

Research Article

LEAD BIOACCUMULATION AND THE IMPOSEX EFFECT OF *VOLEMA (PUGILINA) COCHLIDIUM* IN BACOR BAY, PHILIPPINES

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Abstract:

This study assessed the bioaccumulation of total lead and the imposex effect of the *Volema (Pugilina) cochlidium* gastropods in Bacoor Bay, Philippines. The hepatopancreas was assessed for lead concentrations, and measuring the penis length of the affected female organism assessed the occurrence of the imposex effect of the same organism. Results showed that lead bioaccumulated in the hepatopancreas. The prevalence of imposex in the gastropods in Bacoor Bay is 36.67%. Among the female gastropod examined, about 50% showed the imposex effect. The penis length of the imposex-affected female gastropod increases with the increasing tissue lead concentrations. Likewise, the penis length of normal male gastropods increases with the increasing tissue lead concentrations. However, no significant relationships between the lead concentrations in the tissue to that of the penis length of the normal male gastropods ($R=0.12$; $P=0.59$) and the penis length of those affected female gastropods with the imposex occurrence ($R=0.06$; $P=0.74$) were observed. No significant differences were observed on the total lead bioaccumulated in the hepatopancreas of the male, female and imposex-affected females. Continuous biomonitoring must be undertaken to safeguard the aquatic organisms from the deleterious effects it may acquire from its exposure to the harmful heavy metals emanating in the environment.

Keywords: Imposex, Heavy metal, Gastropods, Bioaccumulation

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Introduction

Heavy metals are continuously discharged from natural and anthropogenic sources and are consequently contributing to the pollution of marine environments (Sia Su et al., 2009). Environmental pollution brought about by heavy metals remained to arouse an extensive concern all over the world. Studies have indicated that the continuous exposure of organisms in marine environments to heavy metals may contribute to the accumulation of the metal in the organism's tissue and potentially bring about deleterious effects that may consequently affect the normal developmental processes of the organisms (Vasanthi et al., 2012; Zapata-Vivenes et al., 2012) and induce morphological, histological, and biochemical alterations (Fadel and Gaber, 2007; Sia Su et al., 2013).

Imposex is a reproductive disorder that describes the presence of penis, vas deferens, and seminiferous tubules on the affected female gastropods (Ketata et al., 2008). It is recognized as an induced response from organotin compounds like tributyltin and triphenyltin (Oetken et al., 2004). In recent years, the heightened concern that certain pollutants in the environment, including heavy metals, may disrupt the normal endocrine functioning of exposed organisms. Tin as an important component of the organotin compounds opens the possibility for heavy metals to induce or express the imposex effect in gastropods. A study (Leung et al., 2005) has indicated that metals may have the potential of expressing the imposex effect, but not all heavy metals may have that potential as no direct relationship of metal exposure to the expression of the imposex effect has been established. The plausibility of a link between heavy metal and the reproductive abnormalities creates gaps in our current knowledge.

In the Philippines, there are no studies that looked into the possibility of heavy metals in the environment to the expression of the imposex effect, and there are limited studies pertaining to the occurrence of imposex in gastropods. This present study aims to assess the bioaccumulation of total lead in *Volema (Pugilina) cochlidium* hepatopancreas and to investigate the occurrence of the imposex effect and the relation of the bioaccumulated total lead to the penis length of the male and imposex-affected female gastropods. Results of this study may significantly contribute to our current knowledge, particularly on the occurrence of imposex in *Volema (Pugilina) cochlidium*. Likewise, this study provides baseline information on the relation of metal exposure through the total lead bioaccumulation to the occurrence of the imposex effect.

Materials and Methods

A hired diver collected the gastropod samples at Bacoar Bay. Bacoar Bay is an inlet of the southeastern part of Manila Bay. It is located south of the Cavite Peninsula. Gastropods collected were placed in oxygenated plastic containers with marine water and immediately brought back to the laboratory. Gastropods collected were weighed (g) using a top loading balance, the length (mm) was measured using a Vernier caliper, and the circumference of the body whorl (mm) was measured using a measuring tape.

Gastropods were sexed based on the appearance of the gonads. Male snail consists of a penis and the absence of sperm-ingesting gland, whereas the female snails have a sperm-ingesting gland. An imposex-affected female gastropod is identified as an organism with a penis and sperm-ingesting gland (Fernandez et al., 2002). The penis length (mm) of male and imposex-affected female gastropods was measured using a Vernier caliper. Four quantitative measures were used to assess the occurrence of imposex, namely the prevalence of imposex, percentage of imposex-affected females, relative penis length index (RPLI), and relative penis size index (RPSI) (Astilla et al., 2005; Fernandez et al., 2002).

About a 1-g sample of the hepatopancreas of the *Volema (Pugilina) cochlidium* dissected was ash, and the wet digestion method was used in the analysis of the heavy metal total lead following the standard procedures (APHA et al., 1998) before using the Shimadzu AA-6300 atomic absorption spectrophotometer (Shimadzu Scientific Instruments, Inc., Kyoto, Japan) with a 0.05 ppb detection limit for Pb. Appropriate controls and standards and calibration curves were prepared for the variables tested in the gastropod. The tested samples were read in triplicates. Significant differences in sex (male, female, and imposex-affected female) and the total lead concentrations of the gastropods were tested by means of one-way analysis of variance. Significant differences between the male and the imposex-affected female penis length were tested by means of an independent *t* test. The Pearson's correlation analysis was employed to establish whether a significant relationship exists between total lead concentrations and the penis length of the males and imposex-affected females. A *P* value of 0.05 is considered significant. All statistical analyses were performed using GNU PSPP open software.

Results

Thirty *Volema (Pugilina) cochlidium* snails were collected in Bacoar Bay. Of the total gastropods collected, 8 were males and 22 were females. The mean \pm SEM length, weight, and whorl of the gastropods collected and examined were 68.56 ± 1.78 mm, 48.37 ± 2.42 g, and 102.03 ± 4.81 mm, respectively. The prevalence of imposex in the examined gastropods was 36.67%, where the percentage of imposex-affected females was 50.00%. The mean \pm SEM penis lengths of males and imposex-affected females were 26.13 ± 3.05 mm and 12.91 ± 0.79 mm, respectively. RPLI and RPSI are 49.41% and 12.06%, respectively. A significantly higher penis length of the male gastropods to that of the imposex-affected female gastropods was observed ($t=2.53$; $P=0.021$).

The mean \pm SEM total lead concentration bioaccumulated in the hepatopancreas of the gastropods was 0.59 ± 0.05 ppm. The mean \pm SEM total lead concentrations bioaccumulated by the male, female, and imposex-affected female gastropods examined were 0.71 ± 0.08 ppm, 0.49 ± 0.06 ppm, and 0.59 ± 0.11 ppm, respectively. No significant differences on the total lead bioaccumulated in the hepatopancreas of the male, female, and imposex-affected females were observed ($F=1.124$; $P=0.340$). No significant correlations between the penis length of the male gastropods and total lead concentrations ($R=0.115$; $P=0.593$) and between the imposex-affected female gastropods and total lead

concentrations ($R=0.061$; $P=0.74$) were observed as shown in Figure 1.

Discussion

This was a cross-sectional study, and its scope is limited to assessing the occurrence of imposex, examining the total lead bioaccumulated within the organism, and relating the penis length to the total lead bioaccumulation of the male and imposex-affected females. Although considerable knowledge has been gained over the effect of organotin in the occurrence of imposex among gastropods, it is unclear whether other pollutants may bring about the potential of endocrine-disrupting effects. No case has considered the possibility that total lead may have an influence in the occurrence of imposex. This study would like to generate information exploring the possibility of heavy metals, particularly total lead. The *Volema (Pugilina) cochlidium* are important gastropods used in the craft industry and are eaten as a delicacy in the country (Chan, 2009). This study has shown that imposex occurs among the *Volema (Pugilina) cochlidium* gastropods. A previous study (Swennen et al., 2009) has also shown that imposex can occur in these gastropods. The imposex occurrence usually affects the female gastropod species as a penis is observed in the imposex females. The penis length of the male gastropods is observed to be significantly greater than the penis length of the imposex-affected females. A previous study (Power and Keegan, 2001) corroborates with our findings where the penis length of the normal male gastropods is longer than the imposex-affected females. A study by Horiguchi (2006) indicated that the penis length of the male gastropods is longer due to the male sex hormones responsible for inducing the development of the secondary sexual characteristics such as the penis.

Considerable amounts of total lead bioaccumulated in the hepatopancreas of the gastropods examined. The organisms examined showed no significant differences in terms of the total lead bioaccumulated. However, intraspecific differences in terms of the total lead bioaccumulated in the hepatopancreas were observed. It is likely that the intraspecific differences in the total lead concentrations within the organism may be attributed to the organism's uptake strategy. Some organisms may either be a strong

or a weak accumulator of the total lead, and the accumulation patterns of the metal vary depending on the regulatory mechanism (Sarkar et al., 2008) and metabolic activity of the organism (Yap et al., 2009). The penis length and the total lead concentrations were tested for correlation, and no significant relationship can be established. It is worth noting though no significant correlation exists, the penis length increases as the total lead concentrations consequently increases. The limitation in the samples obtained in the study may be contributory to the insignificant relationship; however, a study (WHO/UNEP, 2013) has indicated that there is still a weak relationship that exists between linking metals from the endocrine disruption effects like the imposex occurrence in the gastropods.

Conclusion

Lead bioaccumulated in the hepatopancreas of the gastropods examined. A low prevalence of imposex in the gastropods in Bacoor Bay was observed. The penis length of the male gastropods was longer than the imposex-affected female gastropods. The penis length of normal male and imposex-affected female gastropods increases with the increasing tissue lead concentrations. However, no significant relationships between the lead concentrations in the tissue to that of the penis length were observed. Considerable biomonitoring is required to facilitate our understanding on how heavy metals may potentially influence the occurrence of endocrine disrupting effects.

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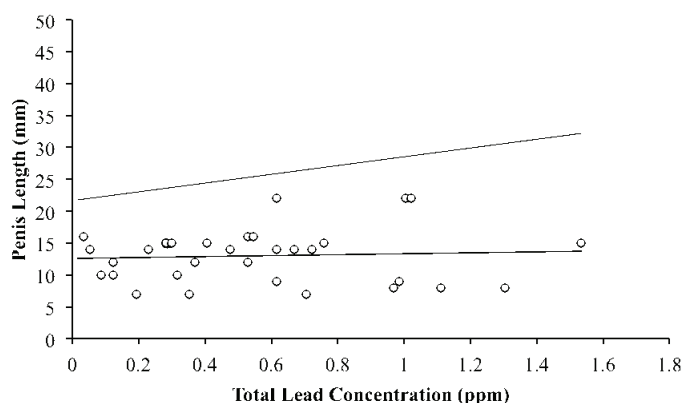


Figure 1: Total lead concentration and penis length relationship of the male and imposex-affected female *Volema (Pugilina) cochlidium* in Bacoor Bay, Philippines.

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