g lw) in homogenates of whole earthworms from three localities in Sweden. DDE was present in quantifiable amounts in all samples. The lowest levels were found at Grimsö (village) and Grimsö (Bergshyttan). See Appendix, table 2.

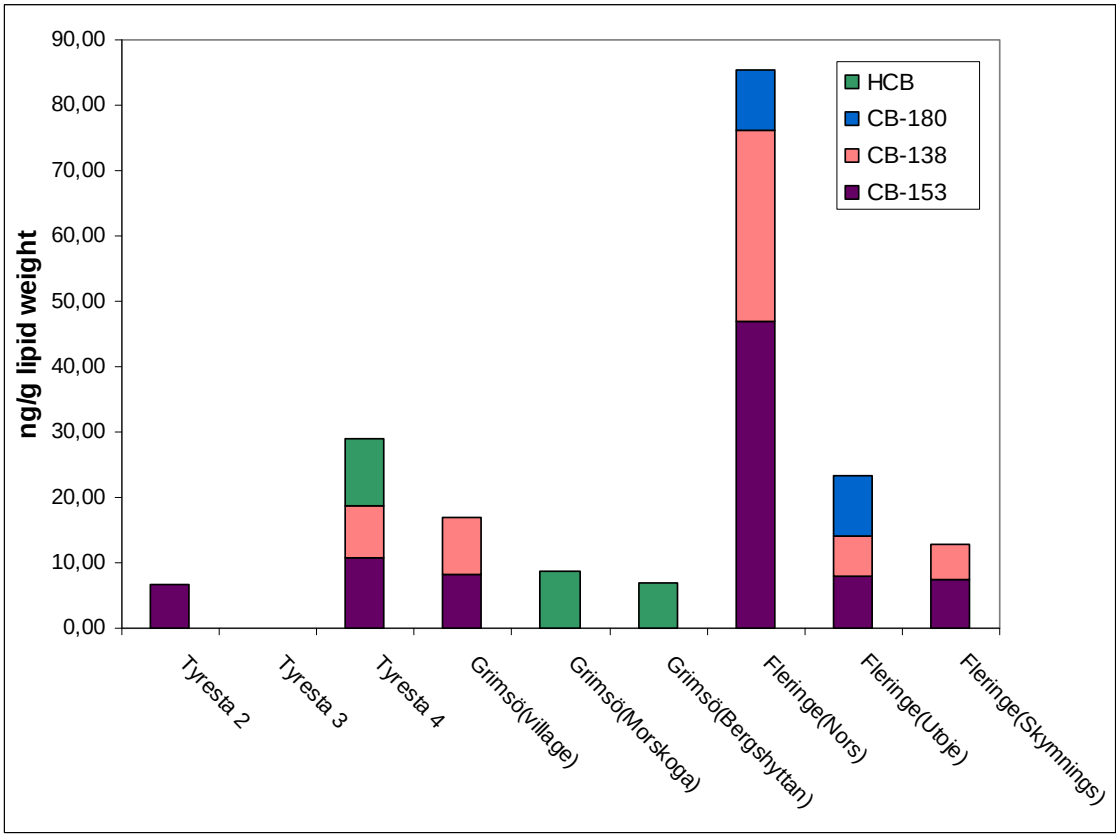

Figure 8. HCB, CB-138, CB-153 and CB-180 (ng/g lipid weight) in homogenates of whole earthworms from three localities in Sweden.

Summary chlorinated compounds

The most conspicuous is the high levels of DDT and the break-down products (DDE, DDD) of DDT found in the Grimsö (Morskoga) sample. ΣDDT for this sample was 11375,9 ng/ g lw and the distribution was 47% DDT, 41% DDE and 12% DDD. In the sample from Fleringe (Nors) that also contained high levels of DDE, ΣDDT was 3653,5 ng/g lw and the distribution was 98,5% DDE and 1,5% DDD. No DDT was found in this sample. DDE was the only chlorinated compound that was found in quantifiable concentrations in all of the analyzed earthworm samples. That DDT was found in relatively high amounts, far higher compared to any of the other pesticides analyzed in earthworms in the Morskoga sample is remarkable. The use of DDT in agriculture was banned in 1970 and in forestry, a few years later and the present results indicate that, forty years later, it is still present in significant amounts in certain areas.

Chlorinated compounds was analysed in bank voles collected in 2001 from five different localities in Sweden. Except for HCB, the levels were generally lower in bank voles compared to the levels in earthworms found in the present study (Lind and Odsjö 2010).

Brominated compounds (BRFs)

Seven BFRs were analysed in homogenates of whole earthworms (Appendix, Table 3). HBCD and BDE-154 was not found above LOQ in any of the samples. BDE-47 was found in all of the samples from Tyresta (1,08-1,49 ng/g lw) and BDE-99 was found in all of the samples from Fleringe (0,44-2,23 ng/g lw). BDE-209 (20,9 ng/g lw) was found in one sample from Grimsö (Figure 9). This sample contained none of the other BFRs analyzed in levels above LOQ. One sample from Fleringe (Utoje) contained detectable levels of four of the analysed BDEs (BDE-47, BDE-99, BDE-100, BDE-153).

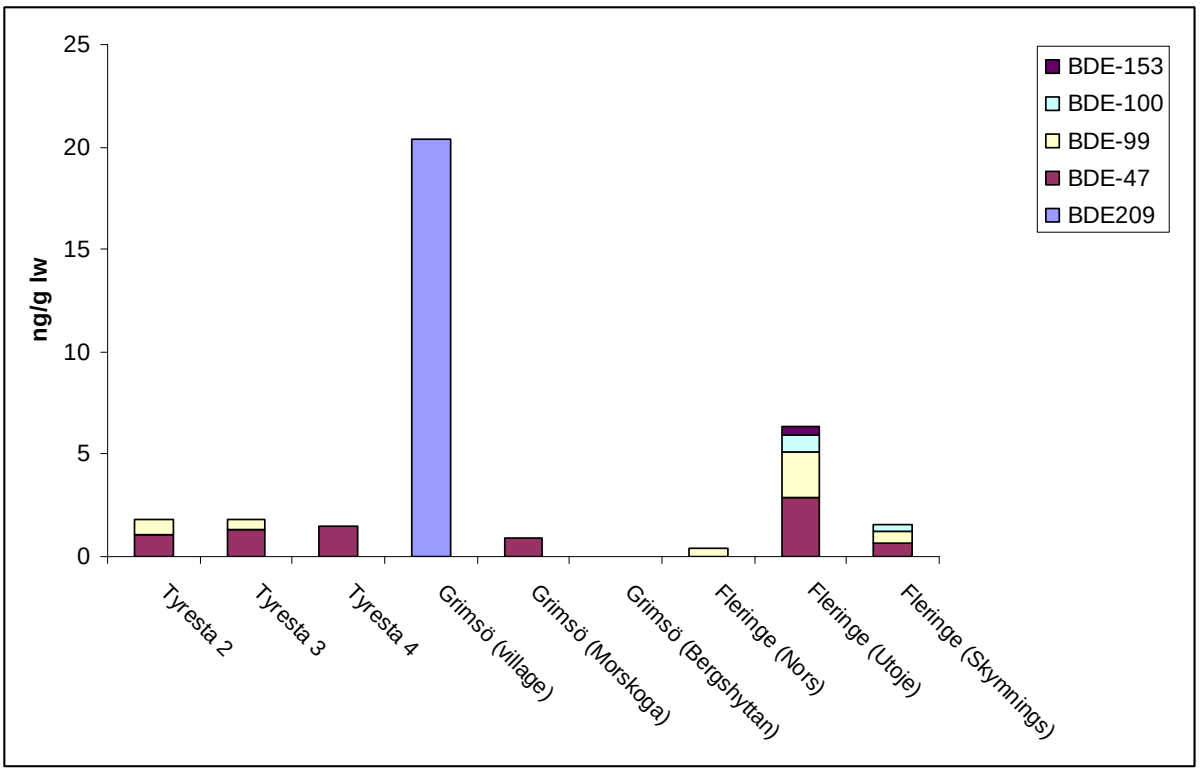

Figure 9. Brominated compounds (BRFs) in homogenates of whole earthworms from three localities in Sweden.

Summary brominated compounds

The most abundant of the analysed BFRs in the earthworm samples was BDE-47 that was present in all of the Tyresta samples, in two of the Fleringe samples and in one of the Grimsö samples. BDE-99 was present in all of the Fleringe samples and in two of the Tyresta samples but in none of the Grimsö samples. The highest level of one brominated compound was found in the Grimsö village sample (Fig 9). This sample contained only one congener, BDE-209 and this was also the only sample where BDE-209 was found.

In starlings from eight localities in southern and central Sweden, all of the analysed BFRs (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154) was found in quantifiable amounts and the most abundant of the BFRs in starlings was BDE-99 (Odsjö et al. 2008). In bank voles, the most abundant BFR congener was BDE-153 that was present in eight of the fifteen analysed samples from five localities. BDE-47 was not found in any of the analysed vole samples and BDE-99 was found in only one of the analysed bank vole samples (Lind and Odsjö 2010). In a study by Sellström et al (Sellstrom et al. 2005) of higher brominated diphenyl ethers in earthworms from reference and sewage sludge treated fields, BDE-47 and 99 was the dominating congeners in the earthworms followed by BDE-209. These congeners

were present in earthworms from both sewage sludge treated fields and reference fields. The level of BDE-209 found in the Grimsö(village) sample (20 ng/g lw) was high compared to the levels found in earthworms from most of the sampling field in the study of Sellström et al. (2005). BDE-47 has earlier been shown to be more abundant in the aquatic environment while BDE-99 and BDE-153 are more abundant in terrestrial environments (Law et al. 2003; Lindberg et al. 2004). In a recently published study on time trends in 1974-2007 of BFRs in peregrine falcon eggs, BDE-47 was the dominating congener in the 1970s, while BDE-153 was the dominating congener after the year 2000 (Johansson et al. 2011). It was also found that BDE-209 increased in peregrine falcon eggs after the year 2000. This indicates that it is both a spatial and a temporal difference in congener patterns of BFRs.

Phenolic compounds

Pentachlorophenol (PCP), n-octylphenol (n-OP), tert-octylphenol (t-OP), nonylphenol (NP), and triclosan were analysed. Triclosan, n-OP and t-OP and were not found above LOQ in any of the analyzed samples. PCP was found in all samples and NP were found in levels at LOQ in two of the Tyresta samples and in one of the Grimsö samples (Appendix, Table 4).

Nonylphenol

Considering the high LOQ (48 ng/g ww corresponding to 1800 ng/g lw) it is somewhat remarkable that that nonylphenol was found in earthworms from three of the sampling spots representing the localities Tyresta and Grimsö. In bank voles collected from five localities in 2001, nonylphenol was found in detectable levels at N:a Kvill and Vålådalen and traces of nonylphenol was found in voles from Ammarnäs (Lind and Odsjö 2010). In that study, however, nonylphenol was not found in voles from Grimsö. LOQ for the analyses of nonylphenol in voles and earthworms were the same on lipid weight basis, 1800 ng/g lw in these two studies. Nonylphenol could not be detected in starlings collected in 2006 (Odsjö et al. 2008). LOQ for nonylphenolic compounds was however very high (8000 ng/ ww) in that study which could be a reason that this compound was not found.

Pentachlorophenol (PCP)

PCP was found in all of the analyzed earthworm samples in the present study (fig 10). The levels was well above LOQ (1,1 ng/g ww) in all samples. The highest levels of PCP was found in one of the samples from Tyresta (18 ng/g ww) and in one of the samples from Grimsö (14 ng/g ww), however not in the same samples where nonylphenol was present

PCP was not found in starlings or bank voles in the previous studies.

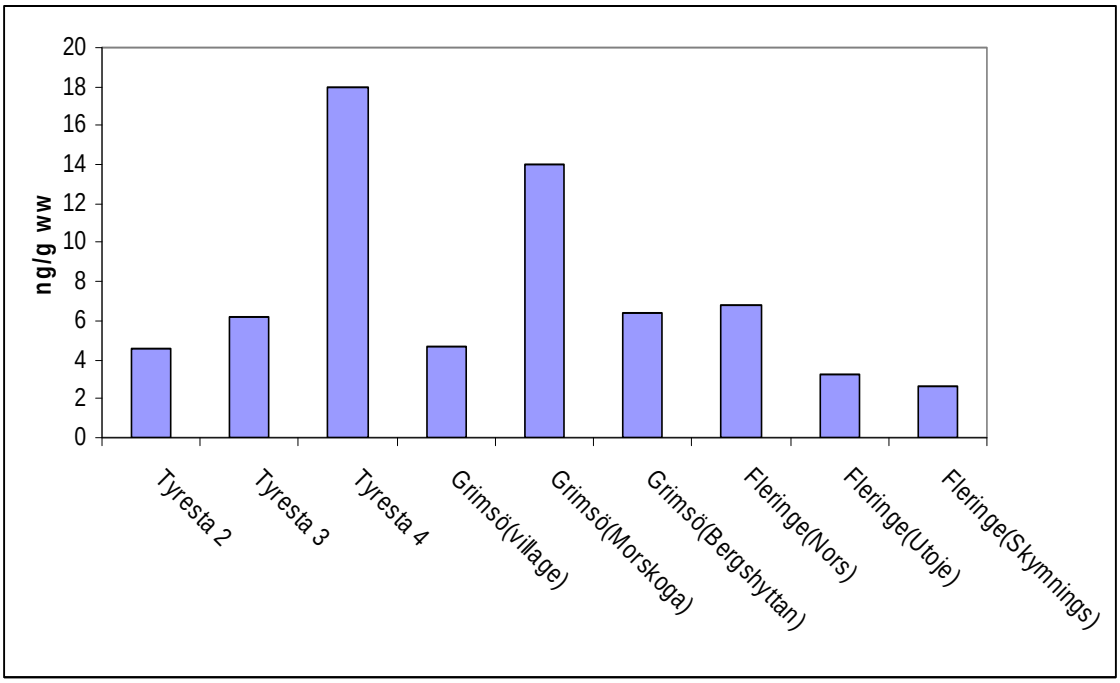

Figure 10. Pentachlorophenol (ng/g ww) in whole earthworms from three localities in Sweden.

Summary phenolic compounds

As the LOQ for some of the analysed phenolic compounds are rather high, it is difficult to get a reliable picture of the levels of these compounds in earthworms. There are also considerable differences in LOQs between different matrices for these compounds. None of the earthworm samples contained triclosan above LOQ (1,6 ng/g ww) while all of the starling samples contained triclosan above LOQ. However, if the levels in starlings are compared to the LOQ for triclosan in earthworms, all of the starling samples fall below LOQ. For PCP the relationship is the opposite with LOQ in earthworms being lower compared to LOQ in starlings and bank voles. This could be the reason that PCP is found in detectable levels in all earthworm samples but in none of the starling or bank vole samples.

This difference in detection and quantification limits between different matrices makes it difficult to compare the levels of phenolic compounds and to get a reliable and comprehensive picture of the levels of these compounds in biota. In earthworms, PCP are found in all of the analysed samples in levels well above LOQ (1,1 ng/g ww) indicating that this compound is ubiquitous in the environment. The large variation between sampling spots from the same localities also indicate that the levels of PCP in the environment is very variable and that the

sources probably are of local origin. PCP was not found in muscle tissue of neither bank voles nor starlings in the previous studies (Odsjö et al. 2008; Lind and Odsjö 2010). Nonylphenol is not detected in all samples but in the samples where it is found the levels are rather high as the LOQ is 48 ng/g wet weight. This could imply that nonylphenol also is ubiquitous in the environment and/or a large variation in the environmental levels. Triclosan is found in none of the earthworm samples but it is found in all of the staring samples and in some of the bank vole samples. However, the large difference in LOQ for the triclosan analyses between these matrices makes it difficult to draw any conclusions concerning the levels of triclosan in the environment.

The conclusions that can be drawn from the existing analyses on phenolic compounds are that they exist in biota but there are analytical problems for these compounds that makes it difficult draw conclusion from the existing results. PCP was found in all the analysed samples of earthworms in the present study but no geographical pattern could be detected. On the contrary there was a large variation between the different samples from the same locality indicating local sources for this compound.

Perfluorinated compounds (PFCs)

The levels of PFCs were generally very low and in most cases below LOQ. However, if the levels were above LOD but below LOQ, estimations were made on the concentrations that are considered to be semi-quantitative. These estimations are included in Figure 11. Results from all the analyses of PFCs are shown in Appendix, Table 5. Six of the analysed PFCs (PFHxA, PFDcA, PFBS, PFHxS, PFDcS, PFOSA) were below LOD in all samples. PFTriA was above LOQ in all samples and PFOS was above LOQ in all samples except one from Tyresta. PFNA was present in levels above LOQ in six of the samples (one from Tyresta, in all Grimsö samples, and two samples from Fleringe). PFOS was found in highest levels in the earthworm samples followed by PFTriA (Fig 11).

Within the monitoring programme on contaminants in terrestrial biota, perfluorinated compounds have earlier been analyzed in of bank voles (*Myodes glareolus*) collected from five localities in 2001 and of young starlings (*Sturnus vulgaris*) collected from eight localities in 2005. The mean level of PFCs in liver of bank voles was 12,8 ng/g ww (Lind and Odsjö 2010). The mean level in liver of young starlings was 5,2 ng /g ww (Odsjö et al. 2008). In whole earthworms, the mean level was 3,15 ng/g ww in the present study. In a retrospective study on the levels of PFCs in muscle from young moose in the Grimsö area in 1986-2005

and in three year old reindeer from the Abisko area in 1987-2006, PFOSA, PFOS and PFOA was detected some years but the levels were always below LOQ (Danielsson et al. 2008).
It has been speculated that some PFCs such as PFOS that have a high water solubility but very low degradability are more easily washed out by rain and thus are accumulated in water while other PFCs with very low water solubility are retained in soil (Holmström et al. 2010). This would account for the higher levels in aqueous environment compared to terrestrial environments. This could also imply that the levels in terrestrial environments are more sensitive to short term fluctuation in atmospheric emissions and rainfalls and that the relative distribution between different PFCs could differ between aquatic and terrestrial environments.

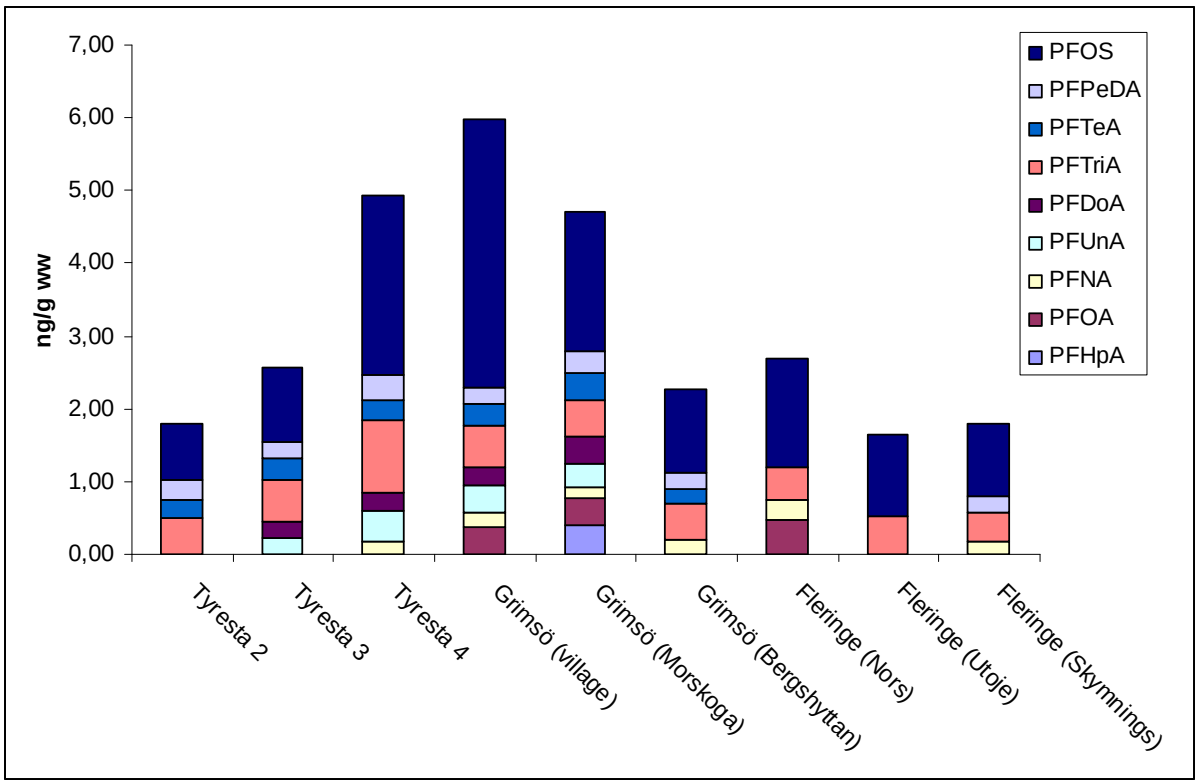

Fig 11. Perfluorinated compounds (BFCs) in homogenates of whole earthworms from three localities in Sweden. The figure includes values below LOQ but above LOD (see text and Table 5 in Appendix for explanations).

Musk substances

Fifteen musk substances were analysed (Table 5). Levels above LOD were not found in any of the analysed samples.

Pesticides

Forty-five pesticides were analysed (Table 6). The levels were generally below detection limits. One sample from Grimsö (Morskoga) contained endosulfan sulfate (70 ng/g ww). Endosulfan sulfate is a stable breakdown product of endosulfan, an insecticide earlier used in Sweden against a variety of insects and mites, mainly in orchards and gardens. The use of endosulfan has been banned in Sweden since 1995 but it is still in use in large parts of the world. Two of the samples from Fleringe (Utoje, Skymnings) contained klorpyrifos just above LOD (0,7 ng/ g ww). One of the samples from Fleringe (Nors) contained low levels of metribuzin (6 ng/g ww) and propikonazol (8 ng/g ww). Klorpyrifos has been used as a broad-spectrum insecticide but no preparation containing klorpyrifos is allowed in Sweden since 2008 (KemI). Metribuzin and propikonazol are still in use in Sweden (KemI).

No pesticides were found in the Tyresta samples.

The clean up method used in the pesticide analyses was not specially adapted to the earthworm matrix. According to the laboratory, the background noise was higher in the earthworm matrix compared to earlier analyses of pesticides in livers of young starlings. This made some of the analyses impossible to perform and the quantification limit became higher for some of the substances compared to earlier analyses made on liver homogenates of young starlings.

Summary and conclusions

It can be concluded that levels of environmental contaminants analysed in this study are, as expected low or very low. There is however some exception to this. DDE and DDD levels were unexpectedly high in one of the Grimsö (Morskoga) sample and in one of the samples from Fleringe (Nors). In the Morskoga sample, there was also a surprisingly high level of DDT. Chlorinated compounds were analysed yearly in muscle of starlings from Grimsö in 1981-1995. Only DDE were analyzed during this period and it was apparent that the Morskoga samples had considerably higher in DDE levels compared to the samples from Grimsö village and Grimsö-Bergshyttan situated 4-5 km from Morskoga, indicating that the source of DDT is local. This can also bee seen as a confirmation that young starlings are raised on locally collected food and reflect the area where they collected. DDE was also analyzed in starlings from Fleringe between 1983 and 1995. In the Fleringe area there was no differences in DDE levels in starlings between the different collection spots but the DDE

levels in starlings from Fleringe were considerably higher (average for all years 1,31 ng/g lw) compared to the other (average for all years analyzed 0,118 – 0,46 ng/g lw).
Obviously, the level of DDT and its metabolites is still high in the Morskoga area and that DDT constituted almost 50% of the ΣDDT in this sample is remarkable. According to a study made on the bioavailability to earthworms (*Eisenia foetida*) of aged DDT, DDE and DDD, the bioavailability is declining and after 49 years, considerably less (<85%) of the compounds that still existed in the soil was taken up by earthworms compared to when the compounds that were not aged (Morrison et al. 2000). This study also showed that DDT can still be present in soils after many years. The result from the present study indicates that the level in the soil can still be rather high at this location.
The levels of nonylphenol found in earthworms in this study are also somewhat conspicuous. Nonylphenol are often found in water and sludge from sewage treatment plants. The sources of these compounds are alkylphenol ethoxylates that are relatively easily broken down to nonylphenols and octylphenols. No pattern could be detected in the levels of nonylphenol that was found and samples with levels above LOD/LOQ were found at two of the sampling locations.
From the present study, it can be concluded that earthworms could be a useful matrix for monitoring of environmental contaminants in certain cases. They are probably more useful for detecting levels of water soluble compounds such as metals and also compounds from local sources. Earthworms are probably less useful in detecting long range atmospheric transport of fat soluble compounds due to their low fat content. The low lipid content can make it difficult to quantify substances analysed on lipid weight basis.
On the other hand could the presence of a compound in earthworm samples, although in low levels give valuable information on the behaviour of contaminants in the environment such as differences in congener patterns between different localities. Also could differences in levels and congener pattern between earthworms and animals higher up in food webs give information on the behaviour of contaminants in ecosystems.

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Fleringe (Utoje) Foto: U.Arnkvist

Appendix

Table 1. Metal and elements (µg/g ww) in homogenates of whole earthworms.

	Ca	Cd	Co	Cr	Cu	Fe	Hg	Mg	Mn	Mo	Ni	Pb	V	Zn
Tyresta 2	654	1,08	0,855	0,651	1,80	344	0,051	217	8,24	0,160	0,270	0,564	0,866	59
Tyresta 3	654	0,961	1,318	0,936	2,46	565	0,028	270	8,80	0,180	0,390	0,194	1,27	188
Tyresta 4	962	1,51	1,065	0,607	1,54	838	0,092	228	9,04	0,140	0,260	0,303	0,738	92
Grimsö (village)	616	1,29	0,442	0,411	2,25	293	0,104	239	11,9	0,280	0,170	0,437	0,515	167
Grimsö (Morskoga)	558	3,01	0,793	0,476	2,62	260	0,125	203	8,92	0,240	0,170	0,509	0,656	136
Grimsö (Bergshyttan)	505	1,92	0,544	0,247	1,50	202	0,040	166	7,04	0,280	0,130	3,22	0,412	131
Fleringe (Nors)	2583	5,25	0,278	0,375	1,81	177	0,050	244	8,97	0,230	0,260	0,430	0,408	287
Fleringe (Utoje)	1770	4,51	0,263	0,468	2,60	168	0,021	219	6,60	0,180	0,250	0,314	0,413	195
Fleringe (Skymnings)	1510	1,52	0,275	0,620	1,64	265	0,032	218	8,04	0,190	0,240	0,612	0,735	146

Table 2. Chlorinated compounds (ng/glw) in homogenates of whole earthworms. Minus (-) denotes values below LOQ. -99.99 denotes values below LOD.

	HCB	α-HCH	β-HCH	γ-HCH (lindan)	DDE	DDD	DDT	CB28	CB52	CB101	CB118	CB153	CB138	CB180
Tyresta 2	-9	-8,5	-11	-11	20,3	-15	-99.99	-9	-9	-9	-9	6,62	-6	-11
Tyresta 3	-9	-9,1	-11	-11	82,1	-16	-99.99	-9	-9	-9	-9	-7	-7	-11
Tyresta 4	10,2	-9,3	-12	-12	17,4	-16	-99.99	-9	-9	-9	-9	10,7	8,12	-12
Grimsö (village)	-8	-7,8	-9,7	-9,7	7,8	-14	-99.99	-8	-8	-8	-8	8,14	8,73	-10
Grimsö (Morskoga)	8,62	-7,8	-9,8	-9,8	4672	1321	5383	-8	-8	-8	-8	-6	-6	-10
Grimsö (Bergshyttan)	6,92	-7,3	-9,1	-9,1	13,1	-13	-99.99	-7	-7	-7	-7	-5	-5	-9
Fleringe (Nors)	-8	-8,0	-10	-10	3599	54,4	-99.99	-8	-8	-8	-8	47,0	29,2	9,19
Fleringe (Utoje)	-7	-6,8	-8,5	-8,5	281	-12	-99.99	-7	-7	-7	-7	7,85	6,14	9,38
Fleringe (Skymnings)	-6	-6,3	-7,8	-7,8	141	-11	-99.99	-6	-6	-6	-6	7,35	5,48	-8

Table 3. Brominated flame retardants (ng/g lw) in homogenates of whole earthworms. Minus (-) denotes values below LOQ.

	BDE209	BDE47	BDE99	BDE100	BDE153	BDE154	HBCD
Tyresta 2	-5	1,08	0,72	-0,45	-0,45	-0,45	-4,0
Tyresta 3	-5	1,31	0,54	-0,48	-0,47	-0,48	-3,8
Tyresta 4	-5	1,49	-0,49	-0,49	-0,48	-0,49	-3,9
Grimsö (village)	20,3	-0,82	-0,41	-0,41	-0,41	-0,41	-3,3
Grimsö (Morskoga)	-4	0,93	-0,42	-0,42	-0,42	-0,42	-3,4
Grimsö (Bergshyttan)	-4	-0,76	-0,38	-0,38	-0,38	-0,38	-3,0
Fleringe (Nors)	-4	-0,83	0,44	-0,41	-0,41	-0,41	-3,3
Fleringe (Utoje)	-3	2,87	2,23	0,82	0,39	-0,34	-2,8
Fleringe (Skymnings)	-3	0,68	0,54	0,38	-0,33	-0,33	-2,6

Table 4. Phenolic compounds (ng/g ww) in homogenates from whole earthworms. <denotes values below LOQ.

	Pentachlorophenol(PCP)	n-octylphenol	t-octylphenol	nonylphenol	Triclosan
Tyresta 2	4,6	<8,9	<3,3	48[1]	<1,6
Tyresta 3	6,2	<8,9	<3,3	<48	<1,6
Tyresta 4	18	<8,9	<3,3	48[1]	<1,6
Grimsö (village)	4,7	<8,9	<3,3	<48	<1,6
Grimsö (Morskoga)	14	<8,9	<3,3	<48	<1,6
Grimsö (Bergshyttan)	6,4	<8,9	<3,3	48[1]	<1,6
Fleringe (Nors)	6,8	<8,9	<3,3	<48	<1,6
Fleringe (Utoje)	3,2	<8,9	<3,3	<48	<1,6
Fleringe (Skymnings)	2,6	<8,9	<3,3	<48	<1,6

1 Due to analytical problems it was impossible to quantify the levels in these samples. These samples have nonylphenol levels that are above the LOQ (48 ng/g ww).

Table 5. PFCs (ng/g ww) in homogenates of whole earthworms. <denotes values below LOD. Numbers in italics denotes values below LOQ.

	PFHxA	PFHpA	PFOA	PFNA	PFDcA	PFUnA	PFDoA	PFTriA	PFTeA	PFPeD	APFBS	PFHxS	PFOS	PFDcS	PFOSA
Tyresta 2	<0,45	<0,25	<0,35	<0,15	<0,25	<0,2	<0,2	0,50	*0,24*	*0,28*	<0,5	<0,4	*0,76*	<0,2	<0,15
Tyresta 3	<0,45	<0,25	<0,35	<0,15	<0,25	*0,23*	*0,22*	0,57	*0,30*	*0,22*	<0,5	<0,4	1,02	<0,2	<0,15
Tyresta 4	<0,45	<0,25	<0,35	0,19	<0,25	0,41	*0,25*	1,02	*0,25*	*0,36*	<0,5	<0,4	2,46	<0,2	<0,15
Grimsö (village)	<0,45	<0,25	*0,38*	0,19	<0,25	0,39	*0,24*	0,56	*0,30*	*0,23*	<0,5	<0,4	3,69	<0,2	<0,15
Grimsö (Morskoga)	<0,45	*0,40*	*0,36*	0,17	<0,25	*0,30*	0,38	0,50	0,38	*0,30*	<0,5	<0,4	1,92	<0,2	<0,15
Grimsö (Bergshyttan)	<0,45	<0,25	<0,35	0,20	<0,25	<0,2	<0,2	0,49	*0,20*	*0,21*	<0,5	<0,4	1,15	<0,2	<0,15
Fleringe (Nors)	<0,45	<0,25	*0,46*	0,29	<0,25	<0,2	<0,2	0,45	<0,2	<0,2	<0,5	<0,4	1,48	<0,2	<0,15
Fleringe (Utoje)	<0,45	<0,25	<0,35	<0,15	<0,25	<0,2	<0,2	0,53	<0,2	<0,2	<0,5	<0,4	1,12	<0,2	<0,15
Fleringe (Skymnings)	<0,45	<0,25	<0,35	0,16	<0,25	<0,2	<0,2	0,41	<0,2	*0,22*	<0,5	<0,4	1,01	<0,2	<0,15