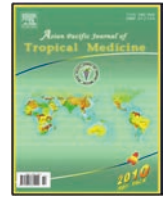


Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

## Asian Pacific Journal of Tropical Medicine

journal homepage: [www.elsevier.com/locate/apjtm](http://www.elsevier.com/locate/apjtm)

## Document heading

# Assessment of micronucleus frequency in exfoliated buccal epithelial cells among fisher folks exposed to mine tailings in Marinduque Island, Philippines

Elena M Ragragio<sup>1</sup>, Celeste P Belleza<sup>1</sup>, Mark C Narciso<sup>1</sup>, Glenn L Sia Su<sup>2\*</sup>

<sup>1</sup>Biology Department, University of the Philippines–Manila, Manila, Philippines

<sup>2</sup>Biology Department, De La Salle University, Manila, Philippines

## ARTICLE INFO

*Article history:*

Received 18 January 2010

Received in revised form 18 February 2010

Accepted 18 March 2010

Available online 18 April 2010

*Keywords:*

Micronucleus frequency

Epithelial cells

Fisher folks

## ABSTRACT

**Objective:** To evaluate the potential toxic effects of mine tailings exposure among the fisher folks residing near and far from the Calancan Bay, Marinduque, using the micronucleus assay as an endpoint. **Methods:** The fisher folks residing near and far from the Calancan Bay were interviewed and the presence and frequency of cells with micronucleus in exfoliated buccal epithelial cells were examined. **Results:** Results showed that the prevalence of cells with micronucleus was higher among the fisher folks who were directly exposed to the mine tailings as compared with those fisher folks who reside in a community without exposure of mine tailings and history of mining ( $P < 0.05$ ). **Conclusions:** The presence and the significant difference in the cells with micronuclei observed near the Calancan Bay could possibly indicate a prolonged chemical stress caused by the toxic heavy metals in the mine tailings and the environment.

## 1. Introduction

Marinduque Island is identified to harbor one of the biggest copper reserves in the country. Since 1969, mining operations extracting copper had been undertaken in Marinduque. In 1975, the dumping of mine tailings into Calancan Bay was permitted, and since then, approximately 200 million tons of mine wastes had been dumped into the Calancan Bay. According to Coumans, the mine tailings were dumped in the causeway and the bay contained high levels of heavy metal concentrations, particularly lead, cadmium, mercury and copper<sup>[1]</sup>.

Despite this occurrence, Calancan Bay remains to be a major source of aquatic resources for the residents nearby. The fisher folks continue to rely on aquatic organisms obtained from the bay, as it provides their sustenance and livelihood. It has been established that people's continuous exposure to environments that are contaminated with harmful chemicals like mine wastes, in particular, threatens the life and health of individuals. There is a real threat of accumulation and magnification of these heavy metals in the human body. Epidemiological studies have established

that levels of exposure of individuals to the pollutants might be measured by the damage to health associated with pollution<sup>[2]</sup>. To clearly understand the health of the fisher folks, the data provide useful lessons on the link between the environment and public health. Micronuclei in exfoliated epithelial cells are widely used as a noninvasive biomonitoring process suitable for the detection of increased cancer risks in man. To date, there are only few Philippine studies about determining the presence of the micronuclei in the buccal epithelial cells. No study has explored and assessed the micronucleus frequency in exfoliated epithelial cells among people exposed to mine wastes in the Philippines. With these concerns and as a preliminary groundwork, this study aims to assess the micronucleus frequency in the exfoliated epithelial cells examined among the fisher folks and whether the presence of micronuclei is significantly different between the fisher folks who are situated near and far from the Calancan Bay, where mine tailings are present.

## 2. Materials and methods

Marinduque Island, particularly the communities near the Calancan Bay in the Philippines, was identified as the study area, where history of mining and exposure to mine tailings are present, while the Sorsogon Province was chosen as the

\*Corresponding author: Glenn L Sia Su, 12D Cleveland Tower, Asiaworld City, Paranaque City, Philippines.  
Tel: +63 2 9946626  
Fax: +63 2 5360228  
E-mail: [gls76@yahoo.com](mailto:gls76@yahoo.com)

control area) to represent the community with no history of mining and exposure to mine tailings. The study area is located at about 160 km south of Metro Manila, whereas the control area is a province situated at the southern–most tip of Luzon. The fisher folks in the town of Sta. Cruz along the Calancan Bay were sampled as the exposed group, while the fisher folks in the town of Bulusan, Sorsogon, were sampled as the unexposed group. All the subjects in the study were purposively selected to be those individuals who relied on the sea for their sustenance and with similar socioeconomic characteristics.

The participants' selection in this study was dependent on the inclusion–exclusion criteria established. Fisher folks who gave their informed consent to join the study, who were willing to be interviewed and who were willing to be scraped for exfoliated buccal epithelial cells from their mouths were included, and those who did not give their consent to join the study, who were not willing to be interviewed and who were not willing to be scraped for exfoliated buccal epithelial cells from their mouths were excluded. Approval for this study was obtained from the ethics board committee of the institution where authors are connected. Data were kept confidential, and the fisher folks were informed about the findings obtained.

The participants of the study were interviewed using an interview schedule, wherein the participants were asked about their demographic characteristics, drinking of alcoholic beverages and smoking activities. Exfoliated cells were obtained from the mouth of the individuals and examined using the micronucleus assay. The mouths of the participating individuals were initially rinsed with tap water twice, and the buccal mucosa was scraped with a wooden tongue depressor to obtain the exfoliated epithelial cells. The buccal epithelial cells were smeared on a sterile precleaned glass slide, air–dried and fixed with methanol and stained using the Feulgen reaction technique[3]. For each individual, 1 000 buccal epithelial cells were examined under a light microscope at 400× magnification. Quantification was done using a tally counter, as the slides were systematically scanned. The presence of micronuclei in the buccal epithelial cells was confirmed, as the cells were viewed at 1 000× magnification under an oil power immersion objective of a light microscope. The micronucleus frequencies were reported based on the number of micronucleated cells per 1 000 cells examined. The micronuclei in the cells were classified according to the criteria established by Celik *et al*[4]. Cells that were binucleated, fragmented, smeared, clumped and contained shattered nuclei were not counted. Cells examined to have undergone degenerative processes, such as karyorrhexis, karyolysis, and fragmentation of the nucleus or pycnosis, were noted but were not included in the micronucleus frequency counting.

The factors, as indicated by exposure to location, smoking, drinking and age, were analyzed to indicate whether significant differences exist within the comparisons of micronucleus frequencies of the participants. The Kruskal–Wallis test was used to establish the statistically significant difference. When the *P* value <0.05, the results were significant. All statistical analyses were performed using the Statistical Package for Social Sciences.

### 3. Results

A total of 81 fisher folks gave their consents to join the study, were interviewed and were assessed using the micronucleus assay. About 54 (66.7%) fisher folks

included in the study reside at the coastal town of Sta. Cruz, Marinduque (study area), whereas 27 (33.3%) fisher folks reside at the coastal town of Bulusan, Sorsogon (control area). The mean±standard deviation age of the fisher folks residing in the study area and control area was (33±17) years old and (31±16) years old, respectively. There were fewer smokers in the exposed group (13%) as compared with those in the unexposed group (22%). Likewise, there were fewer alcohol drinkers (38.9%) in the exposed group as compared with the unexposed group (74.1%) (Table 1).

**Table 1**

Micronucleus frequency of the fisher folks groups (mean±SD).

Index	Exposed group	Unexposed group
Smokers	9.71 ± 1.98 (n=7)	4.00 ± 2.76 (n=6)
Nonsmokers	7.79 ± 2.98 (n=47)	2.29 ± 1.52 (n=21)
Alcohol drinkers	8.23 ± 3.28 (n=21)	3.57 ± 2.88 (n=7)
Nonalcohol drinkers	7.91 ± 2.73 (n=33)	2.35 ± 1.46 (n=20)
Age range (years)	8–23	7.48 ± 3.01 (n=23)
	24–39	6.30 ± 2.16 (n=10)
	40–55	9.38 ± 3.01 (n=13)
	56–70	9.62 ± 1.60 (n=8)
		2.33 ± 2.19 (n=12)
		3.00 ± 2.00 (n=7)
		3.25 ± 2.22 (n=4)
		2.50 ± 1.00 (n=4)

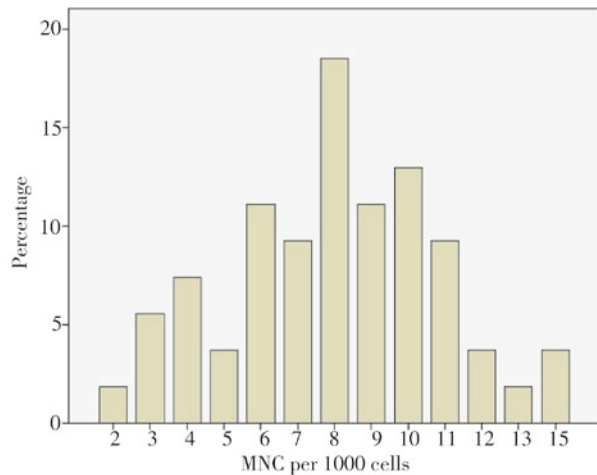
Among the exposed group, the frequency of micronucleus in exfoliated epithelial cells examined per 1 000 cells ranged from 2 to 15. Most individuals in the exposed group had eight micronuclei per 1 000 cells, whereas those in the unexposed group ranged from none to eight micronuclei per 1 000 cells. The frequency of micronucleus per 1 000 cells in exfoliated buccal epithelial cells of exposed individuals (8.04±2.93) were found to be higher than the unexposed individuals (2.67±1.94)(Figure 2&3).

Using the Kruskal–Wallis test, at  $\alpha = 0.05$  level of significance, a significant difference ( $P < 0.05$ ) was found between the exposure of the fisher folks to mine wastes and their micronucleus frequency. No significant differences ( $P > 0.05$ ) between smoking and alcohol drinking and micronucleus frequency were found. There was significant difference between age and the micronucleus frequency of the fisher folks examined ( $P < 0.05$ ).

### 4. Discussion

This is a cross–sectional study, and its scope is limited to assessing the micronucleus frequency in exfoliated epithelial cells of fisher folks residing near or far from an area with a mining history and exposed to mine tailings. The micronuclei are cytoplasmic chromatin masses with the appearance of small nuclei and are formed during the metaphase or anaphase transition of cell division. The presence and frequency of micronucleus in exfoliated epithelial cells have been used to evaluate genotoxic effects of heavy metal exposure. Workers exposed to heavy metals had higher micronuclei mean score than the workers not exposed[5]. Other health impacts induced by the mine tailings exposure such as poisoning and cancer were not covered in the study. This study only looked into factors like exposure to mine wastes, smoking, age and alcohol drinking to the micronuclei occurrence among the fisher folks examined.

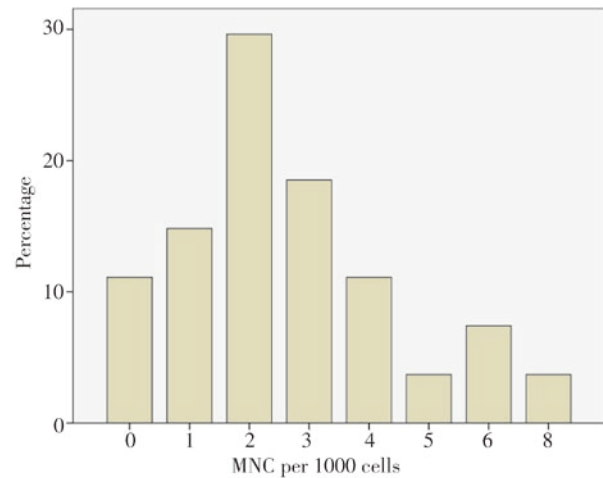
Our findings showed that fisher folks residing in the study area where mining history existed and are



**Figure 1.** Micronucleus frequency distribution of the fisher folks in the study area. MNC, number of micronuclei.

constantly exposed to the mine tailings had higher risks of micronucleus occurrences in their exfoliated epithelial cells as compared with those residing in the control area with no mining history and no mine tailings exposure. The results of this study agree with Stoia *et al*[5] as they likewise observed that the mean micronucleus score in workers exposed to pollutants in their workplace had significantly higher micronucleus occurrences as compared with the controls. This study also showed that the results of micronucleus assays in exfoliated buccal epithelial cells of smokers and alcohol drinkers were slightly higher than those observed in nonsmokers and nonalcohol drinkers. Although much higher micronucleus frequency was found, no significant differences were seen. Bohrer *et al*[6] likewise found no effect of alcohol consumption on micronucleus formation. The results in this present investigation were consistent with Nersesyan *et al*[3], wherein we found that smoking has a statistically significant effect on the micronucleus formation in the fisher folks examined. This study also presented that age had an effect on the micronucleus formation in the buccal epithelial cells. The micronucleus formation in the fisher folks increases with age[7]. According to Bukvic *et al*[8], the increase in aneuploidy with increasing age is correlated with an increase in the micronucleus formation. This study also showed that the frequency of micronucleus in the exfoliated epithelial cells of the fisher folks examined varied in the individuals for both the exposed and unexposed groups. This variability in the individual micronucleus occurrences among the groups may be attributed to the intraindividual homogeneity and interindividual variability[9] brought about by differences in body responses from individual to individual, gene composition and lifestyle habits[10] of the fisher folks examined.

Our investigation indicates that the frequency of micronucleus in exfoliated epithelial cells among fisher folks examined varied in individuals from both the exposed and unexposed groups. Higher frequency of micronucleus was observed among those fisher folks residing in an environment exposed to mining and its mine wastes. This study has likewise showed that smoking and alcohol consumption have no association with the induction of micronuclei in the exfoliated oral mucosa cells. The increased risks of micronucleus formation increased with the age of the fisher folks.



**Figure 2.** Micronucleus frequency distribution of the fisher folks in the control area.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgments

We would like to express our sincerest gratitude to all who have assisted and supported this study.

### References

- [1] Coumans C. Summary of findings from environmental studies of Calancan Bay related to surface disposal dumping of mine tailings into the bay from the Tapian Pit of the Marcopper Mine. Available at: [www.miningwatch.ca/.../summary-findings-environmental-studies-calancan-bay-related-surface-disposal-dumping-mine-tailings](http://www.miningwatch.ca/.../summary-findings-environmental-studies-calancan-bay-related-surface-disposal-dumping-mine-tailings).
- [2] Sia Su G. Impact on drinking water sources in close proximity to the Payatas dumpsite, Philippines. *J Publ Health* 2007; **15**: 51–5.
- [3] Nersesyan A, Kundi M, Atefie K, Shulte–Hermann R, Knasmuller S. Effect of staining procedures on the results of micronucleus assays with exfoliated oral mucosa cells. *Cancer Epidemiol Biomarkers Rev* 2006; **15**(10): 1835–40.
- [4] Celik A, Cavas T, Ergene G. Cytogenetic biomonitoring in petrol station attendants: micronucleus test in exfoliated buccal cells. *Mutagenesis* 2003; **18**(5): 417–21.
- [5] Stoia M, Oancea S, Obreja DC. Comparative study of genotoxic effects in workers exposed to inorganic lead and low dose irradiation using micronucleus test. *Rom J Leg Med* 2009; **4**: 289–94.
- [6] Bohrer PL, Filho MS, Paiva RL, da Silva IL, Rados PV. Assessment of micronucleus frequency in normal oral mucosa of patients exposed to carcinogens. *Acta Cytol* 2005; **49**: 265–72.
- [7] Rekhadevi PV, Sailaja N, Chandrasekhar M, Mahboob M, Rahman MF, Grover P. Genotoxicity assessment in oncology nurses handling anti-neoplastic drugs. *Mutagenesis* 2007; **22**: 395–401.
- [8] Bukvic N, Gentile M, Susca F, Fanelli M, Serio G, Buonadonna L, et al. Sex chromosome loss, micronuclei, sister chromatid exchange and aging: a study including 16 centenarians. *Mutat Res/Genet Toxicol Environ Mutagen* 2001; **498**(1): 159–67.
- [9] Moller P, Wallin H, Holst E, Knudsen LE. Sunlight–induced DNA damage in human mononuclear cells. *FASEB* 2002; **16**: 45–53.
- [10] Benites CI, Amado LL, Vianna RAP, Martino–Roth MG. Micronucleus test on gas station attendants. *Genet Mol Res* 2006; **5**(1): 45–54.