POST-SIEGE GENOTOXIC HAZARDS IN LAKE LANAO, PHILIPPINES BY MICRONUCLEUS ASSAY

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ABSTRACT

Massive war pollutants brought by Marawi Siege in the Philippines have contaminated the city environs and the surrounding ancient Lake Lanao. Munition residues including heavy metals are reportedly genotoxic hence this study was conducted to determine the post-siege genotoxic hazards posed by the munition pollutants through micronucleus (MN) assay on the slow-moving edible gastropod snails *Vivipara angularis* Muller (locally known as "suso") thriving in the lake. MN, a biomarker of genotoxicity was examined and counted in the hemocytes of exposed juvenile and adult snails. Results revealed that MN frequencies (no. of MN/1000 hemocytes) were significantly higher in snails sampled in Lake Lanao lakeshores than in the reference site Lake Dapao. Among adult samples, there was a decreasing trend of MN frequencies with increasing distance from lakeshore fronting warzone 'ground zero' where the battle was heaviest (site A) to sampling sites away from it: sites B, C, and D (Lake Dapao) which are 8.15 km, 24.41 km, and 34.45 km, respectively. Moreover, varied patterns of micronucleation were observed between age groups and sites, i.e., in all sites except C, adults had greater MN counts than juveniles which were significant in site A only. It is a generally recognized observation that MN frequency increases with age; unexpectedly juveniles displayed significantly higher MN counts than adults in site C. The elevated MN frequencies in the snail hemocytes exposed to war pollution could be attributed to genotoxic munition residues eroded and washed into the lake water. Moreover, heavy metals which are common components of weaponries were also detected in the snail muscles, although at concentrations within safe levels but continued consumption may be cautioned to avoid biomagnification. Other genotoxins must be present in site C other than munition residues predisposing the juvenile snails. The results are baseline data on the MN frequencies in *V. angularis* exposed to war pollutants in Lake Lanao which need further investigation.

Keywords: genotoxicity, lake lanao, marawi siege, micronucleus assay

INTRODUCTION

The devastating Marawi siege, an armed conflict that lasted for five months in 2017 was the longest urban warfare in the Philippines, polluting the air, soil, and water environment and destroying biodiversity. Adding to the insurmountable damage are the numerous ill-health effects that could be suffered by the inhabitants, especially humans. High-tech ammunitions utilized both by the terrorist and the government troops included bombs, mortars, grenades such as rocket-propelled grenades (RPG), hand-held, anti-tank grenade launchers, and the likes aside from the conventional rifles, improvised explosive devices (IEDs), and carpet, and aerial bombs (Kapoor, 2017, Pareño, 2017, & Miller, 2017). These are depots of toxic pollutants such as high-melting explosives (HMX), hexahydro-1,3,5-trinitro-1,3,5- triazine (RDX), trinitrotoluene (TNT), and heavy metals with potential genotoxic threats such as lead (Pb), arsenic (As), nickel (Ni), copper (Cu) and zinc (Zn), strontium (Sr), magnesium (Mg), and barium (Ba), mercury (Hg), cadmium (Cd) antimony (Sb), iron (Fe) and chromium (Cr) (IARC 2021, Broomandi *et al.* 2020, Skalny *et al.* 2021, Chatterjee *et al.* 2017). Known components of weaponries listed in IARC (2021) are established mutagens and carcinogens causing cancers affecting the respiratory tract, liver, bone, blood, and other parts of the human body. The magnitude of weaponries used during the siege

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heavily contaminated the city environs and its surrounding lake with genotoxic pollutants posing health hazards to the local inhabitants as well as to the organisms thriving in the second largest lake in the country and one of the ancient lakes in the world, that is, Lake Lanao, in the province of Lanao del Sur (LDS).

Genotoxicity is the ability of chemicals to damage the genetic information of the cells thereby resulting in mutations and eventual carcinogenesis (Seukep *et al.*, 2014). One reliable method to assess the genotoxic potential of some substances (such as heavy metals, pesticides including gamma radiation), is the micronucleus (MN) assay and it is used in various test organisms including man, mice, land and aquatic snails, mussels, and fishes (Silva *et al.* 2011, da Silva 2013, Bolognesi & Fenech 2012, Gentile *et al.* 2012, Luzhna & Kovalchuk, 2013, Beedanagari *et al.* 2014, da Rocha *et al.* 2016, Hayashi 2016, El Safty *et al.* 2018, Nikdehghan *et al.* 2018, and de Vasconcelos Lima 2019, Fenech *et al.* 2020). The present study made use of the freshwater gastropods *Vivipara angularis* Muller (locally known as "suso") in the MN assays instead of *B. glabrata* because they are abundant in Lake Lanao and utilized as food by the Meranaw (people of the lake). The strategic use of the snails in MN assays is anchored on their sedentary lifestyle crawling on soil and rock substratum. They use their gills as filter feeding apparatus and respiratory organs and are the first barrier to potential contaminants and the ideal target for genotoxic studies. They are tolerant to heavy metals and bio-accumulate them (Baroudi *et al.*, 2020). Importantly, snails are less mobile compared to other organisms like fishes, thus they cannot easily escape from toxic substances present in their habitat. This study therefore primarily aimed to estimate the post-siege genotoxic hazards posed by munition residues in the lake through micronucleus assay using the *V. angularis* Muller thriving in the lake. It also examined the micronucleation pattern among exposed snails sampled in Lake Lanao and non-exposed snails in the reference site, Lake Dapao, LDS. Factors like the distance of the sampling sites from the war zone and the age groups of the sampled snails were considered in the MN assays.

MATERIALS AND METHODS

Study Design and Sampling Sites

Descriptive cross-sectional study design and the purposive sampling collection (accessibility of the sites and age groups of snails) were conducted in assessing the genotoxicity of the war residues by MN assay in the hemocytes of the exposed freshwater gastropods *V. angularis* of Lake Lanao. Figure 1 shows the map of the Philippines and the sampling sites, location coordinates, and the distance between sites. The first site was the lakeshore fronting Marawi City marked as ground zero (Site A) where the battle was fiercest and where residues of war were substantially deposited; the second (B) and third (C) sites were 8.15 km and 24.41 km away from site A. These three sites represent munition residues exposed sites. Site D is the reference lake (non-exposed site), Lake Dapao, LDS, 34.45 km from site A. The coordinates were determined using Garmin(R), GPSMAP 64x, and the actual sites were generated by plotting the coordinates through Google Earth. The distance of the sites from site A was calculated through Movable Type Scripts (https://www.movable-type.co.uk/scripts/latlong.html).

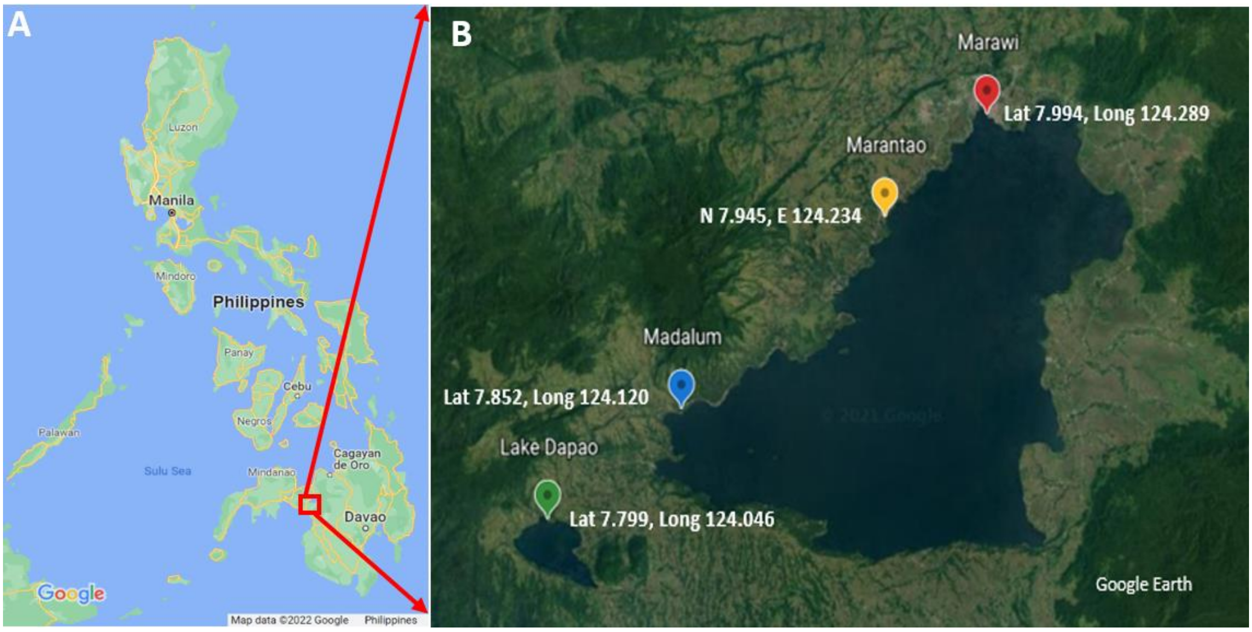

Figure 1A) Map of the Philippines showing the location of Marawi City and Lake Lanao in the red inset. B) Sampling sites (balloons) in Lake Lanao shores and their corresponding coordinates: red, site A, ground zero in Marawi city; yellow, site B; blue, site C; and green, reference site (Lake Dapao)

Test Organisms, Collection, and Identification

The test organisms were *V. angularis* Muller (class Gastropoda, phylum Mollusca, Fig. 2) Color, description, and identification of the snails were based on the work of Pagulayan & Cepillo (1991), Moneva *et al.* (2012), and Torres *et al.* (2014). Sub-adult and adult *V. angularis* have 4 whorls with shell colors ranging from dark greenish brown or greyish yellow. It is striated but not with a hammer pattern. Immature or juvenile *Vivipara* is usually up to 14-16 mm while adult length ranges from 18-31 mm. The height of the shell is 25–35 mm. The width of the shell is 20–26 mm. The shell has 5.5-6 weakly convex whorls. The last whorl is relatively large compared to that of other *Vivipara* species. The umbilicus is narrow.

Vivipara snails were randomly collected within 100 m along the lakeshores and 5-50 m perpendicular to the shores. At least 20 juveniles and 20 adult snails were purposively handpicked, systematically washed to remove mud, placed separately in containers with lake water, and transported to Research Laboratory, Biology Department, Mindanao State University, Marawi Campus. They were temporarily kept in the aerated aquaria with lettuce leaves. Micronucleus assay proceeded immediately as soon as they were brought to the laboratory.

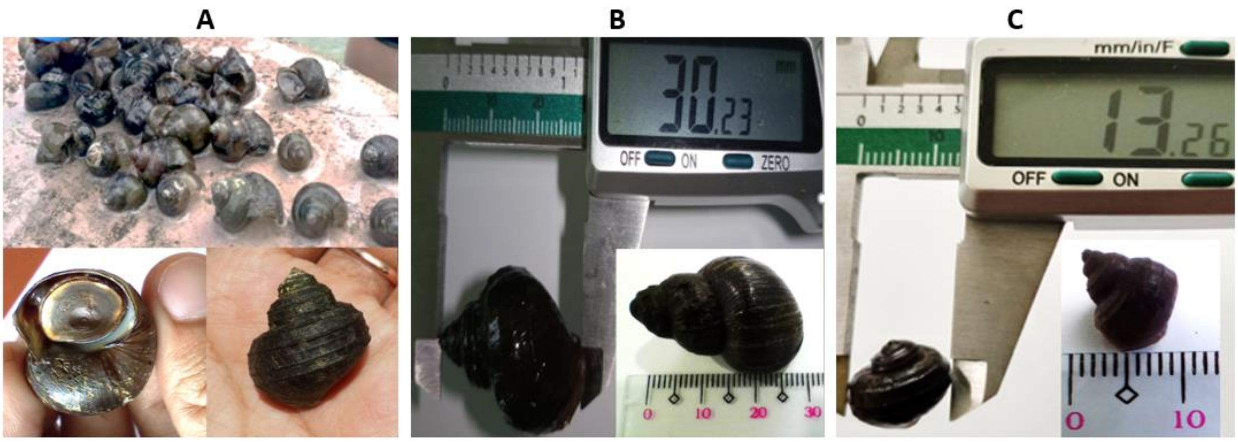

Figure 2 *Vivipara angularis* Muller snails collected from Lake Lanao (A); adults (B); and juvenile (C) samples

Micronucleus Assay

Twenty (n=20) *V. angularis, i.e.,* ten (n=10) juvenile and ten (n=10) mature gastropods from each site, including the reference site, were randomly selected, and processed for MN assay following the work of da Silva *et al.*, 2013, de Vasconcelos Lima, 2019. To collect 0.1 ml of hemolymph, the operculum of the snail was gently tapped by a micropipette tip triggering the snails to retract and release hemolymph. The hemolymph was smeared into clean slides and air-dried for 10 min, followed by adding 0.1 ml of Ringer's solution, and 0.1 ml of the 10 mM EDTA solution. The smears were placed in a moist chamber for 30 min before fixing with glutaraldehyde for 5 min, rinsed with Ringer's solution, stained with Giemsa solution (Giemsa Set, Medic) for 7 min then finally washed with distilled water and air-dried. Each slide was viewed for blind scoring of MN under a phase contrast microscope (Olympus, CKX53). Blind scoring was employed. With the help of a hand-held counter, 1000 hemocytes (cells) were examined per individual gastropod, such that, (10 adults + 10 juveniles) x 1000 cells = 20,000 cells were examined per site. Only hemocytes with an intact nucleus and cellular distinction were considered and the MN was identified and counted when its diameter was smaller than one-third the size of the nucleus, its color and texture should be similar to that of the nucleus and not in direct contact with the main nucleus. A digital camera (XCAM1080PHB, Toup Tek, China) was used to aid in counting the MN in the monitor and in taking photomicrographs.

Detection of Heavy Metals in the Snail Muscles

Muscles from adults and juvenile snails (150 g x 3 replicates) from each sampling site were harvested and immediately analyzed for heavy metals utilizing atomic absorption spectrophotometry (AAS) for Cd, Cr, and Pb, silver diethyldithiocarbamate-colorimetric (modified) for As and cold vapor AAS for Hg.

Statistical Analysis

The resulting MN frequencies between exposed and non-exposed sites and between juvenile and adult samples were analyzed statistically using Statistical Package for the Social Sciences (SPSS) for Windows. Using the Shapiro-Wilks Test, the means of the MN frequencies for sites and age groups were found not normally distributed, hence Kruskal-Wallis and the Mann-Whitney U tests were used to analyze the differences of the MN frequencies in various sites and age groups.

RESULTS AND DISCUSSION

Micronucleation by Sampling Sites

Micronucleation (Figure 3) was observed in the hemocytes of *V. angularis* in all sampling sites. Table 1 shows the mean frequencies of the MN per site and the variation was highly significant by the Kruskal Wallis test, [$P=.000$, $H(3, N=80)=28.0326$]. Indeed, MN frequencies were significantly higher in Lake Lanao sites compared to the reference site, Lake Dapao. The highest mean was recorded in site A, the ground zero where the encounter was heftiest, and the lowest in site D, the reference lake. A pairwise comparison of the mean ranks by the Mann-Whitney U test illustrated that site A was significantly higher than other sites ($P<0.5$) except for site C ($P=0.37346$). Conversely, the reference site was significantly lower than the rest of the sites ($P<.05$).

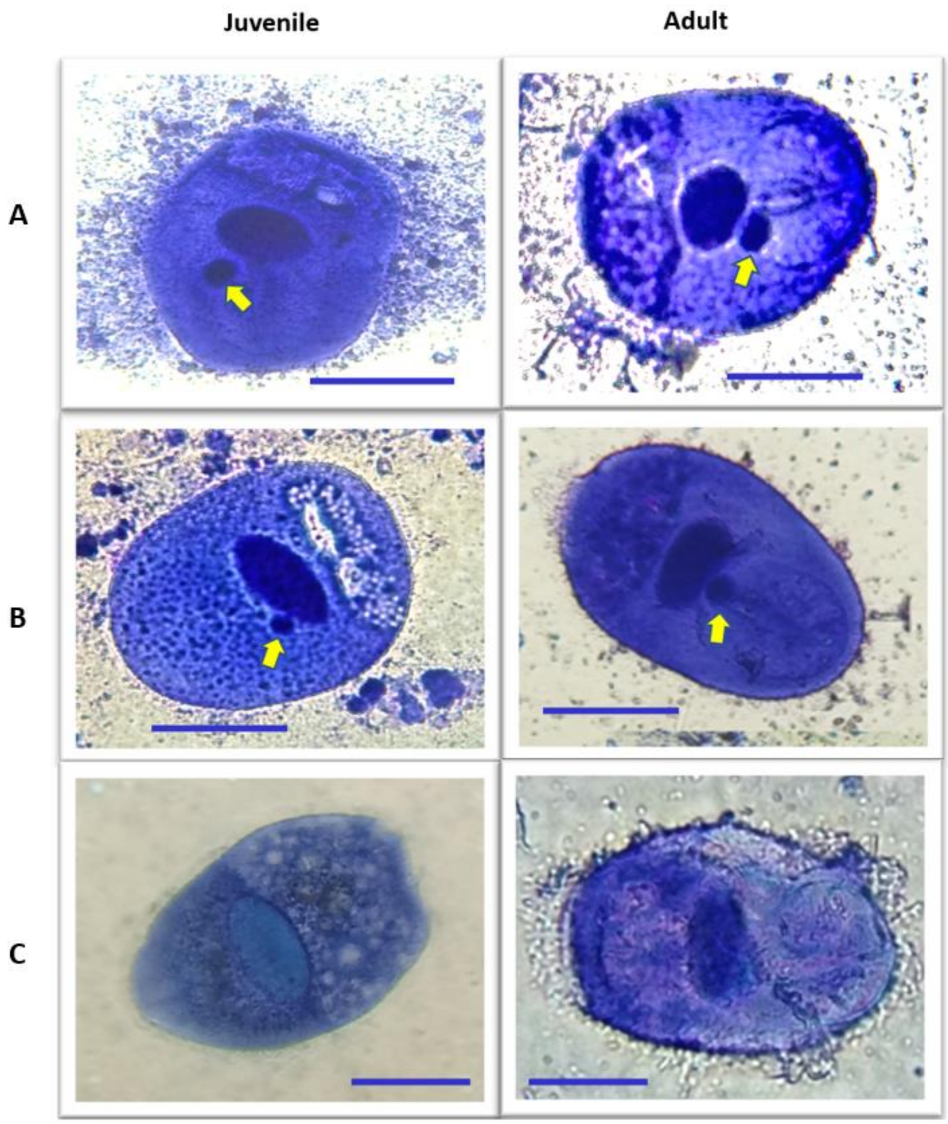

Figure 3A-B Photomicrographs of *V. angularis* hemocytes showing micronuclei (yellow arrows) in adult and juvenile samples. C, hemocytes without micronuclei are shown in C. Scale bars, 5μm

Table 1 MN frequencies in the hemocytes of adult and juvenile *V. angularis* sampled from the different sites in Lake Lanao and in Lake, Dapao, Philippines

Sampling Sites	MN frequency* Mean			SD		
	Juvenile	Adult	Total	Juvenile	Adult	Total
Site A	3.6	10	6.8	1.776	6.6	5.736
Site B	2.4	2.4	2.4	2.319	1.506	1.903
Site C	6.8	3.1	4.95	3.938	2.885	3.859
Site D	1.5	0.8	1.15	1.65	0.422	1.226
Total	3.58	4.08	3.82	3.21	5.025	4.197

*Frequency values (MN observed per 1000 hemocytes)

Spontaneous micronuclei formation can happen in any eukaryotic cell as it divides. It is often brought about by a broken chromosome fragment or rarely an entire chromosome that lags and remains outside the nucleus after cell division (Beedanagari *et al.* 2014). These are extra-nuclear bodies that contain damaged chromosome fragments or whole chromosomes that are not incorporated into the nucleus after cell division (Luzhna & Kovalchuk 2013). It can be induced by defects in the cell repair machinery and accumulation of DNA damages and chromosomal aberrations. This explains why MN was also observed in the reference lake although the count was significantly lower than the exposed sites.

As mentioned earlier, MN frequencies were shown to increase in cells of various organisms that were exposed to carcinogens and genotoxins, thus, MN assay is one of the most widely used genotoxicity biomarkers, providing an efficient measure of chromosomal DNA damage. The elevated MN counts in site A could be attributed to genotoxic chemicals most probably from war pollutants washed into the lake water where the snail samples were thriving. It must be noted that site A is fronting "ground zero" in Marawi where the encounter was densest. IARC (2021) provided a list of eroded components of munitions that are carcinogenic including heavy metals, such as As, Hg, Cd, Cr, and Pb. These heavy metals were detected in the muscle tissues of *V. angularis* from sampling sites (Table 6), and Jalova *et al.* (2021) also reported heavy metals in commonly consumed fishes sampled from Lake Lanao, though the levels detected were mostly lower than the standard limits (WHO/FAO 2010, 2011, Factor *et al.* 2012, Perelonia *et al.* 2017, and Gbogbo *et al.* 2017).

A closer look at Table 2 revealed that a higher concentration of Ar, (0.27 mg/L) from snail muscles was detected in site A, which was higher than the national acceptable limit in drinking water set at 0.010 mg/L, although the safe limit for mollusk as food is set at 1mg/kg. Nevertheless, this detection may call for some concerns due to the continued consumption of the snails by the locals because this trace metal, aside from being highly toxic and carcinogenic (Apostol *et al.* 2022), can biomagnify along with other toxic pollutants, that is, to increase in concentration in the living organisms successively at higher levels in the food chain (Hepp 2017).

The study by Sanderson *et al.*, (2017), reported that HMX and trace levels of RDX explosives compounds were found in the fiddler crabs from the live impact area (LIA) in the island of Vieques in Puerto Rico which also recorded higher levels of some metals such as arsenic. Moreover, the study by Dong *et al.* (2019) also published that arsenic-exposed human populations through drinking contaminated water had increased micronucleus formation from the buccal cells, lymphocytes, and urothelial cell samples. Moreover, Gbogbo *et al.* (2017) detected levels of arsenic in freshwater fish and shellfish from 0.2 to 2.2 mg/L in Ghana rivers and recommended in their study that the maximum quantities of the organisms considered safe for consumption ranged from 375 to 5250 g per week.

Table 2 Mean concentration of As, Cd, Cr, Pb, Hg (mg/L) detected in muscle tissues of *V. angularis* collected from the different sampling sites

Sampling Sites	Heavy Metals (mg/L)				
	Arsenic (As)	Mercury (Hg)	Cadmium (Cd)	Chromium (Cr)	Lead (Pb)
Site A	0.27	≤ 0.01*	≤ 0.025*	≤ 0.025*	0.350
Site B	≤ 0.025*	≤ 0.01*	≤ 0.025*	0.893	0.647
Site C	≤ 0.025*	≤ 0.01*	≤ 0.025*	0.92	0.507
Site D	≤ 0.025*	≤ 0.01*	≤ 0.025*	≤ 0.025*	0.54

*Machine reporting limit

Micronucleation by age groups and increasing distance from ground zero

Figure 1 shows the relative distance of the sampling sites from site A where the fiercest fight occurred. Looking at Figure 4, adult snails obviously displayed a decreasing trend of MN frequencies with increasing distance from site A but not among the juvenile samples. Comparing the MN counts between age groups, it is clearly seen that the exposed adults in site A marked significantly high MN frequencies compared to their juvenile counterparts (Table 3). These results implied that the genotoxic contaminants in these sites affected the adult snails more than the juvenile samples. It makes sense knowing that the average life expectancy of freshwater snails is 3 to 5 years (https://foliargarden.com/ life-expectancy-of-a-freshwater-snail/), and some of the adult samples must have already been at the site during the siege and thus were the most exposed to war pollutants at the highest magnitude which may have resulted in DNA damage as indicated by higher MN frequency compared to the juveniles.

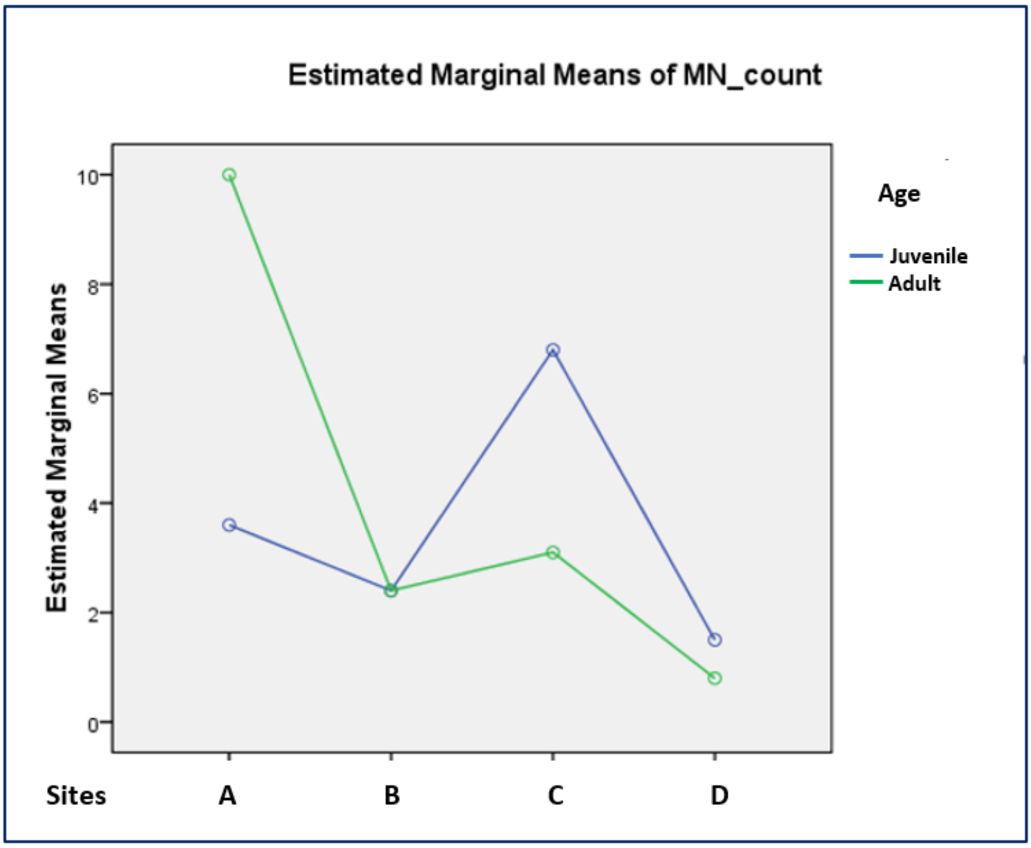

Figure 4 Profile plots of the estimated marginal means of MN frequencies in different sites and age groups showing a downward trend among adults from sites A, and B to D, except in site C which showed an unexpected increase

Table 3 Pairwise comparison of MN frequencies between age groups at different sites by Mann-Whitney U

Sites	Comparison of MN frequencies between juvenile and adult	
	Sig. level	Remarks
Site A	0.011	Significantly different (adult has higher count)
Site B	0.853	Not significantly different
Site C	0.023	Significantly different (adult has lower count)
Site D	0.579	Not significantly different

On the other hand, in site C, which is 24.41 km away from site A, a different MN pattern was observed wherein juveniles displayed higher MN counts than adults. These results were unexpectedly alarming and need further studies. This must be attributed to some genotoxic substances other than the munition residues due to war which particularly predisposed the young snails. One possible explanation is the nearby brassware industry which utilizes brass, a metal alloy primarily composed of Cu and Zn. Copper alloy with a higher amount of Cu is known to cause 'contact killing', possibly via redox cycling and production of reactive oxygen species (ROS) groups that can cause lethal damage to cell membranes and oxidative damage to DNA (Grass *et al.* 2011 and Bille *et al.* 2013). DNA has a higher affinity to Cu2+ which may affect the integrity of DNA and the normal process of DNA replication and transcription (Govindarajua *et al.* 2013) and consequently micronucleus formation (Nikdehghan *et al.* 2018)Substantial evidence has shown the possible genotoxicity of Cu and Zn in aquatic organisms. Moreover, looking back at Table 2, Cr was detected highest in site C although it was closely followed by site B. The study by El Safty *et al.* (2018) demonstrated that Cr- and Ni-exposed workers of the electroplating industry had increased MN signifying cytogenetic damage caused by these heavy metals.

The observed MN frequencies in this study are comparable to the works of Silva *et al.* (2011) and de Vasconcelos Lima (2019) who tested the genotoxicity of gamma radiation and heavy metals and domestic sewage sludge, respectively against freshwater snails *B. glabrata* using the MN assay. The assay was also utilized by da Silva *et al.* (2013) when they tested tobacco leaves in the land snails *Helix aspersa*. Their results considered MN assays as a reliable parameter to measure genotoxic hazards in the environment using snails. Although the MN frequencies in the present study are baseline data to approximate genotoxicity in the munition-laden Lake Lanao using *V. Angularis*. Further studies must be conducted to verify the genotoxic chemicals in crucial sites.

CONCLUSION

Post-siege genotoxic hazard in Lake Lanao, LDS, Philippines brought by Marawi Siege in 2017 was assessed through MN assay using V. angularis snails sampled from different sites in Lake Lanao and in the reference site Lake Dapao. Results demonstrated a higher MN frequency in snails sampled from munition-exposed sites in Lake Lanao lakeshores than in the reference site, Lake Dapao. Among the adult samples, there was a decreasing trend of MN frequencies with increasing distance from lake shore fronting ground zero at Marawi City where the siege was heaviest. A remarkable difference was also found in the MN frequency patterns between the age groups. In all sites, except site C, adults had higher MN frequencies than juveniles and the difference was significant in site A. Moreover, it is generally accepted that MN frequency increases with age, but unexpectedly, MN frequencies were significantly higher among the juvenile samples than adult samples in site C. The post-siege genotoxic hazards possibly posed by the war pollutants in Lake Lanao near ground zero cannot be overlooked. Local leaders must be informed of the results for possible interventions to protect the health and well-being of the populace around Marawi and Lake Lanao.

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REFERENCES

Alborz A. 2013. Environmental characteristics and prevalence of birth defects among children in post-war Iraq: implications for policies on rebuilding the Iraqi education system. Med Confl Surviv 29(1):26-44. https://doi.org/0.1080/13623699.2013.765197

Apostol GLC, Valenzuela S, Seposo X. 2022. Arsenic in Groundwater Sources from Selected Communities Surrounding Taal Volcano, Philippines: An Exploratory Study. Earth 3: 448-459. https://doi.org/10.3390/earth3010027

Baroudi, F., Al-Alam, J., Fajloun, Z., & Millet, M. (2020). Snail as sentinel organism for monitoring the environmental pollution; a review. *Ecological Indicators*, *113*, 106240. https://doi.org/10.1016/j.ecolind.2020.106240

Beedanagari S, Vulimiri S, Bhatia S, Mahadevan B. 2014. Chapter 43 – Genotoxicity biomarkers: Molecular basis of genetic variability and susceptibility. In: Gupta R, editors. Biomarkers in Toxicology, Academic Press. p 729-742. https://doi.org/10.1016/B978-0-12-404630-6.00043-9.

Bille T, Grimes M, Podini D. (2013). Copper Induces DNA damage on unfired brass cartridge casings. https://www.promega.com/-/media/files/resources/conference-proceedings/ishi-24/poster-abstracts/21-daniele-podini.pdf . Retrieved May 5, 2021.

Bolognesi C, Fenech M. 2012. Mussel micronucleus cytome assay. Nat Protoc 7: 1125-1137. https://doi.org/10.1038/nprot.2012.043.

Broomandi P, Guney M, Kim JR, Karaca F. 2020. Soil contamination in areas impacted by military activities: A critical review. Sustainability 12(21): 9002.

Camama CG, Torres MA, Manting MM, Gorospe JG, Demayo CG. 2014. Compartmentalization in the shells of four lake populations of *Vivipara angularis*. Ann Biol Res 2014, 5(3): 16-21.

Chatterjee S, Deb U, Datta S, Walther C, Gupta DK. 2017. Common explosives (TNT, RDX, HMX) and their fate in the environment: Emphasizing bioremediation. Chemosphere 184:438-451. https://doi.org/10.1016/j.chemosphere.2017.06.008

da Rocha SM, da Rocha CAM. 2016. Micronucleus test in bivalve mollusks as an important tool for xenobiotic exposure risk assessment. Acta Fish Aquat Res 4(1):70-79. https://doi.org/10.2312/ActaFish.2016.4.1.70-79

da Silva FR, Erdtmann B, Dalpiaz T, Nunes E, Ferraz A, Martins, TL, Dias J F, da Rosa DP, Porawskie M, Bona S, da Silva J. 2013. Genotoxicity of Nicotiana tabacum leaves on Helix aspersa. Genet Mol Biol 36(2):269-275. https://doi.org/10.1590/S1415-47572013005000020

de Vasconcelos Lima M, de Siqueira WN, Silva HAMF, de Melo Lima Filho J, de França EJ, de Albuquerque Melo AMM. 2019. Cytotoxic and genotoxic effect of oxyfluorfen on hemocytes of *Biomphalaria glabrata*. Environ Sci Pollut Res Int (4):3350-3356. https://doi.org/10.1007/s11356-018-3848-3

Dong J, Wang JQ, Qian Q, Li GC, Yang DQ, Jiang C. 2019. Micronucleus assay for monitoring the genotoxic effects of arsenic in human populations: A systematic review of the literature and meta-analysis. Mutat Res Rev Mutat Res 780: 1-10.

Earth 2022, 3, 448–459 Arsenic in Groundwater Sources from Selected Communities Surrounding Taal Volcano, Philippines: An Exploratory Study. https://doi.org/10.3390/earth3010027

El Safty AMK, Samir AM, Mekkawy MK, Fouad MM. 2018. Genotoxic Effects Due to Exposure to Chromium and Nickel Among Electroplating Workers. Int J Toxicol 37(3): 234-240. https://doi.org/10.1177/1091581818764084

Factor CJB, de Chavez ERC. 2012. Toxicity of Arsenic, Aluminum, Chromium and Nickel to the Embryos of the Freshwater Snail, Radix quadrasi von Möellendorf 1898. Philipp J. Sci 141 (2): 207-216.

Fathi RA, Matti LY, Al-Salih HS, Godbold D. 2013. Environmental pollution by depleted uranium in Iraq with special reference to Mosul and possible effects on cancer and birth defect rates. Med Confl Surviv 29(1): 7-25. https://doi.org/10.1080/13623699.2013.765173

Fenech M, Knasmueller S, Bolognesi C, Holland N, Bonassi S, Kirsch-Volders M. 2020. Micronuclei as biomarkers of DNA damage, aneuploidy, inducers of chromosomal hypermutation and as sources of pro-inflammatory DNA in humans. Mutation Research/Reviews in Mutation Research 786: 108342. https://doi.org/10.1016/j.mrrev.2020.108342

Gbogbo F, Otoo SD, Asomaning O, Huago RQ. 2017. Contamination status of arsenic in fish and shellfish from three river basins in Ghana. Environ Monit Assess 189(8): 400. https://doi.org/10.1007/s10661-017-6118-9.

Gentile N, Mañas F, Bosch B, Peralta L, Gorla N, Aiassa D. 2012. Micronucleus assay as a biomarker of genotoxicity in the occupational exposure to agrochemicals in rural workers. Bull Environ Contam Toxicol. 88(6): 816-22. doi: 10.1007/s00128-012-0589-8

Govindaraju M, Shekar HS, Sateesha SB, Vasudeva Raju P, Sambasiva Rao KR, Rao KSJ, Rajamma AJ. 2013. Copper interactions with DNA of chromatin and its role in neurodegenerative disorders. J Pharm Anal. 3(5): 354-359. doi: 10.1016/ j.jpha.2013.03.003.

Grass G, Rensing C, Solioz M. 2011. Metallic copper as an antimicrobial surface. Appl Environ Microbiol 77(5): 1541-1547. https://doi.org/10.1128/AEM.02766-10.

Hans Sanderson, Patrik Fauser, Ricky Steven Stauber, Jesper Christensen, Per Løfstrøm & Thomas Becker (2017) Civilian exposure to munitions-specific carcinogens and resulting cancer risks for civilians on the Puerto Rican island of Vieques following military exercises from 1947 to 1998, Global Security: Health, Science and Policy, 2:1, 40-61, DOI: 10.1080/23779497.2017.1369358

Hayashi M. 2016. The micronucleus test—most widely used in vivo genotoxicity test—. Genes and Environ 38(1): 1-6. https://doi.org/10.1186/s41021-016-0044-x

Hepp LU, Pratas JA, Graça MA. Arsenic in stream waters is bioaccumulated but neither biomagnified through food webs nor biodispersed to land. Ecotoxicol Environ Saf 139: 132-138. https://doi.org/10.1016/j.ecoenv.2017.01.035

International Agency for Research on Cancer (IARC) 2021. IARC monographs on the identification of carcinogenic hazards to humans [Internet]. Int. Agency Res. Cancer. URL https://monographs.iarc.who.int/list-of-classifications (accessed 3.23.21).

Jalova MC, Lomantong AD, Calibo LG, Camarin MM. 2021. Assessment of Heavy Metals Found in Commonly Consumed Fishes from Lake Lanao, Philippines. Isr J Aquac Bamidgeh 73: 1-9. https://doi.org/10.46989/001c.24939.

Kapoor K. 2017. Snipers, bombs, mortars-Philippines troops battle against Islamists. World News. [accessed 2017 Dec 29]. Available at: https://sg.news.yahoo.com/snipers-bombs-mortars-philippine-troops-battle-against-islamists-125808644.html

Luzhna L, Kathiria P, Kovalchuk O. 2013. Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. Front Genet 4:131. https://doi.org/10.3389/fgene.2013.00131

Moneva CS, Torres MA, Demayo CG. 2012. Applications of geometric morphometric analysis in describing sexual dimorphism in shell shapes in Vivipara angularis Muller (Family Viviparidae). Anim Biol Anim Husb 4(1): 14-19.

Nikdehghan N, Kashiri H, Hedayati A. 2018. CuO nanoparticles-induced micronuclei and DNA damage in Cyprinus carpio. Aquac Aquar Conserv Legis (AACL) Bioflux 11(3): 925-936.

Pagulayan IF, Cepillo JC. 1991. The Reproductive Biology and Laboratory Maintenance of Vivipara angularis Muller (Prosobranchia: Viviparidae). Transactions NAST PHL 13: 535-568.

Pareño R. 2017. US delivers weapons for PAF amid Marawi siege. Philippines: philstar.com; [accessed 2017 July]. Available from:https://www.philstar.com/headlines/2017/07/29/1722681/us-delivers-weapons-paf-amid-marawi-siege

Perelonia KB, Abendanio, CC, Raña, JA, Opinion AG, Villeza JT, and Cambia, FD. 2017. Heavy Metal Contamination in Water and Fishery Resources in Manila Bay Aquaculture Farms. The Philipp J Fish 24(2): 74-97. https://doi.org/10.31398/tpjf/24.2.2016A0014

Savabieasfahani M, Ali SS, Bacho R, Savabi O, Alsabbak M. 2016. Prenatal metal exposure in the Middle East: imprint of war in deciduous teeth of children. Environ Monit Assess 188(9): 505. https://doi.org/10.1007/s10661-016-5491-0

Seukep AJ, Noumedem JA, Djeussi DE, Kuete V. 2014. Genotoxicity and teratogenicity of African medicinal plants. In: Toxicological survey of African medicinal plants: Elsevier. p. 235-275.

Silva L, Silva B, Melo A, Silva R, Amancio F. 2011. Micronuclei as biomarkers of genotoxicity of gamma radiation in aquatic environments. INAC 2011: International nuclear atlantic conference Nuclear energy: new jobs for a better life, Brazil. [Accessed 2017 August 14]. Available at: https://inis.iaea.org/collection/NCLCollectionStore/_Public/42/107/42107631.pdf

Skalny AV, Aschner M, Bobrovnitsky IP, Chen P, Tsatsakis A, Paoliello MMB, Buha Djordevic A, Tinkov AA. 2021. Environmental and health hazards of military metal pollution. Environ Res 201:111568. https://doi.org/10.1016/ j.envres.2021.111568

World Health Organization/Food and Agriculture Organization (WHO/FAO). (2010). Joint Expert Committee on Food Additives (JECFA) Meeting (72nd: Rome, Italy), Pp. 16. https://apps.who.int/iris/handle/10665/44520

WHO/FAO (2011). JECFA Meeting (73rd: Switzerland). Evaluation of certain food additives and contaminants: Technical Report Series 960: 228. https://apps.who.int/iris/handle/10665/44515