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Department of Sanitary Engineering
Applied Environmental Measurement Techniques

MASTER THESIS

Platinum Levels in Raptor Populations troughout Sweden

ALIA STEVENS

Master Thesis 1998:1

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voltammetry

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ABSTRACT

The goal of this project was to establish the concentration of platinum in raptor populations throughout Sweden. Several habitats and food chains were investigated to determine this level and whether or not platinum is bioaccumulating. To accomplish this the amount of platinum in four raptor species, six populations (*Falco peregrinus,* Northern-wild, southern-wild, and captive; *Falco rusticolus*, wild northern; *Falco tinnunculus,* wild urban; and *Accipiter nisus*, wild-urban) and four different non-invasive biological samples (blood, feathers, fecal matter and egg matter) were analyzed using cathodic stripping voltammetry (CSV). The levels of platinum were determined for several of the biological samples coming from the same site and eggs from the same female. The amounts of platinum found in the different populations were tested against each other using the Mann-Whitney test. There were significant differences found as follows (the first sample is the one with the higher amount of platinum): Captive Peregrin eggs vs southern peregrine eggs 0.021, Sparrowhawk egg vs northern peregrine egg =0.046, Sparrowhawk egg vs southern peregrine=0.011, southern fecal vs gyrfalcon fecal=0.0485, southern peregrine fecal vs southern peregrine egg = 0.0001, southern peregrine blood vs southern peregrine egg = 0.0008. The mean amount found in the egg samples ranged from 0.51 ngg-1 in the Sparrowhawk to 0.043 ngg-1 in the southern peregrine. The mean blood levels were 0.15 ngg-1 for the northern peregrine population and 0.29 ngg-1 for the southern peregrine population. The method failed for the feather samples. Raptors were used for this project for several reasons. 1. They are found at a high trophic level allowing for biomagnification in toxins. 2. They are found in several habitats in Sweden. 3. They have different food chains. Due to the large decrease in the peregrine falcon population in the 1970's, there has been wide research completed on them and their given biological sample. This is why the peregrine was a focus of this study.

Key Words

Falco peregrinus, Falco rusticolus, *Falco tinnunculus, Accipiter nisus,* Platinum, bioaccumulation, cathodic stripping voltammatry.

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To my partner in life, Henrik, you have been more than I thought possible for any one person to be. You are my best friend, my love and my inspiration. With you anything is possible and we will change the world. Remember Phyllis.

Prayer of the Good Green Boy

Before we're all wrecked
By the greenhouse effect
Could you kindly protect us from frying?
Help wetlands endure.
Make rivers run pure
So the fishes who live there quit dying.

Please clean up the air
And arrange for repair
Where the ozone layer's torn to tatters.
Let coral reefs thrive
And all species survive
Because even the measliest matters.

Un-acid the rain.
Tell polluters: Refrain!
Help the rainforests gain, not grow smaller.
Make the ecosphere sing.
And-oh yes- one more thing.
Could you please make me four inches taller?

Written by Judith Viorst in Sad underwear ...and other complications.

I. Introduction

Platinum (Pt) is being released into the environment from the catalytic converters used in the automotive industry. It is a non-essential trace element (Lyengar 1989). Platinum is not noted as a toxin. This does not mean platinum is not toxic; it has not been investigated at levels known to cause toxicity for other metals noted as environmental concerns, i.e. lead, cadmium, and mercury. There was a flurry of investigations on platinum in the mid 1970's before these converters were mandated in the United States (Holbrook *et al.* 1975, Moore *et al.* 1975, Brubaker *et al.* 1975, and Johnson *et al.* 1975). The goal of these groups was to establish a background value for the amount of Pt in the environment and its toxicity. All of these groups found low levels of platinum and low levels of toxicity. Also, none of these groups discovered if Pt is biotransferable.

The idea of bioaccumulation is not new. The best method used to investigate the level of accumulation is a top predator of the selected ecosystem. If a high amount of the investigated toxin is found, the toxin must be traced through the environment. The tracing is done by starting with the soil, it gives nutrients to the plants. Along with the nutrients, the soil will also transfer toxins that have accumulated there into the plants. These toxins then flow to the primary herbivore, and from there to the primary carnivore. This is explained in figure 1. The diagram shows the energy flows through the food chain. This flow is what allows toxins to bioaccumulate.

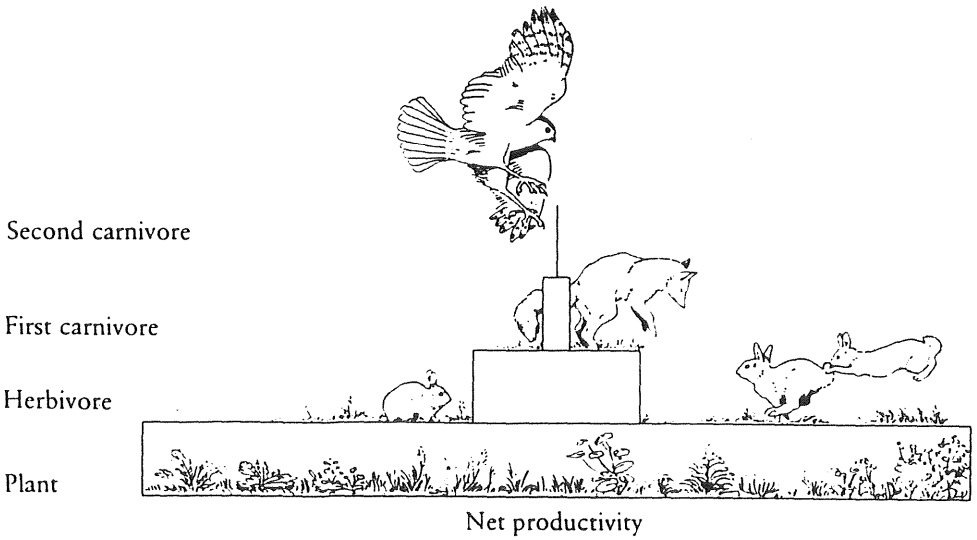

Figure 1. This diagram depicts the net productivity of each trophic level through the food chain. The length of each bar represents the amount of net productivity for that level (Ricklefs 1993).

The easiest way to measure bioaccumulation is to find a species that exhibits traceable characteristics or predictable reactions to given toxins. Birds are used to monitor many heavy metals. Mercury, cadmium, DDT and lead are some of the most frequently analyzed toxins in bird populations (Appelquist *et al.* 1984, Braune 1987, Court *et al.* 1990, Peakall *et al.* 1990, Burger and Gochfield 1995, Esselink *et al.* 1995, Rumbold *et al.* 1997). For example, peregrine falcons [(*Falco peregrinus),* figure 2] have been used to monitor DDT (Dichlorodiphenyltrichloroethane). In the DDT example, DDE, one of the breakdown products of DDT, is predicted from the eggshell thickness. The thickness of the eggshell is measured and then compared to an index, thus determining the amount of DDE in the female in question (Lindberg *et al.* 1985).

During this study, four raptor species, all with different food choices, were selected for analysis. Six populations of raptors were examined by using non-invasive biological samples from the individuals. The four species were peregrine falcon (*Falco peregrinus)*, gyrfalcon *(Falco rusticolus)*, kestrel *(Falco tinnunculus)*, and sparrowhawk (*Accipiter nisus)*. These species are shown in figures 2- 5. The populations of these birds were located throughout Sweden. There were three populations of peregrines examined here; wild northern Sweden, wild southwestern Sweden, and captive individuals in southwest Sweden. The gyrfalcon population was located in the same area as the northern peregrines. The kestrel and sparrowhawk populations were located in an industrialized urban area in southwest Sweden.

The biological samples were eggs from all of the populations except the gyrfalcon. Fecal samples were taken from both of the wild peregrine populations and the gyrfalcons. Blood samples were taken form the wild peregrine populations only. Feathers were collected from the sparrowhawk females only.

I.II. Aims and objectives

This study attempted to establish the level of platinum in four raptor species in Sweden. That was accomplished by examining the level of platinum in four different biological samples, egg matter, fecal matter, blood and feathers. To the best of our knowledge this level has not been investigated before. Therefore, there will be no references upon which to base comparisons.

There were a few questions this project attempted to answer: Is platinum bioaccumulating?; If it is accumulating, is it following the same pathways as known for bioaccumulating metals?; Are there varying amounts of platinum in the given biological samples?; Are there differences in the location of the population, i.e. urban versus rural and the amount of platinum found in the biological samples? Attempts were made to answer these questions by the evaluation of egg matter, fecal matter, blood, and feathers of raptors in Sweden. These raptor populations were located in rural and urban settings. These samples were analyzed using cathodic stripping voltammetry (CSV) and the results were statistically tested.

Figure 2. The peregrine falcon *(Falco peregrinus)*

Figure 3. The sparrowhawk (*Accipiter nisus*)

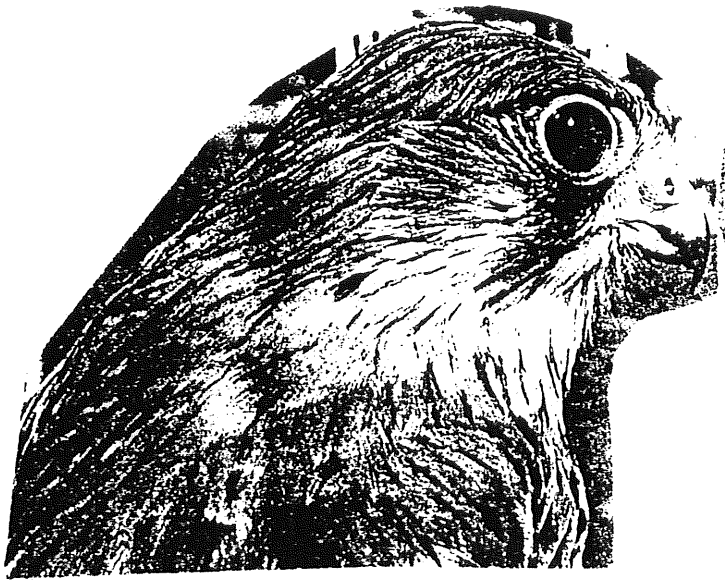

Figure 4. The European kestrel (*Falco tinnunculus)*

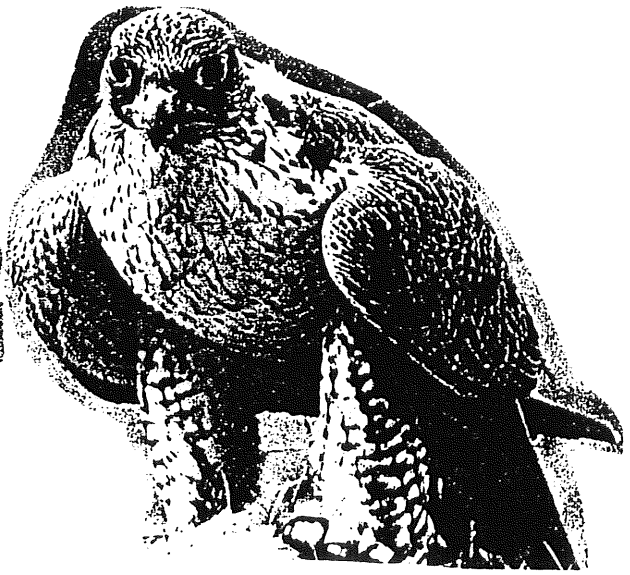

Figure 5. The gyrfalcon (*Falco rusticolus)*

II. Background

II.I. Platinum.

The metal platinum (Pt) is a very scare element in the Earth's crust (Alt. *et al.*1997). However, the use of platinum is increasing. The increased amount of usage means an increased amount in the biosphere (the part of the atmosphere and earth capable of supporting life). Due to this increase, there is much interest in examining the interactions between platinum complexes and biological systems (Di Notor *et al.* 1995).

The majority of the production of platinum is in South Africa (Cohn 1993). The amount of Pt produced is approximately 1000 tones per year (Alt *et al.*1997). The main increase in the use of platinum is in the automotive industry. There are new technologies using larger amounts of platinum. The main automotive use of Pt is the catalytic converter. They are used to remove nitrogen oxides, carbon monoxide and hydrocarbons from the exhaust (Brubaker *et al.* 1975, Moore *et al.* 1975, Nygren *et al.*, 1990, Vaughan and Florence 1992, Alt *et al.* 1993, Wei and Morrison, 1994, Vetz *et al.*, 1996, Alt. *et al* 1997). These converters contain 0.9-2.3g Pt/car and there are concerns about the amount of Pt these catalysts are allowing to enter the environment (Alt *et al* 1997). It has been shown that as automobiles are driven at faster speeds more Pt is released (König 1992). Also, along those same lines, Schierl and Fruhman (1996) demonstrated that there is an increase in the amount of Pt measured in high traffic areas as opposed to low traffic areas. Pt is also used in cancer therapy and can be introduced to the environment through hospital effluents, though this is not the major source (Kummerer 1997). The amount of platinum in the environment is on the rise. A study done by Wei and Morrison (1994) showed an increase in platinum in road dust over several years. The question needing to be answered is whether or not the benefits received when using Pt catalysts is worth the introduction of platinum into the environment.

There are more and more demands being placed on platinum supplies. There has been a new catalyst developed by the Engelhard corporation of the United States. This catalyst is a clean air catalyst called PermAir (Fineberg 1995b). PermAir involves a coating, which is platinum based, to be applied to the air conditioning condenser and to the radiator (Fineberg 1995a). As the air passes over the coated areas, ground level ozone is converted into oxygen while carbon monoxide is converted into carbon dioxide (Fineberg 1995b). There is some question about PermAir's, durability and efficiency (Fineberg 1994). Claims have been made that the catalyst converts 90% of the ozone and/or carbon monoxide coming in contact with it (Fineberg 1995a). As this new catalyst is not limited to automobile condensers, it is perceivable that it can be used on commercial and private air conditioning units. This could double the demand for platinum (Fineberg 1994).

Automotive manufacturing companies are favoring the use of the PermAir catalysts as the environmental regulations strengthen. The European Union is demanding lower vehicle exhaust emissions and all diesel cars and off road machines must have a converter fitted by 1997 (LaRue 1996). The Ford Motor Company is planning on all of its models having this converter by 1998 or 1999 (Fineberg 1995a). Also, General Motors has designed a platinum tipped spark plug that is supposed to last twice as long as conventional plugs put in all of its 1997 models (Fineberg 1994). With all of this increase in the use of platinum, the amount in the environment will probably increase dramatically.

The determination of platinum as a human health hazard has not been established. Direct exposure can lead to a condition termed as "Platinosis" in humans and allergic reactions, such as cold like symptoms and skin irritations in those already sensitive (Brubaker *et al.* 1975). Platinosis is caused by continual exposure to Pt salts, which can lead to an asthmatic like response (Brubaker *et al.* 1975). There is little information about long-term low-level exposure and biotransformation, a process allowing platinum to enter the food chain (Nygren *et al.*1990).

The amount of Pt in ocean water has been studied by several groups (Hodge et al. 1986, van den Berg and Jacinto 1988, Jacinto and van den Berg 1989, Adeloju et al. 1990, Barefoot 1997). Ocean water is important to study when dealing with Pt because of the movement of Pt through the environment. Pt is injected into the environment along the roadside. The closer the sample was taken to the road, the higher the amount of platinum found when examined (Barefoot 1997 and Messerschmidt *et al.* 1994). As these soil particles wash away with erosion the Pt is also removed into the aquatic system; therefore, a high amount will be entering the ocean waters from the inland streams. The ocean could be acting as a sink for platinum however, the behavior of Pt in ocean waters is unpredictable (Jacinto and van den Berg 1989).

The transformation of platinum, either from the metallic form or from compounds, into a bioavailable species could take, primarily, one of two paths. The first path is chemical oxidation. This occurs with oxygen and other complexes connected to the ligands, binding sites, in the soil. Within 21 days, up to 3% of the metal is dissolved by platinophile complexing elements in the oxygenated atmosphere (Lustig *et al* 1997).

The second path is through biological transformation by way of microorganisms. This pathway is similar to the pathway followed by mercury. This pathway includes the possiblity that platinum is biomethylated by bacteria. These methylated compounds would be very stable (Lustig *et al.* 1997). There has not been adequate investigations completed to conclude if one of these pathways is the actual path of platinum.

There have been several techniques used for determining platinum in biological samples. The following is a list of some of those techniques: X-ray fluorescence, atomic absorption spectroscopy, anodic stripping voltommetry, differential pulse polography, neutron activation analysis, high performance liquid chromatography with

electrochemical detection and with UV detection or interfaced to an inductively coupled plasma spectrometer (Vaughan and Florence 1992).
It is important to begin finding whether or not platinum is bioaccumulating and the speciation of the metal.

II.II. Bioaccumulation.

Bioaccumulation is the result of passive or active accumulation of a substance in a biological system. This accumulation can be either through a food choice, or through the environment (Streit 1992). Bioaccumulation is what allows for toxins, or whatever substance is in question, to increase in amount as it travels up the trophic levels.

The first large-scale examination of bioaccumulation was a forty-year process. It began in the 1930's in Minimata Bay, Japan. The Chisso Corporation of Japan opened a factory in the bay. The factory produced vinyl chloride and formaldehyde, the by-products contained mercury. These by-products were then emptied into the bay waters. Subsequently, the methyl-mercury chloride levels in the fish and shellfish in the area rose. These marine species were the main staple of the diet of the people living in Minimata.

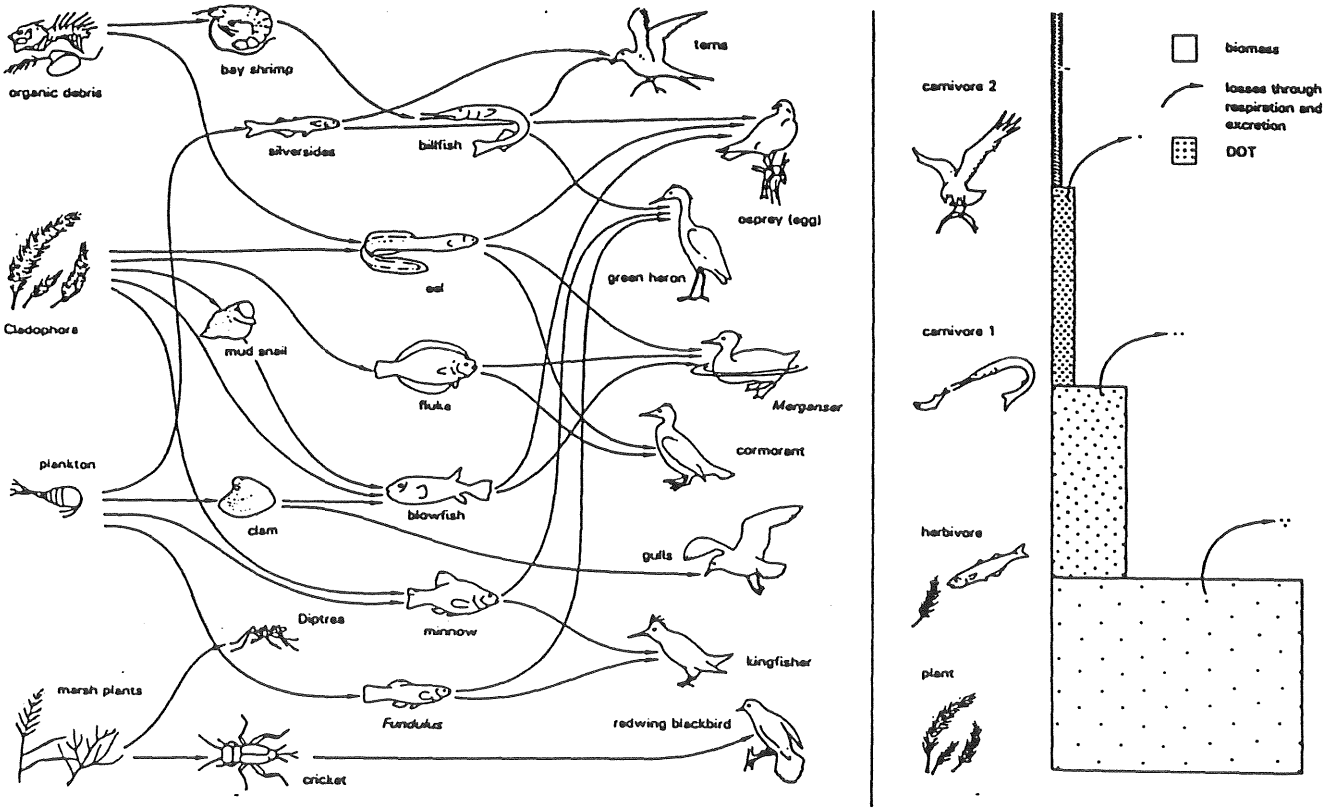

Figure 6. The left side of the diagram shows the traditional food web in the eastern US. It shows the path DDT would follow while bioaccumulating. The right half of this diagram shows the bioconcentrations of DDT at the given trophic levels(Streit 1992).

15 years later a permanently disabling neurological disorder named "Minimata Disease" was diagnosed. It occurred mostly in the children in the area. In 1959 the link between mercury and the disorder was established. It was not until 1960 that it was understood mercury was coming from the factory. In the 1970's the government of Japan finally regulated the emissions by this factory. After this incident many other nations also set restrictions on the emission of mercury (Nybakka1993).

A second, well-known example of bioaccumulation is the maturation of DDT. From the 1940's through the 1970's, the most widely used pesticide in the world was DDT. It was popular because it was very effective and it had a long half-life. Therefore, the farmers were applying it less than any other pesticide and having better results. However, DDT's resistance to biological decomposition soon caused problems for the wild life. The chemical was following the run off of the soil into the water systems and was assimilated by the plankton eaten by fish. Figure 6. is a diagram of a regular aquatic food chain and the flow of DDT through it. The accumulation, at this level, was not harmful for the fish, but almost caused the extinction of several top predators (*Ardea, Pandion, Phalacrocorax, Mergus, and Alcedo*) in their ecosystem. The fish were being eaten by birds and assorted mammals. Not until the chemical reached a higher trophic level did it almost wipe out entire species (Court 1990). For example, there were large declines in the peregrine falcon populations throughout the world. The majority of this decline was seen in the Northern hemisphere that contains some of the most important breeding areas. This decline was occurring because DDE, a breakdown component of DDT, was magnifying in the falcons. This magnification caused eggshell thinning. The thin eggshells made it impossible for the parent to incubate. Subsequently, DDE caused a lowering in the reproduction rate of the peregrines. The use of DDT is now banned in many parts of the world. Unfortunately, those parts do not include many of the counties, where the majority of migrating birds winter, laying in the southern-hemisphere. It will be many lifetimes until the biosphere is clear of DDT and its components.

II.III. The Specimens.

II.III A. Peregrine Falcon

There are approximately 60 pairs of wild peregrine falcons in Sweden and their average production of young is around 1.7 chicks per year (Kjellèn 1997). The peregrine falcon was once a widespread species covering Sweden, but it experienced a large decline in the 1950's and 1960's. The species was endangered and on the brink of extinction in the 1970's and its distribution was restricted to two main areas in Sweden. One area is in the Southwest, in the provinces of Halland, Bohuslän, Dalsland and Västergötland. The second area is in the north in the province of Norrbotten. The populations in the north and in the south have different food chains and migratory patterns. This study used several samples from both of these populations as residue

levels have been found to be two to ten times higher in the Peregrine than in its prey (Lindberg and Odsjö 1983).

This study also used samples from a captive peregrine population that was established to aid in the recovery of the dwindled wild population. This group of peregrines is fed 3-week-old chickens raised on site.

The southern, wild population feeds mainly on pigeons (*Columbidae*), gulls (*Laridae)*, starlings (*Sturuidae*), waders (*charadriidae*), and thrushes (*Turdidae*) during the breeding season. The average weight of the prey taken is 242g. This population is on the top of a terrestrial based food chain (Lindberg 1983).

The third group is a wild, northern population. This population feeds mainly on ducks (*Anatidae*) and waders the average weight of prey taken by this falcon is 232g. Therefore, this population is on top of an aquatic food chain (Lindberg 1983).

II.III B. Gyrfalcon

The gyrfalcon (*Falco rusticolus*) lives in the same mountainous region of Sweden as the Northern wild peregrine population (Lindberg, 1983). However, these two populations do not compete for prey or cliff-side nest sites to any great extent. Much of the competition is relieved because the gyrfalcon begins its breeding season one month before the peregrine (Lindberg 1983). The gyrfalcon is a residential member of the alpine community and preys mainly on grouse (*Lagopus lagopus/ mutus)*. The grouse is approximately 70% of their prey (Lindberg 1983).

The gyrfalcon has been shown to have the lowest level of mercury and organochlorines of all raptor populations that have been investigated (Lindberg 1984). They were used to help determine the amount of toxin in the fecal samples of the northern populations.

II.III C. Kestrel

The kestrel, *Falco tinnunculus,* used in this study live in and around the city of Göteborg, Sweden. There are approximately 3,000 pairs with a 2.5 average of young produced in Sweden (Kjellèn 1997). The majority of the Kestrel population was found close to the highway going north from Göteborg. This city is the second largest city in Sweden. It also has an extensive industrialized area. Therefore, this population is exposed to high levels of urbanized pollutants.

They prey on small mammals, such as voles and mice (Olsen 1992). These mammals often live in fields and near roadways. Consequently the kestrel also lives

near roadways and fields. This proximity to highways makes the kestrel a prime candidate for platinum studies.

II.III D. Sparrowhawk

The final population examined in this study was that of the sparrowhawk, *Acceptor nisus*. There are approximately 20,000 pairs breeding in Sweden and they have an average production of chicks of 2.0 per year (Kjellèn 1997). The birds used in this study live in many areas of Göteborg. A few of the individuals live in the downtown area.

This bird feeds on insectivorous and grainivorous (eating only grains) passerines living in Göteborg. Some of these passerines are the pied flycatcher (*Ficedula hypoleuca*), the robin (*Erithacus rubecula*), house sparrows (*Passer domesticus*), and chaffinch (*Fringilla coelebs*) (Newton 1986 and Solonem 1997).

II.V. Biological samples

For this investigation four non-invasive biological samples from the raptor populations were used. Those samples were egg matter, fecal matter, feathers, and blood. These samples were selected because they do not affect the population being studied, and they are all used in other studies about toxins in avian populations. These are all common elements used in examining levels of pollutants in the populations of birds. They are favored because of having been proven to be representative of relative amounts of questioned toxins in the avian population. However, the most preferred characteristic of these elements is that they can be collected and analyzed with no effect on the population being studied.

II.V.A. Feathers

Feathers are used as a simple, reliable way to determine the amount of total mercury in a given population (Lindberg and Odsjö 1983, Appelquist *et al.* 1984, Lindberg *et al.* 1985, Noble and Elliot 1990, and Burger and Gochfield 1995). They show the amount of a substance, for example mercury, in the blood during the formation of the feather (Iyengar 1989). However, feathers do not show lead levels that are in relation to the amount of lead the individual has been exposed to (Pain *et al.* 1994).

The feathers used during this study were sparrowhawk feathers. They were collected during the breeding season of 1995 from female birds around the city of Gothenburg, Sweden.

II.V.B. Fecal Matter

The second sample material analyzed was fecal matter. This is important because it should show the amount of exposure to a given toxin the bird has experienced. The amount of lead in the fecal matter and in the liver of Great tit (*Parus major*) nestling had a positive correlation when investigated (Nyholm *et al.* 1995).

The fecal samples were collected around the nests of the wild peregrine falcons, from both locations, and from the gyrfalcon during the 1997 breeding season. The fecal sample were come mainly from the chicks because it was easiest to collect. It forms a calcified whitewash surrounding the nest.

II.V.C. Blood

The third sample examined was blood. The blood samples were taken from wild peregrine chicks, both northern and southern locations, during the 1996 and 1997 breeding season. Metal and organochloride studies in the blood show the amount of toxin directly within the specimen (Court *et al.* 1990 and Pain *et al.* 1993).

II.V.D. Egg Matter

The fourth sample analyzed was egg matter. The eggs being used here were all addled eggs. This means they did not hatch after a natural incubation period because they were either infertile, abandoned, or the development of the fetus was aborted. Every precaution was taken to make sure that viable eggs were not taken from the nest.

The egg was important because the female will sequester much of the toxin in her body into the egg. Therefore, the eggs should have relatively high amounts of the given substance. Actually egg matter has been shown to be a better representative of the amount of total mercury in a population than blood (Court *et al.*1990). If the level in the egg matter is high then it is safe to assume that the population may be at risk. It is good to remember that the eggs used were ones that were not successful in hatching. If there was a high amount of a toxin in the egg matter, maybe that it was what prevented the embryo from developing. If prolonged interference occurs the population affected will dwindle. Eggs were collected from all of the populations except the gyrfalcon.

From several of the wild peregrine nest sites all three samples, egg, from the adult female, and fecal and blood, from the chicks, were analyzed. This was to examine the amount of platinum in different samples from the same nest. Also, a few sets of eggs coming from the same clutch of the captive peregrines were analyzed to examine variability in the female.

III. The Experiment

III.I. Sample Preparation

Peter Lindberg and the team of Gothenburg University collected the samples. The samples were then placed in either a deep freezer or an airtight plastic bag where they were kept until analyzed. The blood samples were treated with a preservative, APS-buffer.

The egg samples were the samples requiring extra preparation before being analyzed. To begin the process, the eggs were shaken to cause the yolk and albumin to homogenize. The eggs were drained of their contains though of two holes. The holes (2-3 mm) were drilled in two locations, one at the equator of the egg and the other at the bottom. The bottom hole was placed over an acid rinsed jar while air pressure was added through the equatorial hole. The samples were then frozen until analyzed.

The fecal matter samples were ground in an acid washed mortar and 20 to 40 mg of the samples were used. This procedure is outlined in Vaughan and Florence (1992).

Of the feather, only the portion of the shaft that grew internally was used. This section was cut off from the feather using stainless steel scissors. This section was used because it was internal to the bird and would then not have platinum adhering to it from an outside contamination. The removed sections were placed in quartz crucibles and weighed. The same digestion was then followed for the feather samples as it was for the others.

III.II. Sample Digestion

The digestion was the same as followed in Nygren *et al* (1990). A sample of 500-250 *m*l, for the blood and egg samples, or the weighed amount of the other samples, was placed into a quartz crucible along with 0.3 ml of HCl at 12 M concentration. The crucible was then covered and placed on a hot plate where it was slowly brought to dryness. The sample was then ashed in a muffle furnace as described below. After ashing, adding 1.5 ml of the following aqueous solution dissolved the sample: 1 ml of concentrated HNO_3 and 0.5 ml of 12 M HCl. This was allowed to stand for four hours. After the incubation period, the sample was again slowly brought to dryness on a hot plate. After this procedure was completed 1.2 ml of HCl was added. The sample was then brought to volume (25 ml) using the required amount of nanopure water and either stored in an acid washed, polyethene container, or placed directly into the voltammetric cell to be analyzed. To the cell 0.2 ml of each of the following were added, 0.4% hydrazine and 3.2% formaldehyde.

All of the digested samples were placed in a muffle furnace after the wet ashing procedure. The program on the furnace was the same as in Nygren *et al.* (1990): 1 h at 200° C, 30 min at 250° C, 1h at 350° C, 30 min at 425° C, and 3h at 800° C with the heat rising 20° C per minute. Also, the crucibles were acid washed for 24 hours between each digestion. The voltammetric cell was also acid washed whenever contamination was detected. When the samples were being prepared, a new, sterilized tip was used for each automatic pipette. There were many precautions taken to control contamination.

III.III. Analysis

The analytical instrement used in this procedure was 646 VA processor and 647 VA stand (Metrohm Ltd., Herisau, Switzerland). This apparatus is described in detail in Nygren *et al.* 1990, and in Vaughan and Florence, 1992. When analyzing, the electrode used was a static mercury drop electrode. After the sample was placed in the voltammetric cell, the electrode was lowered into the sample. This lowering causes the cell to be sealed. The sample was then de-aerated for five min (360 seconds). When the oxygen had been removed the sample was pre-electrolyzed for three minutes at -800 mV vs. Ag/AgCl. After this three minute interval the stirrer was stopped while the stripping was recorded. This procedure was repeated three times for each sample. Plotting known standards on a best-fit curve and then comparing the resulting value determined the amount of platinum.

III.IV. Statistical Analysis of the Data

For all nine set of data the following descriptive statistical analyses were performed: Average, standard deviation, and coefficient of variance. The average, or mean, is the number used in most of the data presentation, i.e. graphs. The standard deviation shows the variation of the data set. The coefficient of variation will show how far the data set varies from the mean. These two analyses will be used to discuss the quality of the data sets themselves.

All of the related data sets were tested against each other using the Mann-Whitney U-test (two tailed) (Siegel 1956). This test will determine the amount of significant difference between each set. The set must have a "P" value equal to or less than 0.05 to be considered to have significant differences between them.

IV. Results

The three samples presented here are egg matter, fecal matter, and blood. Feathers were also analyzed, but amounts were not obtained. Also, the five populations will be compared. The results of the descriptive statistics are shown in table 1. The results of the Mann-Whitney test are in table 2. The comparisons of the different samples for the same female are in table 3 and the intraclutch (within the same clutch of eggs) comparisons are in table 4.

Table 1. Level of Pt (ngg-1) in egg, blood, and fecal matter for four different raptor species in Sweden. The sample size, mean, standard deviation, and coefficient of variation are given. N/A = Not Analyzed.

A. *Falco peregrinus* Captive Population	EGG (ngg-1)	BLOOD (ngg-1)	FECAL (ngg-1)
Sample Size (n)	9	N/A	N/A
Mean ($\bar{x}$)	0.17	N/A	N/A
Standard Deviation(SD	0.26	N/A	N/A
Coefficient of Variation (CV)	1.5	N/A	N/A

B. *Falco peregrinus* Wild, North Sweden	EGG	BLOOD	FECAL
N	9	8	7
$\bar{x}$	0.102	0.15	0.44
SD	0.13	0.10	0.89
CV	1.4	0.68	2.01

C. *Falco peregrinus* Wild, South Sweden	EGG	BLOOD	FECAL
N	12	8	13
$\bar{x}$	0.043	0.29	0.72
SD	0.041	0.204	1.01
CV	0.95	0.72	1.4

D. *Falco rusticolus* Wild, North Sweden	EGG	BLOOD	FECAL
N			6
$\bar{x}$	N/A	N/A	0.17
SD	N/A	N/A	0.17
CV	N/A	N/A	0.99

E. *Falco tinnunculus* **Wild, SW Sweden** N	EGG 9	BLOOD	FECAL
$\bar{X}$	0.14	N/A	N/A
SD	0.24	N/A	N/A
CV	1.7	N/A	N/A

F. *Accipiter nisus* **Wild, SW Sweden** **Urban** N	EGG 10	BLOOD	FECAL
$\bar{X}$	0.51	N/A	N/A
SD	0.69	N/A	N/A
CV	1.4	N/A	N/A

The following figures are a graphic representation of this data, excluding the coefficient of variation. Figure 7 is the graph illustrating the means and deviations of the given biological samples for the Northern, wild falcon and the Southern, wild falcon and gyrfalcon populations. The standard deviations are shown in bar form. Figure 8 is the graphical representation of the means of the Pt found in the eggs of all of the tested populations. Each vertical data set in the graphs represents the mean of the values, the middle mark, and the highest variation along with the lowest, the upper and lower marks, respectively. In the second graph, figure 8, the sparrowhawk egg appears to have the highest value. The other values seem to have very little difference. In the first graph the trends are difficult to see. The deviations of the values are so large that the possible regions of the data fall with in each other.

From table 2 (below), six significant differences are shown. The amount of platinum in the captive egg was higher than the amount of Pt in southern peregrine egg. The sparrowhawk egg had a greater amount of Pt than of the wild peregrine (North and South) eggs did. The blood of the southern peregrine had a higher amount of Pt than the southern peregrine egg. The southern peregrine fecal sample also had a higher level of platinum than the southern peregrine egg samples. And the southern peregrine fecal sample contained more platinum than the fecal sample of the gyrfalcon.

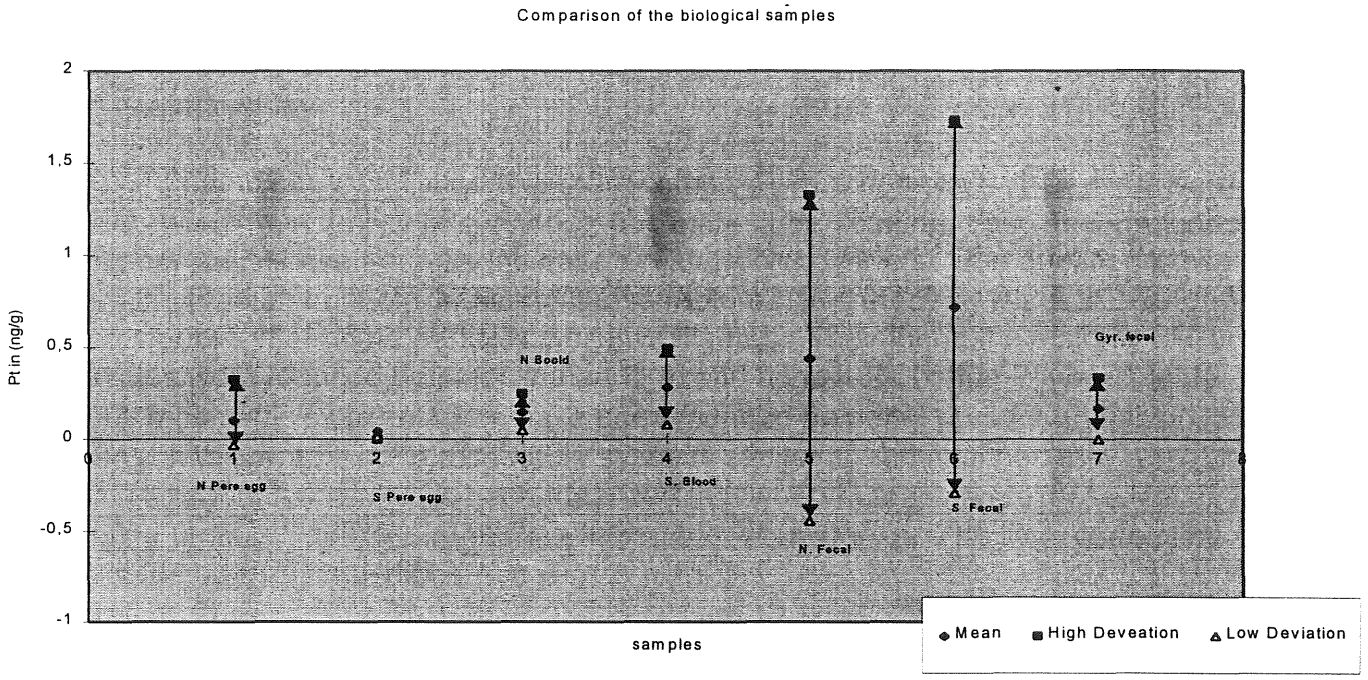

Figure 7. The graphical illustration of the different sample types, grouped by population, plotted against the amount of platinum (ngg^{-1}).

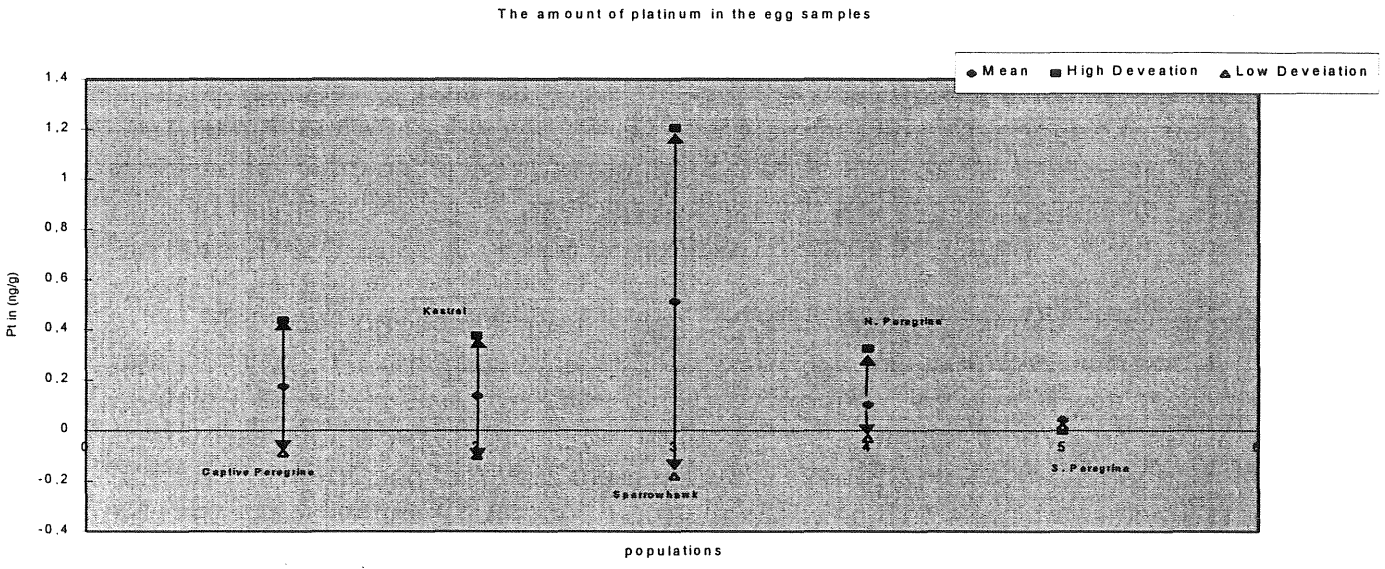

Figure 8. The graphical illustration of the amount of platinum (ngg^{-1}) in the egg samples for the given populations.

Table 2. The results of the non-parametric Mann-Whitney U-test considering the four groups of raptors and the three types of biological material. The codes for the following table are the group assignments from table 1. The number of (*)s after the p value will indicate the value's significance. The value must be below 0.05 for it to be considered significant (Siegel 1956).

A= Captive peregrine falcons, B= Northern peregrine, C= Southern peregrine, D= gyrfalcon, E= kestrel, F= sparrowhawk, Bf= wild N. peregrines fecal samples, Bb= wild N peregrine blood samples, Cf= wild S. peregrine fecal, and Cb= wild S. peregrine blood.

Egg Comparisons **A versus (vs) B**	**0.077**
A VS C	**0.021****
A VS E	**0.11**
A VS F	**0.65**
B VS C	**0.86**
B VS E	**0.93**
B VS F	**0.046***
C VS E	**0.92**
C VS F	**0.011****
E VS F	**0.094**

EGG VS BLOOD	**P value**
B VS Bb	**0.075**
C VS Cb	**0.0008*****

BLOOD VS BLOOD	**P value**
Bb VS Cb	**0.32**

FECAL VS BLOOD	**P value**
Bf VS Bb	**0.95**
Cf VS Cb	**0.18**

FECAL VS EGG	**P value**
Bf VS B	**0.11**
Cf VS C	**0.0001*****

FECAL VS FECAL	**P value**
Cf VS Bf	**0.15**
Cf VS D	**0.049***
Bf VS D	**0.94**

Table 3. Comparison of Pt levels (ngg-1) in samples from the same nest site. These are from both groups of wild peregrine falcons. Ranking is the order from greatest to least. N/A= not analyzed.

Name of bird And location	Ranking	Fecal(F)	Blood(B)	Egg(E)
DN south	F>E	0.49	N/A	0.019
HH south	B>E>F	0.015	0.094	0.041
KU south	B>F>E	O.12	0.42	0.051
PD south	F>B	1.5	0.060	N/A
ML south	B>F>E	0.301	0.52	0.00104
SB south	F>E	0.79	N/A	0.036
NN south	F>B	0.51	0.083	N/A
OH south	B>E	N/A	0.16	0.039
PL south	B>F	0.15	0.49	N/A
LAI north	B>E	N/A	0.37	0.032
PIA north	B>F	0.15	0.19	N/A
P3 north	F>B	0.48	0.11	N/A
SARA north	F>B	2.4	0.18	N/A

This table shows the amount of platinum found in the blood, fecal matter, and egg from populations in the same location. Not all of the locations have all three samples, most have two of the three. The ranking shows the order of the amount of platinum. The amount of Pt found in blood was always higher than the amount found in the egg. The amount of Pt found in the fecal was higher than that found in the blood a little less than half of the time. The fecal level was higher than the egg level of Pt all but one time.

Table 4. the amount of platinum (ngg-1) found in egg sample from the same clutch of captive peregrine falcons. Mean, standard deviation and coefficient of variation are all listed. The bird 223 had two clutches examined for variation.

Bird Number	N	$\bar{x}$	SD	CV
60	2	0.11	0.025	0.24
85	3	0.035	0.026	0.75
132	3	0.054	0.029	0.55
223 (1)	2	0.023	0.0076	0.34
223 (2)	3	0.22	0.12	0.56

This table lists the values found for eggs from the same clutch. The means are listed along with the standard deviation and the coefficient of the variance. The CV is the most important value shown here as it shows the amount of variation within the data set. All of these sets have CVs of less than one so the variation is considered low.

V. Discussion

V.I. Comparison of data.

The data providing the most information was that data shown to have significant differences in the Mann-Whitney test. These significant comparisons (comparing the amount of Pt in two different samples) were as follows: Sparrowhawk vs the wild peregrine eggs; the southern peregrine egg vs the southern peregrine blood; wild southern peregrine eggs vs captive eggs; the southern peregrine fecal samples vs the southern peregrine egg; and the southern fecal samples vs the gyrfalcon samples.

Table 1 and the figures 7-8 illustrate the means, and standard deviations of the given data sets. The levels found here are very small. At first glance, there seems to be some obvious trends. In figure 7, it appears that the N. Peregrine should have some difference in the biological samples. Also, in figure 8, the graph seems to show more differences than what were found. However, the large variation in the data sets does not allow for much comparison. The complete data set is offered in appendix 1. The results have a wide range, both from within and without the data sets.

Table 3, the comparison of the different biological samples from the same site, showed the amount of Pt found in the fecal matter was higher than the amount of Pt in the egg matter in almost every case. The amount of platinum in the blood samples were higher then the amount of Pt in the egg matter in every case.

The final table illustrated the low variation of platinum in the female captive falcons. The variation was shown in the CV value. If the value is lower than 1, then the data set being analyzed has a low variation. The egg samples were representative of the female's clutch because one egg does not have higher amounts than any of the other eggs laid by that female.

V.II. Implications of the data.

The comparison between the sparrowhawk and the peregrine (both of the wild populations) is the most striking for this project. This comparison suggests that there is a difference in the amount of Pt in the ecosystem of the two species. These three populations have different food chains. However, the southern wild peregrine's food choice is not much different than the sparrowhawk's food choice. The northern population has an aquatic based food chain having the food chain most different from the sparrowhawk. There are several possible explanations for these differences.

The differing levels of platinum caused by the various food chains seem obvious; however, there was no significant difference found in the platinum levels of the

northern and southern peregrine populations. The Southern peregrine population and the sparrowhawk population have a more closely related food chain than that of the N. and S. wild peregrine populations. So the most favorably explanation is the habitat. This is favorable because the sparrowhawk lives in an urbanized area where the peregrine populations live far from man's influence.

The sparrowhawk is part of an urban population. The two peregrine populations are rural. The most obvious difference between rural areas and urban areas is the presence of humans. The abundant presence of humans is associated with the presence of automobiles. These cars emit Pt from the catalytic converters. The relationship between the amount of Pt in the air and the amount of traffic has been found to have a positive correlation (Schierl and Fruhman 1995). This means as the amount of traffic increases, so does the amount of airborne Pt. Therefore, the amount of Pt in the urban area is higher than that in the rural area. Figure 9 is a graph from the project that determined the correlation.

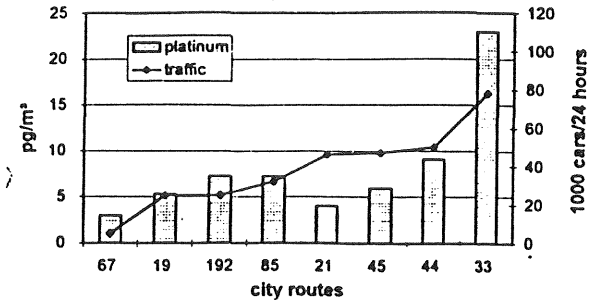

Figure 9. Mean platinum concentrations compared to the amount of cars passing in 24 hrs along different bus routes through out Munich. (Schiel and Fruhman 1995).

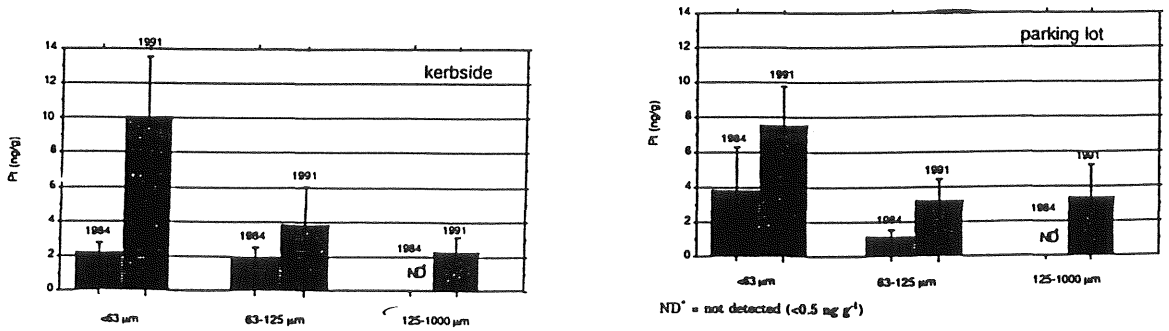

Figure 10. The amount of Pt found in the sediments of a car parking area and a curbside area comparing levels in 1984 and 1991 (Wei and Morrison 1994).

In laboratory experiments, Pt was found to be emitted in larger amounts at higher speeds than slower speeds (König *et al.*1992). Since this is true, the amount of Pt released by cars near highways should be higher than amount of Pt found near smaller roads with slower moving traffic (assuming the same traffic intensity for both roads). Highways converge around cites. Cities are a source for members of an urban population to be exposed to a higher amount of Pt than the members of a rural population. Also, the amount of Pt has been increasing in recent years. In a study by

Wei and Morrison (1994) they found Pt has increased in all three of the categories they measured; river sediment, curbside dust, and car park dust. Figure 10 is a comparison of the amount of Pt found in the two dust areas. The increase in Pt was evident in all areas of the study.

It has been established that there is more Pt in urban areas than in rural areas due to the higher density of cars operating in the urban setting. Also, the amount of Pt has been on the rise from the increase in catalytic converters. The question is; would this affect the amount of Pt found in these raptors? In a study by Alt *et al.*(1993) the amount of Pt in airborne particles was investigated. They found the majority of Pt particles were found to be smaller than 8μm and up to 43% of that Pt was soluble. The problem is particles up to 10μm have been found to be dangerous for humans (Camner 1994). If the raptors have respiratory systems similar to humans then they are able to gain Pt by inhalation. It has been theorized that when humans are exposed to air with varying amounts of Pt then the Pt in their urine would vary in a positive correlation (Schierl and Fruhman 1996).

However, the threat in the possibility of the sparrowhawk collecting platinum through inhalation is not the only threat. The sparrow, the sparrowhawk's favorite prey, is also an urban bird. This bird lives in highly populated areas near roadways. Moreover, they are in the habitat of dust bathing. This may not seem harmful, but they dust bath near roads, in the area having the most platinum deposited. These birds could have very high levels of platinum in their system and on their feathers. The sparrowhawk does ingest a low proportion of feathers from sparrows. This is another possible pathway for platinum to be entering this population that is not seen in either of the wild peregrine populations. It also offers another area for possible research. The kestrel eggs were gathered from roughly the same area as the hawk eggs and kestrel egg did not have any significant relationships with any of other eggs tested. This supports that there could be another pathway besides inhalation.

Another significant difference was found between the southern peregrine's egg and its blood. The blood sample was found to have a higher level of Pt than the egg sample. It was expected to be the other way around. If platinum was accumulating in the bird's systems then it should be higher in the egg matter than it is in the blood samples. The reason for this is the female will sequester toxins from her body into the eggs she is delivering (Court *et al.*1990). In many studies about the amount of mercury in these populations, the eggs are used to monitor the amounts of the toxin (Lindberg and Odjsö 1983, Appelquist *et al.* 1984, and Noble and Elliot 1990). A possible explanation for this is that the chicks were exposed to a higher amount of Pt than the female. If the prey in the breeding area has a higher amount of Pt in them than the prey in the wintering grounds, then the blood samples would have a higher level of platinum than the egg samples.

The feathers all failed during analysis.

Also, the amount of Pt in the southern wild peregrine blood was higher than the amount in its fecal samples. In a previous study on environmental pollutants in birds, the amount found in the fecal samples was much higher than that found in any other biological samples (Nyholm *et. al.* 1995). This study proposed the use of fecal matter to monitor the amount of total exposure as they found a positive correlation between the amount in the fecal samples and the amount in the liver. Another possible theory is that the absorption time for platinum is faster than the metal in the Nyholm *et al.* (1995) project. This suggests that as platinum moved through the digestive system the bird quickly absorbed it. This would explain why there was a lower level of platinum found in the fecal matter samples than in the blood samples.

A fourth significance involving the southern peregrine population was the comparison with the gyrfalcon fecal samples. The gyrfalcon had a significantly lower amount of Pt than the southern population. The major difference in these populations, besides habitat, is migration. However, the northern peregrine population also migrates. If the difference in platinum level was due to migration than the N. peregrine would have also had a significantly higher amount of platinum than the gyrfalcon. The N. peregrine population and the gyrfalcon population live in the same habitat. This is not easily explained. Perhaps the difference is in the prey. Most likely the varying levels of platinum are due to a combination of habitat, migration, and prey.

The most unexpected result of this test was finding the captive peregrines have a significantly higher amount of platinum in their eggs then the southern population. This was surprising because the captive population is fed a strict diet of three-week-old chickens that are raised at the breeding station. The only explanation for this finding is offered in a paper done by Vaughn and Florence (1992). They tested many foodstuffs for platinum levels and found chicken and chicken eggs to have the high amounts. They did not offer any explanation for the high levels seen in chicken.

The table describing the differences in the biological samples is interesting because it suggests Pt is not following the path of other toxins which are known to accumulate. In mercury investigations the highest level of the metal is found in the egg. In this table the egg samples were shown to have the lowest level of platinum when compared to the other samples. There are nine sites that had both blood and fecal, four of those have higher fecal results leaving five favoring blood levels. The platinum level in the fecal samples should have been showing exposure rates. There are many factors still unknown about platinum that could explain these differences. Not enough research has been completed on platinum to be able to assume the biological pathway it would follow. The absorption rate, the solubility, the speciation, and low level accumulation are all aspects of platinum with many unanswered questions.

The final table is also interesting. It shows that the eggs laid in the same clutch have low variation. This means the females were not exposed to high amounts of this metal. If they had been then the females would have been sequestering Pt into the eggs. This means the first egg laid would have a noticeably higher level of platinum

than the later laid eggs. As this procedure went on, the amount of Pt would decrease in the female and in the latter laid eggs. This would then show a variation in the amount of platinum in the first egg as compared to the last egg laid. However, in this study, there is a low variation in the levels found throughout the breeding season by the same female. In fact, bird number 223 had two clutches examined here. The first had a much lower amount than the second did. If the female had been accumulating Pt through out the year she probably would have sequestered as much as possible into the first eggs. That did not happen so it is doubtful that Pt is bioaccumulating.

V.III. Evaluating The Threat

The EC50, where 50% of the subjects die, of *photobacterium phosphoreum* was found to be 25 μg/L (Wei and Morrison 1994). When compared to the highest mean in this study,(9.6 ng/L) the EC50 value is approximately 2,600 times larger. This suggests that platinum is not of direct toxicity for these populations. It is not in high enough concentrations to be the reason for any of these eggs have not developed. That does not mean it would not be wise to continue work in this field.

The amount of platinum is increasing in the environment and very little is known about the behavior of this metal. The species of platinum found in this study is not even known. If platinum is methylating then it may prove to be more dangerous than thought so far.

The threat of platinum will be easier to define when the danger to low level exposure has been estimated. Also, the absorption rates along with the speciation and solubility will all have to be evaluated concerning biological systems to more accurately evaluate the threat this metal posses to the biosphere.

VI. Conclusions

This study attempted to establish the level of platinum in four raptor populations in Sweden using four different types of biological samples. The amounts of platinum found were very low. It was found that the blood has a significantly higher amount of platinum than the egg matter or the fecal matter. This is the first study of this type so there was no reference for comparison. Also, the species of platinum found here is undetermined. More research needs to be completed on the speciation of platinum in the biological community. However, this study did provide a starting point for future research to be based upon.

There were a few questions this project attempted to answer: Is platinum bioaccumulating?; If it is accumulating, is it following the same pathways as known for bioaccumulating metals?; Are the difference in the location of the population, i.e. urban versus rural?; Does the amount of platinum found in the given biological samples vary? Platinum has not followed the pathways of known bioaccumulating metals. Most of the known accumulating metals have a significantly higher metal levels in the egg sample than the blood sample. There is a positive correlation between the amount of the metal in the fecal samples and in the amount in the other biological samples. In this study neither of these were true. The blood had a significantly higher amount of Pt than the egg and there was no correlation of the fecal samples. There does appear to be a difference in the amount of Pt found in the populations that is dependent on the proximity to an urban area. The Sparrowhawk had significantly higher amounts of platinum than either of the wild peregrine populations. There is a possibility for some interesting research to come from these ideas. Such as discovering if the frequency of roadway usage is a factor in the amount of Pt in the sample. Or, if the population of an urban dwelling species has higher levels than a rural population of that same species.

The question of bioaccumulation is still unanswered. This project did not offer evidence to support the theory of platinum bioaccumulating. However, this project did not offer any evidence of platinum not bioaccumulating. There are many aspects of platinum that are still unknown. Absorption rates, speciation, and the effect of low level exposure are some of the areas that will need to be further investigated before the bioaccumulation of platinum can be determined.

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Appendix 1

This is the data set used for this project.

Name	Egg Sampel	Blood sampel	Fecal sampel
CAPTIVE P. FALCONS	ngg^{-1}	ngg^{-1}	Ngg^{-1}
06-1-1-94	0.0837		
9-2-3-88	0.175		
60-1-2-97	0.130		
60-1-3-0	0.798		
85-1-97	0.0258		
85-2-97	0.0713		
85-3-97	0.00965		
94-1-1-0	0.865		
132-2-1-97	0.0869		
132-2-2-97	0.425		
132-2-3-97	0.0318		
223-1-1-97	0.0151		
223-1-2-97	0.0303		
223-2-1-97	0.0572		
223-2-2-97	0.0326		
223-2-3-97	0.272		
377-3-1-97	0.0984		
WILD NORTH P FALCON			
AP-1-93	0.0174		
BPAB-1-940714	0.0307		
BPS-960710	0.0341		
BPS-2-940711	0.0416		
KARIN-970707	0.0146		
KB2-970707	0.0779		
LAI-960718	0.0324	0.365	
LUS-9707	0.353		
MALIN-9707			0.151
MÀRTA-970715		0.0413	
MK-970711		0.123	
NIK-970711		0.0746	
P3-970713		0.114	0.0476
PIA-9707		0.185	0.152
RUT-9607	0.317		
SARA-970619		0.184	2.442
TM-970714			0.0394
VERA-970711		0.101	
WILD SOUTH P FALCON			
BB-1-4-95	0.0511		
DN-970626	0.0193		0.497
FF-3-97	0.0253		
HH-970623	0.418	0.0953	0.105
HÅL-970611			0.108

JP-970616			0.0847
KU-970603	0.0508	0.421	0.115
ML-970618	0.0104	0.512	0.301
NDB-970612			0.508
NN-970603		0.813	0.508
NVG-1-92	0.0529		
NVK-3-95	0.0884		
OH-970604	0.0387	0.156	
OM-3-97	0.00781		
PC-970612		0.465	
PD-970616		0.060	1.502
PL-970613		0.492	0.136
SB-970617	0.304		0.788
WIK-970604			3.790
GYRFALCON			
N4B-9706			0.0681
NIA-970610			0.256
SC-970609			0.331
SH-970606			0.119
SL-970609			0.422
SN-970612			0.0656
SPARROWHAWK			
HO-291695	0.0175		
SA-950701	1.386		
SB-950619	0.8055		
SH-950710	0.491		
SKA-9505	0.0586		
SK-950602	2.021		
SP-950701	0.158		
SS-950619	0.0837		
SVL	0.0608		
VS-950627	0.0457		
KESTREL			
ST-5-90	0.00773		
STT-6-90	0.246		
C79/7048	0.0496		
C82/7072	0.7402		
C84/7114	0.0337		
VH-950729	0.0255		
VHM.950729	0.0222		
TF-102-2	0.265		
TI-950710	0.0845		