# Comparison of life cycle toxicity assessment methods for municipal wastewater treatment with the inclusion of direct emissions of metals, PPCPs and EDCs

**Siti Safirah Rashid, Yong-Qiang Liu** \*

Faculty of Engineering and Physical Sciences, University of Southampton, Southampton, SO17 1BJ, United Kingdom

## ABSTRACT

The occurrence of various micropollutants such as pharmaceuticals personal care products, endocrine disrupting chemicals (PPCPs/EDCs) and metals in municipal wastewater, and their poor removal efficiencies can lead to toxicity impact on humans, and freshwater and terrestrial ecosystems. Life cycle assessment is an efficient and effective tool to evaluate the environmental impact of wastewater treatment plants, but guidelines for toxicity assessment are lacking due to the complexity. This study aims to evaluate both life cycle inventory by including metals and PEC, and life cycle toxicity assessment (LCIA) methods namely CML-IA, Recipe, USEtox, EDIP 2003 and IMPACT 2002+ in midpoint category with a large centralised wastewater treatment plant in Malaysia as a case study. The removal efficiencies of metals and PPCPs/EDCs in the wastewater ranged from 9% to 99% and no clear patterns were found about occurrence and removal efficiencies of metals and PPCPs/EDCs in developing and developed countries. The inclusion of metals and PPCPs/EDCs in effluent resulted in 76% increase in freshwater ecotoxicity potential (FEP) and 88% increase in terrestrial ecotoxicity potential (TEP) while only 4% increase in human toxicity potential (HTP). The results indicate the importance of including direct emissions such as metals and PPCPs/EDCs even in low-strength municipal wastewater for environmental toxicity assessment. The comparison of five LCIA methods suggests that HTP assessment is more challenging due to inconsistency between five LCIA methods while CML-IA, Recipe, and IMPACT 2002+ achieved consistent human toxicity and ecotoxicity assessment results in the WWTP. The results highlight the importance of sampling and inclusion of metals and PPCPs/EDCs data especially prioritised micropollutants for life cycle toxicity assessment and recommend LCIA methods for ecotoxicity assessment of WWTPs in the current scientific development situation on toxicity studies, which can provide guidance to researchers for life cycle toxicity assessment of wastewater treatment.

*Keywords:*Wastewater treatment plants; Toxicity assessment; Metals; Pharmaceuticals and personal care products; Endocrine disrupting chemicals; Life cycle impact assessment method; Characterisation factors

\*Corresponding author.

*E-mail address*: [Y.Liu@soton.ac.uk](mailto:Y.Liu@soton.ac.uk)

## 1. Introduction

Toxicity impact assessment from municipal wastewater treatment has attracted great attention in recent years especially when more and more contaminants of emerging concern (CEC) are detected from municipal wastewater. According to European Economic Community 1991 (EEC, 1991), municipal wastewater treatment plants contribute a considerable amount of pollutants to the natural environment through disposal of sludge and discharge of effluent to water bodies. In the year 2000, the EU framework directive identified 33 priority pollutants in the aquatic environment including metals such as nickel, chromium, lead and mercury. In addition, pharmaceuticals such as carbamazepine and ibuprofen, triclosan, and bisphenol-A were identified by EU framework directive 2007 as emerging priority substances for future control (Archer et al., 2017). Although these priority pollutants are present in very low concentrations, their continued release from wastewater effluent to the environment is believed to have potential to cause long term hazards to human and the environment (Bolong et al., 2009; Alfonsín et al., 2014). Typical sewage treatment plants are usually designed to remove major pollutants such as organic matters and nutrients but not micropollutants such as pesticides, metals, endocrine disrupting chemicals (EDCs) and pharmaceuticals (Gallego-Schmid and Tarpani, 2019). Therefore, toxicity assessment of sewage treatment plants increasingly gain attention in the last decade to see what degree of hazards that micropollutants or other priority pollutants might cause, and if measures need to take particularly in vulnerable and sensitive areas. To achieve this purpose, life cycle assessment (LCA) has been used as a potential approach.

A wide range of factors influence the types and quantities of PPCPs/EDCs and metals in wastewater such as catchment size, lifestyles, economic development level, local medical and farming practice. In addition, wastewater treatment technologies adopted in sewage treatment plants can also affect the concentrations of PPCPs/EDCs and metals in effluent because it has been reported that some PPCPs/EDCs such as acetaminophen and caffeine could be removed efficiently by microbial biodegradation or sorption to sludge even though they are not targeted at (Sin et al., 2009). It is thus expected that toxicity effects from sewage plants might vary region by region. So far, most studies on toxicity impacts of sewage plants by LCA were carried out for developed countries (Lorenzo-Toja et al., 2016; Shimako et al., 2017; Emara et al., 2018). Only recently a LCA study on toxicity was conducted in China by Li et al. (2019), but all the inventories or databases including metals and 126 pharmaceuticals and personal care products (PPCPs) were from secondary data (i.e. from various literature in developed and developing countries). As highlighted by Gallego-Schmid and Tarpani (2019), the key parameters such as influent, effluent and sludge produced, and the technologies adopted for wastewater treatment have medium to high influence on the LCA of wastewater treatment. The lack of such real key information would make LCA of wastewater less representative for local situations. Therefore, sampling campaign is more preferred than computing or modelling particularly for PPCPs/EDCs and metals as they might vary significantly with wastewater treatment technologies, locations and seasons (Luo et al., 2014). According to a recent review paper concerning wastewater strength in developed and developing countries by Gallego-Schmid and Tarpani, (2019), average influent BOD, COD and SS concentrations are 251, 551 and 252 mg/L, respectively, in developed countries, while they are 209, 410 and 190 mg/L, respectively, in developing countries, indicating a low strength of sewage in developing countries. In addition, Rashid and Liu (2020) shows that wastewater in Malaysia is very diluted and almost at the lower limit of the ranges of these parameters in developing countries as described by Gallego-Schmid and Tarpani (2019). This poses interesting questions, which are: i) are PPCPs/EDCs and metals as highly diluted as major pollutants such as BOD, COD and nutrients in highly diluted wastewater, Malaysia? ii) for highly diluted municipal wastewater, if the inclusion of micropollutants in life cycle toxicity assessment can lead to noticeable difference, and iii) if necessary measures need to take in the future for more stringent discharge standards of treated wastewater or sludge. To answer these questions, sampling campaign has to be carried out because the data from literature especially from developed countries could not represent the situation in Malaysia. Meanwhile, information about PPCPs/EDCs and metals in Malaysian municipal WWTPs is completely missing.

Compared with impact categories such as acidification potential (AP) and global warming potential (GWP), the study on toxicity with LCA is relatively new and more challenging. Large discrepancies between the life cycle impact assessment (LCIA) methods regarding toxicity impact were reported (Renou et al., 2007; Pizzol et al., 2011) and the comparison between different models was carried out to identify the sources of differences (Renou et al., 2007; Niero et al., 2014; Piao et al., 2016). The Society for Environmental Toxicology and Chemistry (SETAC) and United Nations Environment Program (UNEP) have introduced the Life Cycle Initiative for LCA users to apply more effective life cycle practice (Rosenbaum et al., 2008). As a result, USEtox was developed and recommended as a scientific consensus model after a comparison between several models such as IMPACT 2002+, WATSON, USES-LCA, EDIP, BETR, EcoSenee, and CalTox for assessing toxicity-related effects in LCA (Rosenbaum et al., 2008; European commission, 2013). However, due to the complexity of computing characterization factors (CFs), it was clearly pointed out in the USEtox that CFs provided are only interim instead of recommended for metals, dissociating and amphiphilic substances (Rosenbaum et al., 2008). In addition, available CFs of PPCPs/EDCs in existing USEtox model are very limited and some of the modelling on fate, exposure and impact pathways of chemicals is inaccurate (Emara et al., 2018). Thus, for the emissions of PPCPs/EDCs (some of them are dissociating and amphiphilic chemicals) and metals, the comparison between different models are still necessary in the current situation to enhance the understanding regarding different LCIA methods for WWTP-LCA. Even though the above mentioned problems exist, toxicity assessment can still not only provide indicative effects of micropollutants on human and ecosystems (Pedrazzani et al., 2019), but also prioritise/rank metals or PPCPs/EDCs for removal. The life cycle toxicity assessment by considering emissions of both PPCPs/EDCs and metals in highly diluted wastewater, however, is limited (Sin et al., 2009). This highlights a great challenge to understand the toxicity impacts in developing countries with different wastewater quality and technologies adopted. Toxicity impact is more important to regions than global warming as toxicity can directly cause impact to human and ecosystems in more vulnerable and disadvantaged areas. Furthermore, it is still not very clear about the contribution of PPCPs/EDCs and metals to toxicity categories compared with indirect emissions such as electricity and chemicals used for wastewater treatment. Therefore, this study aims to i) assess toxicity impact of emissions of PPCPs/EDCs and metals from a large centralised wastewater treatment plant in Malaysia with low wastewater strength, and ii) provide useful information for LCA toxicity assessment practice by identifying the importance and contribution of PPCPs/EDCs and metals and comparing LCIA models.

## Materials and methods

### *2.1 The selection and description of the case study*

A large centralised municipal wastewater treatment plant (Malaysian STP) in Georgetown, Penang, Malaysia, operated for 662,002 population equivalents on 14 hectares including residential and commercial areas, was selected as a case study to investigate the life cycle toxicity impacts of diluted wastewater treatment. The Malaysian STP adopts sequencing batch reactor (SBR) technology, which is typical in Malaysian cities for municipal wastewater treatment. Its detailed process for wastewater and sludge treatment can be referred to our previous work (Rashid et al., 2020) and **Figure 1**.

The selection of 9 metals and 10 PPCPs/EDCs as toxic pollutants in this study was based on their occurrence in Malaysia found by Al-Odaini et al., (2010) and Tan at el., (2015), their importance worldwide as highlighted by Ustun, (2009),Lorenzo-Toja et al., (2016), and Yoshida et al. (2014), and the availability of their analytical methodology in Malaysia. The list of all selected substances including organic matters, suspended solids, nutrients, metals and PPCPs/EDCs and their respective analytical methods are shown in **Table 1**.

Apart from the operating data provided by the Malaysian STP, we conducted sampling practice in August 2017 to get data including major pollutants such as COD, TN, and TP as well as micropollutants such as PPCPs/EDCs and metals in influent, effluent and sludge. The inventory data in three scenarios with/without inclusion of metals and PPCPs/EDCs are shown in **Table 2**. To avoid possible reaction of PPCPs/EDCs and metals with holding containers during sample storage periods, polytetrafluoroethylene (PTFE) bottles were used for samples intended for the analysis of metals because there might be chemical interaction between metals ions and glass. Amber crystal bottles were used for the relevant analysis of PPCPs/EDCs to minimize photo-degradation of PPCPs/EDCs when exposed in the light. All samples were kept in an ice box at low temperature to prevent biodegradation when transferred from the site to the lab for analysis.

### *Life cycle assessment*

#### 2.2.1 Goal and scope

The goal of this research was to investigate the life cycle toxicity impact of PPCPs/EDCs and metals of a centralised wastewater treatment plant in Malaysia. Since this study focused on toxic pollutants to the life cycle environmental impact from the plant’s operation, construction and demolition stages were not considered because these two stages contribute negligible pollutants to water. ‘Gate-to-gate’ assessment was adopted by restricting the boundary to wastewater and sludge treatment as a foreground system while chemical and material transport, electricity and chemical production are considered as background system.

#### Functional unit

1 m3 of treated wastewater was used as Functional unit (FU), which has been widely adopted in LCA of wastewater treatment. The system boundary of this toxicity study is shown in **Figure 1**.

#### 2.2.3 Life cycle inventory (LCI)

All collected data were converted to LCI, which can be classified into three groups. The first group includes the volume of treated wastewater, wastewater characteristics such as TCOD, TN, TP, TSS, metals, PPCPs/EDCs in influent and effluent, and sludge quantity and metals in sludge. The second group consists of direct gas emissions such as CO2, CH4 and N2O from wastewater/sludge treatment processes, which were calculated by referring to the Intergovernmental Panel on Climate Change (IPCC) guidelines (IPCC, 2006). The third group comprise indirect emissions from resources consumed such as electricity, chemicals and fuels for transport for the operation of the wastewater treatment plant. The indirect emissions were taken from the Ecoinvent v3.3 with details described in our previous study (Rashid et al., 2020).

Including PPCPs/EDCs and metals in toxicity assessment means that LCI was expanded with unregulated emerging micropollutants. To evaluate if the expansion of LCI by including PPCPs/EDCs and metals to different extents can cause obvious different toxicity results, we set up three LCI scenarios for LCA assessment. Scenario 1 did not consider metals and PPCPs/EDCs in LCI, which was typical in the most studies on LCA of wastewater treatment plants. Scenario 2 included ten PPCPs/EDCs and nine metals in the liquid effluent, and scenario 3 encompassed both metals and PPCPs/EDCs in the effluent and metals in the sludge. LCI data for all three scenarios are shown in **Table 2**.

#### 2.2.4 Life cycle impact assessment (LCIA) and interpretation

CML-IA method was chosen because it had been widely used in LCA assessment of WWTPs for midpoint impact categories including toxicity impact (Renou et al., 2007; Munoz et al., 2008; Lorenzo-Toja et al., 2016). Although USEtox is recommended for toxicity analysis, CFs of dominant metals and PPCPs/EDCs provided in USEtox are still interim (Pizzol et al., 2011). Furthermore, only human toxicity and freshwater ecotoxicity potentials are considered in this model. Thus, given the importance of metals to terrestrial impact especially when biosolids are disposed to landfills or used in agriculture lands, CML-IA was selected for the main analysis because it provides terrestrial ecotoxicity and it is easier to compare with other researchers’ results. For the comparison between different scenarios, eight characterisation impact categories such as GWP, AP, HTP, FEP, TEP, eutrophication potential (EP), abiotic depletion (fossil fuel) potential (ADFP) and ozone layer depletion potential (OLDP) were assessed. The results were interpreted according to the contribution of different components such as electricity consumption, chemicals consumption, transportation and direct emission of pollutants including greenhouse gases (GHGs) to each environmental impact category.

For the comparison of models for toxicity assessment, two LCIA methods namely CML-IA and USEtox were used to investigate and compare effects of the selected metals and PPCPs/EDCs on toxicity impact categories, i.e. HTP, FEP and TEP, with the inclusion of local data of metals and PPCPs/EDCs in the inventory. USEtox was selected for this analysis because it is the latest developed consensus-model for toxicity categories. The impact values obtained by different methods cannot be compared directly because different units are used in different models. For example, kg 1.4-dichlorobenzene equivalent (kg 1.4-DB eq.) is used in CML-IA, whilst USEtox shows results with the comparative toxic unit (CTU). Therefore, only relative scores as percentages within each model were calculated for the comparison purpose. CF values of metals and PPCPs/EDCs by using USES-LCA and USEtox are tabulated in **Table S1** and **Table S2**, which were referred to Alfonsin et al., (2014); Ortiz de Garcia et al., (2017); Li et al., (2019) and the databases in CML-IA and USEtox.

To further study effects of LCIA methods on toxicity impacts, five LCIA methods namely CML-IA, Recipe, IMPACT 2002+, EDIP 2003 and USEtox were used to compare results of midpoint toxicity impact categories using LCI in scenario 3 (i.e. the most extended data including metals and PPCPs/EDCs in water and sludge). These methods were selected due to the availability of suitable toxicity impacts categories in each method and their wide use in LCA-WWTP research for toxicity related studies (Pizzol et al., 2011; Lorenzo-Toja et al., 2016; Munoz et al., 2008; Li et al., 2019).

CFs are available in several life cycle impact assessment (LCIA) methods, such as the International Reference Life Cycle Data System (ILCD) 2011, Impact 2002+, CML-IA, USEtox or EDIP 2003. CFs models are built based on mechanisms of cause-effect chains starting from emissions to impacts to calculate CF values, which are the total results from environmental fate, exposure and the effects on the receiving compartment such as human, freshwater and terrestrial (Huijbregts et al., 2005). CFs were thus calculated as shown in **Eq.** (**1)** (Hou et al., 2020; Rosenbaum et al., 2008; Hedberg et al., 2019). Fate factor (FF) represents the mobility of pollutants to the receiving compartments such as through ingestion by human, runoff to freshwater or adsorption in soil. Exposure factor (XF) relates to concentrations of substances taken by the receiving compartment while Effect factor (EF) is associated with effect levels in the receiving compartment. CFs are multiplied with the inventory data (LCI) that emitted to air, water and soil compartments, to get the potential toxicity impact as shown in **Eq.** **(2)** (Ortiz et al., 2013). CFs are expressed in specific unit contribution (**Table S8**).

The life cycle inventory (ICI) was calculated by dividing daily input collected from WWTP such as electricity (kWh/day), chemicals (kg/day), transportation (t.km/day), and calculated direct emissions of CO2/CH4/N2O (kg/day) with inflow rate of wastewater (m3/day) to WWTP.

Calculation of CF:

(1)

where

Calculation example of FEP by CML-IA:

(unit: kg 1,4-DB eq.) (2)

#### Sensitivity analysis

Throughout the operation years of Malaysian STP, there is possibility of variation in pollutant concentrations, pollutant loads to the plant, electricity and chemical consumption. Furthermore, concentrations of metals and PPCPs/EDCs might fluctuate with seasons, and lifestyle change. Thus, primary data such as metals in sludge, metals and PPCPs/EDCs in effluent, and electricity consumption were varied by ±10% to evaluate how toxicity impact results are sensitive to these priority data. For metals in effluent and sludge, the most toxic pollutants such as nickel, zinc and copper were selected. For PPCPs/EDCs in effluent, the top 3 pollutants such as 17α-ethinylestradiol, 17β-estradiol and triclosan which contribute most to toxicity potential levels were selected. Each of the selected pollutant was analysed for sensitivity to identify if the increase/reduction in concentration of each pollutant can cause more or less changes in toxicity potential results.

## Results and discussion

### *Occurrence and removal of metals and PPCPs/EDCs by wastewater treatment*

The removal efficiencies of PPCPs/EDCs and metals in this study with the SBR system varied from 9% to 99% except for lead, zinc, cadmium, and trimethoprim as shown in **Table 3**. Metals could be removed from wastewater by precipitation or adsorption by activated sludge, which leads to metal transfer from water to sludge. A large variation in metals removal efficiencies ranging from 8.8% to 73.0% was found in this study. The results of metals removal efficiencies in this study were generally lower than those reported by Ustun et al., 2009. They examined the removal of metals such as cadmium, chromium, zinc, nickel and lead in an activated sludge process with a continuous aeration tank in Turkey and found that the removal efficiencies ranged between 47% and 95%. Nonetheless, Lorenzo-Toja et al. (2016) found a larger variation in removal efficiencies of metals in Spain, ranging from 33% to 97%.

**Table S3** shows the comparison of metals concentrations and removal efficiencies in developing and developed countries with different wastewater strengths. Unlike BOD and nutrient removal efficiencies, which are usually lower in developing countries, no clear patterns in metals concentrations and removal efficiency are found between developing and developed countries. For example, the removal efficiency of copper in our study is 55.4%, higher than 32.8% obtained in one WWTP, Spain, by Lorenzo et al. (2016). while the removal efficiency of nickel in our study is 8.8%, significantly lower than 43.5% achieved in Lorenzo’s study. This highlights the high variability of metals concentrations in sewage and their associated removal efficiencies. Thus, it is imperative to do sampling for metals studies to better understand the pollution caused by metals locally. In addition, in this study, it was found that concentrations of lead, zinc, and cadmium in the effluent were higher than those in the influent, resulting in negative removal efficiencies. This phenomenon might be due to the solubilisation/hydrolysis of particulates in biodegradation process, desorption of soluble metals from particulates in the wastewater treatment process, or dissolution from metals precipitates in wastewater treatment conditions. These multiple possibilities suggest complexity and unpredictability of metals removals from aqueous solutions. In terms of toxicity, the transfer of metals from aqueous wastewater to sludge means that toxicity could transfer from wastewater to land if sludge is finally disposed of in a landfill or applied in agriculture land, which can transfer back to freshwater by runoff.

PPCPs/EDCs were chosen as contaminants of emerging concern to analyse in this study due to their continuous release into aquatic ecosystems where their effects may go unnoticed (Lorenzo-Toja et al., 2016). In addition, a study by Munoz et al. (2008) concluded that PPCPs contributed more environmental impact than priority pollutants such as polycyclic aromatic hydrocarbons (PAHs), biocides, and organic priority pollutants due to the higher product values of concentrations of PPCPs and their characterization factors. PPCPs/EDCs could be degraded by activated sludge to certain extents or removed from aqueous solution through adsorption by activated sludge or air stripping (Sagban, 2014). **Table 3** shows that all tested PPCPs/EDCs were removed with efficiencies ranging from 46.9% to 99.2%, except for trimethoprim. The removal efficiencies of micropollutants vary from one study to another as it greatly depends on influent concentrations, treatment technologies adopted (Kasprzyk-Hordern et al., 2009), and operation conditions of treatment facilities (Ustun, 2009). For instance, Kasprzyk-Hordern et al. (2009) reported that a local WWTP in the UK with an extended aeration oxidation ditch had over 85% removal efficiencies for 37 pharmaceuticals, 13 personal care products, 3 illicit drugs, and 2 endocrine disruptors, indicating that this type of treatment provided good removal efficiencies for PPCPs/EDCs. In addition, a review by Yang et al. (2017) highlighted that conventional wastewater treatment in seven different countries removed 21 PPCPs/EDCs with efficiencies ranging from 74% to 99% with an exception of trimethoprim, which had negative removal efficiency with a higher concentration in the effluent than in the influent, which is the same as our study. A similar phenomenon of negative PPCPs/EDCs removals was reported by Üstün, (2009); Sun et al., (2014); Wang et al., (2015). Higher concentrations of PPCPs/EDCs in effluent than influent may be attributed to the deconjugation (Gardner et al., 2013), desorption of pollutants from particulates during treatment and low biodegradability levels (Üstün, 2009). In developed countries, more advanced processes are used for nutrients removal as well, which requires longer sludge retention times (SRTs) and thus might be beneficial for degradation of PPCPs/EDCs due to long explosion to activated sludge. By contrast, WWTPs in developing countries usually do not have nutrient removal, resulting in shorter SRTs. It is reasonably speculated that long SRTs generally benefit to PPCPs/EDCs degradation, i.e. after WWTPs are upgraded for nutrient removal with extended SRT (Rashid et al., 2020). However, similar to metals, concentrations and removal efficiencies of PPCPs/EDCs between different wastewater treatment plants in developed and developing countries do not show any clear patterns (**Table S4**), suggesting unpredictability of PPCPs/EDCs concentrations and their removal efficiencies in sewage treatment plants.

**Table S5** furthercompares concentrations of metals and PPCPs/EDCs in Spain and Malaysia with similar wastewater strength, i.e. an average COD concentration of 378 mg/L in influent (year 2017) in this study (Malaysia), and an average COD concentration of 424 mg/L in influent in the Betanzos WWTP in Spain (Lorenzo-Toja et al., 2016). This comparison revealed no pattern in terms of the concentrations of metals and PPCPs/EDCs in both WWTPs although wastewater strengths are similar. Unlike lower nutrient concentrations in low strength sewage, some concentrations of metals and PPCPs/EDCs were even higher in the Malaysian STP than those in the Spanish WWTP, indicating the unrelated relationship between concentrations of metals and PPCPs/EDCs with wastewater strength. They might be closely related to local lifestyles and commodities, demographics and receiving of partial industrial and commercial wastewater in municipal wastewater treatment plants. Kookana et al. (2014) reported that the use of pharmaceuticals is dependent on countries. Thus, the levels of PPCPs in wastewater has no obvious relationship with wastewater strength in developed and developing countries. Unlike general pollutants such as COD, BOD and nutrients, metals and PPCPs/EDCs show much specificity in each wastewater treatment plants. This further highlights the significance of sampling for studies of toxicity caused by PPCPs/EDCs and metals in a specific wastewater treatment plant instead of obtaining data from the published literature or simulation to represent the real toxicity effects of metals and PPCPs/EDCs. Although most PPCPs/EDCs and metals concentrations can be reduced to low levels in aqueous solution, they still have potential toxicity impact. In addition, the migration of pollutants from aqueous solution to sludge poses a challenge for safe sludge disposal. Therefore, the inclusion of local PPCPs/EDCs and metals into toxicity assessments is necessary to provide a more relatively accurate and holistic view of impact from a wide range of contaminants.

### *Inclusion of PPCPs/EDCs and metals for LCA toxicity assessment using CML-IA*

Three scenarios with different inventories (i.e. scenarios 1, 2 and 3) were studied to investigate the effects of inclusion of PPCPs/EDCs and metals on toxicity impact. As seen in **Figure 2**, the results of impact categories such as ADFP, EP, AP, OLDP and GWP were similar in all three scenarios with or without metals and PPCPs/EDCs, indicating no impact from micropollutants on these five impact categories. However, including PPCPs/EDCs and metals in liquid and sludge changed results of HTP, FEP and TEP.

Human toxicity impact category by including metals and PPCPs/EDCs in scenarios 2 and 3 was 3.2% and 4.0%, respectively, higher than that in scenario 1. The small difference is mainly because the indirect emissions from electricity production are the dominant contributors, accounting for 94%. This result is in agreement with the review results from Zang et al. (2015) on 56 studies related to LCA-WWTPs in both developing and developed countries, which reported that fossil fuel-based electricity, sludge incineration (if there is) and chemical consumption were the main sources of HTP.

However, both FEP and TEP increased significantly by including direct emissions of metals and PPCPs/EDCs. FEP increased by 74% in scenario 2 and 76% in scenario 3 compared with that in scenario 1. The increased values are straightforward to understand because the emissions of PPCPs/EDCs and metals from the effluent have a direct impact on freshwater ecotoxicity. Although the wastewater in this study was much diluted, micropollutants in the effluent at the Malaysian STP were not necessarily lower than those in higher strength wastewater reported in Literature (**Tables S3 and S4**). Metals in the effluent and from electricity generation contributed 65.3% and 23.7% to FEP, respectively, whereas PPCPs/EDCs only contributed 11%. According to the LCA study by Lorenzo-Toja et al. (2016) for the WWTP with medium strength wastewater in Spain using activated sludge with the extended aeration, the impact of 11 metals and 19 PPCPs/EDCs in the effluent to FEP was only 10%, whereas electricity and sludge composting contributed 35% and 55%, respectively. Notably, the removal efficiencies of all toxic pollutants in their study were between 62% and 99%, much higher than those in this study. This indicates that good removal efficiency of toxic pollutants resulted in less contribution of effluent to FEP, linking wastewater treatment technology with FEP.

TEP increased by 88% from scenario 2 to scenario 3 when metals in sludge were considered in LCA. This finding highlights the importance of including data of sludge disposal in terrestrial ecotoxicity analysis. Gallego et al. (2008) reported that seven metals in sludge for agricultural land application from 13 WWTPs with extended aeration, biodenpho, and aerobic-anoxic technologies for less than 20,000 PEs in Spain were the main contributors to terrestrial ecotoxicity, with mercury and chromium contributing 51% and 31%, respectively. The high contribution of sludge metals to TEP indicates the importance of including metals in sludge to terrestrial ecotoxicity impact assessment regardless of the sizes of treatment plants and technologies used. Nevertheless, it needs to point out that the impact of metals in sludge and effluent on ecosystems is very complicated which is dependent on many factors such as the mobility and dilution of the contaminants in the ecosystems. LCA toxicity assessment can be used as a screening method before a more sophisticated toxicity assessment.

Overall, the results indicate that the inclusion of PPCPs/EDCs and metals lead to significant difference in terms of FEP and TEP assessment results even with highly diluted municipal wastewater but negligible HTP. Thus, micropollutants and metals should not be omitted from studies of toxicity impacts particularly freshwater toxicity and terrestrial toxicity when sludge is landfilled or applied to the land.

### *Contribution of PPCPs/EDCs and metals to toxicity impact categories using CML-IA*

It is well known that wastewater treatment is energy intensive. Meanwhile, chemicals are consumed in different operation units such as for denitrification, dewatering, or phosphorus removal. The processes for electricity generation and chemical production emit toxic substances, which cause indirect toxicity to humans and the environment. **Figure 3a** shows the contributions of direct emissions such as PPCPs/EDCs and metals and indirect emissions such as electricity, sludge transportation and chemicals used in the wastewater treatment plant in this study to toxicity impact categories. As seen, the indirect emissions from electricity consumption accounted for 94.4% of HTP, indicating negligible contributions from direct emissions such as PPCPs/EDCs and metals.

**Figure 3b** shows the contribution of different processes to HTP, FEP and TEP. Secondary treatment is the highest contributor with an average 60% to all three toxicity categories. This result highlights the importance of reducing energy consumption in the secondary treatment for a reduced HTP, FEP and TEP. The main toxic substances emitted from electricity generation in Malaysia were barium, hydrogen fluoride, and nickel. This is because that 93% of electricity in Malaysia is generated from fossil fuels, with 40% of it from coals (https://energypedia.info/wiki/Malaysia\_Energy\_Situation). As we know, although carbon and hydrogen are two main elements contained in fossil fuels, there are also other elements such as metals, chlorine, fluorine, sulphur and nitrogen. The combustion of fossil fuels results in the emissions of metals, and other harmful gases. Piao et al. (2016) reported that 70-89% of the impact to HTP by WWTPs in Korea was attributed to the emissions of nickel, barium, and nitrogen oxides from the generation of electricity. In Korea, 74% of electricity generated is from fossil fuels. As reported by Hospido et al. (2008) on the HTP by the CML2000 method for 4 municipal WWTPs in Spain, energy consumption contributed to 85%, and the remaining 15% was from direct pollutants in effluent and sludge. All these consistent results indicate that the consumption of fossil fuels for electricity generation is much related to HTP results by WWTPs. Therefore, moving fossil fuels to clean energy such as natural gas and hydrogen, solar and wind energy can reduce human toxicity potential effectively. In addition, if WWTPs could achieve energy neutrality, i.e. energy recovered from wastewater can cover electricity consumed for treatment, HTP would be significantly reduced.

Currently, energy recovery from wastewater and improving energy efficiency in WWTPs are being intensively studied. It is expected that the current trend to shift electricity generation from fossil fuels to renewable energy would mitigate HTP. Since the WWTP in this study was designed and operated for suspended solid and COD removal only, no chemicals were needed for denitrification or phosphorus removal. The only chemical used was acrylonitrile, which was added to enhance sludge dewatering. Acrylonitrile used in this WWTP had a negligible impact on toxicity. If more or different types of chemicals were used in different units in WWTPs, the toxicity impact of chemicals would be different from this study. For example, Nieroet al. (2014) reported that ferric chloride consumption in WWTPs for phosphorus removal contributed 20% - 40% to HTP. This comparison indicates a trade-off between toxicity and eutrophication impact category when chemicals are used for nutrient removal. Thus, the technology selection for phosphorus removal should not be just focused on nutrient removal and chemical cost only. Toxicity can also be used as one of the important criteria for technology selection from a holistic point of view. Finally, the transportation contribution to toxicity was also negligible, which is in agreement with the study by Piao et al., 2015. This highlights that transportation is not the main concern to toxicity impact categories.

The FEP results are shown in **Figure 3a** and the dominant pollutants that contribute to FEP are shown in **Table 4**. Direct emissions of metals and PPCPs/EDCs contributed 76.3% to FEP with the toxicity caused mainly by nickel (34.0%), zinc (14.2%), and 17β-estradiol (7.0%) from the effluent. Hospido et al. (2008) reported that seven metals in both effluent and sludge contributed 75% to FEP. However, their research did not include PPCPs/EDCs due to the lack of relevant information. It is found in this study that the contribution of PPCPs/EDCs in the effluent to FEP was 11%, mainly from 17β-estradiol, triclosan, and 17α-ethinylestradiol, suggesting more concerns about the negative impact of metals on water bodies. It needs to point out that 10 PPCPs/EDCs were investigated in this study according to Malaysian local situation and only 3 of them shows obvious contribution. If expanding PPCPs/EDCs number can result in higher FEP and TEP depends on the concentrations and CF values of the newly included chemicals in effluent and sludge. For example, Li et al. (2019) included 126 PPCPs/EDCs for life cycle toxicity assessment in wastewater treatment using the USEtox model. They found that PPCPs/EDCs contributed only 5% to FEP with 17α-ethinylestradiol and 17β-estradiol as main contributors while the remaining 95% contribution to FEP was mainly from electricity consumption and metals emissions in effluent. Results from both studies indicate that the number of PPCPs/EDCs considered is not critical, but the prioritised (i.e. top ranked) PPCPs/EDCs such as 17α-ethinylestradiol and 17β-estradiol are more important in the toxicity study involving micropollutants. Thus, more research is needed for the identification of PPCPs/EDCs with higher toxicity potential to guide LCA toxicity assessment. In addition, with the current knowledge, the main concern regarding FEP is still from metals although the inclusion of PPCPs/EDCs can lead to a more relatively accurate FEP assessment.

On the other hand, the limitation and inaccuracy regarding CFs of PPCPs/EDCs and metals might be another concern, which needs further investigation. Firstly, the inaccuracy of CFs of PPCPs/EDCs and metals is from inconsistent input variables (e.g. physicochemical properties, degradation rates, human exposure and ecotoxicity rate of pollutants), which determines the CF value of each substance in each model. Secondly, CFs of the same PPCPs/EDCs and metals in different models might be different due to different modelling methods for CFs calculation (**Tables S1, S2 and S8**). For example, melting temperature of chemicals is not required in the USEtox model but required in the USES-LCA model, leading to different magnitudes of CF values of each chemical by different models. Thirdly, chemicals with CFs values in the existing models are limited. Thus, for those chemicals without CFs values provided in the existing models, researchers have to look for the above-mentioned input data from various literatures and put these data into toxicity models for CF calculation. In this way, different researchers might get different CF values even with the same model. To obtain more accurate results, further research is required not only to improve toxicity models, but also to provide more accurate toxicity input parameters of more chemicals to models. This is quite challenging because there are thousands of PPCPs/EDCs now emitted to the environment due to urbanisation. In the short term, it might be worth focusing on priority pollutants first. In addition, it has been suggested that CFs should be regionalised to represent the real toxicity impact in the local environment (Yang et al., 2017). Santos et al. (2018) highlighted that the regionalised CFs with consideration of local specific criteria (e.g. soil properties and climate) could reduce the uncertainty of terrestrial ecotoxicity impact result corresponding to the spatial variability. All above-mentioned uncertainty makes toxicity assessment challenging. Thus, this study aims to evaluate the importance of including metals and PPCPs/EDCs with current knowledge and models. From this study, it can be seen that considering the large contribution of metals and non-negligible contribution of PPCPs/EDCs to FEP, both metals and PPCPs/EDCs should not be ignored in FEP assessment. Further studies in particular on PPCPs/EDCs removal efficiency in conventional biological treatment plants and model improvement for computing CFs of PPCPs/EDCs are needed for more accurate toxicity results.

Direct emissions to the soil are the main contributors to TEP. Because only metals in sludge were considered, metals such as nickel, zinc, mercury, copper, and lead contributed 88.2% to TEP. This result is comparable to that reported by Niero et al. (2014) who found that six metals and one pharmaceutical in soil contributed more than 90% to TEP, with mostly copper and zinc. Thus, further treatment such as chemical immobilisation is required to reduce metals concentration in sludge before sludge disposal or land application (Suh and Rousseaux, 2002). As shown in **Figure 3a**, TEP was found to be less significant impact category compared with FEP (97% less than FEP) and HTP for WWTPs with sludge landfilled. It is because sanitary landfills are engineered to minimise the environmental impact on the soil by leachate. If sludge is used in agriculture land, TEP results could be very different because CF values of metals in agricultural land are higher than those to landfills.

### *Comparison of CML-IA and USEtox for toxicity assessment with the inclusion of direct emissions of metals and PPCPs/EDCs*

**Figure 4** shows the comparison of three toxicity impacts including metals and PPCPs/EDCs assessed by CML-IA and USEtox methods. For HTP, the results from the USEtox method suggest that direct toxic pollutants from Malaysian STP is dominant on human toxicity potential. While, CML-IA method identified electricity consumption as the main contributor to HTP accounting for 94.4%. With USEtox, direct emissions by metals and PPCPs/EDCs in effluent and sludge contributed 74.0% and 91.8%, to HTPcancer and HTPnon-cancer, respectively. **Table S6** shows the detailed contributions of different sources in percentages to each toxicity impact by using two LCIA methods, respectively. PPCPs/EDCs in the effluent of the Malaysian STP contributed less than 0.23% to HTP with any of LCIA methods, indicating a more important role that metals contribute to HTP.

For FEP, direct emissions including metals and PPCPs/EDCs contributed 76.3% by CML-IA and 96.6%, by USEtox, with a less than 23.7% contribution from electricity consumption. As shown in **Table S6**, although specific percentages from each source to FEP were different by each LCIA method, the general trends by using two LCIA methods were similar, i.e. the largest contribution were from direct emissions of metals followed by electricity and PPCPs/EDCs. The difference of PPCPs/EDCs contributions to FEP are mainly due to the differences in CF value between the two models as shown in **Table S2**. Among PPCPs/EDCs, 17β-estradiol is the pollutant which contributes most to FEP in both models (**Table S7**). Although 97.6% of influent 17β-estradiol in Malaysian STP was removed in wastewater treatment process with an effluent concentration as low as 0.001 µg/L, but the high CF value of this substance (representing high toxicity) led to the high contribution of this substance to FEP. From this aspect, LCA analysis is an efficient tool to help identify the toxicity contribution of a specific type of pollutant to guide the setting of WWTP discharge consents. Meanwhile, metals in effluent and sludge contributed 65% by the CML-IA and 94% by USEtox method, respectively, suggesting similar trends by two methods. In addition, sludge disposal is important for LCA in WWTPs in terms of TEP assessment, but USEtox cannot do TEP assessment although USEtox was recommended as a scientific consensus for toxicity assessment in general areas.

Thus, for toxicity assessment in wastewater treatment plants, either CML-IA or USEtox could be used considering their similar result trends, but CML-IA is more suitable for TEP from the practical perspective. Alfonsin et al. (2014) compared USEtox and CML methods by recalculating 13 new CF values of HTP and FEP for PPCPs/EDCs, but the assessment only focused on PPCPs/EDCs without the inclusion of direct emission of metals in effluent. The specific toxicity impact comparison between different LCIA methods (e.g. CML-IA and USEtox) involving both metals and PPCPs/EDCs in wastewater was not conducted in any previous research. Thus, our results here can provide useful information to LCA practitioners for the selection of toxicity models for LCA in WWTPs with focus on toxicity assessment involving micropollutants.

### *Comparison of different LCIA methods for toxicity assessment with the inclusion of direct metals emissions*

Because metals are the main contributors to toxicity potentials and PPCPs/EDCs are not measured as commonly as metals, the contributions of metals from four different inputs, namely direct emissions from effluent and sludge, electricity, chemicals, and transportation, were further assessed using five different LCIA methods, i.e. CML-IA, Recipe, IMPACT 2002+, EDIP 2003, and USEtox, to investigate how various LCIA methods affect the impact results by considering direct metals emissions. As shown in **Figure 5a**, HTP results from direct metals emissions (i.e. effluent and sludge) and indirect emissions (i.e. electricity use) were inconsistent between the five models (including cancer and non-cancer categories by IMPACT 2002+ and USEtox). Impact percentages due to direct metals emissions using CML-IA and Recipe were only 2.1% and 6.5%, respectively, suggesting unimportance of expanding inventory data to direct metals emissions from effluent and sludge by using these two methods. Instead, metals such as barium, nickel, and chromium from electricity generation were the main pollutants, contributing 92.1% and 97.3% to HTP, respectively. However, the other models including IMPACT 2002+, EDIP 2003, and USEtox indicate the necessity of including direct metals emissions from the Malaysian STP such as zinc, cadmium, antimony, and arsenic, with the contribution of 31-92% to HTP. Renou et al. (2007) compared five different LCIA methods namely CML2000, Eco indicator99, EDIP96, EPS, and Ecopoints97 for five impact category assessment of wastewater treatment including EP, AP, HTP and GWP. They found that all impact category results by different methods were almost similar except for the HTP result. This highlights that HTP assessment in WWTP is the most challenging.

Unlike HTP, FEP impact caused by direct pollutants including metals between models ranged between 48% and 97%. If EDIP was not considered, the difference could be as small as 23%. This is due to more consistent CF values of metals for FEP when discharging to water bodies as shown in **Table S8,** although CFs have different units in different LCIA methods. The dominant pollutants from these methods were almost similar, which were nickel, zinc, cadmium, and copper. Halleux et al. (2006) reported that two methods, CML-IA and IMPACT 2002+ produced almost similar results in terms of freshwater ecotoxicity potential, with nickel and zinc as the main pollutants. Additionally, a study by Lorenzo-Toja et al. (2016) indicated that copper, zinc, and nickel were the main contributors in a Spanish WWTP treating high strength wastewater. The high contribution from direct metals emissions to FEP in all methods indicate that the metals in the effluent of the Malaysian STP have a more significant impact on the water body than indirect emissions, regardless of wastewater strength. Thus, more attention needs to be paid to nickel, zinc, copper and cadmium if sludge will be used in agriculture land or treated water will be reused. Meanwhile, electricity consumption contributes 3%-52% to FEP in all methods, indicating that electricity is a moderate contributor to FEP. Similar to HTP, chemicals consumption and transportation in this study had a negligible impact on FEP. It is noted that CFs from different LCIA methods for all metals are inconsistent. This means the difference of toxicity impact were also caused by different CFs of pollutant in each method. For example, CF of nickel (3.24E+03) is 35 times higher than zinc (9.17E+01) in CML-IA method. By contrast, CF of zinc (3.86E+04) is 3 times higher than nickel (1.49E+04) in USEtox (**Table S8**). This highlights that CFs from these methods is not consistent and varies in the rankings of metals although the overall contribution is quite similar. Overall, except EDIP, this study found that CML-IA, Recipe, IMPACT2002+, and USEtox produce consistent FEP results. Thus, any of them could be selected for future LCA studies when including direct metals emissions in the inventory for FEP assessment.

As shown in **Figure 5c**, three models namely CML-IA, Recipe, and IMPACT 2002+ demonstrated less than <13.5% difference. The main contributors to TEP were copper, zinc, nickel, and cadmium. By contrast, the EDIP model in this study showed that 81% of TEP was from electricity consumption, followed by 17% from chemical consumption. Considering that FEP and TEP results by EDIP have a very obvious difference compared with other methods in this study, EDIP is not recommended for toxicity study in WWTPs. In overall, this study shows that CML-IA, Recipe, and IMPACT 2002+ produce consistent results of HTP, FEP, and TEP when including direct metals emissions, whereas EDIP and USEtox provide different results. This model comparison provides useful information and guideline for future LCA practice on the selection of LCIA methods according to specific aims.

### *Sensitivity analysis*

Toxicity impact assessment results in Malaysian STP highlight that metals in sludge, metals and PPCPs/EDCs in influent/effluent, and electricity consumption are the key factors that affect toxicity impact results. **Table 5** shows how toxicity and ecotoxicity impacts from Malaysian STP were affected by changing ±10% of main inventory values from scenario 3 by using CML-IA method. The result shows that HTP, FEP and TEP changed by ±9.89%, ±3.95% and ±2.75%, respectively, to correspond to ±10% variation in electricity consumption. HTP is more sensitive to electricity compared with FEP and TEP, which is in agreement with that reported by Piao et al. (2016) stating HTP was the most sensitive to electricity consumption. Metals in wastewater (i.e. nickel, zinc and copper) mainly affected FEP result (±4.40% variance) with nickel as the most influential pollutant while HTP’s variance with metals was negligible with less than ±1.0% variance. PPCPs/EDCs in wastewater only affected FEP result with the most influential PPCPs/EDCs as 17β-estradiol. Finally, metals in sludge show more effects on TEP by nickel and zinc, and there is negligible change in HTP and FEP results. In general, 10% variation in concentration of metals in sludge, concentration of metals and PPCPs/EDCs in wastewater, and electricity consumption cause less than 10% environmental impact change.

## 4 Conclusions

In this study, inclusion of direct pollutant emissions of metals and PPCPs/EDCs was investigated by LCA to investigate their contribution to toxicity impact levels. In addition, different LCIA methods were compared to assess their suitability for toxicity assessment. The main findings from the assessment in this study are summarised below.

* Unlike major pollutants in wastewater concentrations of PPCPs and metals in wastewater and their removal efficiencies in treatment processes in developing and developed countries with high and low strength wastewater showed no any clear patterns. Thus, sampling work is recommended for the acquisition of PPCPs and metals data to ensure accuracy and reliability of toxicity assessment.
* The inclusion of PPCPs/EDCs and metals from effluent and sludge caused a 76% increase in FEP and an 88% increase in TEP, respectively, but negligible in HTP. The contribution from direct metal emissions to FEP is 65.3%, indicating that metals play more important roles to FEP than PPCPs/EDCs. The contribution of PPCPs/EDCs in the effluent to FEP by the CML-IA method is only 11%, which is mainly from 17β-estradiol, triclosan, and 17α-ethinylestradiol. Thus, the inclusion of prioritised PPCPs/EDCs is far more important than a large number of PPCPs/EDCs for freshwater ecotoxicity studies.
* For ecotoxicity assessment in WWTPs with the inclusion of both direct metals and PPCPs/EDCs emissions, CML-IA and USEtox methods can get similar results.
* When including direct metal emissions only, CML-IA, Recipe, and IMPACT 2002+ produced consistent HTP, FEP and TEP results, whereas EDIP and USEtox provided different results.
* In the sensitivity analysis, the ±10%, variation in concentration of top 3 metals in sludge, and top 3 metals and PPCPs/EDCs in wastewater only led to less than ±5% variation in HTP, FEP and TEP.

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