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Spermatogenesis stage sensitivity and change in testicular stress oxidative profile against leachate genotoxic component

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ABSTRACT. Exposure to environmental waste such as landfill leachate has the potential to cause genotoxic effects and oxidative stress impacting the mammalian reproductive system. This study examined the effects of Sarimukti Landfill Leachate (LTS) on sperm DNA damage and testicular oxidative stress in male Wistar rats. Rats were exposed to LTS at concentrations of 0%, 35%, and 63% during distinct spermatogenesis stages: mitosis (MTS), meiosis (MSS), and spermiogenesis (SMGS). Sperm DNA damage was assessed using the Sperm Chromatin Dispersion (SCD) assay, while testicular oxidative stress was measured by quantifying Malondialdehyde (MDA) and total protein levels. The results showed dose-dependent sperm DNA damage across all spermatogenesis stages, with the most significant damage observed at the 63% LTS dose. Sperm DNA damage levels were highest in the MTS group (93.53%), followed by MSS (87.5%) and SMGS (86.8%). The 63% LTS dose also increased testicular lipid peroxidation, as indicated by elevated MDA levels (4.63 μM/mL). This suggests that LTS can damage lipid components in testicular tissue. Additionally, the 63% LTS dose reduced total protein levels in testicular tissue to 63.55 μg/mL, compared to 108.31 μg/mL in the control group. Heavy metals (arsenic, plumbum, nickel, chromium) and organic pollutants in LTS generated reactive oxygen species (ROS) in germ cells, disrupting cellular repair mechanisms also inducing lipid and protein oxidation. This oxidative stress further exacerbated sperm chromatin fragmentation. These findings highlight the reproductive toxicity of landfill leachate and emphasize the need for protective measures during early spermatogenesis stages. This study provides insights into the environmental impacts on male fertility and underscores the risks of genotoxic exposure.

Keywords: leachate; oxidative stress; sperm DNA damage; spermatogenesis; SCD assay

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INTRODUCTION

The quality of the male reproductive system is largely determined by spermatogenesis, the process by which male germ cells (spermatogonia) transform into mature spermatozoa within the seminiferous tubules (Gilbert & Baressi, 2018; Carlson, 2016). The process comprises three stages: mitotic proliferation of germ cells, meiosis to produce spermatids, and spermiogenesis, during which spermatids differentiate into spermatozoa (Aitken & Baker, 2020; Aitken & Lewis, 2022). Each stage is tightly regulated and interconnected through cytoplasmic bridges that help maintain synchronization during spermatogenesis (Carlson, 2016; Chaigne & Brunet, 2022).

The testes contain high levels of polyunsaturated fatty acids (PUFAs), making them particularly vulnerable to oxidative damage from toxins such as heavy metals and xenobiotics (Botella *et al.*, 2021; Salihu *et al.*, 2021). PUFAs are highly susceptible to oxidation by peroxides, generating reactive oxygen species (ROS) that infiltrate seminiferous tubules and bind to germ cell DNA (Aitken & Lewis, 2022; Collodel *et al.*, 2022). This oxidative damage can lead to DNA fragmentation, impaired mismatch repair mechanisms, and ultimately contribute to infertility, recurrent miscarriages, and birth defects (Tan, 2019; Aitken and Baker, 2020; Li *et al.*, 2025; Gkeka *et al.*, 2023).

One significant source of environmental genotoxicants is municipal landfill leachate, which contains heavy metals (plumbum, cadmium, arsenic, chromium) and hazardous organic compounds (Bakare *et al.*, 2013; Eleawa *et al*, 2013; Aziz & Mojiri, 2015; Ademola *et al.*, 2020). In West Java, the Sarimukti landfill leachate has been found to contain arsenic, lead, nickel, cadmium, and could

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induce micro nucleous formation in bone marrow (Yusuf & Kinanti, 2015; Sembiring, 2024). While these contaminants are known to cause sperm abnormalities and reduced sperm quality (Akintunde *et al*., 2015), their specific effects on sperm DNA integrity and testicular oxidative stress profiles remain unstudied. Sperm DNA damage in the form of single strand breaks DNA fragmentation can be observed using the sperm chromatin dispersion method (Vandekerckhove *et al*., 2016; Ragosta *et al*., 2024). Sperm DNA damage is detected by measuring the 'halo' (chromatin extension) size. A larger halo indicates intact DNA, while a smaller halo suggests DNA fragmentation (Vandekerckhove *et al*., 2016; Ragosta *et al*., 2024).

Different stages of spermatogenesis indicates that each stage has a different probability of sperm DNA damage levels. In addition, changes in the testicular oxidative stress profile that can be identified through lipid and protein peroxidation levels also need to be measured as supporting data. This study provides the first evidence of stage-specific sperm DNA damage and testicular oxidative stress profile changes induced by Sarimukti landfill leachate, identifying mitosis as the most sensitive phase. Our findings advance understanding of how environmental genotoxicants disrupt spermatogenesis at molecular and cellular levels, with implications for male fertility risk assessment.

MATERIALS AND METHODS

Sampling location and leachate test. Leachate samples were taken from the leachate discharge outlet of Sarimukti Municipal Landfill, 6º48’6”S, 107º20’54”E. Sampling was conducted alongside measurements of physical and chemical parameters of Sarimukti Landfill Leachate (LTS), in the form of pH, conductivity, dissolved organic mass, dissolved oxygen levels, and water temperature. Samples were filtered with Whatman filter paper No. 42 and then stored at a temperature of -4ºC (Ademolla *et al*., 2020). Testing of heavy metal levels is carried out using the AAS (Atomic Absorption Spectroscopy) method and testing of organic contamination levels is carried out using the GCMS (Gas Chromatography Mass Spectroscopy) method at the PT. Sucofindo Laboratory. AAS function is measures the absorption of light by free atoms in the gaseous state. While GCMS function is combines gas chromatography (separation of volatile compounds) with mass spectrometry (identification via mass-to-charge ratios). Sampling location map using ArcGIS can be seen on Fig. 1.

Fig. 1. Sarimukti municipal landfill site and its leachate pool

Test solution. Solution like PBS, SCD acid buffer solution (HCL 0.08 N), SCD lysing solution (0.5 EDTA; 0.01 M Trisbase; 2.5 M NaCl; 1% Triton X-100; 0.025 M DTT), Giemsa, TBARs assay solution (Tris Base, RIPA's Buffer, Malondialdehyde, BHT and SDS), and Bradford's reagent are obtained from SigmaAldrich. For alcohol, aquadest, and ethanol obtained from PT. Bratachem, Bandung.

Test animals. Forty-five male Wistar rats (10 weeks, 225–275 g) from PT. Biofarma were housed in standard cages (21–27°C, 60–75% humidity, 12h light/dark cycle) and acclimatized for 7 days. They received standard feed (10% per body weight) and water ad libitum, following OECD 478 guidelines (OECD, 478). The acclimatization process lasted for 7 days before the administration of test materials.

Research design and leachate administration process. Rats were exposed to Sarimukti landfill leachate (LTS) at 0% (control, i.v. aquadest), 35%, and 63% doses via intraperitoneal injection (once daily). Groups were divided by spermatogenesis stage: (a) MTS (Mitosis): 14 days LTS + 32-day waiting time, (b) MSS (Meiosis): 22 days LTS + 20-day waiting time, and (c) SMGS (Spermiogenesis): 22 days LTS + 3-day waiting time. After treatment, all rats were euthanized (CO_2 chamber, OECD 478), and sperm DNA damage was assessed via Sperm Chromatin Dispersion Assay (Vandekerckhove *et al*., 2016; Ragosta *et al*., 2024). In addition, SMGS testes were cryopreserved for lipid peroxidation & total protein analysis.

Sperm chromatin dispersion (SCD) assay. SCD Assay was performed by mixing 60 µL of rat epididymal semen with 140 µL of 1% LMP agarose, incubating at 37°C, and homogenizing. The homogenate (50 µL) was placed on agarose-coated glass, covered, and chilled (4°C, 5 min., dark). After removing the cover glass, samples were treated with acid buffer (15 min., dark) and lysing solution (35 min., light), then dehydrated in ethanol (70%, 90%, 100%), rinsed, and air-dried. Staining used Giemsa (15 min.), and sperm were examined under a microscope (40x100). 500 sperm per sample were counted (hemocytometer/Image-J) and classified into five Halo types: Big Halo, Medium Halo (both as normal DNA) and Small Halo, No Halo Degraded Halo (as sperm with fragmented DNA) (Vandekerckhove *et al*., 2016; Ragosta *et al*., 2024), as illustrated in Fig. 2.

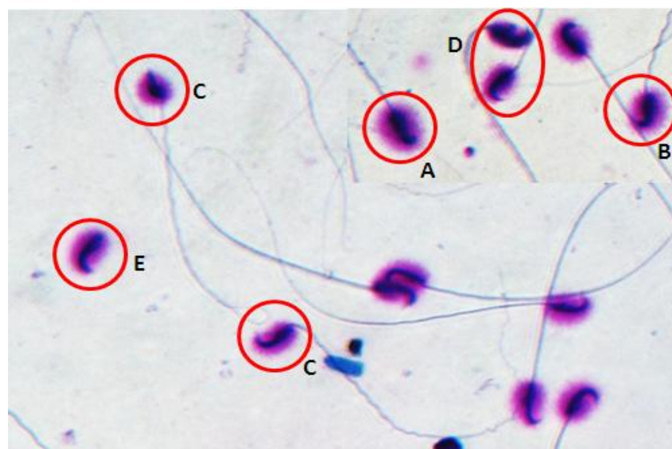

Fig.2. Type of Halos in sperm sample, (A) Big Halo (B) Medium Halo (C) Small Halo (D) No Halo (E) Degraded Halo

The process of calculating the amount of sperm chromatin dispersion, referring to the formula of Vandekerchkhove (2016) and Ragosta (2024). The details are as follows:

$$\textbf{\% Sperm with DNA Damage} = \frac{\sum\% \textit{ (Small Halo + No Halo + Degraded Halo)}}{\sum\% \textit{ (Big Halo + Medium Halo + Small Halo + No Halo + Degraded Halo)}}$$

Preparation of testicular tissues sample. A total of 100 mg of testicular tissue was washed using PBS. The tissue was then immersed in 1 mL of RIPA's buffer and homogenized using an ultrasonic homogenizer (on:off interval 30 sec:30 sec, 100% power, 15 cycles). The samples were centrifuged at 10,000 rcf for 15 minutes to separate the supernatant and pellet. The supernatant was stored at -80°C, while the pellet was stored at -20°C (Nedecky *et al*., 2013; De Leon *et al*., 2022).

Quantification of testicular malondialdehyde (MDA) levels. Lipid peroxidation levels, represented as MDA levels in testicular homogenates, were measured by the reaction of thiobarbituric acid (TBA) using the modified Ohkawa procedure. This method spectrophotometrically measures the color produced from the reaction of TBA with MDA at a wavelength of 532 nm. Tissue supernatant (100 µl) was added to a test tube containing 1% butylated hydroxytoluene (BHT) in methanol, followed by 200 µl of 8% SDS, 250 µl of acid reagent (15% trichloroacetic acid), and 250 µl of TBA solution. The mixture was incubated for 60 min at 95°C, then centrifuged at 10,000 × g for 3 min. The absorbance of the supernatant (75 µl) was read using a plate reader at 532 nm. MDA levels were expressed in nmol/mg protein (Nedecky *et al*., 2013; De Leon *et al*., 2022).

Quantification of total testicular protein levels. Total protein levels in testicular pellets were measured using a modified Diagenode protocol (Diagenode, 2010) which detects the color of the reaction product of Coomassie Brilliant Blue G-250 with protein at 495 nm. Tissue pellets (20 mg) were dissolved in 0.1 N NaOH to a concentration of 100 mg/mL. A total of 10 µl of homogenate was added to the microplate, then mixed with 100 µl of Bradford reagent. Absorbance was measured using an ELISA reader at 495 nm, and protein levels were expressed in mg/mL of homogenate.

Data analysis. In this study, the main data obtained were the levels of sperm DNA damage & testicular oxidative stress profile analyzed using IBM SPSS Statistics 24. Statistical analysis began with a data normality test using Kolmogorov-Smirnov and a homogeneity test with Levene. Followed by ANOVA analysis, and post hoc with TUKEY test. All of these statistical tests aimed to identify significant differences in the levels of sperm DNA damage both between doses and between stages of spermatogenesis with a 95% confidence level.

RESULTS AND DISCUSSION

Leachate component. Analysis of the Sarimukti landfill leachate (LTS) by PT Sucofindo using AAS revealed elevated concentrations of heavy metals, including arsenic (As), lead (Pb), nickel (Ni), and cadmium (Cd), all exceeding WHO safety thresholds. GCMS testing further identified pentachlorophenol levels above WHO limits. The result of AAS and GCMS can be seen in Table 1.

Table 1. The concentrations of heavy metals and pentachlorophenols in Saminukti Municipal Landfill Leachate (LTS)

Parameter	Unit	Detection Limit	Result	WHO Recommendation	Methods
Arsenic	mg/L	0.003	3	0.5	US EPA SW-846-7061
Cadmium	mg/L	0.006	0.9	0.15	US EPA SW-846-6010 B
Lead	mg/L	0.029	3	0.5	US EPA SW-846-6010 B
Nickel	mg/L	0.032	21	3.5	US EPA SW-846-6010 B
Pentachlorophenol	mg/L	0.001	2.7	0.45	US EPA SW-846-8270 C

These five contaminants are established genotoxicants associated with sperm DNA damage in experimental studies (Botella *et al*., 2021; Salihu *et al*., 2021; Aitken *et al*., 2022). Additionally, these compounds may induce oxidative stress in testicular tissue, leading to lipid peroxidation and protein oxidation (Ademola *et al*., 2020).

Sperm DNA damage. Based on the research results, it can be seen that LTS with doses of 35%, and 63% caused increasing number sperm DNA damage, both at the mitosis (MTS), meiosis (MSS) and spermiogenesis (SPMS) stages. All spermatogenesis stage formed different percentage of each "*Halo*" types and the distribution can be seen in Fig. 3.

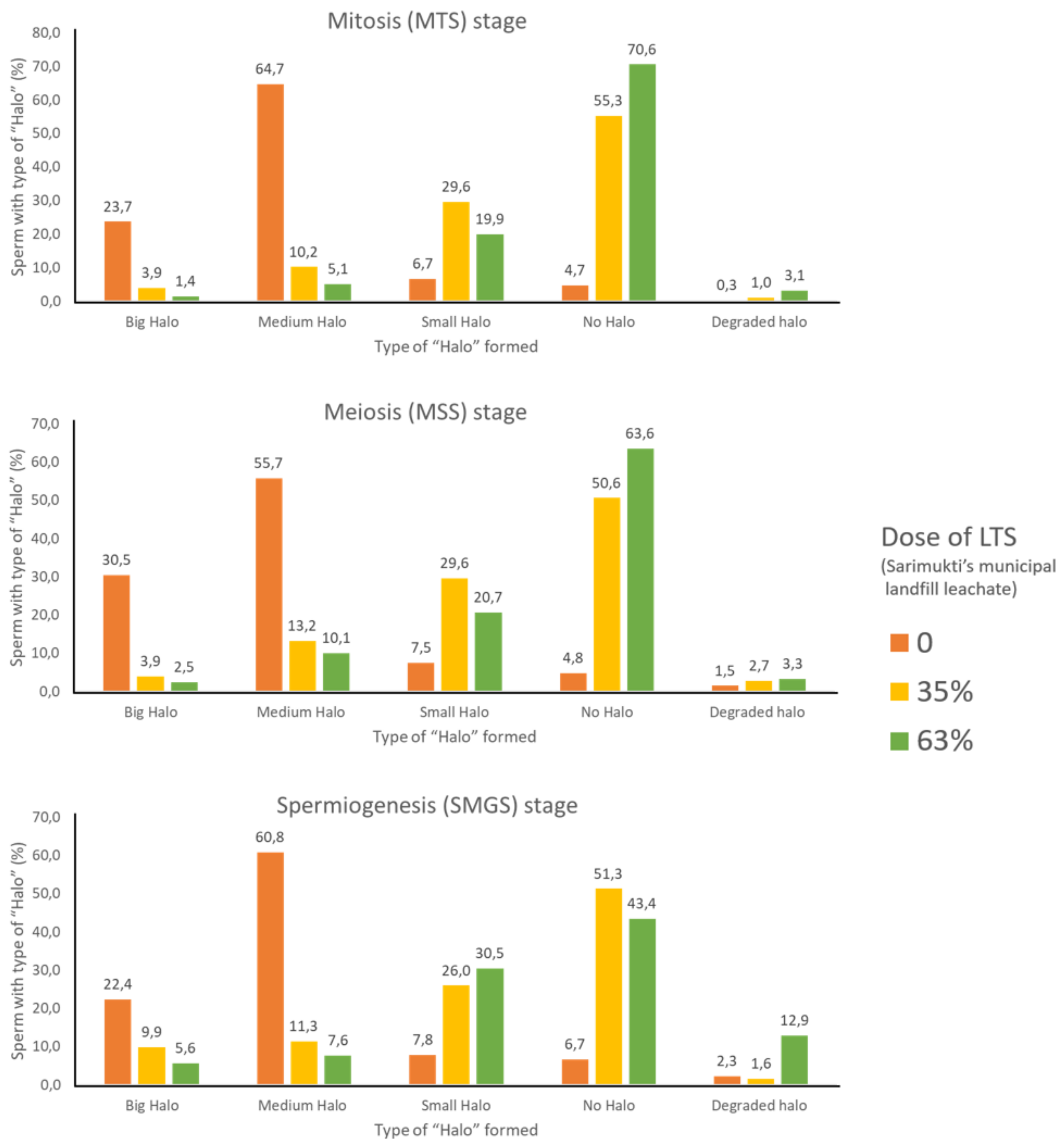

Fig. 3. Percentage of each type of "*Halo*" in every stage of spermatogenesis stages induced by Sarimukti Muncipal Landfill Leachate (LTS)

As we knew form the formulas, sperm with damaged DNA can be known by calculating percentage of sperm with small halo, no halo, and degraded halo. Percentage of sperm with DNA damage in each group as shown in **Figure 4**.

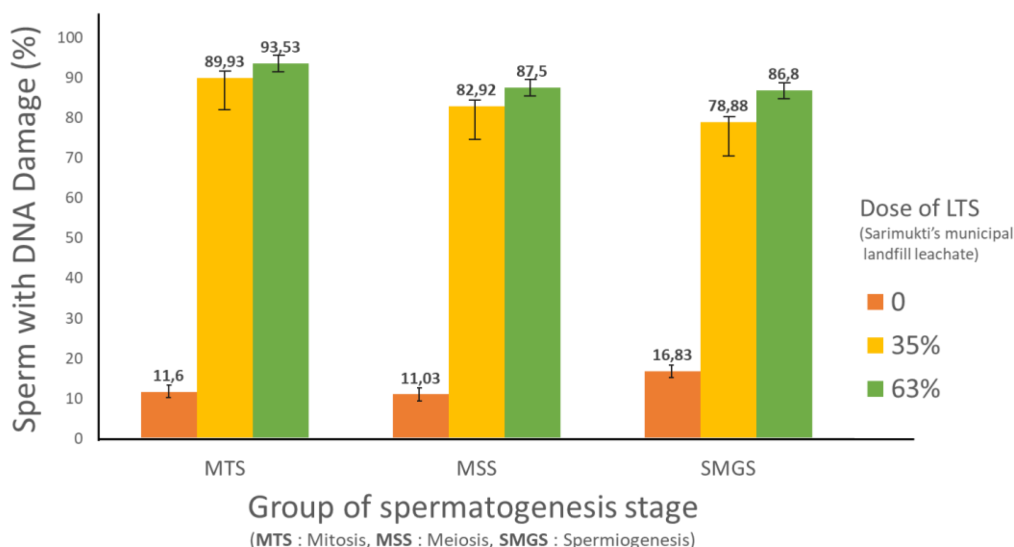

Fig. 4. Percentage of sperm with DNA damage in all spermatogenesis stages induced by Sarimukti Municipal Landfill Leachate (LTS)

In addition, along with the increasing dose of LTS given, the higher the DNA damage found. From the three stages of spermatogenesis observed, it was found that the mitosis stage (MTS) experienced the highest DNA damage (LTS dose 63%, 93.53% damaged sperm), followed by the meiosis stage (MSS) and the lowest sperm DNA damage was found at the spermiogenesis stage (LTS dose 35%, 78.88% damaged sperm).

Testicular oxidative stress measure by MDA levels and total protein quantity. Based on Fig. 3 and 4, it demonstrate that the mitosis stage (MTS group) of spermatogenesis exhibits the highest susceptibility to DNA damage from genotoxic components in LTS. This conclusion is supported by the significantly higher prevalence of Small Halo, No Halo, and Degraded Halo sperm in this stage compared to the MSS and SGMS groups.The observed DNA damage likely results from LTS-induced alterations in the natural reactive oxygen species (ROS) profile of testicular tissue. This hypothesis is further corroborated by elevated lipid and protein peroxidation levels, as evidenced in Fig. 5.

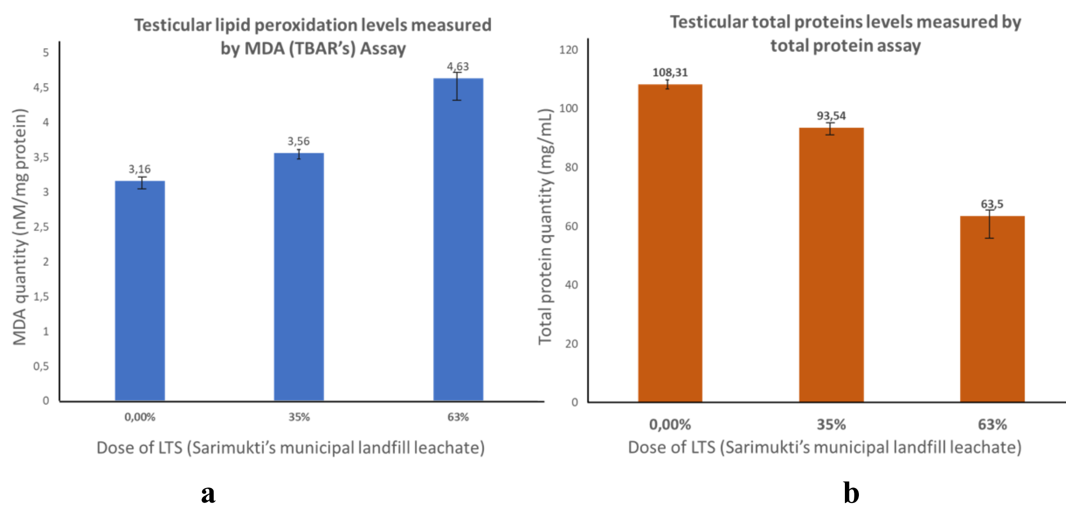

Fig. 5. (a) total MDA levels and (b) total protein quantity in testicular tissue induced by Sarimukti Municipal Landfill Leachate (LTS)

Atomic Absorption Spectroscopy (AAS) analysis revealed that Sarimukti's municipal landfill leachate (LTS) contains elevated concentrations of heavy metals (arsenic, lead, and tin) exceeding WHO safety thresholds. Additionally, Table 1 shows phenolic compound levels six times higher than WHO recommendations. These contaminants are known to induce reactive oxygen species (ROS) production in testicular tissue and semen (Agarwal *et al*., 2014; Muenyi *et al*, 2015; Anyanwu *et al*., 2019; Aitken & Lewis, 2022). The ionic forms of these metals (As^{2+}, Pb^{2+}, Cd^{2+}, Cr^{2+}, Hg^{2+}) readily interact with cellular components, particularly through the Haber-Weiss reaction in germ cells. This process generates hydroxyl radicals (OH*) and hydroxide ions (OH^-) as by products of dehydrogenase activity. Testicular tissue, rich in polyunsaturated fatty acids (PUFAs), is exceptionally vulnerable to ROS-mediated oxidation (Aitken & Lewis, 2022; Collodel *et al*., 2022). PUFA oxidation in cellular and organelle membranes facilitates ROS penetration into the nucleus, where hydroxyl radicals directly damage DNA. The binding of OH- and OH* by DNA causes fragmentation, and results in the chromatin structure becoming incomplete or shorter. This aligns with the findings of Vandekerckhove (2016), who noted that fragmentation of sperm DNA by ROS causes the formation of "Halo" that is smaller or shorter in length.

Spermatogenesis process requires simultaneous and uninterrupted regulation between spermatogonia. This regulation is facilitated by the presence of a cytoplasmidal bridges, so that signaling from one cell to another is always the same (Carlson, 2016; Chaigne & Brunet, 2022; Maroto *et al*., 2025). However, the cytoplasmidal bridge is very susceptible to damage, one of which can be caused by ROS (Maroto *et al*., 2025). ROS could be formed from the presence of phenol and heavy metal ions cadmium, lead, nickel, chromium and arsenic in leachate (Ademola *et al*., 2020). The presence of ROS in germ cells can oxidize PUFA and damage the existing cytoplasmidal bridge (Kimura, 2015; Chaigne & Brunet, 2022). Damage to the cytoplasmidal bridge will ultimately disrupt or even damage signaling between germ cells. The damage in cell signalling, could lead to incomplete mitotic or meiotic process, resulting in sperm with un repaired DNA damage.

Binding of PUFA by ROS in testicular tissue will produce a derivative compound in the form of MDA. The change in MDA levels in the testicular tissue of the experimental animals administered a 63% LTS dose showed an increase compared to the control group. This indicates that MDA can serve as a biomarker for altered lipid peroxidation levels in testicular tissue. Several studies have stated that the presence of MDA in testicular tissue can result in the failure of the DNA repair process. Naturally, cells will respond to fragmentation in DNA through BER (Base Excission Repair) (Ayala *et al*., 2014; Gohil *et al*., 2023; Li *et al*., 2025). The presence of MDA in cell nucleous, could interact with guanine in DNA, and cause fragmentation. Normally, DNA fragmentation in germ cells triggers DNA glycosylase to initiate base excision repair (BER). However, MDA-DNA adducts form deoxy-guanosine compounds that evade glycosylase detection, preventing BER. This leads to unrepaired mutations and exacerbated DNA fragmentation (Gohil *et al*., 2023; Li *et al*., 2025).

BER mechanism normally occurred the most especially in Mitosis. In germ cells, mitosis stage has G2 phase as a quality control of the integrity of cell DNA (Gilberts & Barresi, 2018; Carlson, 2016). In the G2 phase, germ cells will perform quality control in the form of ensuring that all DNA is completely replicated, and all proteins in the cell are formed according to their size. If DNA damage occurs in the G2 phase, repairs will be carried out by stopping the cell cycle in the G2 phase. Then the BER (Base Excission Repair) mechanism is carried out to repair germ cell DNA. However, the presence of MDA and ROS causes damaged DNA to not be identified by the DNA Glycosylase enzyme. This causes germ cell DNA to remain fragmented. This supports the finding that the mitosis stage is the most sensitive stage to DNA damage.

CONCLUSION

The toxic components in Sarimukti landfill leachate have been shown to induce sperm DNA fragmentation as the dose given increases. Each stage of spermatogenesis has a different sensitivity, indicated by the difference in the percentage of sperm that experience DNA damage, with the mitosis

stage as the most sensitive stage. These findings highlight the importance of early stage protection during spermatogenesis against environmental genotoxicant.

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REFERENCES

Ademola OJ, Alimba CG, Bakare AA. 2020. Reproductive toxicity assessment of Olusosun municipal landfill leachate in Mus musculus using abnormal sperm morphology and dominant lethal mutation assays. *Journal of Environmental Analysis Health and Toxicology*. vol 35(2): 1-10. doi: https://doi.org/10.5620/eaht.e2020010.

Agarwal A, Virk G, Ong C, du Plessis SS. 2014. Effect of oxidative stress on male reproduction. *World Journal of Mens Health*. vol 32(1): 1-17. doi: http://dx.doi.org/10.5534/wjmh.2014.32.1.1.

Aitken RJ, Lewis SEM. 2022. DNA damage in testicular germ cells and spermatozoa. When and how is it induced? How should we measure it? What does it mean? *Journal of Andrology*. vol 11(8): 1545-1557. doi: https://doi.org/10.1111/andr.13375.

Aitken RJ, Baker MA. 2020. The role of genetics and oxidative stress in the etiology of male infertility—A unifying hypothesis? *Journal of Frontiers in Endocrinology*. vol 11: 1-22. doi: https://doi.org/10.3389/fendo.2020.581838.

Akintunde JK, Oboh G, Akindahunsi AA. 2015. Sub-chronic Exposure to EOMABRS leachate induces germinal epithelial cell abnormalities and oxidative damage in rats. *Asian Pacific Journal of Reproduction*. vol 4(4): 288-297. doi: https://doi.org/10.1016/j.apjr.2015.07.006.

Anyanwu A, Ekong W, Offor S, Orji O, Umoh E, Owhorji J, Emetonjor F, Usoro C. 2019. Heavy metals, biomarkers of oxidative stress and changes in sperm function: A case-control study. *International Journal of Reproductive BioMedicine*. vol 17(3): 163-174. doi: https://doi.org/10.18502/ijrm.v17i3.4515.

Ayala A, Munoz M, Aguelles S. 2014. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Journal of Oxidative Medicine and Cellular Longevity*. vol 2014: 1-31. doi: https://doi.org/10.1155/2014/360438.

Aziz SQ, Mojiri A. 2015. Composition of leachate in Hamidi Abdul Aziz & Salem Abu Amr (Eds.) *Control and treatment of landfill leachate for sanitary waste disposal*. *IGI Global*. doi: https://doi.org/ 10.4018/978-1-4666-9610-5.ch007.

Bakare A, Alabi O, Gbadebo A, Ogunsunyi O, Alimba G. 2013. In vivo cytogenotoxicity and oxidative stress induced by electronic waste leachate and contaminated well water. *Challenges Journal*. vol 4(2): 169-187. doi: https://doi.org/10.3390/challe4020169.

Botella AL, Velasco I, Acien M, Espinosa P, Torro JLT, Romero RS, amd Torres MJG. 2021. Impact of heavy metals on human male fertility—An overview. *Journal of Antioxidants*. vol 10(9): 1-24. doi: https://doi.org/10.3390/antiox10091473.

Carlson MB. 2016. Developmental Biology. Saunders, Philladephia, USA: Elsevier.

Chaigne A, Brunet T. 2022. Incomplete abscission and cytoplasmic birdges in the evolution of eukaryotic multicellularity. *Journal of Current Biology*. vol 32(8): R385-R397. doi: https://doi.org/10.1016/j.cub.2022.03.021.

Collodel G, Moretti E, Noto D, Corsaro R, Signorini C. 2022. Oxidation of polyunsaturated fatty acids as a promising area of research in infertility. *Journal of Antioxidant*. vol 11(5): 1-14. doi: https://doi.org/10.3390/antiox11051002.

De Leon JAD, Borgest CR. 2022. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *Journal of Vis. Exp.*: 1-22. doi: https://dx.doi.org/10.3791/61122.

Eleawa S, Alkhateeb M, Alhashem F, Jaliah I, Sakr H, Elrefaey H, Elkarib A, Alessa R, Haidara M, Shatoor A, Khalil M. 2013. Reservatrol reverses cadmium chloride-induced testicular damage and subfertility by downregulatig p53 and Bax and upregulating gonadotrophins and Bcl-2 gene expression. *Journal of Reproduction and Development*. vol 60(2): 115-127. doi: https://doi.org/10.1262/jrd.2013-097.

Gkeka K, Symeonidis E, Tsampoukas G, Moussa M, Issa H, Kontogianni E, Almusafer M, Katsouri A, Mykoniatis I, Dimitriadis F, Papatsoris A, Buchholz N. 2023. Recurrent miscarriage and male factor infertility: Diagnostic and therapeutic implications. A narrative review. *Central European Journal of Urology*. vol 76(4): 336-346. doi: 10.5173/ceju.2023.74.

Gilbert FS, Barresi JFM. 2018. Developmental Biology. Philladelphia, USA: Elsevier.

Gohil D, Sarker AH, Roy R. 2023. Base excision repair: Mechanisms and impact in biology, disease, and medicine. *International Journal of Molecular Sciences*. vol 24(18): 1-29. doi: https://doi.org/10.3390/ ijms241814186.

Kimura H. 2015. Signaling molecules, hydrogen sulfide and polysulfide. *Antioxidants and Redox Signaling*. vol 22(5): 362–376. doi: https://doi.org/10.1089/ars.2014.5869.

Li N, Wang H, Zou S, Yu X, Li J. 2025. Perspective in the mechanisms of repairing sperm DNA damage. *Journal of Reproductive Sciences*. vol 32: 41-51. doi: https://doi.org/10.1007/s43032-024-01714-5.

Maroto M, Torvisco S, Merino C, Gonzale RF, Pericuesta E. 2025. Mechanisms of hormonal, genetic, and temperature regulation of germ cell proliferation, differentiation, and death during spermatogenesis. *Biomolecules*. vol 15(4): 1-35. doi: https://doi.org/10.3390/biom15040500.

Muenyi C, Ljungman M, States C. 2015. Arsenic disruption of DNA damage responses-potential role in carcinogenesis and chemotherapy. *Journal of Biomolecules*. vol 5(4): 2184-2193. doi: https://doi.org/10.3390/biom5042184.

Nedecky R, Nejdi L, Gumulee J, Zitka O, Masarik M, Eckschlager T, Stiborova M, Adam V, Kizek R. 2013. Protein extraction for tissues and cultured cells using BioRuptor Standard Plus.ZDiagenode Protocol, Amerika Serikat of metallothionein in oxidative stress. *International Journal of Molecular Sciences*, vol 14: 6044-6066.

OECD. 2016. OECD guidelines for testing of chemicals. Organization for Economic Co-operation and Development.

Protocol Diagenode. 2011 Protein extraction for tissues and cultured cells using BioRuptor Standard Plus Diagenode Protocol, USA.

Ragosta ME, Traini G, Tamburrino L, Degl'Innoceti S, Fino MG, Dabizzi S, Vignozzi L, Baldi E, Marchiani S. 2024. Sperm chromatin dispersion test detects sperm DNA fragmentation mainly associated with unviable spermatozoa and underestimates the values with respect to TUNEL assay. *Journal of Molecullar Science*, vol 25(8): 1-12. doi: https://doi.org/10.3390/ijms25084481.

Salihu SI, Yusuf IL, Abba A, Tijjani MB, Maina UA, Shamaki BU. 2021. Heavy metal toxicosis and male fertility: The role of pentahydroxyflavone quercetin; a Review. *Journal of Applied Sciences and Environmental Management*. vol 25(7): 1277-1287. doi: https://dx.doi.org/10.4314/jasem.v25i7.25.

Scott FG, Michael JFB. 2018. Developmental Biology. Philladelphia, USA: Elsevier.

Sembiring E. 2024. Pengelolaan sampah dalam perspektif ekonomi sirkuler dan upaya pengurangan kebocoran sampah ke laut. Orasi Ilmiah Guru Besar Institut Teknologi Bandung.

Tan J, Taskin O, Albert A, Bedaiwy MA. 2019. Association between sperm DNA fragmentation and idiopathic recurrent pregnancy loss: a systematic review and meta-analysis. *Reprod. Biomed. Online*. vol 38(6): 951-960. doi: https://doi.org/10.1016/j.bmo.2018.12.029 1472-6483.

Vandekerckhove FWRC, Croo ID, Gerris J, Abbeel EV, Sutter PD. 2016. Sperm chromatin dispersion test befor sperm preparation is predictive of clinical pregnancy in cases of unexplained infertility treated with intrauterine insemination and induction with clomiphene citrate. *Journal Frontiers in Medicine*. vol. 3(63): 1-8. doi: https://doi.org/10.3389/fmed.2016.00063.

Yusuf A, Kinanti A. 2015. GO Protection Against Leachate at TPAS Sarimukti causing DNA damage in bone marrow and peripheral blood of male rat. *Academic Journal of Sciences*. vol 4(1): 259–265.