AIM

Persistent organic pollutants (POPs) are groups of synthetically produced organic chemicals that persist in the environment and are highly lipophilic in nature (World Health Organization, 2008). In the Philippines, few reports and studies on POPs and its effect on aquatic and marine organisms are available. Thus, the study aimed to measure the residue levels of POPs in the tissues of Nile Tilapia from two Laguna Lake sites (Los Baños and Santa Rosa) and in agricultural site in Bilar, Bohol in the Philippines.

PROCEDURE

Nile tilapia were collected from February 22 – March 4, 2013 in Sta. Rosa and Los Banos, Laguna, and in Bilar, Bohol, Philippines. The animals were immediately dissected on board after measurement of biometry (body length, body weight, etc.). Organ (liver, spleen, muscle, and kidney) and fat tissue samples were removed, and weight were measured. The subsamples were frozen in liquid nitrogen, transported to Ehime University, Japan, and stored at − 80°C for further analysis. PCBs, PBDEs, and organochlorine pesticides were measured from the lipid and muscle tissue samples.

RESULTS

The analysis and determination of the levels of certain persistent organic pollutants present in the liver samples revealed the presence of certain polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine (OC) metabolites, either in elevated levels and/or minimum levels. Figures 1 - 3 summarizes the observed amounts of these Persistent organic pollutants (POPs) observed from the fish liver samples obtained from the three sampling sites.

Polychlorinated biphenyl Polychlorinated biphenyls are synthetically produced chemicals used as insulating fluids and coolant in electrical equipment, transformers and capacitors due to their volatile nature (United States Environmental Protection Agency, 2015). They can exist in the environment as vapors or settle in the soil and water systems when disposed improperly or by burning of incinerators (US Department of Health and Human Services, 2000). Theoretically, there can be 209 PCB congeners however, only few are largely detectable.

In the present study, it was found out that Bohol (141.419 ± 20.364 mg/g lipid) had the highest concentration of PCBs measured followed by Los Baños (114.857 ± 29.629 mg/g lipid), then Santa Rosa (59.286 ± 3.400 mg/g lipid) as shown in Figure 1. In Bohol, the top three PCB congeners found were CB153> CB118 > CB28. In Los Baños, the top three PCB congeners were CB153 > CB138 >CB180 and in Santa Rosa, CB153 > CB118 > CB28. The most common PCB congeners found among the three sampling sites were CB28, CB138 and CB 153. Boyer et al. (1991) stated the Maximum Contaminant Level (MCL) or the tolerable amount of PCBs in fish is 2 parts per million (ppm) or 0.0002 mg/g. This showed that the observed amount of PCBs from the three sampling sites were significantly higher than the tolerable amount in fish tissues. Because of PCB's ability to resist biodegradation, it is most likely that these compounds would become highly concentrated as it moves higher in the food chain (Agency for Toxic Substances and Disease Registry, 2014). Filipkowska (2013) stated that these PCB congeners (alongside with CB52, CB101 and CB118) were the most used and highly recommended congeners to be measured in biomonitoring and determining total PCBs present. The Agency for Toxic Substances and Disease Registry (2014) stated that PCB congeners with numerous number of conjugated chlorines tends to become more concentrated in the lipid tissues, which might explain why the above-mentioned congeners accumulated in the fishes obtained from the three sampling sites. Statistical analyses showed that PCB content measured between Los Baños and Bohol had no significant difference ($p = 0.276$), while between Santa Rosa and Bohol, there is a significant difference ($p = 0.0225$).

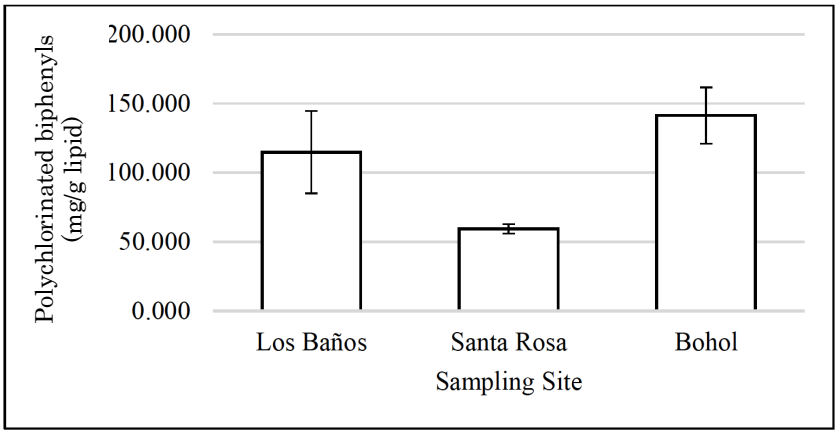

Figure 1. Polychlorinated biphenyls (PCB) concentration in the livers of the Nile tilapia sampled from the three sampling sites. PCB concentrations were highest in Bohol followed by Los Baños and Santa Rosa.

Polybrominated diphenyl ethers

Polybrominated diphenyl ethers (PBDEs) were members of the brominated flame retardant (BFR) compounds which provide plastics and foams the property of being difficult to burn (Agency for Toxic Substances and Disease Registry, 2004). Similar to PCBs, they also exist in the environment as a mixture of congeners. Since they are mixed with the plastics and foams and are not bound to them, they can be easily dissipated in the environment. Gilbert (2014) stated that there are 209 PBDEs identified, which are structurally different on the basis of the number of bromine atoms attached to the central atom. Among these 209 PBDE compounds, three were commercially common formulations: the deca-, octa- and penta- PBDEs.

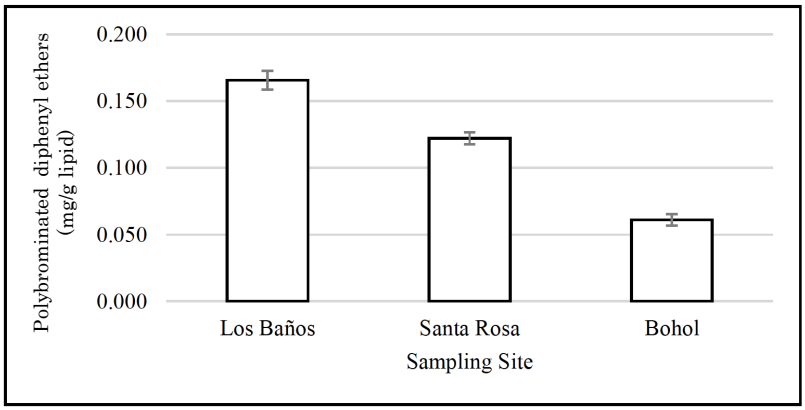

Figure 2. Concentration of polybrominated diphenyl ethers (PBDEs) of the Nile Tilapia liver samples obtained from the three sampling sites. Los Baños fish samples had the highest PBDE concentration followed by Santa Rosa then Bohol.

Figure 2 shows the concentration of PBDE in the liver samples obtained from the three sampling sites. From the data obtained, it was found out that Los Baños had the highest amount of PBDE (18.943 ± 0.883 mg/g lipid). This was followed by Santa Rosa with PBDE content of 5.157 ± 0.256 mg/g lipid. Bohol had the lowest PBDE content accounting to 2.849 ± 0.203 mg/g lipid and 0.061 ± 0.004 mg/g liver. The determined PBDE congener for each sampling site existed in varying levels. The top three congeners found in Los Baños were BDE47 > BDE209 > BDE 155 while in Santa Rosa, the top three PBDEs were BDE209 > BDE47 > BDE49. Lastly, in Bohol, BDE47 > BDE209 > BDE154 where the congeners that highly accumulated in the liver samples. It was observed that BDE47 and BDE209 congeners were commonly found among the three sampling sites. These two congeners were massively produced and were largely found in the aquatic environment (Sha et al. 2015). Statistical analyses showed that the amount of PBDE observed in Los Baños were significantly higher than Bohol ($p = 0.022$). Santa Rosa and Bohol, on the other hand did not show significant differences ($p = 0.197$).

Organochlorines

Organochlorine (OC) compounds are pesticides which are known to be slow-degraders and are very soluble in the lipid tissue (Newman, 2010). Similar to PCBs and PBDEs, they had the ability to bioaccumulate and increase concentration as it goes higher in the food chain. Among fishes, OCs enter the tissues of the fish via diffusion and/or intake of water or sediments. While it easily diffuses through the fish's tissues, the probability of the OCs being diffused outwards of the tissues or being excreted is relatively very low. The reason of which would be due to the high molecular weight and high octanol-water partition coefficient (Kow) of the OCs (Alani et al. 2012).

In the study, each sampling sites had different levels of OC compounds observed (Fig 3). The most notable compounds found to be in very high concentrations were the dichlorodiphenyldichloroethylene (4,4' DDE), oxychlordane and *trans* nona-chlor. In Los Baños, the four highly accumulating OC metabolites were *cis* chlordane (7.286 ± 2.230 mg/g lipid), *cis* nona-chlor (6.187 ± 1.862 mg/g lipid), dichlorodiphenyldichloroethane and (4,4' DDD) (5.700 ± 1.033 mg/g lipid). In Santa Rosa sampling site, 4,4' DDD (19.429 ± 1.307 mg/g lipid), *trans*-nona-chlor (1.657 ± 0.127 mg/g lipid) and 4,4' DDD (2.071 ± 0.144 mg/g lipid) had the highest concentrations. Lastly, in Bohol, six OC compounds were found to be significantly high in Bohol. These were 4' DDE (80.429 ± 12.438 mg/g lipid), oxychlordane (34.143 ± 3.820 mg/g lipid), *trans* nona-chlor (18.957 ± 2.879 mg/g lipid), β-hexachlorocyclohexane (β-HCH) (0.951 ± 0.144 mg/g lipid), hexachlorobenzene (HCB) (0.7614 ± 0.039 mg/g lipid) and α-hexachlorocyclohexane (α-HCH) (0.414 ± 0.040 mg/g lipid). There were no γ-hexachlorocyclohexane (γ-HCH) found among the three sampling sites. In Bohol, despite the duck farming observed to lessen the use of pesticides and herbicides, the measured concentrations of the OC compounds suggests that there were still pesticides used.

In summary, POPs are present in varying concentrations in Nile Tilapias from the Philippines. Nile Tilapias from Los Baños and Bohol had high amounts of PCBs, PBDEs and organochlorines such as oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, 4,4'-DDE, 4,4'-DDD and 4,4'-DDT. Meanwhile, Nile Tilapias in Santa Rosa were contaminated with PCBs and PBDEs and some organochlorines but in lower concentration compared with Los Baños and Bohol. Presence of these in the tissues of the fish may have contributed to the disruption of the pathways of xenobiotic metabolism, antioxidant defense, and glucose and fatty acid metabolism based on earlier studies.

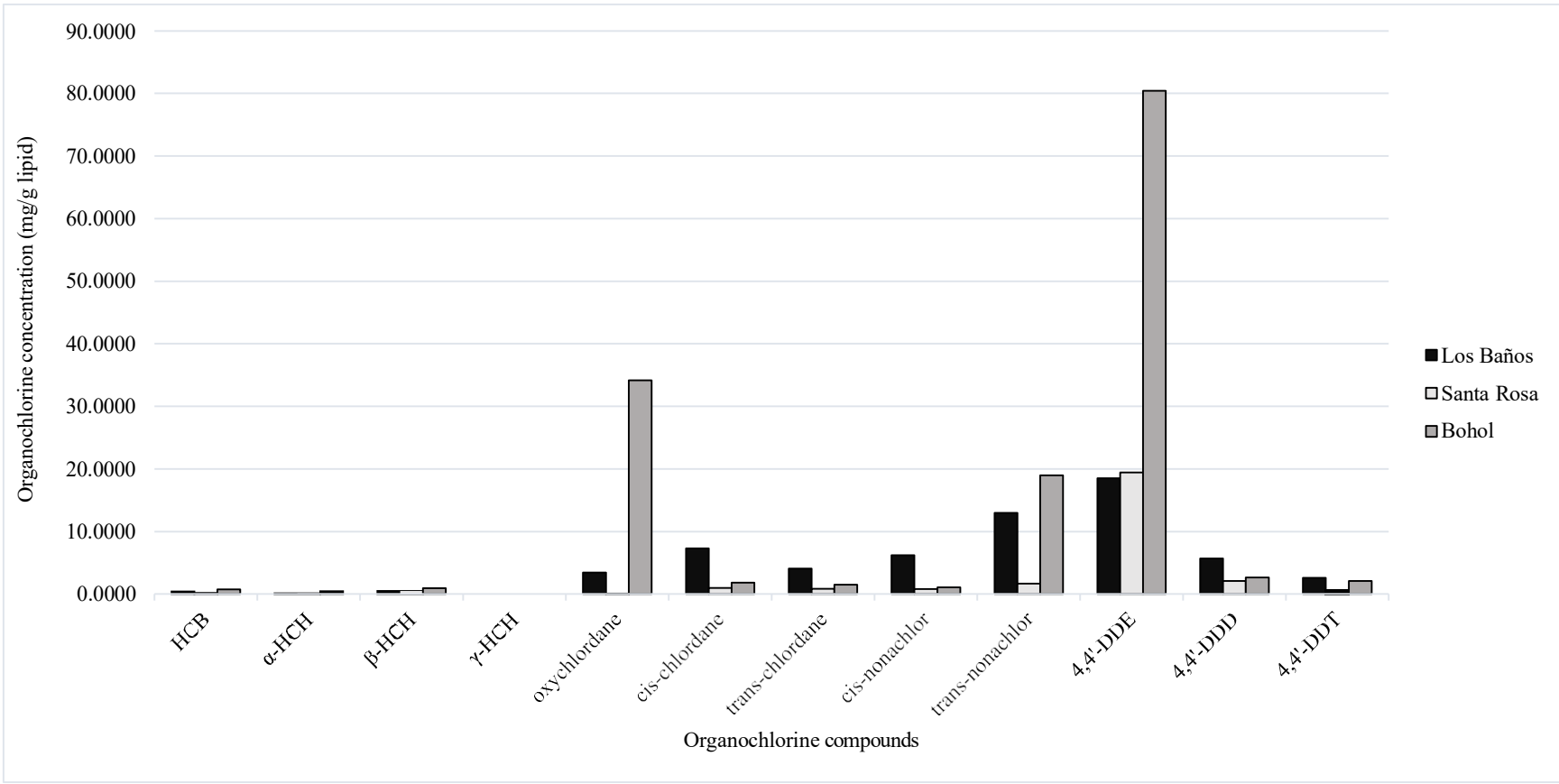

Figure 3. The concentration of organochlorine compounds in the liver tissues of the fish samples obtained from the three sampling sites.

FUTURE PERSPECTIVES

Results from this study will be presented in conferences and published as a journal article. Further studies to analyze and correlate the expression of selected genes involved in different metabolic pathways is recommended. Preliminary *in silico* molecular docking is also recommended to be done to investigate the possible interaction between the known pollutants and Nile tilapia proteins at the atomic level to possibly allow understanding the behavior of small molecules in the binding site of target proteins.

References:

BOYER IJ, KOKOSKI CJ and BOLGER PM. 1991. Role of FDA in establishing tolerable levels for dioxin and PCBs in aquatic organisms. Journal of Toxicology and Environmental Health. 33(1): 93-101

AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY. 2014. Toxic Substances Portal – Polychlorinated biphenyls (PCBs). https://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=140&tid=26.

FILIPOWSKA A. 2013. Determining PCBs in fish and sediment samples related to intercomparison studies. 22(5): 1341-1347

GILBERT S. 2014. Polybrominated diphenyl ethers (PBDEs). http://www.toxipedia.org/pages/viewpage.action?pageId=296.

SHA JJ, WANG Y, LU JX, WANG H, CHEN HM, QI LL and TANG XX. 2015. Effects of two polybrominated diphenyl ethers (BDE-47, BDE-209) on the swimming behavior, population growth and reproduction of the rotifer *Branchionus plicatilis*. Journal of Environmental Sciences. 28: 54-63

NEWMAN MC. 2010. Fundamentals of Ecotoxicology. 3ed. USA: CRC Press. pp 29.

ALANI R, DROUILLARD K, OLAYINKA K and ALO B. 2012. Bioaccumulation of organochlorine pesticide residues in fish and invertebrates of Lagos Lagoon, Nigeria. American Journal of Scientific and Industrial Research. 4(1): 22-30

WORLD HEALTH ORGANIZATION. 2008. Persistent Organic Pollutants (POPs). WHO Training Package for the Health Sector

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 2015. Polychlorinated biphenyls. https://www.epa.gov/sites/production/files/2015- 05/documents/biomonitoring-pcbs.pdf.

US DEPARTMENT OF HEALTH AND HUMAN SERVICES. 2000. Toxicological profile for polychlorinated biphenyls (PCBs). https://www.atsdr.cdc.gov/toxprofles/tp17.pdf.