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First Determination of the Levels of Platinum Group Metals in *Manta birostris* (Manta Ray) Caught Along the Ghanaian Coastline

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Abstract Tissues from *Manta birostris* caught by fishermen from Dixcove in the western part of Ghana were analyzed for their Platinum, palladium and rhodium concentrations (PGM). The use of chondrichthyan fish has permitted the study of trace levels of Platinum group metals (PGMs) which have travelled very far into the sea. The analysis showed that Ghana's coastline is fairly polluted with these platinum group metals (PGMs). PGM concentration in manta ray recorded a range of (0.15–0.85) μg/g for Pt, (0.033–0.67) μg/g for Pd and (0.007–0.145) μg/g for Rh. Comparing these values to the UK dietary intake of 0.2 μg/day for Pt and Rh and 1.0 μg/day for Pd, its indicates that the values obtained from the analysis for Pt was above the required level. This is the first study to show the accumulation of PGM in chondrichthyan fish, although the sources of this pollution are not clear as manta birostris is migratory and therefore need to be investigated further. The presence of the PGM is very significant, since manta ray meat is consumed in Ghana. This may presents a health risk, due to a possible accumulation of PGMs in humans.

Keywords Dixcove · Neutron activation analysis · Chondrichthyes · Myliobatidae · Manta birostris and Kako

Over the last decade, chemical contamination of aquatic sediment has been recognized as a serious problem in some US coastal waters. The hot spots of toxic chemicals have been shown to alter and reduce the bottom-dwelling community, to interfere with cellular and physiological processes, and to cause disease in fish. Most hot spots are in areas of high vessel traffic, industrial activities, or poor flushing and are often located near urban centres (Mashiatullah et al. 2004). Anthropogenic sources of platinum group metals (PGMs) in the environment include smelting and gold mining operations, dentistry, platinum drugs, and chemical industry. The major source, however, is from automobile emissions (Kristine et al. 2004). Most of the anthropogenic sources of PGMs in the environment come from catalytic converters that have been designed in car exhausts to control air pollution. The increasing use of catalytic converters based on PGMs has raised much concern as recent investigations has seen increases in the accumulation of the metals in roadside dust, airborne dust, soil, water bodies, grass and in the food chain (Caroli et al. 2000, 2001; Palacios et al. 2000; Petrucci et al. 2000, 2004; Gómez et al. 2002; Moldovan et al. 2002; Bocca et al. 2003, 2004; Gyula et al. 2004; Lesniewska et al. 2004).

Contaminants on land can be carried by rivers either in dissolved, colloidal or particulate form to estuaries and finally to coastal oceans (Clark 1989), where they enter into the food chain and become concentrated in fish and other edible organisms (known as bioaccumulation), particularly in near-shore areas (His et al. 1999; Essumang et al. 2008). Many substances pollute the marine environment, but non-biodegradable compounds (heavy metals) are the most problematic due to their innate ability to constantly remain with the ecosystem (Hernández-Hernández et al. 1990); Tyler 1972). Heavy metals are noted for their high toxicity (Kristine et al. 2004).

The manta ray or giant *Manta birostris* which is popularly called 'madai' belongs to a class of Chondrichthyes and the family Myliobatidae. It is the largest of the rays, with the largest known specimen having nearly 7.6 m across its pectoral fins (or "wings") and weighing about

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3,000 kg. This species is found in temperate and tropical waters around coral reefs, seamounts and islands. Mantas have been given a variety of common names, including Atlantic manta, Pacific manta, devil ray, devilfish, and just manta. The most common rays have black above and white below, but some also do have blue on their backs. Manta rays are largely plankton-feeding, but also feed on fish larvae and small organisms such as tiny crustaceans. They funnel the food into their mouth while they swim, using two large flap-like cephalic lobes which extend forward from the eyes. Manta rays have reduced and non-functional teeth, which do not enable them to consume larger species. These creatures use their 'horns' to direct plankton and water into their mouth. They are known to migrate all over the world in search of up-welling plankton-rich waters. Like other rays, the manta has five pairs of gills on the underside (Marshall et al. 2006).

Mantas generally eat plankton, fish larvae and small organisms that are filtered out from the water by their gill rakers; a type of filter feeding that is called ram-jet feeding. Assuming that these organisms have some amount of heavy metals in their body tissue, then it is expected that elevated concentrations may be found in the manta ray, their predator (Col 1998–2007).

Manta rays serve as a common nutritional fish for some people in Ghana. It is locally eaten as either "kako" (salted fish) or smoked as well as fresh fish. At least about 10 g of the fish is used in stew and soup preparations. Some also use it as a flavour especially, in preparation of stew. Consumption of manta ray (muscle) tainted with elevated levels of heavy metals (PGMs) could cause some health problems (Kalavrouziotis and Koukoulakis 2009).

Although these particles known as PGMs are not yet considered a serious health risk, evidence suggests that they could potentially pose a future danger (Kalavrouziotis and Koukoulakis 2009) as worldwide car sales is projected to increase from an estimated 50 million in 2000 to more than 140 million in 2050. The availability of Pd and Pt should be of great concern, as they are known to have mutagenic and toxic effects even at very low concentrations (Hoppstock and Sures 2004). In addition, platinum in the aquatic environment may biomethylate in a similar way to that of mercury (CORDIS 2003).

The long-held belief that PGM's are generally harmless stems from their chemical inertness. On the other hand, they are sensitizers of allergenic pathologies such as: asthma, conjunctivitis, dermatitis; rhinitis and urticaria (Rosner and Merget 1990). Furthermore, Pt complexes are well-known cytotoxic agent, which are widely used in several anticancer drugs (e.g. Cisplatin and Carbo-platin), for the treatment of a number of malignancies (Dominici et al. 1989, Ensslin et al. 1994). Tumour cells are more prone to attack by Pt-containing substance than normal cells as a consequence of their higher permeability of the former to low-molecular weight Pt species formed in vivo, as well as to the high dose, short-term conditions of administration (acute exposure) which leave healthy cells practically unaffected. This situation may dramatically change as the permanent presence of much lower concentrations of Pt in the environment completely switches the scenario to that of chronic exposure, the impact of which on the healthy cells of living organisms is partly unknown (e.g. increase in mutagenic effect on health care personnel manipulating Pt containing drugs) (Vaughan and Florence 1992).

This is the first study to show the accumulation of PGM in chondrichthyan fish, although the sources of this PGM pollution are not clear and therefore need to be investigated further as manta birostris are migratory. The objective of this study is to determine the levels of PGM's in manta rays caught along the Ghanaian shores and to make recommendations on the findings.

Materials and Methods

The study involved six manta ray samples which might have migrated from afar, that were caught (at different days) by local fishermen in drift gill-nets and landed at Dixcove. Dixcove (4°47′21.65″N and 1°57′01.11″W) is the site where the manta rays were caught by the fishermen between January and May, 2008. These manta rays inhabited the deep seas. There's however the possibility of these manta ray migrating from somewhere very far from the fishing area within the Atlantic Ocean. Dixcove is noted for its seasonal harvest of manta rays (Debrah 2000). The ages and other information about the animals sampled were difficult to ascertain, since they were all from the wild. Their sizes and masses did not give much information either, since they were approximately of the same size, except for manta number M1, which was a bit smaller than the others.

About 30 g sub-samples of muscle, kidney and liver of each manta ray were obtained from these fishermen who skilfully cut them out of their catch. They were carried in a portable ice chest, at a temperature of 4.0°C to the laboratory, where the analysis on the various tissues was made.

About 200 mg of each part of the manta ray samples was weighed by Mettler Electronic Balance AE 163-BDH into a clean polyethylene film. The films were wrapped and heat-sealed. Two replicate sub-samples were prepared for each sample. The samples were packed into 7 mL volume rabbit capsules for irradiation. IAEA standard Reference material Platinum ore NIM; SARM-7 (certified standard for Pt, Pd and Rh) were also prepared and treated as the main sample and irradiated for one hour (IAEATECDOC-

1443 2005). The bagged samples were then packed into rabbit vials and heat-sealed for further analysis.

Trace PGMs were analysed by neutron activation analysis (NAA) using thermal neutron from a low flux Am–Be radioisotope. Theoretically, neutron activation analysis is based on the measurement of characteristic gamma-rays from a radionuclide formed from the specific neutron reaction which can be used to measure the amount of element, using the usual radioactive decay law (Tolgyessy and Kyrs 1989; Essumang 2008). In this case, the unknown concentration of the element in the sample can be obtained by comparing the activities of the gamma-ray peaks (Tolgyessy and Kyrs 1989; Essumang et al. 2008).

The irradiation source is a 20 curie Am–Be radioactive neutron source. It is cylindrically shaped and is fixed in a holder at the centre of a fibre-glass tank, filled with deionized water. The deionised water served a dual purpose of moderator and also absorber of neutrons. Extra shielding is provided by concrete blocks arranged round the tank. Transfer of sample to and from the neutron source is by means of a flexo-rabbit pneumatic transfer system operating under a pressure of 15 psi, given a sample transfer time of 1.3 s as cited in Essumang (2008). The thermal neutron flux at the irradiation site is 1.124×10^5 ns^{-1} cm^{-2} as followed in Essumang et al. (2008).

Each of the samples was sent by the pneumatic transfer system into the Am–Be source for irradiation. The irradiation schemes were chosen so as to take into account the half-lives of the radioactive nuclides. In this regard, one hour was chosen for all the samples because all the metals in question are medium lived. At the end of each irradiation, the sample was returned for counting. These samples were irradiated for one hour and left overnight for cooling or decaying process to take place. They were then counted the next day for 600 s and its intensities saved for analysis.

The detector type used for the counting of signals was an ENERTEC High Germanium (HPGe) detector of 3,000 (+ve) bias and a resolution of 2.55 keV for 1,332 keV photo peak of Co-60. The signals from the detector were passed through the spectroscopy amplifier, and then accumulated by the Canberra multi-channel analyzer (MCA) for a preset time. The spectra from the MCA were transferred to a DEC 350 microcomputer for analysis using Gamma spectrum analysis software (Ortec multi-channel buffer (MCB)). This software identifies the various photo peaks and works out the areas under them. By means of equation from radioactive decay law, the concentration of each element was calculated (Essumang et al. 2008).

Validation of the analytical procedure was undertaken by irradiating an IAEA standard reference material Platinum: platinum ore NIM, SARM 7, 3.74 μg/g; Palladium: palladium Ore NIM, SARM-7 1.53 μg/g; Rhodium: rhodium Ore, SABS-SARM-7, 0.24 μg/g with 5% uncertainty and counted under identical experimental conditions.

Results and Discussion

Table 1 report the values of merit for PGM determination by neutron activation analysis (NAA) in a certified reference material (NIM, SARM 7), which, however, has a certified values of 3.7, 1.53 and 0.24 μg/g of platinum, palladium and rhodium respectively. The detection power offered by the technique in the investigated matrices was checked by analyzing independent samples of NIM, SARM 7 standard reference materials.

The instrumental precision was evaluated and monitored over the entire working range by performing replicate analyses of the standards. As regards to the test for accuracy, unfortunately, no reference materials certified for PGMs in the said matrices are commercially available. Consequently, reliability of measurements was checked through recovery tests by using standard reference material NIM SARM 7.

The above results suggest that the analytical method used was very reliable Table 2 below show the average concentration (μg/g) of Platinum, Palladium and Rhodium in manta ray samples.

The results obtained were compared with the values of daily dietary intake of PGM in Table 3 below as stated by Ysart et al. (1999). However, the upper limit were all above the dietary requirements of the PGMs especially Pt and may not be too good for consumers of the fish product.

From the results (Table 2) the levels (±SE) of the listed PGMs for manta rays had concentration of Pt (0.847 μg/g) which was the highest recorded in the kidney followed by

Table 1 Result of the quality control analysis of PGM's standard SARM 7

PGM's	Standard. conc. SARM 7	Results obtained after NAA analysis of the standards			Mean	% Recoveries
		1	2	3		
Pt μg/g	3.74	3.60	3.71	3.77	3.67	98.0
Pd μg/g	1.53	1.49	1.50	1.50	1.50	98.0
Rh μg/g	0.24	0.23	0.20	0.19	0.21	87.5

Table 2 The average levels of PGM in the manta ray samples

Sample	Pt/μg/g (±SE) wet weight	Pd/μg/g (±SE) wet weight	Rh/μg/g (±SE) wet weight
MK1	0.847 ± 0.127	0.145 ± 0.0217	0.015 ± 0.002
MK2	0.345 ± 0.0517	0.033 ± 0.005	0.008 ± 0.001
MK3	0.399 ± 0.059	ND	ND
MK4	ND	0.232 ± 0.0347	ND
MK5	0.169 ± 0.026	0.15851 ± 0.0238	0.017 ± 0.003
MK6	0.280 ± 0.042	0.665 ± 0.099	0.145 ± 0.022
ML1	0.148 ± 0.022	ND	0.010 ± 0.002
ML2	0.436 ± 0.065	0.357 ± 0.054	0.007 ± 0.001
ML3	ND	ND	0.017 ± 0.003
ML4	0.172 ± 0.026	0.087 ± 0.013	0.012 ± 0.002
ML5	ND	0.036 ± 0.005	0.008 ± 0.001
ML6	0.228 ± 0.034	0.117 ± 0.018	ND
MM1	0.825 ± 0.124	0.184 ± 0.028	ND
MM2	0.425 ± 0.064	0.563 ± 0.085	ND
MM3	0.468 ± 0.072	0.314 ± 0.047	0.027 ± 0.004
MM4	0.416 ± 0.065	0.583 ± 0.084	ND
MM5	0.284 ± 0.043	0.2134 ± 0.032	ND
MM6	0.611 ± 0.092	ND	ND

NB. *MM* muscle, *MK* kidney, *ML* liver, *ND* no detection, detection limit = 0.001 μg/g

SE standard error

Table 3 Levels of manta ray PGM in this study compared with values of daily dietary intake of PGM

Element	Range of values obtained from the study/mg/kg	Experimental value mg/kg/day (Ysart et al. 1999)	
		Mean	Adults
Pt	0.148–0.847	0.0002	0.0031
Pd	0.033–0.665	0.001	0.015
Rh	0.007–0.145	0.0002	0.0031

muscle Pt (0.825 μg/g) with liver recording the least concentration (0.436 μg/g) wet weight (ww). This did not follow what was observed in dolphins (Essumang 2008).

It was also realized from the analysis that the kidney of the manta ray was polluted with Pd (0.665 μg/g) (ww) which was followed by the muscle (0.583 μg/g) (ww). The liver again recorded the least Pd concentration (0.357 μg/g) (ww). With Rhodium, it was again realized that the kidney of the manta ray seemed to be the most highly polluted (0.145 μg/g) (ww) which was followed by the muscle. The liver again had the least concentration. This may be due to the eating habit of the manta species.

Other researchers do not specify as to which organ accumulate more but states that kidney and liver are the organs of accumulation (Hofer and Lackner 1995; Sures and Taraschewski 1999; Sures et al. 1999). Studies on metal accumulation in different fish species has also shown that liver usually contains the highest burdens of various metals compared to other tissues like muscle (Sures et al. 2001). This study did not show a similar trend as muscle was found to have higher PGMs than the liver. In addition, studies by Gill et al. (1992) showed higher concentration of metal accumulation in kidney and liver which is similar to this study in the case of kidney. It is noted that mode of metal transport by fish is still a controversial issue and more research is needed in this area (Sures et al. 2001). Studies have however shown that geographical area influences the PGM accumulation in dolphins (Essumang 2008) and since manta rays are migratory the source of these PGMs is not very clear and needs further studies. Due to the toxic nature of pollutants, organisms with a very high toxicity tolerance must be chosen for bioaccumulation studies (Sures et al. 2001). Manta rays are chosen for this study because they are also bottom-dwelling (at times) animal and gill feeders and therefore may tend to accumulate more heavy metals than other marine mammals (Essumang 2008). The pattern of accumulation suggest a mobility of Pt > Pd > Rh.

Comparing the values from this study to the UK, adult mean dietary intake of 0.003 μg/g/day for Pt and Rh, and 0.015 μg/g/day for Pd (Ysart et al. 1999), it shows that the values obtained from the analysis for Pt was above the required levels. Similar work by Essumang (2008) reported similar levels of accumulation in dolphins. However, the levels found in the manta ray were generally higher than that of dolphin which may be due to the feeding habit of manta rays as stated above. This further goes to confirm that the marine environment is being polluted and calls for the attention of agencies and governmental bodies in-charge to intensify their work (Essumang 2008).

PGM contamination in manta rays can occur via pulmonary, cutaneous and digestive barriers as reported for dolphins (Essumang, 2008). Various researchers (Gaskin et al. 1979; Honda et al. 1983) agree that no influence of sex on PGM concentration is observed in marine mammals.

A careful study of the pattern of bioaccumulation of Pd, Pt and Rh in the tissues of the manta ray samples used, which were found in the Ghanaian coastline, suggests that pollution level of PGMs even in the open sea is on the increase. The presence of the PGM in manta ray tissues is very significant since the meat is consumed in Ghana and other part of the world, hence it is possible that PGM accumulation may occur in humans with its attendant health problems (Kalavrouziotis and Koukoulakis 2009), although its source is not clear and requires further investigation and action. Though the sample size may be too small for any general conclusion, it is however the very

Fig. 1 Chondrichthyan fish at the Dixcove beach

first study to show the accumulation of PGM in chondrichthyan fish (Fig. 1).

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