



Evaluation of Glyphosate Levels in Sediments of Milky Stork Foraging Areas in Kuala Gula Bird Sanctuary, Perak, Malaysia

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ABSTRACT

Glyphosate is the most widely used herbicide in the world since its first introduction in the 1970s. Although its accumulation and direct impact on top predators are unlikely, it has the potential to affect the lower trophic community causing imbalance to the local food web. This paper highlights the background levels of glyphosate in the sediment in a part of the Important Bird and Biodiversity Programme Areas (IBA) in the Matang coast i.e., the Kuala Gula Bird Sanctuary, Perak, Malaysia. Despite housing endangered species like the milky stork and migratory birds, the area is surrounded by massive oil palm plantations that utilize glyphosate-based herbicide. Sediment samples were taken from areas frequently visited by the bird population. The herbicide was analyzed by HPLC-UV detection with previous derivatization using 9-fluorenylmethylchloroformate (FMOC-Cl). In general, the glyphosate levels were between 0.26 and 1.72 ppm. These levels are considered low when compared to other agricultural sites in both local and regional areas. In addition,

no significant relationship was established between glyphosate levels in the study sites with the distance from nearby agricultural activity ($r = 0.2$, $n = 18$, $p > 0.05$). This evidence suggests that the pollutant most likely comes from an inter-connected network of water canals and water-bodies that accumulate the plantations' effluent over time. Due to its resistant nature and low concentration in the field, there is

ARTICLE INFO

Article history:

Received: 18 September 2017

Accepted: 27 May 2019

Published: 19 August 2019

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little concern over its adverse impact on the aquatic ecosystem in the study area. However, continuous monitoring is still emphasized in this paper.

Keywords: Agriculture, glyphosate, Kuala Gula Bird Sanctuary, Malaysia, milky stork

INTRODUCTION

Glyphosate is the most widely used herbicide in the world since its first introduction in the 1970s. Oil palm plantation is one of the most important industries that utilize this herbicide. At least 15 million liters of this herbicide is used annually to control the weed problem (Raman et al., 2010). Although its accumulation and direct impact on top predators are unlikely, it has the potential to affect the lower trophic community causing imbalance to the local food web. In addition, there is growing evidence that glyphosate and its metabolite are harmful. Its widespread use has caused negative effects to the environment (Székács & Darvas, 2012). Although glyphosate itself is only slightly or moderately toxic to the aquatic animals (Giesy et al., 2000; Solomon & Thompson, 2003), the surfactant polyoxyethylene amine (POEA) added in the commercial formulation is considered to be more toxic (Brausch & Smith, 2007; Tsui & Chu, 2003). Thus the combination of the two is expected to be more lethal (Yusof et al., 2014).

Williams et al. (2012) stated that glyphosate was non-volatile and bound strongly to most soils or sediments, and was degraded by microbes before it could

reach any non-target area. The C-P bonds in the phosphonate of this herbicide are resistant to physicochemical factors (Murai & Tomizawa 1976) but they can be cleaved by the microbial enzymatic system under certain conditions (Kononova & Nesmeyanova, 2002). In addition, the degradation of glyphosate is slow in muck soils due to the inaccessibility of any microbial metabolism into the muck (Cheah et al., 1998). Similarly, sediment could represent an alternative transport pathway during monsoonal floods which move vast quantities of sediment with glyphosate in them (Brodie et al., 2012; Kroon et al., 2012).

Malaysia is the second largest oil palm producer behind Indonesia (Malaysian Oil Palm Council [MPOC], 2016) and the industry relies heavily on herbicides to control weeds. Mishandling and excess usage of glyphosate-based herbicides in the country have also been reported (Ali & Shaari, 2015). However, monitoring data pertaining to glyphosate levels in the environment is scarce in Malaysia. Limited study has been conducted so far to address this issue. For instance, high concentration of glyphosate in waterbodies was reported in Tasik Chini particularly during the rainy season (Mardiana-Jansar & Ismail, 2014). However, no other monitoring studies were conducted to highlight this issue. Glyphosate can be easily leached out into the aquatic environment following high rainfall activity. Rainfall in Malaysia is divided into two maximum rainfalls i.e., between October and November as well as April and May for the most part of the peninsular

with an average of 250 cm of rain annually (Malaysian Meteorological Department [MetMalaysia], 2017). Thus, more studies are needed to monitor and understand the impact of the herbicide's input into such an environment. In addition, with the heavy usage of glyphosate-based herbicides in the country, leaching and surface runoff will occur during heavy rainfall activity causing most of it to flow into rivers and finally enter the sea. Interestingly, glyphosate has also been demonstrated to be moderate to highly persistent in marine water thus affecting coral reefs in the ocean (Mercurio et al., 2014).

The Kuala Gula Bird Sanctuary in Malaysia is an important example where intense agricultural activity in its hinterland could potentially cause a negative impact on its aquatic environment. Given the large scale plantations operating with over 7000 ha that reach Kuala Sepetang in the east and Bagan Serai on the north, as well as their proximity to the Kuala Gula's estuary and mudflats, the accumulation of glyphosate in the sediment can potentially be harmful to lower trophic organisms. These include benthic and fish communities that are mostly consumed by top predators like the water birds in the area. Consequently, the decline of their prey would greatly affect the predator population in the long run. However, there are no available studies that highlight this particular issue in this area. Considering the importance of the sanctuary to both resident and migratory birds, including endangered species like the milky stork, there is a need to evaluate the levels of glyphosate in Kuala Gula's aquatic

environment. The findings would also serve as important baseline information for future studies.

MATERIALS AND METHODS

Study Area

The Kuala Gula Bird Sanctuary (4°56'00" N; 100°28'00" E) is located in the northern part of the west coast of Peninsular Malaysia in the Kerian district, 45 kilometres from Taiping, Perak (Figure 1). It is also part of the north area of the Matang Mangrove Forest Reserve (MMFR), the single largest track of mangroves in the country with an area of approximately 40,000 ha. Elevation of the area averages at 2 - 3 metres above sea level and the average rainfall is 2063 mm per annum (Department of Irrigation and Drainage Malaysia [JPS], 2018). Kuala Gula is regarded as one of the important stopovers in the East Asia-Australia flyway and also a sanctuary to a number of endangered species such as the milky stork and lesser adjutant (Ismail & Rahman, 2016). Possessing one of the major estuaries in the Matang coast, it is included in the Important Bird and Biodiversity Area (IBA) Programme, a worldwide initiative aimed at identifying, documenting and working towards the conservation and sustainable management of a network of critical sites for the world's birds. Continuous habitat destruction along the coastal area of the peninsular is considered as one of the primary factors that drive the milky storks in Malaysia close to extinction (Ismail & Rahman, 2012, 2016). As such, Kuala Gula serves as an important and final frontier in which the last population

could thrive. Therefore, monitoring its environment to ensure any development and human activity are being carried out sustainably should remain a top priority. At least five sites frequently visited by the endangered milky stork population were studied. They consisted of both natural and artificial terrains and hydrology which serve as important foraging areas for the milky storks (Rahman et al., 2017). In addition,

some of these sites also overlap with the wintering ground of many shorebirds during the migratory season. Table 1 shows the descriptions of the sampling areas in Kuala Gula. Palm oil plantation is a massive industry in Kuala Gula where most of the inland areas have been converted to support this agricultural activity. It is estimated that at least 7,000 ha of palm oil plantations are active throughout Kuala Gula. The

Table 1
Description of the study sites in Kuala Gula Bird Sanctuary, Malaysia

Site	Coordinates	Site description
S1	4°56015.77N; 100°28003.39E	Water canal that channels both agricultural and domestic effluents into the Gula River's tributary
S2	4°56024.22N; 100°28007.42E	Intertidal mudflat area close to a jetty and fishing village in Gula River
S3	4°57010.24N; 100°29013.66E	Intertidal mudflat turned into shrimp farm
S4	4°55040.03N; 100°27046.54E	Intertidal mudflat of Gula River that also receives effluents from nearby aquaculture farms
S5	4°56014.14N; 100°28006.68E	Intertidal mudflat adjacent to a vacation resort along the Gula River
S6	4°55'52.12"N; 100°29'24.71"E	Mangrove area recently turned into new shrimp farms in Pulau Gula (Gula Island)

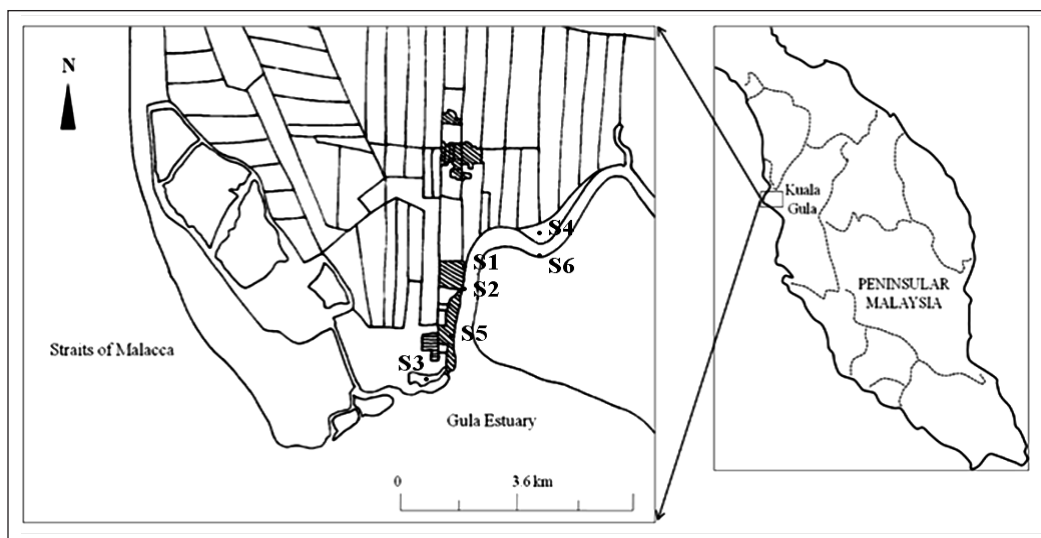


Figure 1. Map of the Kuala Gula Bird Sanctuary, Perak, Malaysia

aquaculture industry is also expanding rapidly and has led to an increased number of reclaimed mangroves in recent years (Ismail & Rahman, 2016; Rahman et al., 2013, 2017).

Laboratory Work and Analysis

Sampling activity was carried out in Kuala Gula's coastal in January 2013. Approximately 50 g of surface sediments (1-5 cm depth) were taken from each site, S1-S6 and kept in an ice box with a temperature of 4°C during transportation. At the laboratory, the samples were dried in an air-circulating oven at 60°C for at least 72 hours or until constant dry weights were achieved (Ismail & Ramli, 1997). The samples were later sieved for further processing (Fisher Scientific Company, mesh size: 63 µm). For the glyphosate extraction procedure, a modified method based on Peruzzo et al. (2008) was employed. The presence of the herbicide in the sediment was determined after the extraction of 15 g of sample with monopotassium phosphate (MKP, KH_2PO_4) 0.1 M; the mixture then undergoes agitation for 15 min followed by centrifugation (1,464 g; 10 min) and later filtration through Whatman No. 1 filter paper. The extraction was repeated twice on solid residue, obtaining a 25 ml extract from each sample. Later, the extracts were filtered through a 0.45 mm cellulose acetate membrane. Finally, the derivatization process was carried out as follows: 0.25 ml of Borate buffer 0.025 M, 0.30 ml of Fmoc-Cl 2 M and chloroform (CHCl_3) were added to 1 ml of each sample. Then the mixture

was left for 24 hours to react in 40°C temperature in the dark. After that, 0.30 ml of Phosphoric acid (H_3PO_4) (2%) was added and then kept in the fridge for further analysis. The derivatized product (Gly-Fmoc) was analyzed in triplicates using High Performance Liquid Chromatography (HPLC, Shimadzu) with Ultra-Violet (UV) detector; 20 µL of acetonitrile was injected as blank solution prior to the samples, after which each sample was injected three times with a similar volume as the blank. The limit of detection for the glyphosate analyzed was 0.00021 nanogram per gram (ng/g), with retention time of 32.6 minutes. The chromatographic conditions used in this study were as follow: Fortis Universal C18 column (5 mm particle size, length I.D.: 15 cm x 4.9 mm); mobile phase 0.05 M phosphate buffer (pH 5.5): acetonitrile (70:30); flow: 0.8 ml/min; and UV detection: 206 nm. The standards used were derived using Sigma PESTANAL® with 99% purity and further diluted using Milli-Q® water. The glyphosate was first dissolved into 100 ppm concentration and later had its concentration diluted to 1 ppm, 0.5 ppm, 0.1 ppm, 0.05 ppm, 0.01 ppm, 0.005 ppm and 0.001 ppm for testing. Figure 2 shows the peak versus the concentration of the glyphosate's standard analyzed for linearity purpose. Other chemicals used are of analytical grade quality. Recoveries of 80-120% were obtained for the glyphosate in the sediments. Pearson's rho correlation was used to understand the relationship between the glyphosate levels and the distance from agricultural activity in the area.

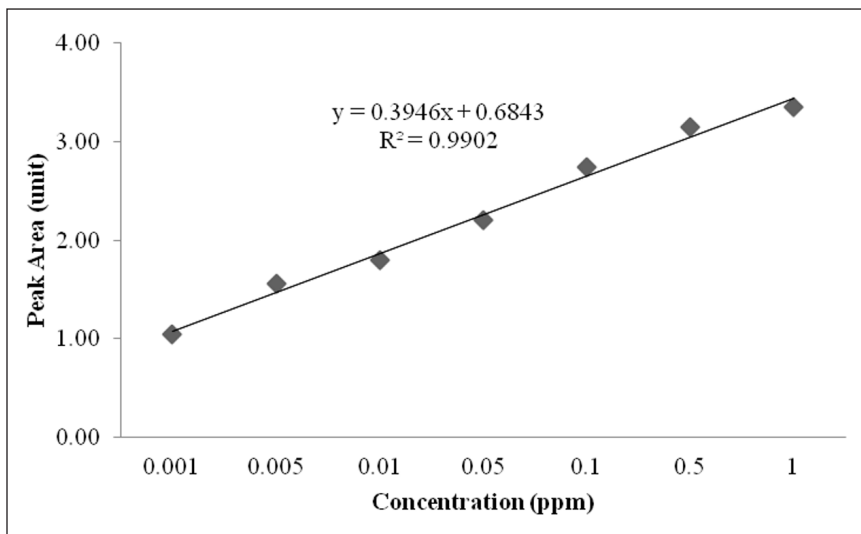


Figure 2. Peak area versus glyphosate's standards linearity

RESULTS AND DISCUSSION

The glyphosate was successfully extracted and analyzed from surface sediments in the foraging areas of Kuala Gula. Figure 3 shows the example of the chromatograms for the standard (0.001 ppm) and one of the samples analyzed. In general, the levels obtained ranged from 0.26 to 1.80 ppm. Figure 4 shows the different levels of glyphosate according to the different sites available. In general, sites 1 to 4 had accumulated higher amounts of glyphosate in the sediment compared to sites 5 and 6. These sites (S1, S2, S3 and S4) are mostly surrounded by massive oil palm plantations and could have directly or indirectly received effluents from the agricultural activity. On the other hand, S6 had the lowest glyphosate level with 0.26 ppm recorded in the sediment. We found no significant correlation between the glyphosate levels and the distance to the oil palm plantations in the area ($r = 0.2$, $n = 18$, $p > 0.05$). The findings suggest that

the pollutant most likely comes from an inter-connected network of water canals and water-bodies surrounding the study sites which accumulate effluents from agricultural activity in Kuala Gula. This could occur either through a leaching process or direct input of the effluent particularly during high rainfall activity. Further study could focus on monitoring the temporal fluctuation of glyphosate levels in different seasons post-application in the field to understand its accumulation and distribution patterns.

According to Smith and Neiman (2012), soils are vertically weathering profiles that develop in place requiring time and stable surface while sediments are particles transported by natural forces like wind or water. Schuette (1998) stated that glyphosate was relatively immobile in most soil environments as a result of its strong adsorption to soil particles. In addition, glyphosate can also last for at least one year following aerial-spray

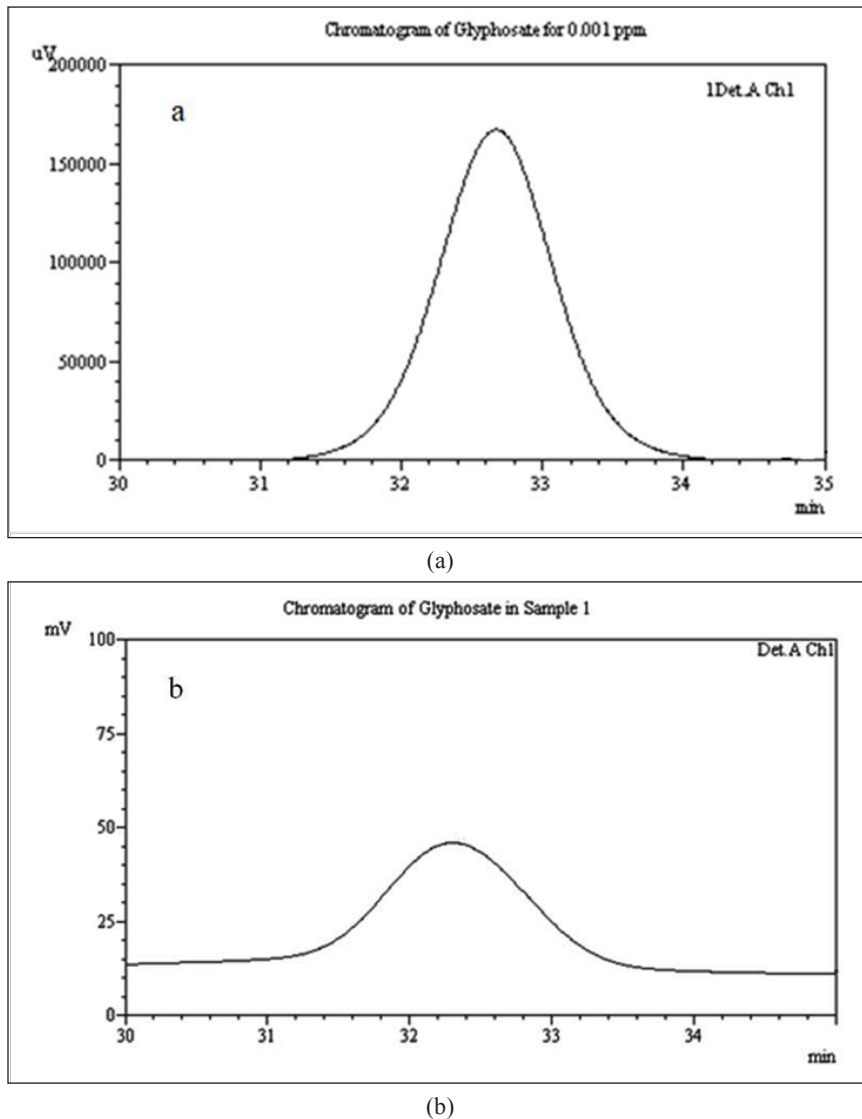


Figure 3. Example of the chromatogram for a) 0.001 ppm standard and b) one of the samples in which glyphosate compound was detected at the 32.6 minutes mark

activity (Major et al., 2003) depending on the soil type and light availability. On the contrary, glyphosate in sediment, although can be very resistant, is highly mobile as it can even be found in areas that have no direct pesticide application which are considered undeveloped and remote (Smalling et al., 2012). Its strong binding

particularly to organic matter allows for long distance transport and being persistent in the environment (Solomon & Thompsom, 2003). Moreover, as sediment appears to be the major sink for glyphosate residue in most of the aquatic systems (Schuette, 1998), this pollutant can reach farther into seawater, even affecting coral reefs such as

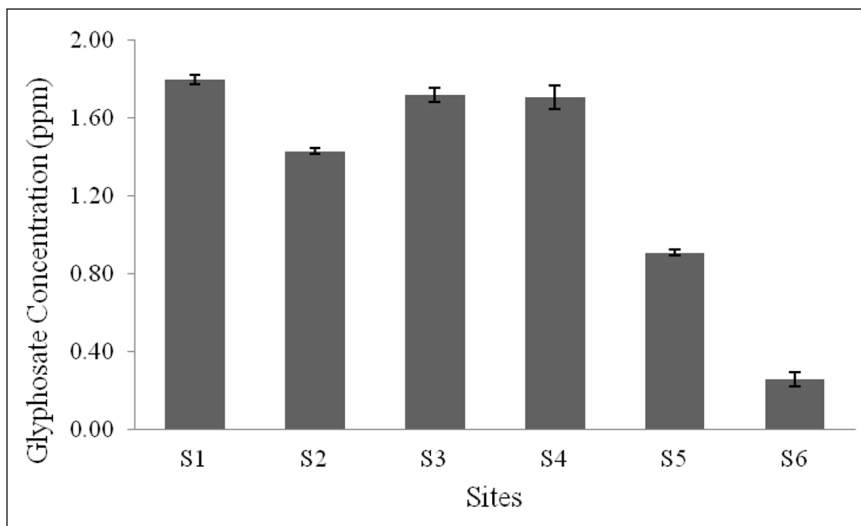


Figure 4. Glyphosate concentrations (mean \pm standard deviation) in sediment in the different sites used by the Milky stork in Kuala Gula

in the case of the Great Barrier Reef studied by Mercurio et al. (2014). In our study, the presence of this agricultural pollutant can be explained by the scale of the oil palm plantations in Kuala Gula, estimated to accommodate more than 7000 ha area. Moreover, given that Malaysia is the second largest oil palm producer after Indonesia, which accounts for 39% of global oil palm production and 44% of global export (MPOC, 2016), the use of such herbicide is heavily relied upon. For instance, a survey conducted by Abdul (2017) estimated that the total amount of glyphosate-based herbicide volume sold in the country in 2017 alone was about 27 kilotons. The rampant mismanagement of the glyphosate-based herbicide in the country involving unlicensed manufacturers and retailers, and inadequate users' information that lead to the mishandling and excess usage of the pollutant further contribute to the problem (Ali & Shaari, 2015). As rainfall activity

in Malaysia is often very high, much of this herbicide will be washed out into the streams and rivers. Thus, the possibility of the pollutant reaching remote areas needs to be studied to provide important information on the pollutant's mobility in the larger part of the Matang Mangrove Forest.

Table 2 shows the levels of glyphosate in sediments in agricultural areas from different countries worldwide using comparable methodology. In general, glyphosate levels recorded in Kuala Gula is much lower compared to other areas with agricultural activity of similar intensity. Soil characteristics may play an important role in the accumulation of glyphosate as glyphosate degradation rates vary considerably across a wide variety of soil types but it does not appear to be largely dependent on soil pH or organic content (Grossbard & Atkinson, 1984). Sprankle et al. (1975) reported that the prime factor in determining the amount of glyphosate adsorbed to soil

particles was the soil phosphate level and that glyphosate was bound to soil through the phosphonic acid moiety. Phosphate (P) in mangrove soils can be immobile and unavailable for plant use (Reef et al., 2010) but the occurrence of phosphate-solubilizing bacteria in association with mangrove roots serve as an important role in P uptake by the plant (Kothamasi et al., 2006; Vazquez et al., 2000). Although the soil properties in the study areas were not studied, the milky stork population was often found foraging close to or along the mangroves which are muddy and have high organic content. The half-life of glyphosate in both water and soil can range from few days to up a year depending on the soil composition (Székács & Darvas, 2012). For instance, soil parameters and temperature differ significantly depending on its composition, making glyphosate half-lives reaching more than 300 and up to 428 days (Borggard & Gimsing, 2008). In addition, due to its adsorption on clay particles and organic matter, glyphosate may remain unchanged in the soil for varying lengths of time (Penaloza-Vazquez et al., 1995). The presence of high organic matter in the mangrove would mean that most of

the available glyphosate residues will bind to them and are less likely to be leached into the aquatic environment. Hence the sediment samples obtained from such areas may explain the low level of glyphosate recorded in this study. There are also many tributaries or rivers nearby in which the glyphosate could escape into eventually reaching and sinking into the estuaries. This may help reduce the amount of glyphosate found in a particular area. As such, to account for the actual amount of glyphosate in the environment in the area, future studies should also consider obtaining samples from other nearby rivers and estuaries.

Water birds are known to be an important indicator for environmental changes and have been used widely around the world (Rahman & Ismail, 2018). Although the high levels of glyphosate may not affect the Milky stork population directly, its indirect effect on the lower trophic organisms including benthic and fish communities have been highlighted by many (Ayoola, 2008; Cuhra et al., 2013; Lajmanovich et al., 2003; Meyer et al., 2014; Paganelli et al., 2010; Relyea, 2005; Schneider et al., 2009). These studies showed that, through

Table 2
Comparison of glyphosate levels in sediments reported in different countries with commercial agricultural activity

No	Country	Agricultural activity	GLYP levels (ppm)	Reference(s)
1	Kuala Gula, Malaysia	Palm oil	0.3 – 1.8	This study
2	Tasik Chini, Malaysia	Palm oil	0.0 – 6.0	Mardiana-Jansar and Ismail (2014)
3	Pampa, Argentina	Soybean	0.5 – 5.0	Peruzzo et al. (2008)
4	Chaco, Argentina	Soybean/ Corn	0.0 – 0.3	Aparicio et al. (2014)
5	Willipa Bay, Washington, USA	NA	1.6 – 2.8	Paveglio et al. (1996)

various modes of actions, even low levels of glyphosate can negatively affected non-target organisms. In addition, Mertens et al. (2018) highlighted that the strong tendency of glyphosate to sorb on minerals through its functional groups might mobilize bound trace metals by chelation and sorbed anions by displacement. High glyphosate input in soils containing elevated concentrations of heavy metals and phosphate have been shown to increased leaching activity of the metals compared to the one with normal background concentrations of heavy metals (Barret & McBride, 2006). Through bio-accumulation and bio-magnification processes, the metals will affect the food chains and ultimately top predators like the water birds. As such, there is an important need to continuously monitor glyphosate activity in the study area. Currently, apart from the mismanagement of the herbicide by irresponsible parties, continuous activity and the expansion of anthropogenic activity in Kuala Gula could lead to the increase of glyphosate levels in the aquatic environment in the future. Such activities would have a negative impact on the water bird population such as the endangered milky storks as they rely on these sites as a food source. Thus, there is a need to increase the awareness and support among the public, land owners and related industries in the area to help conserve the coastal ecosystem of Kuala Gula. Nonetheless, at its current state, glyphosate pollution in Kuala Gula can still be considered as very low and is unlikely to cause any issues to the water birds in the near future.

CONCLUSION

The glyphosate levels in Kuala Gula are still considered safe and are unlikely to cause any negative impact on the endangered species in the area. However, further monitoring is still needed as the mismanagement of the herbicide and the reclamation of the mangrove and its surrounding areas still continue. The current findings also serve as important baseline information for future studies.

ACKNOWLEDGEMENT

This work was financially supported by the Ministry of Higher Education Malaysia under the Fundamental Research Grant Scheme, Vot No. 5524646.

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