Plasma lead, silicon and titanium concentrations are considerably higher in green sea turtle from the suburban coast than in those from the rural coast in Okinawa, Japan

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ABSTRACT. The purpose of this study was to compare the concentration of trace elements in the plasma of sea turtles that inhabited the suburban (Okinawa Main Island, n=8) and the rural coast (Yaeyama Island, n=57) in Okinawa, Japan. Particle induced X-ray emission allowed detection of 20 trace and major elements. The wild sea turtles in the suburban coast in Okinawa were found to have high concentrations of Pb, Si and Ti in the plasma when compared to the rural area but there were no significant changes in the Al, As and Hg concentrations. These results may help to suggest the status of some elements in a marine environment. Further, monitoring the plasma trace and major element status in sea turtles can be used as a bio-monitoring approach by which specific types of elements found here could indicate effects that are related to human activities.

KEY WORDS: marine pollution, Okinawa Main Island, particle induced X-ray emission, sea turtle, Yaeyama Island

Trace and major element pollutants resulting from negative human activities are found in aquatic ecosystems. This remains a problem in marine environments and is an ongoing subject of research. High urbanization and industrialization rates, as well as the rapidly increasing population growth rates over the last few decades have been responsible for the production of huge quantities of wastewater, often disposed of in the environment without any treatment. Excessive amounts of heavy metals are introduced to estuarine and coastal environments through rivers, runoffs, and land-based point sources [12]. De Carvalho et al. [7] reported that 39% of the total number of turtles that they studied had ingested marine debris such as soft plastic, hard plastic, metal, polyethylene terephthalate bottle caps, human hair, tampons, and latex condoms. It is possible that anthropogenic activities in suburban areas pose one of the worst environmental problems in marine ecosystems, acting as sources of contaminants in aquatic systems. Pollution of the ocean due to anthropogenic environmental contaminants has been linked to the emergence of diseases and syndromes in individuals, populations, and ecosystems [1]. In particular, exposure to heavy metals has been linked to the rapid degradation of coastal habitats of sea turtles [3, 13, 14]. In consequence, knowledge about the accumulation of heavy metals and elements in sea turtles is an important focal point to assess the potential impact of these pollutants on this endangered organism.

The western region of the North Pacific including the Coast of Okinawa Main Island can be expected to have a large influence on the distribution of metals in the Pacific Ocean because the rate of deposition of Asian dust is much larger than that in the central or eastern North Pacific region [15].

In most studies on pollution in marine ecosystems using sea turtles, liver, kidney, muscle, heart, and shell tissue samples are obtained during autopsy of animals collected from the beach or caught for commercial purposes [11, 18]. Several researchers [6, 22, 31, 32] have indicated that using blood samples is an excellent method and a relatively non-invasive way to measure...
were compared among groups using the post-ANOVA (with F-test) Tukey’s HSD test. The significance level was set at

dependent variable were compared between the summer and winter sub-groups within the rural group using the Student’s t-test, and

package from IBM, SPSS Statistics, v.21 (IBM Co., Somers, NY, U.S.A.). For normally distributed data, the mean values for each

detected by PIXE analysis are summarized in Table 1. Except for Mg, the spike recovery values and CV for other elements were

Ind., Ltd., Osaka, Japan), and Titanium standard solution (Ti 1000, Wako Pure Chemical Ind., Ltd.) were added to the pooled sea

Moreover, precisions and accuracy of PIXE has been confirmed by using standard materials such as bovine

eq 30, 31] conducted to assess correlations between carapace parameters and trace and major element status in the

weight (BW) of sea turtles and that plasma trace elements in captive sea turtles showed almost no variation with BW and CL [31].

The mean carapace length (CL) of study individuals in the rural coast, suburban coast, and captured groups were 551.3 ± 121.9, 787.0 ± 232.6 and 963.2 ± 35.4 mm, respectively. There were differences in CL among groups.

Our previous study [31] conducted between carapace parameters and trace and major element status in the plasma using captive adult sea turtles weighing more than 27.9 kg, suggested that CL is a suitable indicator to estimate the body weight (BW) of sea turtles and that plasma trace elements in captive sea turtles showed almost no variation with BW and CL [31]. These findings may be relevant in pollution and marine ecosystem studies where physiological aging is not considered. Therefore, monitoring the increase in the concentration of trace and major elements in the plasma in wild sea turtles might be useful in monitoring for possible accumulation caused by marine pollution.

Blood samples, measuring 10 ml, were taken from the dorsal cervical sinus using a sterile plastic disposable syringe and needle, which were immediately placed in a heparinized tube. The samples were then centrifuged at 3,000 g for 10 min at 4°C to harvest the plasma. The plasma samples were stored at −80°C until it was assayed. Wild sea turtles were released to sea after blood samples were collected and measurements noted.

The mean concentrations of total of plasma element of interest were measured by the particle induced X-ray emission (PIXE) method [29–32]. Briefly, 100 µl supernatant was placed on a subtlety Myler membrane and desiccated. The supernatants were directly irradiated with proton beams. A small (baby) cyclotron used for positron nuclear medicine at the Nishina Memorial Cyclotron Center (Iwate, Japan) provided a 2.9 MeV-proton beam on a target after passing through a graphite beam collimator. A Si (Li) detector (0.0254 mm Be window) with 300 and 1,000 µm thick Mylar absorbers was used to select X-rays with energy higher than that of K-K alpha. For lower-energy X-rays, another Si (Li) detector (0.008 mm Be) was used without an absorber.

There are many studies that evaluate the relationships between trace element concentrations in biological samples such as liver [10, 16, 33], bone [9], plasma [5, 31, 32], and serum [10, 19, 20, 33] measured by the PIXE method and the pathophysiological status and in particular, the pathological status. For example, the relationship between trace element concentrations in blood samples measured by the PIXE method and peripartum cardiomyopathy [5], and chronic hepatitis C [16] have been studied.

Analytical precision was confirmed by assessing the results with those obtained from ICP-MS, Neutron Activation Analysis, etc. [29, 30]. The PIXE System at the Nishina Memorial Cyclotron Center was maintained in the same conditions as when initially setting it [29, 30]. Moreover, precisions and accuracy of PIXE has been confirmed by using standard materials such as bovine liver, tomato leaves, city ash, and human serum (National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg, MD, U.S.A.) at regular intervals in accordance to the guideline of the facility.

Regarding the accuracy and precision of the PIXE method, a spike and recovery test using certified reference materials was conducted in addition to the regular maintenance performed at the Nishina Memorial Foundation. Certified multi-element standard (ICP multi-element standard VIII, Merck KGaA, Darmstadt, Germany), Silicon standard solution (Si 1000, Wako Pure Chemical Ind., Ltd., Osaka, Japan), and Titanium standard solution (Ti 1000, Wako Pure Chemical Ind., Ltd.) were added to the pooled sea turtle plasma to get a final concentration of 10 µg/ml. Analysis of the plasma specimens before and after addition of the standard solutions were repeated six times by the PIXE method to measure the respective trace elements. The recovery ratio and coefficient of variation (CV) were calculated for 17 elements (Al, Ca, Cd, Co, Cr, Cu, Fe, Ga, Mg, Mn, Ni, Pb, Sc, Sr, Ti and Zn). The values detected by PIXE analysis are summarized in Table 1. Except for Mg, the spike recovery values and CV for other elements were considered valid. However, since the recovery value and CV of plasma Mg were high, Mg was not evaluated in this study.

The data are shown as mean ± standard deviation (SD). Statistical analyses were performed using a commercial software package from IBM, SPSS Statistics, v.21 (IBM Co., Somers, NY, U.S.A.). For normally distributed data, the mean values for each dependent variable were compared between the summer and winter sub-groups within the rural group using the Student’s t-test, and were compared among groups using the post-ANOVA (with F-test) Tukey’s HSD test. The significance level was set at $P<0.05$. 

K. TSUKANO ET AL.
concentration in sea turtles might be a useful bio-monitoring method to estimate the marine pollution level due to Ti, which can be attributable to their feeding habits, which depends on their living environment. Immature green sea turtles are omnivorous and exhibit more selective feeding patterns.

On comparing individuals from the suburban and rural coastal areas, differences in Pb, Se, Si and Ti concentrations in the plasma were observed. Plasma Pb, Si and Ti concentrations in the green sea turtle inhabiting the suburban areas were significantly higher than those in the captive and rural coastal groups (P < 0.01). These results suggest marine pollution by Pb from fossil materials and from paints and are in agreement with studies using tissue samples obtained from autopsy where high Pb accumulation [3, 27] in wild sea turtles has been reported. In Japan, the use of leaded gasoline for automobiles has been banned since the 1970s, and the particulate matter containing lead compounds from the exhaust of automobiles, before this ban, may have been deposited on the sediments in the coastal area of Okinawa. The sediment concentrations of heavy metals such as Cu, Pb, Zn are reflected in the concentration of the particulate materials containing lead compounds from the exhaust of automobiles, before this ban, may have been deposited on the sediments in the coastal area of Okinawa. The sediment concentrations of heavy metals such as Cu, Pb and Zn are in agreement with previous studies that compare captive and wild sea turtles in Okinawa Main Island [31, 32].

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Titanium dioxide (TiO_2) is used for many household products such as toothpastes, soaps, and detergents; the usage of such products results in the TiO_2 entering the wastewater effluents and eventually reaching the coastal open water. Among the TiO_2 containing compounds, nano particulate-titanium dioxide (nano-TiO_2) is one of the most universally engineered nano-material. Many chemicals of emerging concern (CECs) are frequently used in cosmetic formulations and other products, and these can potentially reach the marine environment to increase concentrations that may harm the marine ecosystem. Since the nano-TiO_2 is reported to cause oxidative stress mediated phototoxic effect due to its photoactive nature, it can cause cytotoxic effects in phytoplankton. Thus, the increased concentration of nano-TiO_2 in marine ecosystems may have negative consequences [25]. Skin care products have the highest quantities of CECs, with titanium dioxide and zinc oxide nano-materials being dominant potential contaminants [8]. They are widely used and present in the aquatic environment [26]. The large production and growing use of nano-TiO_2 has resulted in their continuous release into aquatic systems, which may cause harmful effects to marine organisms either directly or due to their potential interaction with conventional toxic contaminants, which represents a growing concern for biota [28]. The plasma Ti concentration in the sea turtles in the suburban coast was higher than that in the rural coastal area, which is most likely related to the influence of residential and industrial wastewater. Therefore, measurement of the plasma Ti concentration in sea turtles might be a useful bio-monitoring method to estimate the marine pollution level due to Ti, which can
change with changing human activities and the type of industrial processes present.

Labrada-Martagón et al. [21] demonstrated that wild green sea turtles from suburban coastal areas showed higher Si concentrations, but lower antioxidant enzyme activities than individuals from rural coastal area in the Baja California Peninsula. Si is an essential element in human-dominated landscapes where it is used in constructions, electronic equipment, cosmetics, and medical materials. Human impacts on Si cycling are only recently being explored [25]. The majority of Si entering the world’s oceans is from rivers in the form of dissolved Si that originates from rinsing-water and waste-water from various human activities. Maguire and Fulweiler [24] highlight the influences of urban environments in altering the flux of Si from land to sea. For marine vegetation such as a standing stock of eelgrass, *Zostera marina*, Si levels in the leaves has been shown to be strongly correlated with the dissolved silicon concentration in the water column [17]. Since the eelgrass is commonly found in Okinawa area and is known to be grazed by green sea turtles, this could have contributed, along with various human activities, to a significantly higher concentration of Si in the suburban group.

Se is an element related to glutathione peroxidase, which is an antioxidant. It is known to detoxify Hg, a known neurotoxin with no known essential function, in the body. However, Se can become toxic at elevated concentrations. In this study, plasma Se concentration in the green sea turtles inhabiting the suburban coastal was 0.120 ± 0.077 µg/g, which was significantly lower than those in the captive (0.179 ± 0.081 µg/g, *P*<0.05) and rural coastal groups (0.192 ± 0.074 µg/g, *P*<0.05). Therefore, our results for the concentration of Pb, Se and Ti for plasma samples collected from the two different areas indicates that the concentrations are related to the industrial activities in the urban regions of Okinawa.

Since mature green sea turtles in this area remain in the coastal regions instead of migrating to other oceanic regions, the concentration of trace and major elements in their body is a direct reflection of the pollution in these coastal waters. The results of this study confirm that the concentrations of some elements are higher in green sea turtles from the Okinawa Main Island, which is probably related to the high degree of anthropogenic effluent pressure in this coastal basin. Thus, these individuals are more likely to experience adverse effects related to contaminants when compared to individuals from the Yaeyama Island. Data such as those presented in this study are useful to assess marine pollution levels and to motivate local and regional authorities to continue to monitor the level of multiple elements in green turtles. This also is a potential early warning system to avoid adverse exposure, not only for sea turtles but also other marine organisms, to some trace and major elements associated with the suburban areas of Okinawa.

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REFERENCES

1. Aguirre, A. A. and Lutz, P. L. 2004. Marine turtles as sentinels of ecosystem health: is fibropapillomatosis an indicator? *EcoHealth* 1: 275–283.
2. Anan, Y., Kunito, T., Watanabe, I., Sakai, H. and Tanabe, S. 2001. Trace element accumulation in hawksbill turtles (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*) from Yaeyama Islands, Japan. *Environ. Toxicol. Chem.* 20: 2802–2814. [Medline] [CrossRef]
3. Bishop, B. E., Savitzky, B. A. and Abdel-Fattah, T. 2010. Lead bioaccumulation in emydid turtles of an urban lake and its relationship to shell
4. Bjornsdal, K. A. 1997. Foraging ecology and nutrition of sea turtles. pp. 199–231. In: The Biology of Sea Turtles. (Lutz, P. L., Musick, J. A. eds.) CRC, Boca Raton.

5. Cénac, A., Simonoff, M. and Djibo, A. 1996. Nutritional status and plasma trace elements in peripartum cardiomyopathy. A comparative study in Niger. J. Cardiovasc. Risk 3: 483–487. [Medline] [CrossRef]

6. Day, R. D., Keller, J. M., Harms, C. A., Segars, A. L., Cluse, W. M., Godfrey, M. H., Lee, A. M., Peden-Adams, M., Thorvalson, K., Dodd, M. and Norton, T. 2010. Comparison of mercury burdens in chronically debilitated and healthy loggerhead sea turtles (Caretta caretta). J. Wildl. Dis. 46: 111–117. [Medline] [CrossRef]

7. de Carvalho, R. H., Rada, M. P., da Silva Mendes, S., Barbosa, B. C., Paschoalini, M., Prezoto, F. and de Sousa, B. M. 2015. Marine debris ingestion by sea turtles (Testudines): on the Brazilian coast: an underestimated threat? Mar. Pollut. Bull. 101: 746–749. [Medline] [CrossRef]

8. Dhanirama, D., Gronow, J. and Voulvoulis, N. 2012. Cosmetics as a potential source of environmental contamination in the U.K. Environ. Technol. 33: 1597–1608. [Medline] [CrossRef]

9. Eisa, M. H., Shen, H., Jin, W., Alaamer, A. S., Al-Rajhi, M. A. and Idriss, H. 2016. PIXE study on the effects of parathyroid hormone on elemental content in rat bones. Phys. Med. 52: 1615–1620. [Medline] [CrossRef]

10. Fukuda, H., Ebara, M., Okabe, S., Yoshikawa, M., Sugiu, N., Saisho, H., Kondo, F. and Yukawa, M. 2004. Metal contents of liver parenchyma and hepatic injury in chronic hepatitis C. J. Gastroenterol. Hepatol. 19: 1050–1056. [Medline] [CrossRef]

11. Gardner, S. C., Fitzgerald, S. L., Vargas, B. A. and Rodríguez, L. M. 2006. Heavy metal accumulation in four species of sea turtles from the Baja California peninsula. Mexico. Biomarkers 19: 91–99. [Medline] [CrossRef]

12. Gargouri, D., Azri, C., Serbaji, M. M., Jedoui, Y. and Montacer, M. 2011. Heavy metal concentrations in the surface marine sediments of Sfax Coast, Tunisia. Environ. Monit. Assess. 175: 519–530. [Medline] [CrossRef]

13. Godley, B. J., Thomson, D. R. and Furness, R. W. 1999. Do heavy metal concentrations pose threat to marine turtle from Mediterranean Sea? Mar. Pollut. Bull. 38: 497–502. [CrossRef]

14. Green-Ruiz, C. R. and Páez-Osuna, F. 2004. Potential bioavailability of heavy metals in surface sediments from the Altata-Ensenada del pabellon lagoon, SE Gulf of California. J. Coast. Res. 20: 1126–1134. [CrossRef]

15. Greaves, M. J., Elderfield, H. and Sholkovitz, E. R. 1999. Aeolian sources of rare earth elements to the Western Pacific Ocean. Mar. Chem. 68: 31–38. [CrossRef]

16. Hatano, R., Ebara, M., Fukuda, H., Yoshikawa, M., Sugiu, N., Kondo, F. and Yukawa, M. 2004. Accumulation of liver parenchyma after percutaneous ethanol injection or radiofrequency ablation in patients with hepatocellular carcinoma before and after trientine hydrochloride therapy. J. Lab. Clin. Med. 143: 333–339. [Medline] [CrossRef]

17. Gardner, S. C., Fitzgerald, S. L., Vargas, B. A. and Rodríguez, L. M. 2006. Heavy metal accumulation in four species of sea turtles from the Baja California peninsula. Mexico. Biomarkers 19: 91–99. [Medline] [CrossRef]

18. Kampalath, R., Gardner, S. C., Fitzgerald, S. L., Vargas, B. A. and Rodríguez, L. M. 2006. Heavy metal accumulation in four species of sea turtles from the Baja California peninsula. Mexico. Biomarkers 19: 91–99. [Medline] [CrossRef]

19. Kiilholma, P. 1986. Serum copper and zinc concentrations in intrahepatic cholestasis of pregnancy: a controlled study. J. Trace Elem. Electrolytes Health Dis. 1: 111–116. [Medline] [CrossRef]

20. Kriek, N. P., Sly, M. R., du Bruyn, D. B., de Klerk, W. A., Renan, M. J., Van Schalkwyk, D. J. and Van Rensburg, S. J. 1982. Dietary wheaten bran in baboons: long-term effect on the morphology of the digestive tract and aorta, and on tissue mineral concentrations. Br. J. Exp. Pathol. 63: 254–268. [CrossRef]

21. Labrada-Martagón, V., Rodríguez, P. A., Méndez-Rodríguez, L. C. and Zenteno-Savín, T. 2011. Oxidative stress indicators and chemical contaminants in East Pacific green turtles (Chelonia mydas) inhabiting two foraging coastal lagoons in the Baja California peninsula. Mar. Pollut. Bull. 62: 1979–1983. [Medline] [CrossRef]

22. Ley-Quiñónez, C., Zavala-Norzagaray, A. A., Espinosa-Carreón, T. L., Peckham, H., Marquez-Herrera, C., Campos-Villegas, L. and Aguirre, A. A. 2011. Baseline heavy metals and metalloid values in blood of loggerhead turtles (Caretta caretta) from Baja California Sur, Mexico. Mar. Pollut. Bull. 62: 1816–1823. [Medline] [CrossRef]

23. Lyngby, J. E. and Brix, H. 1982. Seasonal and environmental variation in cadmium, copper, lead and zinc concentrations in eelgrass (Zostera marina L.) in the Limfjord, Denmark. Aquat. Bot. 14: 59–74. [CrossRef]

24. Maguire, T. J. and Fulweiler, R. W. 2016. Urban Dissolved Silica: Quantifying the Role of Groundwater and Runoff in Wastewater Influent. Environ. Sci. Technol. 50: 54–61. [CrossRef]

25. Miller, R. J., Bennett, S., Keller, A. A., Pease, S. and Lenihan, H. S. 2012. TiO2 nanoparticles are phototoxic to marine phytoplankton. PLoS ONE 7: e36321. [Medline] [CrossRef]

26. Nigro, M., Bernardeschi, M., Costagliola, D., Dellà Torre, C., Frenzilli, G., Guidi, P., Lucchesi, P., Mottola, F., Santonastaso, M., Scarcelli, V., Monaci, F., Corsi, I., Stingo, V. and Rocco, L. 2015. n-TiO2 and CdCl2 co-exposure to titanium dioxide nanoparticles and cadmium: Genomic, DNA and chromosomal damage evaluation in the marine fish European sea bass (Dicentrarchus labrax). Aquat. Toxicol. 168: 72–77. [Medline] [CrossRef]

27. Páez-Osuna, F., Calderón-Camuzpanuzo, M. F., Soto-Jiménez, M. F. and Ruelas-Inzunza, J. R. 2010. Trace metals (Cd, Cu, Ni, and Zn) in blood and eggs of California sea lion (Zalophus californianus) from Marina L. Bull. Mar. Sci. 83: 627–641. [Medline] [CrossRef]

28. Rocco, L., Santonastaso, M., Nigro, M., Mottola, F., Costagliola, D., Bernardeschi, M., Guidi, P., Lucchesi, P., Scarcelli, V., Corsi, I., Stingo, V. and Frenzilli, G. 2015. Genomic and chromosomal damage in the marine mussel Mytilus galloprovincialis: Effects of the combined exposure to titanium dioxide nanoparticles and cadmium chloride. Mar. Environ. Res. 111: 144–148. [Medline] [CrossRef]

29. Sera, K., Futatsugawa, S. and Matsuda, K. 1999. Quantitative analysis of untreated bio-samples. Nucl. Instrum. Methods Phys. Res. B 150: 226–233. [CrossRef]

30. Sera, K., Futatsugawa, S., Matsuda, K. and Miura, Y. 1996. Standard-free method of quantitative analysis for bio-samples. Int. J. PIXE 6: 467–481. [CrossRef]

31. Suzuki, K., Noda, J., Yanagisawa, M., Kawazu, I., Sera, K., Fukui, D., Asakawa, M. and Yokota, H. 2012. Relationships between carapace sizes and plasma major and trace element status in captive hawksbill sea turtles (Eretmochelys imbricata). J. Vet. Med. Sci. 74: 1677–1680. [Medline] [CrossRef]

32. Suzuki, K., Noda, J., Yanagisawa, M., Kawazu, I., Sera, K., Fukui, D., Asakawa, M. and Yokota, H. 2012. Particle-induced X-ray emission analysis of elements in plasma from wild and captive sea turtles (Eretmochelys imbricata, Chelonia mydas, and Caretta caretta) in Okinawa, Japan. Biol. Trace Elem. Res. 148: 302–308. [Medline] [CrossRef]

33. Van den Hauwe, J. and Maenhaut, W. 1994. Trace element alterations in rat tissues induced by the hepatotoxic agent CC14. J. Trace Elem. Electrolytes Health Dis. 8: 145–150. [Medline] [CrossRef]