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Original Article

Lead (Pb) accumulation in rice and its impact on DNA stability

Runglawan Sudmoon[1], Arunrat Chaveerach[2], Unchaleeporn Ameamsri[2], Penkhae Thamsenanupap[3,4], Natapol Pumipuntu[4,5], Shiou Yih Lee[6], Ong Ghim Hock[6], Tawatchai Tanee[3,4*]

[1]Faculty of Law, Khon Kaen University, Khon Kaen, Thailand
[2]Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand
[3]Faculty of Environment and Resource Studies, Mahasarakham University, Maha Sarakham, Thailand
[4]One Health Research Unit, Mahasarakham University, Maha Sarakham, Thailand
[5]Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham, Thailand
[6]Faculty of Health and Life Sciences, INTI International University, Negeri Sembilan, Malaysia

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**Corresponding author email:*
tawatchai5@hotmail.com

Abstract

Heavy metal contamination, notably lead (Pb), poses a threat to plants and, consequently, human health through the food chain. This study investigates Pb accumulation in rice and how it affects the rice DNA. The rice (*Oryza sativa*) samples were cultivated in the soil supplemented with Pb at concentrations of 0, 15, 30, 60, and 120 mg/kg. Then the samples were harvested and analyzed for Pb accumulation and DNA alterations using the PCR amplification profiles measured through genomic template stability (GTS). The results demonstrate a Pb concentration hierarchy (root > stem > leaves), with Bioconcentration Factor (BCF) and Translocation Factor (TF) rising when soil Pb contents are supplemented. For DNA alterations, observed GTS values ranged from 23.3 to 76.67%, revealed a general decline with increasing Pb. This correlation assured the influence of Pb on rice DNA stability. Our findings suggest that heavy metal concentration, particularly Pb, has a direct influence on the integrity of rice DNA. Understanding these dynamics is vital for unraveling the complexities of heavy metal-induced genetic changes in plants and their potential implications for food safety and environmental health.

Keywords: Bioconcentration Factor, DNA changes, Genomic template stability, Lead accumulation, Translocation Factor

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Introduction

Lead (Pb) is one of the heavy metals extensively utilized and released from agricultural and chemical industries. It is commonly found in animal wastes and manures, and used in various applications, including inorganic fertilizers, pesticides, and herbicides as reported by Modaihsh et al. (2004), Keepax et al. (2011) and Budianta et al. (2022). The non-biodegradable nature of Pb and its excessive

usage contribute to its escalation toxic levels (Nas and Ali, 2018), presence in the environment, and pose potential risks to ecosystems, food chains, and human health. Previous research highlighted Pb's capacity to induce DNA damage, chromosomal aberrations, and genotoxicity, leading to various disorders affecting neurological, nephrological, cardiovascular, haematological, immunological, and reproductive functions (Nagaraju et al., 2022). Consequently, contamination of soil and water with Pb remains a significant environmental concern due to its inherent toxicity and persistent presence in the environment.

In soils contaminated with heavy metals, plants absorb the metal through their roots, subsequently translocating them to the shoot through physiological and biochemical mechanisms within plant cells. The contamination of agricultural soil with Pb not only results in its accumulation in plants but also facilitates its transfer from soil to the human food chain. Sharma and Dubey (2005) revealed that Pb alters hormone status, disrupts water and nutritional balance, and inhibits photosynthesis. Therefore, excessive Pb exposure leads to toxicity-response symptoms in plants, including root blackening, chlorosis, and stunted growth. Furthermore, an abundance of heavy metals leads to harm in various cellular components of plants including membranes, proteins, lipids, especially DNA as reported by Liu et al. (2005, 2009), Sudmoon et al. (2015), Tanee et al. (2016, 2018) and Azar and Vajargah (2023).

Advances in molecular technology have provided essential instruments for DNA analysis in genotoxicology. Genomic template stability (GTS) analysis, achieved through the determination of random DNA amplification profiles, enables the detection of various types of DNA mutations and damage, including rearrangements, point mutations, and insertions or deletions. This analysis has proven successful in investigating DNA changes induced by heavy metals in plants as evidenced by Liu et al. (2005, 2007, 2009), Sudmoon et al. (2015, 2021) and Tanee et al. (2016, 2018).

The adverse effects of Pb on both living organisms and the environment highlight the critical need for sustainable practices and regulations to moderate its impact and protect ecosystems and human health. In this study we utilized rice (*Oryza sativa* L.), which is one of the major foods globally (Rashid et al., 2018), especially in Thailand as a model for assessing Pb-induced genotoxicity. The research is mainly aimed to detect Pb concentrations in the rice tissues, including roots, stems, and leaves, and to assess the extent of its DNA damage induced by Pb using GTS analysis. This will contribute to understanding the interactions between heavy metal exposure, plant physiology, and genomic stability, revealing potential risks associated with heavy metal contamination in edible plants. Thus, leading to the practices and regulations for heavy metal usage, particularly Pb.

Material and Methods

Rice cultivation in Pb treatment

The soil under investigation was obtained from random agricultural sites in Maha Sarakham province, Northeastern Thailand, with initial Pb concentrations determined before the commencement of the study. Surface soil samples (0–30 cm depth) were collected and treated with varying concentrations of Pb ($PbCl_2$; Univar, Australia): 0 (no supplements as controls), 15, 30, 60, and 120 mg/kg. Seeds of *O. sativa* (Farmer, Thailand) were sown in a plastic pot contained one kg of the treated soil, resulting in five seedlings per pot. The pots were placed in an open field to replicate the natural cultivation conditions, with the application of organic fertilizers. The experiment was conducted following a fully randomized design with three replicates. After 90 days, cultivated rice was harvested and soil samples were collected for further examination. The leaf samples were spared for DNA extraction.

Examination of Pb in the rice and the soil

The following assessments were performed in three replicates. The soil samples initially collected from the field (pre-experiment) and experimental pots (post-experiment) were completely dried using an oven (Binder, USA) set at 105°C for 24 hours. Plant components, including roots, stems, and leaves, were dissected, thoroughly rinsed to remove surface impurities, and dried in the same condition. The oven-dried tissues were milled using mortars and pestles. For each sample of soil and plant tissues, one g was used and added to a 12 ml mixture of $HClO_4$: HNO_3 (1 : 3 ratio) in a test tube. The tubes were boiled in a water bath set at 100°C for digestion following the method by Tanee et al. (2013). The resulting transparent solutions were adjusted to a volume of 50 ml using distilled-deionized water and subsequently underwent analysis for Pb levels utilizing the Atomic Absorption Spectrophotometer

(AA 6200, Shimadzu, Japan).

DNA preparation for PCR reactions

The leaves were subjected to DNA extraction using the CTAB method, as described by Porebski et al. (1997) with some modifications (Tanee et al., 2018). The extracted DNA samples were stored at -20°C.

The PCR reaction was carried out in 25-µl reactions containing the PCR mixture (GoTaq Green Master Mix, Promega, USA), with an addition of 0.5 µM primer (purchased from Invitrogen, USA), using a 20-ng DNA. Out of 47 short random sequence primers screened, the ten primers that produced distinct banding profiles with polymorphisms were selected for further analysis. The sequences were as follows (5′-3′): TGCCGAGCTG, GGGTAACGCC, CAGCACCCAC, TGCGCCCTTC, GGTGACGCAG, GTCCACACGG, CTGCTGGGAC, GTGAGGCGTC, GATGACCGCC, and TGTCTGGGTG.

The reaction solutions underwent a 3-min pre-denaturation at 94°C. Subsequently, the 35-cycle amplification was performed by 30-sec denaturation at 94°C, a 30-sec annealing at 40°C, a 2-min extension at 72°C, and a final 7-min extension at 72°C utilizing Swift^{TM} Maxi Thermal Cycler (Esco Micro Pte. Ltd., Singapore). The amplified products obtained were determined using agarose gel electrophoresis. The amplifying profiles were counted and employed for the analysis of GTS.

Data analysis

Pb accumulation: The experiments were carried out in triplicate, and the data were analyzed utilizing Microsoft Office Excel 2010. The means and standard deviations (S.D.) for Pb concentrations in both the soil and the rice tissues were computed. The calculation of the Bioconcentration Factor (BCF) involved determining the ratio of Pb concentrations in the roots to the soil. Similarly, the Translocation Factor (TF) was established as the Pb concentration ratios in the shoots to the roots, in accordance with the method described by Malik et al. (2010) with the following calculation formula:

$$\mathrm{BCF} = \frac{Concentration\ of\ Pb\ in\ the\ roots\ (mg/kg)}{Concentration\ of\ Pb\ in\ the\ soil\ (mg/kg)}$$

$$\mathrm{TF} = \frac{Concentration\ of\ Pb\ in\ the\ stems\ (mg/kg)\ + Concentration\ of\ Pb\ in\ the\ leaves\ (mg/kg)}{Concentration\ of\ Pb\ in\ the\ roots\ (mg/kg)}$$

DNA alterations: The computation of GTS values followed the formula outlined by Liu et al. (2007) with the following calculation formula:

$$\text{Percentage of GTS} = (1 - \frac{a}{n}) \times 100$$

Where 'a' signifies the number of polymorphic loci detected in each experimental rice sample, and 'n' represents the total number of the banding loci in the control rice sample. Polymorphic variant, involving the disappearance and appearance of characteristic bands in the experimental rice sample as compared to the controls, were observed in the amplification patterns.

Results

Pb accumulations in the soil and plant tissues

The pre-experiment field soils were found to contain 16.18 mg/kg of Pb on average. Table 1 presents the quantity and dispersion of Pb in the soil samples and *O. sativa* tissues exposed to varying concentrations of Pb (0, 15, 30, 60, 120 mg/kg) added. The levels of Pb accumulation in the post-experiment soils varied from non-detectable (nd.) to 316 mg/kg. In roots, stems, and leaves of the plant, Pb accumulation ranged from 10.46 to 304.99, nd. to 21.94, and nd. to 31.11 mg/kg, respectively. Notably, the distribution of Pb within the plant showed a diminishing sequence with roots having the highest concentration, followed by stems and leaves. Furthermore, the concentration levels in all rice tissues are aligned to the concentration levels supplemented into the soil samples.

Bioconcentration factor (BCF) and translocation factor (TF)

The BCF assesses a plant's capacity to amass metals from soils, whereas the TF gauges its ability to transfer metals from roots to stems and leaves. Table 1 illustrates the values of BCF and TF for Pb in the five distinct treatments. In the various treatments, BCF values for Pb ranged from nd. to 0.96, signifying the plant's differential uptake of Pb from the soil. Similarly, TF values for Pb ranged from nd. to 0.17, indicating that the metal is transported from the roots to the stems and leaves. The values of both BCF and TF consistently rise with elevated concentrations of Pb in the soil (Table 1).

Table-1. Lead (Pb) accumulations, bioconcentration factor (BCF) and translocation factors (TF), and genomic template stability (GTS) investigated in *Oryza sativa* subjected to Pb treatments.

Pb added (mg/kg)	Pb level (mean mg/kg ± SD)				BCF	TF	GTS (%)
	Soil	Root	Stem	Leaf			
0 (control)	nd.	10.46±11.49	nd.	nd.	nd.	nd.	-
15	44.81±11.42	15.64±5.82	nd.	nd.	0.35	nd.	76.67
30	52.03±24.48	41.39±15.68	nd.	nd.	0.80	nd.	23.3
60	109.53±100.18	95.92±104.04	3.24±6.86	1.66±3.12	0.88	0.05	60.00
120	316.75±141.21	304.99±109.49	21.94±8.92	31.11±7.10	0.96	0.17	56.67

nd.= not detected

Effect of Pb exposure on PCR amplification profiles of the plants

The main observation from the DNA amplification profiles was the variations in the banding patterns of the rice exposed to Pb compared to the control rice. Figure-1 depicts a representative amplifying profile. The set of ten primers generated 37 distinguishable banding loci ranging in size from 100 to 3,000 bp. These patterns revealed noteworthy distinctions between the control rice and the Pb-treated rice, marked by the appearance of new bands or the absence of existing bands in the patterns produced by each primer. In total, 24 alterations in the bands, reflecting the distinct amplified fragments in the experimented and the control samples, were observed.

DNA changes in rice exposed to Pb were expressed as a percentage decrease in GTS, providing a qualitative assessment of alterations in DNA profiles. GTS values varied across different Pb treatments, ranging from 23.30% to 77.67% as shown in Table 1. A consistent reduction in GTS values was noted with an escalation in Pb added (Figure-2). However, at a concentration of 30 mg/kg, the highest reduction level was detected (GTS = 23.30%), this revealed the most substantial DNA changes.

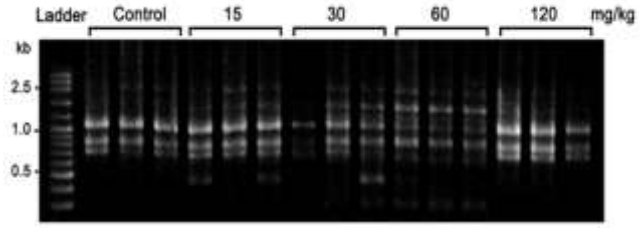

Figure-1. An example of amplifying profile with the primer CTGCTGGGAC showing DNA bands from the rice treated with different concentrations of Pb.

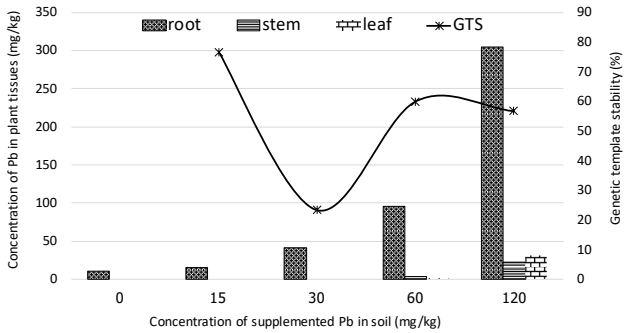

Figure-2. A graph depicts the relationship between Pb content in plant tissues and genetic template stability (GTS) values.

Discussion

Accumulation of heavy metals in consumable plants is a significant issue because it can impair crop yields and pose a health risk via the food chain. This study observed that rice can grow in soil containing diverse levels of Pb and can accumulate Pb in its roots, shoots and leaves. The concentration in the plant increased as concentrations of Pb in the soil rose (see Table 1). These findings align with previous research demonstrating its capability to accumulate Pb from the soil (Ashraf et al., 2017; Khan et al., 2021).

The variance in Pb uptake depends on the chemical and physical characteristics of the soil and the plant's capacity to absorb heavy metals (Bahemuka and Mubofu, 1999; Liu et al., 2005). Metal uptake is often linked to their concentrations in the soil, especially for mobile elements (Bañuelos and Ajwa, 1999). In general, as the Pb content in the soil increased, the plants correspondingly accumulate higher Pb levels.

The BCF level reflects the capability of the plants to uptake Pb from the soil, while the TF level indicates its capability to translocate Pb from its roots to its shoot. In this study, BCF and TF values were nd. to

0.96 and nd. to 0.17, respectively (see Table 1). Consequently, the concentration in the stems and leaves increased with a rise in Pb level in the soil. These findings align with previous reports demonstrating the capacity of plants to accumulate Pb from the soil (Ashraf et al., 2017; Khan et al., 2021). However, the BCF and TF values for Pb in rice were less than 1.0, and TF value was only observed at Pb concentrations of 60 mg/kg and 120 mg/kg, indicating low Pb bioavailability. A previous study noted that the translocation of Pb from soil to the plant was lower than Cd, with a bioaccumulation coefficient less than 1.0 (Kibria, 2012). Retention of heavy metals in the roots plays a crucial role in regulating or facilitating their translocation to the straw and to the grains. The mobility of Pb in roots may rely on adsorption to the cell wall and/or chelation to organic compounds (Kibria, 2012).

Based on the accumulation of Pb in rice, consumption of plants grown in contaminated soil is not recommended. Previous studies have shown that Pb can accumulate in various parts of rice, including the roots, straw, leaves, and grains, in Pb-contaminated soils (Kibria, 2012; Ashraf et al., 2017; Khan et al., 2021; Budianta et al., 2022). These findings emphasize the direct health hazards posed by Pb toxicity through the food chain, resulting in severe damage to organs such as the liver, kidneys, brain, and central nervous and reproductive systems. It can also induce symptoms such as anemia, hypertension, renal impairment, immunotoxicity (Kibria, 2012; World Health Organization, 2023), damage to hematopoietic organs, reduction in their function in animals (Vo et al., 2022), and DNA damage in consumers (Manahan, 2003).

Additionally, previous reports indicate that *O. sativa* can compile various heavy metals like cadmium, chromium, copper, and nickel (Khaniki and Zazoli, 2005; Kibria, 2012; Rashid et al., 2018; Huang et al., 2022; Budianta et al., 2022). However, studies on the genotoxic effects of Pb on *O. sativa* have not been reported. Our study provides further insight into the genotoxic effects of Pb, as evidenced by DNA damage assessed via GTS. While the precise molecular mechanisms underlying Pb-induced genotoxicity are not fully elucidated, previous studies suggest that Pb's genotoxic action primarily occurs through indirect mechanisms, such as the inhibition of DNA repair or induction of DNA oxidative stress (Hemmaphan and Bordeerat, 2022).

Liu et al. (2005, 2007, 2009) and Sun et al. (2009) reported that heavy metal ions induce various responses to cellular stress and plant cells. In genotoxicology, the DNA fingerprinting assay has been employed to detect DNA changes through GTS calculation (Sudmoon et al., 2015; Tanee et al., 2016). The DNA profiles (banding patterns) changes reflect different types of mutations and damage, including point mutations, rearrangements, and insertions or deletions, for examples (Liu et al., 2007).

In this study, the detection of genotoxic effects through DNA fingerprints entailed comparing the DNA profiles of control (unexposed) and treated (exposed) samples exposed to Pb over a 90-day period. The DNA damage induced by Pb was evident through changes in amplification profiles, including the appearance of new amplified fragments, a decrease and increase in band intensity, and the disappearance of bands in the profiles (refer to Table 1 and Figure-1).

Despite the limited total bands in DNA fingerprinting (37 characters), this data set was adequate for the GTS determination, encompassing 24 loci indicative of DNA alterations in comparison to the control sample. This finding supports the notion that alterations in DNA patterns resulting from exposure to genotoxic agents can be explained by a reduction in GTS, as indicated by Liu et al. (2007). Furthermore, GTS values were found to decrease with increasing subjected Pb concentrations (see Table 1 and Figure-2). The results suggest that Pb exposure induced changes in the GTS value in *O. sativa*, reflecting DNA changes in the plant species. Previous studies by Cenkci et al. (2010) and Malar et al. (2014) similarly demonstrated the notable influence of Pb concentrations on GTS in plant species. Additionally, this study proposes that DNA fingerprinting and GTS analysis are valuable protocols for studying ecotoxicology as well as useful biomarkers for detecting genotoxic effects of Pb on plants.

The overall results demonstrated that the elevated level of Pb in the organisms raises concerns, not only due to its potential effects on the environment but also because it causes genomic damage to the plants themselves. This research underscores the importance of informing the public about the implications of heavy metal accumulation in edible plants. Disseminating these findings is crucial for prompting careful consideration of the consumption of such plants. It also emphasizes the need for

guidance aimed at farmers and other stakeholders, specifically addressing the proper use of pesticides, herbicides, and fertilizers to ensure future food safety.

Conclusion

In this research, rice exhibited varying capacities to accumulate Pb across different plant parts, with the highest concentrations observed in roots, followed by stems and leaves. Both BCF and TF values increased proportionally with soil Pb concentrations, indicating the plant's capacity to accumulate Pb. However, BCF and TF values remained below 1.0, signifying relatively low Pb bioavailability. Additionally, DNA damage was found to increase with increasing treated Pb concentrations. These findings designate the impact of Pb on inducing genetic alterations in plants, emphasizing the need for sustainable regulations governing the use of Pb-containing materials to ensure food safety and safeguard human and environmental health.

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Contribution of Authors

Sudmoon R: Contributed to research design, tests, data analysis, manuscript writing and editing.
Chaveerach A & Ameamsri U: Executed the tests and contributed to manuscript editing.
Thamsenanupap P & Pumipuntu N: Contributed to research design and manuscript editing.
Lee SY & Hock OG: Collected the data and contributed to manuscript editing.
Tanee T: Designed the research work, analyzed all the data, wrote and edited manuscript.

All authors critically discussed and finally approved the contents of the manuscript.

References

Ashraf U, Kanu AS, Deng Q, Mo Z, Pan S, Tian H and Tang X, 2017. Lead (Pb) toxicity; physio-biochemical mechanisms, grain yield, quality, and pb distribution proportions in scented rice. Front. Plant Sci. 8: 259. DOI: https://doi.org/10.3389/fpls.2017.00259

Azar H and Vajargah MF, 2023. Investigating the effects of accumulation of lead and cadmium metals in fish and its impact on human health. J. Aqua. Mar. Biol. 12(2): 209–213. DOI: https://doi.org/10.15406/jamb.2023.12.00376

Bahemuka TE and Mubofu EB, 1999. Heavy metals in edible green vegetables grown along the sites of the Sinza and Msimbazi rivers in Dar es Salaam, Tanzania. Food Chem. 66: 63–66. DOI: https://doi.org/10.1016/S0308-8146(98)00213-1

Bañuelos GS and Ajwa HA, 1999. Trace elements in soils and plants: and overview. J Environ. Sci. Health 34(4): 951–974. DOI: https://doi.org/10.1080/10934529909376875

Budianta D, Napoleon A, Merismon and Habi ML, 2022. Save our soil from heavy metals (Pb and Cd) accumulation for rice growth. IOP Conf. Ser.: Earth Environ. Sci. 1005: 012001.

Cenkci S, Ciğerci İH, Yıldız M, Özay C, Bozdağ A and Terzi H, 2010. Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. Environ. Exp. Bot. 67(3): 467–473. DOI: https://doi.org/10.1016/j.envexpbot.2009.10.001

Hemmaphan S and Bordeerat NK, 2022. Genotoxic effects of lead and their impact on the expression of DNA repair genes. Int. J. Environ. Res. Public Health. 19(7): 4307. DOI: https://doi.org/10.3390/ijerph19074307

Huang X, Zhao B, Wu Y, Tan M, Shen L, Feng G, Yang X, Chen S, Xiong Y, Zhang E and Zhou H, 2022. The lead and cadmium content in rice and risk to human health in China: a systematic review and meta-analysis. PLoS ONE 17(12): e0278686. DOI: https://doi.org/10.1371/journal.pone.0278686

Keepax RE, Moyes LN and Livens FR, 2011. Speciation of heavy metals and radioisotopes, pp. 165-199. In S. Aleksandar (ed.), Environmental and Ecological Chemistry Volume II, Encyclopedia of Life Support Systems (EOLSS). Department of Chemistry, University of Manchester, Manchester, UK.

Khan M, Rolly NK, Azzawi TNIA, Imran M, Mun BG, Lee IJ and Yun BW, 2021. Lead (Pb)-induced oxidative stress alters the morphological and

physio-biochemical properties of rice (*Oryza sativa* L.). Agronomy 11: 409. DOI: https://doi.org/10.3390/agronomy11030409

Khaniki GRJ and Zazoli MA, 2005. Cadmium and lead contents in rice (*Oryza sativa*) in the north of Iran. Int. J. Agric. Biol. 7(6): 1026–1029.

Kibria MG, 2012. Uptake of cadmium and lead by rice grown in four contaminated soils of Chittagong, Bangladesh. Hamdard Medicus 55(2): 1–8.

Liu W, Li PJ, Qi XM, Zhou QX, Zheng L, Sun TH and Yang YS, 2005. DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis. Chemosphere 61: 158–167.

Liu W, Yang YS, Li PJ, Zhou QX, Xie LJ and Han YP, 2009. Risk assessment of cadmium-contaminated soil on plant DNA damage using RAPD and physiological indices. J. Hazard. Mater. 161: 878–883.

Liu W, Yang YS, Zhou Q, Xie L, Li P and Sun T, 2007. Impact assessment of cadmium contamination on rice (*Oryza sativa* L.) seedlings at molecular and population levels using multiple biomarkers. Chemosphere 67: 1155–1163.

Malar S, Manikandan R, Favas PJC, Sahi SV and Venkatachalam P, 2014. Effect of lead on phytotoxicity, growth, biochemical alterations and its role on genomic template stability in *Sesbania grandiflora*: a potential plant for phytoremediation. Ecotoxicol. Environ. Saf. 108: 249–257. DOI: https://doi.org/10.1016/j.ecoenv.2014.05.018

Malik RN, Husain SZ and Nazir I, 2010. Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. Pak. J. Bot. 42: 291–301.

Manahan SE, 2003. Toxicological chemistry and biochemistry, 3rd ed. Lewis Publishers, USA.

Modaihsh AS, Al-Swailem MS and Mahjoub MO, 2004. Heavy metals content of commercial inorganic fertilizers used in the Kingdom of Saudi Arabia. Agric. Mar. Sci. 9: 21–25.

Nagaraju R, Kalahasthi R, Balachandar R and Bagepally BS, 2022. Association between lead exposure and DNA damage (genotoxicity): systematic review and meta‑analysis. Arch. Toxicol. 96:2899–2911. DOI: https://doi.org/10.1007/s00204-022-03352-9

Nas F and Ali M, 2018. The effect of lead on plants in terms of growing and biochemical parameters: a review. MOJ Ecol. Environ. Sci. 3: 265–268.

Porebski S, Bailey LG and Baum BR, 1997. Modification of a CTAB extraction protocol for plants containing high polysaccharides and polyphenol component. Plant Mol. Biol. Rep. 15: 8–15.

Rashid R, Muda N, Saaid M, Halim M and Mohamed K, 2018. Determination of Cd, Cu, Ni and Pb in selected rice grain available in Malaysia. Int. J. Engineer. Technol. 7(14): 232–236. DOI: https://doi.org/10.14419/ijet.v7i4.14.27569

Sharma P and Dubey RS, 2005. Lead toxicity in plants. Braz. J. Plant Physiol. 17(1): 35–52. DOI: https://doi.org/10.1590/S1677-04202005000100004

Sudmoon R, Neeratanaphan L, Thamsenanupap P and Tanee T, 2015. Hyperaccumulation of cadmium and DNA changes in popular vegetable, *Brassica chinensis* L. Int. J. Environ. Res. 9(2): 433–438.

Sudmoon R, Thamsenanupap P, Teanma J, Chaveerach A, Kaewduangdee S and Tanee T, 2021. The effect of glyphosate on genotoxicity in *Ipomoea aquatica*. Toxicol. Environ. Chem. 103(3): 245–253. DOI: https://doi.org/10.1080/02772248.2021.1957472

Sun Y, Zhou Q, Wang L and Liu W, 2009. Cadmium tolerance and accumulation characteristics of *Biden spilosa* L. as a potential Cd-hyperaccumulator. J. Hazard. Mater. 161: 808–814.

Tanee T, Sudmoon R, Thamsenanupap P and Chaveerach A, 2016. Effect of cadmium on DNA changes in *Ipomoea aquatica* Forssk. Polish J. Environ. Stud. 25(1): 303–307. DOI: https://doi.org/10.15244/pjoes/60726

Tanee T, Chaveerach A, Narong C, Pimjai M, Punsombut P and Sudmoon R, 2013. Bioaccumulation of heavy metals in fish from the Chi River, Maha Sarakham Province, Thailand. Int. J. Biosci. 3(8): 159–167. DOI: https://doi.org/10.12692/ijb/3.8.159-167

Tanee T, Chaveerach A, Sudmoon R, Teanma J, Ragsasilp A and Sirikhansaeng P, 2018. Heavy metal accumulation and DNA changes in plants around an electronic waste dumpsite suggested environmental management plan. Environ. Claims J. 30(2): 131–141. DOI: https://doi.org/10.1080/10406026.2018.1427961

Vo VT, Long Le TM, Duong TQA, Mai NAT and Thuong NHT, 2022. Assessment of lead toxicity in red tilapia *Oreochromis* sp. through hematological parameters. Asian J. Agric. Biol. 2022(2): 202101016. DOI: https://doi.org/10.35495/ajab.2021.01.016

World Health Organization, 2023. Lead poisoning (https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health).