The 5th International Symposium
Program for Leading Graduate Schools

Recent Advances in Research on Environmental Toxicology by Young Researchers from African Countries and Japan

D a t e : February 28th(Thu), 2013
T i m e : Afternoon 14:30~17:50
Venue : Lecture Hall, Graduate School of Veterinary Medicine, Hokkaido University

Chairperson: Prof. Lawrence Ikechukwu Ezemonye
National Centre for Energy and Environment, Energy Commission of Nigeria, University of Benin, Nigeria

Moderator: Prof. Mayumi Ishizuka
Graduate School of Veterinary Medicine, Hokkaido University, Japan

Joint hosting：JSPS Core-to-Core Program "Establishment of International Toxicology Consortium with 8 African Countries"

Hokkaido University,
Program for Leading Graduates Schools
Fostering Global Leaders in Veterinary Science toward Contributing to "One Health"

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— Program —

14:30 ~ 14:35 Opening Remarks: **Prof. Shigeo Ito**, Dean
Graduate School of Veterinary Medicine, Hokkaido University, Japan

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Session 1 : Field Studies for Ecological and Health Risk Assessment

14:35 ~ 14:55 **Mr. YARED Beyene Yohannes** (Topic from Ethiopia)
Graduate School of Veterinary Medicine, Hokkaido University, Japan
LEVELS OF ORGANOCHLORINE PESTICIDES IN BIRD AND FISH SPECIES FROM ETHIOPIAN RIFT VALLEY LAKE: ASSOCIATION WITH TROPHIC LEVEL

14:55 ~ 15:15 **Mr. Nesta BORTEY-SAM** (Topic from Ghana)
Graduate School of Veterinary Medicine, Hokkaido University, Japan
INCIDENCE OF METALS AND POLY AROMATIC HYDROCARBONS (PAHS) IN SURFACE SOILS FROM THE KUMASI METROPOLIS, GHANA- GIS BASED APPROACH.

15:15 ~ 15:35 **Dr. Abdallah Fikry A. Mahmoud** (Topic from Egypt)
Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt
DETERMINATION OF ORGANOCHLORINE PESTICIDES (OCPS) IN THE EDIBLE OFFAL OF EGYPTIAN BUFFALO

15:35 ~ 15:55 **Mr. Ruan Gerber** (Topic from South Africa)
Department of Zoology, University of Johannesburg, South Africa
THE ECOLOGICAL STATE OF TIGERFISH (HYDROCYNUS VITTATUS) IN THE KRUGER NATIONAL PARK: A STUDY INTO THEIR HEALTH IN TERMS OF BIOACCUMULATION AND BIOMARKER RESPONSES

15:55 ~ 16:05 Coffee Break

Session 2 : Laboratory and Semi-Field Study on Environmental Toxicology

16:05 ~ 16:25 **Dr. Tongo Isioma** (Topic from Nigeria)
Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Nigeria
BIOMARKERS OF STRESS IN AMPHIBIANS (Bufo regularis): ALTERNATIVE ENDPOINTS IN ECOLOGICAL RISK ASSESSMENT OF PESTICIDES

16:25 ~ 16:45 **Dr. Emmanuel Temiotan Ogbomida** (Topic from Nigeria)
Ecotoxicology and Environmental Forensic Unit, National Centre for Energy and Environment, Energy Commission of Nigeria, University of Benin, Nigeria
ECOTOXICOLOGICAL ASSESSMENT OF ANTIFOULING BIOCIDE (TRIBUTYLTIN) IN WARRI HARBOURS

16:45 ~ 17:05 **Dr. Nowaki Hijikata** (Topic from Japan)
Graduated school of Engineering, Hokkaido University, Japan
FEASIBILITY OF GREYWATER REUSE FOR IRRIGATION: THE IMPACT OF LAUNDRY DETERGENT AND ITS SURFACTANT ON PLANT GROWTH AND SYMBIOTIC BACTERIA

17:05 ~ 17:25 **Dr. Ken Ushijima** (Topic from Japan)
Graduated school of Engineering, Hokkaido University, Japan
ENDOTOXIN REMOVAL BY SOIL AQUIFER TREATMENT FOR WASTEWATER REUSE.

17:25 ~ 17:45 **Mr. Kensuke Watanabe** (Topic from Japan)
Graduate School of Veterinary Medicine, Hokkaido University, Japan
AVIAN FEATURES OF CYP 1-3 GENES

17:45 ~ 17:50 Closing: **Prof. Motohiro Horiuchi**, Program Coordinator
Graduate School of Veterinary Medicine, Hokkaido University, Japan

Levels of Organochlorine Pesticides in bird and fish species from Ethiopian Rift Valley Lake: Association with Trophic Level

YARED Beyene Yohannes, Yoshinori Ikenaka, Aksorn Saengtienchai, Kensuke Watanabe, Shouta M.M. Nakayama and Mayumi Ishizuka

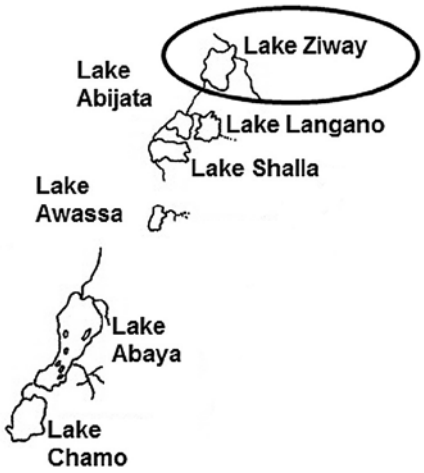
Laboratory of Toxicology, Department of Environmental Veterinary Science Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan

[BACKGROUND and PURPOSE]

The Ethiopian Rift Valley region which encompasses seven lakes is an important area for agricultural, commercial and industrial development of Ethiopia. It is a densely populated area and one of the focal regions for large scale agricultural development in the country. At the same time, it is one of the most environmentally vulnerable areas in Ethiopia. Anthropogenic sources of OCPs from urban and agricultural sources lead to unprecedented environmental contamination to the ecosystem. Thus, this situation puts an increasing claim on the fresh water ecosystem and threatens the surroundings environment. To our knowledge, no information is available concerning the bioaccumulation levels of OCPs by bird and fish species from Ethiopian Rift Valley Lakes. Therefore, the aim of this work is to investigate the levels and bioaccumulation pattern of OCPs in different bird and fish species from Lake Ziway in association with trophic level.

[MATERIALS and METHODS]

Study area: Lake Ziway is a shallow freshwater lake situated in the most northern section of the Ethiopian Rift Valley, which is surrounded by lands that are under continuous cultivation throughout the year. The lake is fed by two rivers, Meki from the north-west and Katar from the east and drains to Lake Abiyata via Bulbula River in the south.



Location of Lake Ziway in the Ethiopian Rift Valley

Samples: BIRD: Scopus umbretta (Hamerkop: H, 5), Threskiornis aethiopicus (African sacred ibis: S, 7), Leptoptilos crumeniferus (Marabou stork: M, 6) and Pelecanus onocrotalus (Great White Pelican: P, 5) FISH: Oreochromis niloticus (Tilapia: O, 28), Tilapia zillii (Zillii: Z, 19) Carp spp. (Carp: C, 27), Clarias gariepinus (Catfish: G, 27) and Barbus intermedius (Barbus: B, 5). Muscle tissues were taken for analyses.

Stable isotope analysis: After removal of lipid from muscle samples using a mixture of chloroform:methanol (2:1 v/v), analysis was carried out using an isotope ratio mass spectrometer equipped with an elemental analyzer.

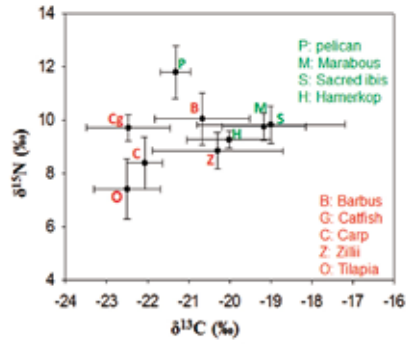
OCPs analysis: Ten gram of muscle tissue was mixed with sodium sulfate and extracted with 3:1 (v/v) hexane:acetone and subjected to clean up on a glass column packed with activated

florisil. Twenty two kinds of OCPs were analyzed by Shimadzu gas chromatography (GC-2014) equipped with a ^{63}Ni electron capture detector and ENV-8MS capillary column (30 m x 0.25 mm x 0.25 μm film thickness).

[RESULT and DISCUSSION]

Stable isotope analysis

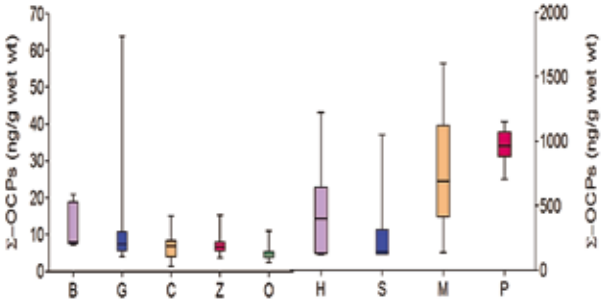
The stable isotope ratios analyses were used to evaluate the trophic positions and carbon source in the food web. Values of δ^{15}N and δ^{13} C in all bird and fish species ranged from 8.91‰ to 13.25‰ and -21.84‰ to -15.39‰, and 5.25‰ to 10.93‰ and -24.83‰ to -16.78‰, respectively. Significant differences ($p < 0.05$) of δ^{15}N and δ^{13}C amongst the studied bird and fish species were observed. The mean δ^{15}N values in catfish and barbus were significantly higher than that of tilapia, zillii and carp species, indicating a higher trophic level of the two carnivorous species. Regards to birds, pelican showed high trophic level due to its piscivorous feeding habits. According to δ^{13}C values, barbus and zillii showed wide range of δ^{13}C values, utilizing carbon source from the benthic to pelagic origin.



Mean and std. of δ^{15}N and δ^{13}C signatures

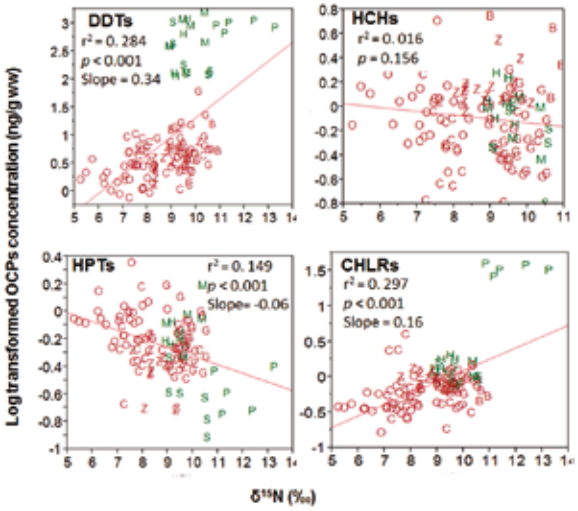
OCP residues

OCPs, including DDTs, HCHs, HPTs and CHLRs were detected in all samples analyzed in this study with varying concentrations among species. The levels of ∑OCPs in the muscle tissues ranging from 1.41-63.82 ng/g ww (average of 7.72 ng/g) in fish species and from 118.77 to 1603.22 ng/g ww (average of 616.77 ng/g) in birds. Among the OCPs analyzed, DDTs were the predominant pollutants in all samples. The possible reasons for predominance of DDTs may be attributed to the run-off and atmospheric deposition from DDT which is used for agricultural and malaria control activities. Contamination pattern of OCPs was generally in the order of DDTs > HCHs > CHLRs ≅ HPTs.



Biomagnification of OCPs

The relationship between log transformed OCPs and δ^{15}N showed a significant correlation ($p < 0.001$) for DDTs and CHRLs, where the slope of regressions were 0.34 and 0.16, respectively. This significant correlation indicated that these two compounds are biomagnified in the food web of the lake. The total HPTs showed negative correlation (slope = -0.06, $p < 0.001$). Nevertheless HCHs showed no significant correlation with δ^{15}N values ($p = 0.156$); largely



owing to their low octanol-water partition coefficients (log K_{ow} ~ 4).

[CONCLUSION]

To the best of our knowledge, this is the first study reporting on the concentrations of OCPs and their bioaccumulation trend in different fish species from Lake Ziway, Ethiopia. Our results indicated the presence of HCHs, HPTs, CHLRs and DDTs with varying concentrations. The present study also demonstrates a biomagnification of DDTs and CHLRs in trophic lakes.

Incidence of Metals and Poly Aromatic Hydrocarbons (PAHs) in Surface Soils from the Kumasi Metropolis, Ghana- GIS Based Approach

Nesta BORTEY-SAM[1], Osei AKOTO[2], Yoshinori IKENAKA[1], Elvis BAIDOO[2], Beyene YARED[1], Shouta M.M. NAKAYAMA[1], Mayumi ISHIZUKA[1]*

[1]Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan
[2]Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[Background and Purpose]

Environmental pollution due to rapid economic development in Ghana has become an issue of concern. Heavy metals and PAHs as ubiquitous environmental pollutants found in water, soil, air, plants and animal tissues. As one of the most industrialized and economically significant cities in Ghana, Kumasi has been subject to heavy anthropogenic influences due to the fast economic growth and urbanization leading to greater fuel combustion rate. In addition garbage, paper, cloth, wood are burned in the open, and all these are potential sources of PAHs contamination. Many heavy metals and PAHs are highly toxic, which gives rise to concerns over their occurrence in the environment. Soil system is an important long-term repository for these contaminants and is considered to be a steady indicator for its environmental pollution state. Metals and PAHs in soils may accumulate in biota via food chains which leads to direct or indirect exposure in humans. Therefore, knowledge of soil contamination is needed to avoid any food production risk and to restrict the deleterious effect of these contaminants. From literature no such comprehensive study has been conducted to address the accumulation and distribution of heavy metals and PAHs in surface soils from Kumasi. The objectives of the study were to determine the concentrations of heavy metals and PAHs in surface soil samples from the Kumasi Metropolis; establish the possible sources of these contaminants and develop distribution maps throughout the city using the Geographic Information System (GIS).

[Materials and Methods]

A total of 129 soil samples were randomly collected from 36 communities within the Kumasi metropolis. Global positioning system (GPS) was used to locate the sampling positions. Prior to chemical analysis, the soil samples were air dried at room temperature and passed through a 2 mm sieve. For metal analysis, 1.0 g soil sample was digested with microwave digester using 10 ml of 60% nitric acid. The concentrations of Cd, Cr, Co, Ni, Pb, As, Cu and Zn were analyzed by AAS. Concentration of Hg was determined by Mercury Analyzer (MA-3000).
10 g of soil was extracted for PAHs with 1:2; v/v acetone-hexane mixture using soxhlet extractor. The extracts were dehydrated and cleaned-up with a column packed with 5 % water content silica gel to remove contaminants. PAHs analyses were carried out using AS3000 Gas Chromatograph (GC) coupled with a Thermo scientific Mass Selective Detector operating in the electron impact mode (GC-MS). Concentrations 22 PAHs (Nap, Ace, Acy, Fle, Phe, Ant, Flu, Pyr, B(a)A, Chry, B(b)F, B(k)F, B(a)P, IDP, Db(a,h)A, B(g,h,i)P, benzo(e)pyrene, Tri, Me Phe, 1-Me-Phe and Ret) were measured in each sample.

[Results and Discussion]

[Metals]

Mean concentrations of metals in soil samples from Kumasi decreased in the order Zn, Cr,

Pb, Cd and Hg with mean values 104.58±65.99, 94.53±46.03, 51.47±38.14, 0.14±0.15 and 0.05±0.05 mg/kg dw respectively. The metal levels from some communities exceeded the benchmark values from the US EPA (2003, 2004) and the reference site. Enrichment factor (EF) and Geo-accumulation index (Igeo) indicated that Zn, Pb and Cr were significantly enriched in the soils from Kumasi metropolis and was highly influenced by anthropogenic factors. Igeo values for Cd and Cr from Yennyawoso and Aboabo respectively were in class 6, showing extreme contamination. Based on Igeo values, soils from the vicinity can therefore be described as uncontaminated to extremely polluted. The high concentrations may be due to heavy vehicle traffic, large metal scraps and tannery industries within the area. The several hot-spots identified from the geochemical map indicated high metal concentrations from Suame, Aboabo, Yennyawoso, Adum, Kejetia, Suntreso, Asafo. These communities are densely populated with vehicles and light industries.

[PAHs]

The mean concentration of total PAHs in the surface soils ranged from 10.85±4.84 at Ahinsan to 2084.87±2210.05 ng/g dw at Adum. According to Maliszewska-Kordybach (1996) classification the communities severely polluted with PAHs were Adum, Romanhill, Mbrom, Suame, Suntreso, Sofoline. These communities constitute the city centre. Fluoranthene was the most abundant PAH in the surface soil from the Kumasi metropolis with mean concentrations of 61.70±86.86 ng/g dw. From this study, it was observed that surface soils from the Kumasi metropolis were dominated by high molecular weight PAHs. Chemical Mass Balance and diagnostic ratios of the results showed that PAHs in the surface soil samples were mainly from fuel combustion. GIS coordinates and concentrations of PAHs in surface soils were used to create the distribution maps. Fluoranthene and pyrene the two most abundant PAH showed the same distribution pattern with extremely high concentrations at Suame, Mbrom, Adum, Suntreso, Romanhill and Sofoline. The high levels of PAHs in the soil samples from these communities may come from the high vehicular traffic and the high human population who may use firewood or charcoal for domestic heating.

[Conclusion]

EF and Igeo values showed that soil was significantly enriched with Zn, Pb, and Cr. Maps of metal distribution were constructed for all communities and this indicated that Suame is the most polluted community with heavy metals in the Kumasi metropolis.
The most abundant PAHs in soils were fluoranthene and Pyrene. Highest concentration of BaP in the surface soils from the Kumasi metropolis was recorded in Adum and the distribution maps indicated that PAHs spread throughout the Kumasi metropolis but Suame, Mbrom, Romanhill, Suntreso, Sofoline, Adum were the most polluted communities.

[References]

Maliszewska-Kordybach B (1996) Polycyclic aromatic hydrocarbons in agricultural soil in Poland, preliminary proposals for criteria to evaluate the level of soil contamination. Appl Geochem 11:121 127

United States Environmental Protection Agency, 2003. Guidance for Developing Ecological Soil Screening Levels. OSWER-Directive 9285.7-55. http://www.epa. gov/ecotox/ecossl/.

United States Environmental Protection Agency, 2004. Framework for Inorganic Metals Risk Assessment. Draft EPA/630/P-04/068B, 20460. US EPA, Washington DC.

DETERMINATION OF ORGANOCHLORINE PESTICIDES (OCPS) IN THE EDIBLE OFFAL OF EGYPTIAN BUFFALO

Abdallah Fikry A. Mahmoud, Wageh Sobhy Darwish, Alaa Eldin M.A. Morshdy, Elsaid A. Eldaly

Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt

[Background and objective]

Environmental contamination by OCPs has a great concern, since most of these pesticides are classified as possible carcinogenic, teratogenic, and neurotoxic. They are of considerable concern to human, animal and environmental health, causing an array of adverse effects and death. Some studies have shown extreme persistence of these pollutants in the world-wide environment and accumulation in human and animal tissue. Substantial amounts of OCPs are still being illegally used for agriculture and animal production programs in many areas in Egypt. As a result, contamination of food stuffs, especially those having a high fat content such as meat and meat products recorded. One of the great challenges in food safety is the control of risks associated with mixtures of contaminants, which are changing continuously. Therefore, this study aimed to investigate the current situation of OCPs residues in livers, kidneys and tongues of the Egyptian buffalo collected from three locations (Ismailia, Zagazig and Mansura) in a comparative way.

[Materials and methods]

A total of 135 random samples of livers, kidneys and tongues were collected from male Egyptian buffalo (Bubalus bubalis) from 3 locations (Ismailia, Zagazig and Mansura, 45 sample for each location divided as 15 for each of liver, kidney and tongue) in the period of October, 2010 to June, 2011. The samples were collected directly after inspection at Ismailia, Zagazig and Mansoura slaughter houses. After sample extraction and clean up of the extracts, concentrations of twenty two OCPs were determined by gas-chromatography equipped with Ni electron capture detector (GC-ECD).

[Results and discussion]

In the current investigation, the concentration of OCPs expressed as ng/g lipid weight (lw) in tissues of buffalo from Ismailia, Zagazig and Mansura slaughterhouses. Among the detected OCPs in this study, HCHs were the most dominant compounds in the examined tissue samples of buffalo followed by Drins. However, the use of DDTs, HCB and CHLRs is scarce. Additionally, data reported in this study showed that DDTs are still in use in Mansura city which is mainly agricultural area compared with both Zagazig and Ismailia. The reported values in this study showed that, the residual concentration of all OCPs detected in buffalo tissues samples were lower than Egyptian recommended maximum permissible limits for HCHs (1μg/g), Drins (600ng/g), DDTs (5μg/g), CHLRs (200ng/g) and HCB (200ng/g).

[Conclusion]

Examined samples from Mansura city had the highest OCPs contamination load. Tongues had the highest concentration of these toxic residues in a comparison to livers and kidneys in the examined samples. The overall results showed that OCPs residues did not exceed the Egyptian maximum permissible limits in all of the analyzed samples from the three different locations.

The Ecological state of tigerfish (Hydrocynus vittatus) in the Kruger National Park: A study into their health in terms of bioaccumulation and biomarker responses

Ruan Gerber[1], Nico Smit[2] & Victor Wepener[1, 3]

1) Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park 2006, South Africa
2) Water Research Group (Ecology), School of Environmental Sciences and Development, Potchefstroom Campus, North West University, Private Bag X6001, Potchefstroom 2520, South Africa
3) Director: School of Biological Science, North West University, South Africa

Even though ecological requirements have been set for most of the rivers in the Kruger National Park (KNP), no monitoring programme is in place to determine whether they are being met. Tigerfish (Hydrocynus vittatus) were selected for this project because they are the top fish predators in African freshwater systems and the most iconic fish species throughout Africa. However tigerfish are considered to be rare in South Africa and as of 2008 are classified as a protected species. The aims of this study were to investigate the suitability of the tigerfish as an indicator of unacceptable changes in environmental quality in the Olifants and Luvuvhu Rivers. To use tigerfish as a model organism to set Threshholds of Potential Concern for fish in the rivers of the KNP and to determine whether applying multiple endpoints is effective in identifying environmental changes due to altered environmental quality. For the purposes of this presentation the bioaccumulation and biomarker response endpoints in tigerfish as indicators of altered environmental quality will be discussed. Tissue samples for bioaccumulation and biomarker studies were collected during the high and low flow periods from September 2009 to May 2011. Bioaccumulation is used to relate environmental contaminants (metals and organic pollutants) levels in water and sediments to the bioavailable fraction in the muscle tissue of the tigerfish. Digestion and metal analysis in muscle were conducted using ICP-OES and ICP-MS techniques. Organic contaminant extraction (solid phase matrix dispersion) and analyses (GC-MS) were carried out using standardised techniques. The biochemical markers measured were: biomarkers of exposure; Acetylcholinesterase (AChE), Cytochrome P450 activity (CYP450) and metallothioneins (MT) and biomarkers of effects; lipid peroxidase (LP), catalase (CAT) activity, protein carbonyls (PC) and cellular energy allocation (CEA). The biomarkers of exposure for organophosphate pesticides (AChE and MT) were greater in the Olifants River when compared to the Luvuvhu River, whereas the organochlorine biomarker of exposure (CYP450) was more pronounced in the Luvuvhu River. The oxidative stress biomarkers reflected this exposure to organochlorines whereas protein damage was observed in the Olifants River fish. The biomarker responses are reflected through the exposure and subsequent bioaccumulation of predominantly organic pollutants in the Luvuvhu and metals in the Olifants Rivers. Thus the application of a multi endpoint approach was successful in elucidating pollutant exposure and subsequent biological responses in tigerfish.

BIOMARKERS OF STRESS IN AMPHIBIANS (Bufo regularis): ALTERNATIVE ENDPOINTS IN ECOLOGICAL RISK ASSESSMENT OF PESTICIDES.

***Tongo Isioma[1], Ezemonye Lawrence[2]**

[1] Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Edo State

[2] National Centre for Energy and Environment, Energy Commission of Nigeria, University of Benin, Benin City, Edo State

E-mail contact: isquared27@yahoo.com, isioma.tongo@uniben.edu

Abstract

The environment is currently flooded with a cocktail of chemicals including fertilizers, herbicides, insecticides and fungicides released by various agricultural and industrial activities. Up to 90% of pesticides applied never reach the intended targets; as a result, many other organisms sharing the same environment as pests are accidentally poisoned. One of the non-target biological groups mostly affected by pesticides are amphibians. Recent widespread amphibian declines call for better techniques to assess population dynamics. This paper therefore assesses sensitive biological markers of stress in the African Toad (Bufo regularis) as alternative endpoints in the ecological risk assessment of pesticides (Endosulfan and Diazinon). The assessed biomarkers were biomarker of oxidative stress (Glutathione-s-transferase (GST), biomarker of neurotoxicity (Acetylcholinesterase AChE) and indicators of alteration in carbohydrate metabolism (Cortisol, Glycogen, Glucose, Total proteins). Biomarkers were assessed in Serum, Brain, Lungs, Gastrointestinal tract (GIT) and Liver. Other biomarkers studied include histopathological biomarkers. The biomarker responses were measured and related to measured concentrations of the pesticide in the toads. More pronounced poisoning symptoms were observed for Diazinon exposed toads at higher concentrations.Toads exposed to varying concentrations of pesticides (Endosulfan and Diazinon) exhibited signs of stress as expressed in elevated levels of GST and glucose, reduction in AChE, Glycogen, Cortisol and Total protein levels. Histopathological aberrations such as dilation of sinusoids and necrosis (Liver), degenerative and necrotic changes in the intestinal mucosa (GIT), interstitial fibrosis and peribronchial lymphoid infiltration (Lungs) and vacuolar changes with empty spaces appearing as "moth eaten" areas (Brain) were exhibited. The data suggests that pesticides may affect amphibian communities and the use of stress biomarkers in toads may be useful tools in evaluating the residual effects of pesticide toxicity to amphibians.

Keywords Amphibians, Bufo regularis, Pesticides, Biomarkers, Stress

ECOTOXICOLOGICAL ASSESSMENT OF ANTIFOULING BIOCIDE (TRIBUTYLTIN) IN WARRI HARBOURS

Ogbomida T Emmanuel[1] and Ezemonye I N Lawrence[2]

[1, 2]Ecotoxicology and Environmental Forensic Unit, National Centre for Energy and Environment, Energy Commission of Nigeria, University of Benin, Nigeria.

E-mail contact: ogbomida2000@justice.com

Tributyltin (TBT) is an antifouling biocide used on ship hulls to prevent attachment of marine organisms. Due to its widespread use, TBT has been considered as the most deliberately introduced contaminant into the environment. This study assessed effects of TBT and its derivatives, monobutyltin (MBT) and dibutyltin (DBT) under field and laboratory conditions. TBT and its derivatives were analyzed in surface water, sediment and periwinkle samples in three harbours and two Creeks from Warri, Delta State, Nigeria. Under laboratory conditions, acute and sublethal effects of TBT to Periwinkle snails (Tympanotonus fuscatus var radula) were assessed. Accumulation, survival and testosterone levels were used as assessment endpoints. Periwinkles snails were exposed for 10 days to varying concentrations of TBT, 0, 20, 30, 40 and 50µg/l (acute test) and 0, 0.1, 1.0, 10 and 20µg/l for 30 days (sublethal test). For the field study, results showed that TBT concentrations were predominant in water, sediment and periwinkle samples than its derivative. However, its derivatives were not observed in the periwinkle samples. The mean concentrations of TBT in the matrices exceeded the acceptable ecotoxicological benchmark of 0.01µg/l recommended by US Environmental Protection Agency (USEPA) which indicates potential environmental risk. Acute toxicity results showed increased mean percentage mortality with increase in concentrations and exposure duration. Estimated lethal concentration (LC_{50}) was 27.98µg/l while derived safe concentration was 2.8µg/l. For the sublethal test, accumulation of TBT in periwinkle and testosterone levels increased with increasing TBT concentrations and exposure duration. Increased testosterone levels in molluscs may have the potentials for imposex expressions leading to reproductive failure. It is therefore imperative that harbour water and sediment quality be assessed for TBT and it derivatives for the protection of sensitive benthic invertebrates in the Niger Delta ecological zone.

Key words : Tributyltin, Antifouling biocide, Periwinkle snail, Testosterone

Feasibility of greywater reuse for irrigation: the impact of laundry detergent and its surfactant on plant growth and symbiotic bacteria

Nowaki Hijikata, Emi Nakaoka, Abe Takayuki, Ken Ushijima, Naoyuki Funamizu

Graduated school of Engineering, Hokkaido University, kita13-nishi 8, Kita-ku, Sapporo-shi, Hokkaido 060-628, Japan

INTRODUCTION The reuse of greywater (non-toilet wastewater from household) offers an attractive option in arid and semi-arid zone since it could reduce potable water use by up to 50% [1] and the area has unstable irrigation due to fluctuate rainfall. The greywater usually contains varying levels of suspended solids, salts, nutrients, organic matter and pathogens which arise from washing with household detergents. Although public health risks associated with reuse of greywater are well studied [2, 3], information on agricultural ecosystem consisted plant and soil microorganisms is still limited. Laundry greywater, one of predominant of the greywater, include surfactants, builders, bleaching agents, auxiliary agents and salts. While some of the salts presented in laundry greywater can be beneficial to plants as nutrients, the extra accumulation in soil, particularly sodium and boron [3, 4], may adversely affect the plant growth in continuous cultivation with the greywater. Furthermore, it has been known that liner-alkylbenzen sulfonate, major surfactant in laundry detergents, inhibits plant growth, activity of soil enzymes and nitrification bacteria [5-7]. However, the effect of its continuous cultivation and the impact on symbiotic nitrogen fixation bacteria has not been studied well. In the present study, therefore, impact of continuous cultivation with laundry detergent on plant growth and impact of LAS on soybean-nitrogen fixation bacteria were observed.

METHOD Komatsuna (Brassica rapa var. perviridis) seeds were sawed in a plastic pot (about 800 mL capacity as 1/10000a) filled with a mixture of sandy soil and small gravel particles (< 2 mm). 0.02, 0.06, 0.2 and 0.6 g/L of LAS solution was applied in the pot as irrigation. Corresponded to the LAS concentration, 0.1, 0.3, 1 and 3 g/L of laundry detergent was also applied (20% of the laundry detergent was LAS). 50-100 mL of the irrigation water was approximately applied in one pot every day. After one month cultivation, Kamatsuna was harvested and measured dry weight. Using same soil, the cultivation was conducted again. In the case of nitrogen fixation bacteria, soybean (Glycine max) was used as host plant. –NPK soil for 80 years was used as the bacteria inoculum. River sand (< 2 mm), small gravel particle (< 2 mm), field soil (-N, P, K soil for 80 years) were mixed at the ratio of 5:3:2 as soil mixture. 700 mL (840 g) of the soil mixture was filled in the pot without any chemical fertilizer. 0 (blank), 0.02, 0.06, 0.2, and 0.6 g/L of LAS were applied in the pot.

RESULT & DISCUSSION In continuous cultivation with LAS in soil, 200 mg/L of LAS inhibited Komatsuna biomass in first time cultivation however, the impact was mitigated in second cultivation and the significant inhibition was observed at 600 mg/L of LAS (Fig. 1A&B). Compared to LAS and laundry detergent, laundry detergent inhibited more significantly at high concentration, 0.6 g/L LAS base, in second time cultivation (t-test, $p<0.05$) (Fig. 1B). This would be influenced by soil pH, since the soil pH exceeded 9.0 at that time. These results suggested that LAS impact was mitigated in long term cultivation, but long term application of

laundry detergent indirectly inhibited plant growth due to alkaline soil. Similar to Komatsuna, soybean was significantly inhibited at 600 mg/L of LAS. Although nodule number on root was significantly low at 600 mg/L of LAS but nitrogen mass in the pot was decreased to minus value from 200 mg/L LAS (Fig. 2 & Table 1). Therefore, it was suggested that nitrogen fixation activity was inhibited in 200 mg/L of LAS, since plus value in nitrogen mass was considered to

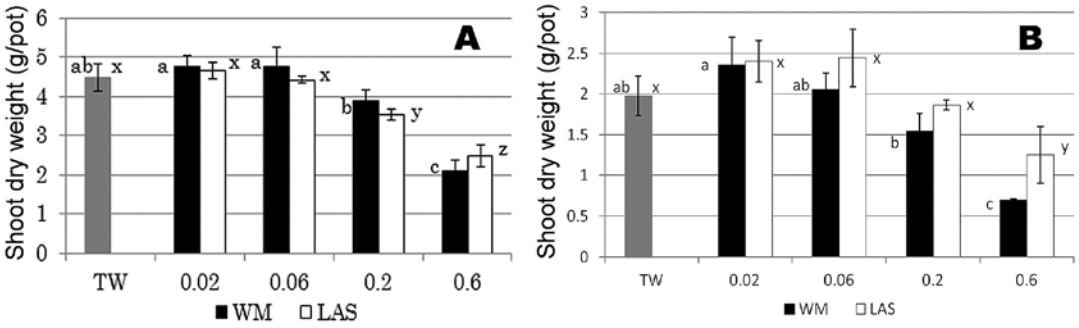


Figure 1 Shoot dry weight of Komatsuna in 1st time cultivation (A) and 2nd cultivation (B) with laundry detergent (WM) and LAS.

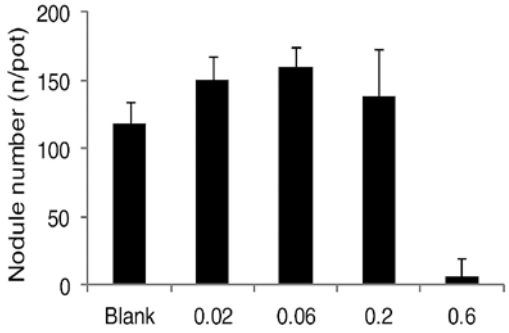


Figure 2 nodule number on soybean root

Table 1 nitrogen balance during soybean cultivation

	mass of nitrogen (mg/pot, mean)				
	Blank	0.02	0.06	0.2	0.6
before cultivation					
seeds	38	38	38	38	38
soil	481	481	481	481	481
after cultivation					
Plant	86	94	88	82	21
Soil	454	455	453	412	419
nitrogen balance	22	30	23	-25	-79

CONCLUSION It was concluded that impact of LAS was observed from 200 mg/L on plant growth and nitrogen fixation activity but continuous impact was mitigated for plant growth. On the other hand, alkaline agents from laundry detergent significantly increased soil pH in continuous cultivation and the high pH inhibited plant growth indirectly.

ACKNOWLEDGEMENT
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REFERENCES

[1] DHWA, Draft Guidelines for Reuse of Greywater in Western Australia, Department of Health, Perth, Australia, 2002, p.37.
[2] Ottoson, J., Stenstom, T.A., 2003, Fecal contamination of greywater and associated microbial risks, Water Res, 37, 645-655
[3] Gross, A., Azulai, N., Oron, G., Ronen,Z., Arnold, M., Nejidat, A. 2005. Environmental

impact and health risks associated with greywater irrigation: a case study Water sci Technol, 52, 161-169
[4] Mortensen, G.K., Egsgaard, H., Ambus, P., Jensen, E.S., Gron, C. 2001. Influence of Plant Growth on Degradation of Linear Alkylbenzene Sulfonate in Sludge-Amended Soil. J. Environ. Qual. **30**, 1266-1270
[5] Misra, R.K., Sivongxay, A., 2009 Reuse of laundry greywater as affected by its interaction with saturated soil, J. Hydrol, **366**, 55-61
[6] Elsgaard, L., Petersen, S.O., Debosz, K. 2001. Effedt and risk assessment of linear alkylbenzene sulfonates in agricultural soil. 1. Short-therm effects on soil microbiology. Environmental Toxicology and Chemistry, **20**, 1656–1663.
[7] Brandt, K.K., Pedersen, A., Sorensen, J., 2002. Solid-Phase Contact Assay That Uses a lux-Marked Nitrosomonas europaea Reporter Strain To Estimate Toxicity of Bioavailable Linear Alkylbenzene Sulfonate in Soil. Appl. Environ. Microbiol. **68**, 3502-3508.

Endotoxin Removal by Soil Aquifer Treatment for Wastewater Reuse

M. Guizani*, H.Kato*, K. Ushijima*, M. Dhahbi, N. Funamizu***

*Graduated school of Engineering, Hokkaido University, kita13-nishi 8, Kita-ku, Sapporo-shi, Hokkaido 060-628, Japan
**University of Tunis Carthage, Tunisia
Contact: uken@eng.hokudai.ac.jp (K. Ushijima)

1. Introduction

In wastewater reclamation and reuse practices, potable reuse is gaining interest due to increasing stress on water resources[1,2]. With respect to the bacterial by-products or lipopolysaccharide endotoxin, which is an outer-membrane component of gram-negative bacteria, several clinical problems may arise due to the risk of exposure to the environments contaminated with lipopolysaccharide (LPS). Endotoxin assessment and removal in drinking water is well documented in the literature[3, 4, 5], however it is not the case in wastewater where soluble microbial products (SMP) have attached many researchers due to the fouling effects that it generates to membranes. Eckenfelder[6] reported that SMP are detectable in the effluent as chemical oxygen demand (COD) and that 5 to 10% of COD degraded in the treatment process. Endotoxins are SMP which are still poorly investigated in wastewater.

This study conducted one year long term experiment of endotoxin removal by soil aquifer treatment, and observed feature of endotoxin removal in sand filtration columns.

2. Material and methods

Four sand filtration columns were prepared with filling silt of 0.125-0.45 mm, sand of 0.125 – 0.45, 0.45 – 0.85 mm and 0.85 – 1.4 mm. Soil depth were 2.0 m of upper unsaturated zone and 0.5 m of saturated zone in the bottom. Treated wastewater from wastewater treatment plant was continuously supplied in flow rate flux of 4.4 – 6.5 mL h^{-1} cm^{-2}. Experimental period was 1 year from October 2009.

Water samplings from every 0.1 m depth were done every one or two weeks. Filtered samples by 0.45 μm were analyzed on DOC (Shimazu, TC-5000A) and Endotoxin (LAL test). Molecular sizes of hydrophilic and hydrophobic organic matter were analyzed. Organic matters in the samples from 0.4 m, 0.8 m and 2.2 m and supplied water at 8th month were first separated into hydrophilic and hydrophobic by HPLC cartridge and HPBC cartridge, and again separated by molecular size by membrane dialysis.

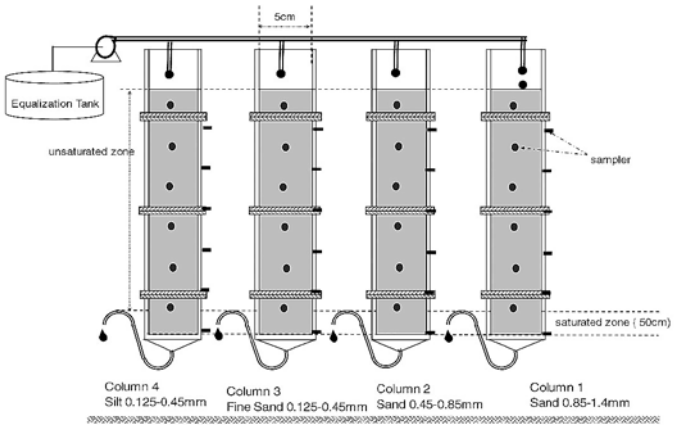

Fig.1 Experimental apparatus for long term experiment

3. Results and Discussion

3.1 Removal efficiencies

Averaged DOC removal efficiency until 3rd month of column 1 (coarse sand), 2 (middle sand), 3 (fine sand) and 4 (silt) were 12.5%, 17.5%, 20.0% and 22.5% respectively. Silt and fine sand showed relatively higher removal rate but these are only around 20%. On the other hand, endotoxin removal efficiencies at the same time were 65.5%, 78.3%, 78.2% and 88.5% respectively. These differences between DOC removal and endotoxin removal imply that removal mechanisms are different between DOC and endotoxin.

Figure 2 and 3 show vertical variation of DOC concentration and endotoxin concentration. DOC was removed gradually throughout 2 m depth, on the other hand, endotoxin was removed rapidly at the upper 0.5 m. These removal features were not change through one year (Fig.4 and 5).

3.2 Characteristics of removed organic matters

Through sand aquifer treatment, hydrophobic (captured by HPBC) large molecules (100 kD – 0.45μm) were reduced, though hydrophilic large molecules shows almost no reduction (Fig.6). This results and above mentioned removal feature of endotoxin implies that adsorption is an important mechanism for endotoxin removal.

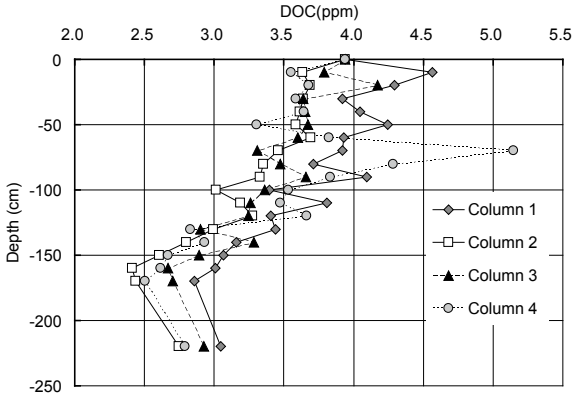

Fig.2 Vertical profiles of DOC concentration in soil column at 3rd month.

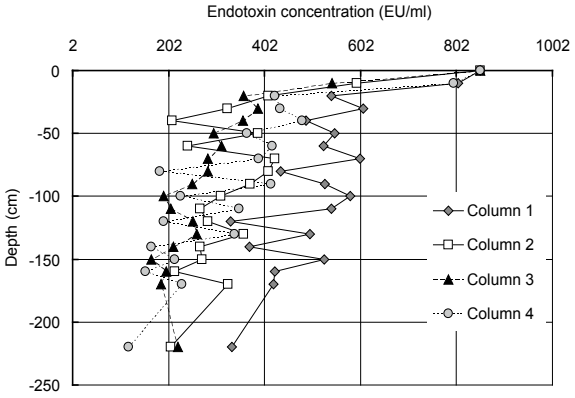

Fig.3 Vertical profiles of Endotoxin concentration in soil column at 3rd month.

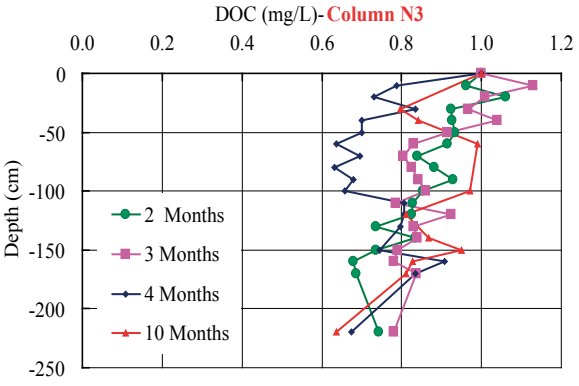

Fig.4 Comparison of DOC vertical profiles in soil column from 2nd month to 10th month in column 3 (fine sand).

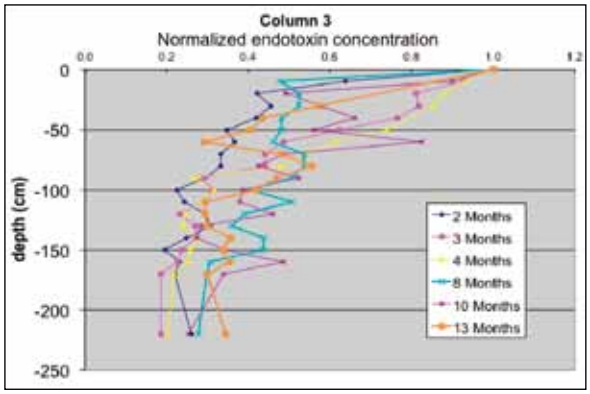

Fig.5 Comparison of endotoxin vertical profiles in soil column from 2nd month to 13th month in column 3 (fine sand).

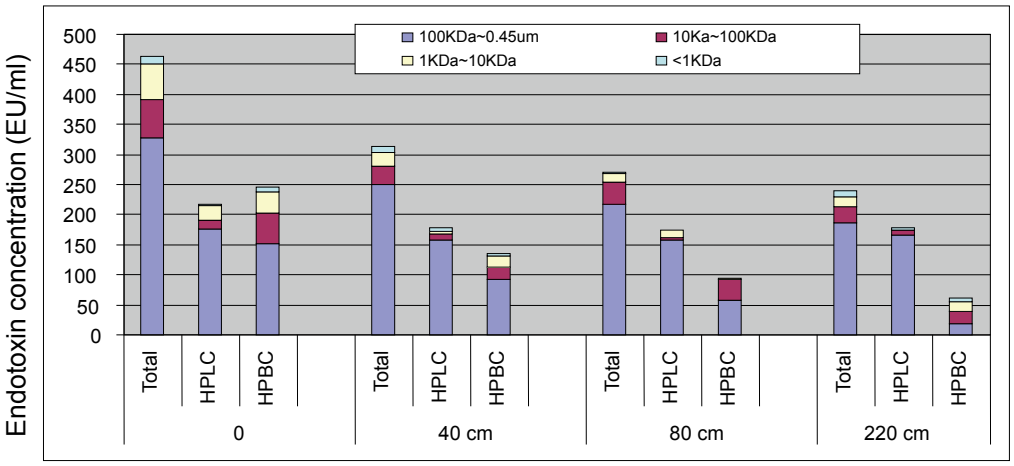

Fig.6 Change in the characteristics of organic matters through soil aquifer treatment at 8th month.

Reference

1. T. Asano and A. D. Levine. Wastewater Reclamation and Reuse. Wastewater Reclamation, Recycling, and Reuse: An Introduction. Technomic Publishing Co. Inc. Lancaster, PA. 1998.
2. Katsuki Kimura, Takuro Naruse and Yoshimasa Watanabe, Water Research Changes in characteristics of soluble microbial products in membrane bioreactors associated with different solid retention times: Relation to membrane fouling, 2009, 43(Issue 4), 1033–1039.
3. C. E. Reed and D. K. Milton, Journal of Allergy and Clinical Immunology, Endotoxin-stimulated innate immunity: A contributing factor for asthma., 2001, 108(Issue 2), 157–166.
4. William B. Anderson, M. Robin and Colin I. M, Canadian Journal of Microbiology, A review of drinking water-associated endotoxin including potential routes of human exposure., 2002, 48, 567–587.
5. William B. Anderson, Colin I. Mayfield, D. G.D. and P. M. Huck, Endotoxin inactivation by selected drinking water treatment oxidants, Water Res., 2003, 37(19), 4553–4560.
6. W. W. Eckenfelder Jr, Water Science and Technology Alternative strategies for meeting stringent effluent guidelines, 1994, 29(8), 1–7.

Avian features of CYP 1-3 genes

Kensuke WATANABE, Yusuke Kawai, Minami Kawata,
Yoshinori IKENAKA, Mayumi ISHIZUKA

Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Kita18, Nishi9, Kita-ku, Sapporo, 060-0818, Japan

[Introduction]

Cytochrome P450 (CYP) 1-3 families are xenobiotic metabolizing enzymes, which determines the sensitivity to chemical compounds. CYP-mediating metabolic activities are known to have a wide interspecies difference among animals. In avian species, we previously reported a large interspecies difference of warfarin metabolic activity, which partially explained the drastic difference of LD_{50} among birds.

Although avian CYPs have been tensely studied for the induction by chemical compounds or for the potential as biomarkers of environmental pollution, all the CYP isoforms have not yet been clarified even in chicken or quails. This lack of the information has been obstacles in many aspects, i.e., the extrapolation in birds, and the identification of the dominant isoform in avian xenobiotic metabolism.

In this study, we aimed to clarify all the CYP 1-3 genes of three bird species, i.e., chicken (Gallus gallus), zebra finch (Taeniopygia guttata) and turkey (Meleagris gallopavo), using available genome databases. We revealed the genetic interspecies differences of CYP 1-3 genes among avian and human, by analyzing the evolutionary history and the orthologous relationships. We also determined the dominantly-expressed gene in chicken liver.

[Materials and methods]

We searched for CYP 1-3 genes of chicken, zebra finch and turkey, from the genome databases. The retrieved genes were analyzed for the phylogeny and synteny to clarify the evolutionary relationships among genes. Selected genes, which had been reported to show high expression levels in mammal species, were analyzed and compared for the mRNA expression levels in chicken liver.

[Result and discussion]

We identified 22 to 27 CYP 1-3 genes in avian species, while human possessed 23 genes. Avian species had several unique features compared to mammals. Phylogeny and synteny analysis suggested no clear orthologous relationships of CYP2C genes between avian species and mammals, while most other CYP 1-3 genes had clear correspondence among species. Avian species possessed several uniquely duplicated genes, such as five CYP2AB genes while human possessed only one pseudogene. Lack of CYP2ABFGST cluster was suggested in avian species, which showed avian species do not possess CYP2A or CYP2B genes. These differences suggested that avian species had unique evolutionary history, which may result in different xenobiotic metabolism systems. Among avian species, a duplication of CYP2C23 genes was observed only in chicken. The loci of CYP2C23 gene were not well conserved between avian and human, and even among avian species, which suggested frequent genomic structural variation of CYP2C23 loci.

qPCR analysis showed that CYP2C genes, i.e., CYP2C23a, CYP2C23b and CYP2C45, showed relatively high mRNA expression levels in chicken liver. Although they had been less studied for the enzymatic characters, these isoforms were suggested to be dominant isoforms in avian xenobiotic metabolism.

[Conclusion]

In this study, we established a basic understanding of avian CYP 1-3 genes. Further study is needed to clarify the roles of dominantly-expressed CYPs, and also to understand the interspecies difference among avian species for the better extrapolation.

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Contact

Graduate School of Veterinary Medicine, Hokkaido University

Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818 Hokkaido, Japan

Email：leading@vetmed.hokudai.ac.jp

http://www.vetmed.hokudai.ac.jp/onehealth/