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**Mercury speciation in soils from mining sites in the Czech
Republic**

Master's thesis

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Engineering**

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Declaration

I hereby declare that I have authored this master's thesis carrying the name "Mercury speciation in soils and plants from a long-term contaminated area" independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I further state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on 21.04.2023

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Mercury speciation in soils from mining sites in the Czech Republic

Summary:

Mercury contamination in the environment is characterized by high toxicity and bioaccumulation potential. The main objective of this thesis is to determine the concentration of some mercury species present in soils from two mining sites in the Czech Republic, and observe how they interact with some soil properties (particularly organic matter) based on the Hg origin in order to determine the fate of Hg in these soils, and assess the threat it poses. Upon the completion of this thesis, there should be a better understanding of 1) concentration of some Hg species in the soil, especially in comparison to the organic matter present, 2) how these different Hg species behave physically and chemically in the environment (and how toxic they can become), and 3) to use this information to ultimately find solutions to remediate these areas.

This objective is being fulfilled by the comparison of two sites in the Czech Republic with different origins of mercury; Libčice (with anthropogenic Hg sources from gold mining) and Horní Luby (with anthropogenic and geogenic Hg sources following abandoned cinnabar mining). The two sites also have similar soil types, vegetation, and climate, so most environmental conditions are quite similar between the two locations. Soil samples were taken from both locations and analysed in the lab for specific soil properties such as pH, cation-exchange capacity, carbon species, carbon-nitrogen ratio, and sulfur. Hg was extracted and speciation was performed using mass spectrometry. All observed Hg species observed were compared and evaluated for correlation with the specific soil properties mentioned. Statistical analyses were done on all parameters using such methods as the Shapiro-Wilk test for normality and the Spearman correlation, in addition to general descriptive statistics for all soil and Hg parameters.

Despite most environmental conditions being comparable between the sites, it was found that conditions such as higher pH, higher cation-exchange capacity, and higher carbon species content found in Libčice did not favour high Hg methylation. However, methylated and mobile forms of Hg were observed almost equally observed between the sites. This poses some questions regarding the origins of the Hg contamination, and why it could be behaving differently based on that origin. These results suggest that despite the overall indicators of higher organic matter which should immobilize mercury in soils, mercury from the gold mining is not immobilizing as expected; more research would need to be done to explore best remediation techniques for situations such as this.

Keywords: mercury; soils; pollution; contamination; speciation; toxicity; mobility; risk assessment; extraction; remediation

Speciace rtuti v půdách z těžebních lokalit v České republice

Souhrn:

Rtuť jako kontaminant prostředí se vyznačuje vysokou toxicitou a vysokým potenciálem bioakumulace. Hlavním cílem této práce je zjistit koncentraci vybraných sloučenin rtuti přítomných v půdách ze dvou těžebních lokalit v České republice a sledovat, jak interagují s některými půdními vlastnostmi (zejména organickou hmotou) na základě původu Hg. Je důležité popsat cyklus Hg v těchto půdách a zhodnotit hrozbu, kterou tento prvek představuje. Výsledky práce by měly napomoci k lepšímu pochopení 1) zastoupení vybraných sloučenin druhů Hg v půdě v závislosti na obsahu půdní organické hmoty, 2) jak výskyt těchto sloučenin Hg závisí na fyzikálně-chemických vlastnostech prostředí (a jaké mohou představovat riziko) a 3) využít tyto poznatky pro případnou remediaci daných lokalit.

Pro experiment byly vybrány dvě lokality v České republice s různým původem rtuti; Libčice (s antropogenními zdroji Hg z těžby zlata) a Horní Luby (s antropogenními a geogenními zdroji Hg po bývalé těžbě cinabaritu). Tyto dvě lokality mají také podobné typy půdy, vegetaci a klima, takže většina podmínek prostředí je mezi těmito dvěma lokalitami docela podobná. Vzorčky půdy byly odebrány z obou míst a analyzovány v laboratoři na specifické vlastnosti půdy, jako je pH, kationtová výměnná kapacita, sloučeniny uhlíku, poměr uhlík-dusík a síra. Hg byla extrahována a speciace byla provedena pomocí hmotnostní spektrometrie. Všechny pozorované sloučeniny Hg byly porovnány a vyhodnoceny pomocí korelace s uvedenými specifickými vlastnostmi půdy. Pro statistické hodnocení dat byl použit Shapiro-Wilkův test na normalitu a Spearmanova pořadová korelace a základní popisná statistika pro všechny parametry půdy a sloučeniny Hg.

Přestože většina podmínek prostředí je mezi lokalitami srovnatelná, bylo zjištěno, že podmínky jako vyšší pH, vyšší kationtová výměnná kapacita a vyšší obsah druhů uhlíku zjištěné v Libčicích nevedou k vysoké míře methylace Hg. Nicméně methylované a mobilní formy Hg byly na obou lokalitách porovnatelné. Zdá se, že rozdílný původ rtuti na obou lokalitách má za následek rozdílné chování sloučenin rtuti. Tyto výsledky naznačují, že navzdory celkovým ukazatelům vyšší organické hmoty, která by měla imobilizovat rtuť v půdách, není rtuť z těžby zlata imobilizovaná podle očekávání; bylo by třeba provést další výzkum zaměřený na případnou imobilizaci Hg na této lokalitě.

Klíčová slova: rtuť; půdy; znečištění; kontaminace; speciace; toxicita; mobilita; odhad rizika; extrakce; náprava

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1 Introduction

Mercury contamination in terrestrial environments has long been a concerning form of environmental pollution, having potentially devastating effects to ecosystems and their functions, as well as human health, with just trace levels of contamination. Hg is highly toxic, volatile, and can be introduced in soil by natural sources (geogenically), as well as human-caused sources (anthropogenically). Geogenic Hg derives primarily from geological sources, such as volcanic eruptions, and from land and ocean surfaces in its elemental form, Hg^0 (Selin, 2009). Anthropogenic sources of Hg can derive primarily from coal fired power plants, or by-products of the mining process for metal ore (e.g., gold or cinnabar) (Selin, 2009). In addition to direct mercury production, other industrial activities that might release mercury into the environment include the manufacturing of cement, nonferrous metals, pig iron and steel, caustic soda, and waste disposal (Selin, 2009). It is estimated that there is an accumulated 86 gigagrams of anthropogenically sourced Hg pollution worldwide (O'Connor et al., 2019).

Mercury can take on different forms in the soil; primarily as inorganic, elemental, or organic forms, all of which vary in their physical, mobilizable, and toxicological properties (Davis et al., 1997). When present in soil, volatile Hg species, such as Hg^0 and dimethyl-Hg have potential to be released into the atmosphere (O'Connor et al., 2019). All forms of Hg are known to be classified as toxic (Hylander & Goodsite, 2006). Chemically, Hg in soils can take the following forms: elemental Hg^0 , iron (oxyhydr)oxides, Hg chlorides (such as calomel, Hg_2Cl_2), Hg in sulfide minerals (such as metacinnabar, $\beta\text{-HgS}$), inorganic Hg(II) adsorbed to the surfaces of clay minerals, or soil organic matter (collectively known as “matrix-bound Hg(II)”), and methylated Hg (MeHg) (Gilli et al., 2018). The speciation of Hg can be determined by various environmental factors, such as the original Hg species used during factory chemical production, methods of transportation from source locations, time since deposition of the contaminant, seasonal temperature, among other variables (Gilli et al., 2018).

Depending on the soil's pH, temperature, and humic content, Hg is vulnerable to a variety of chemical and biological transformation processes after deposition, including elemental Hg^0 oxidation, ionic Hg^{2+} reduction, and methylation (Bollen et al., 2008). As Hg^{2+} and its inorganic compounds have an affinity for functional groups containing sulfur (S), the production of organic Hg^{2+} complexes are known to be the predominant process (Schuster, 1991). Most mercury is found in soils poor in organic matter as reactive, ionic mercury species like Hg_2Cl_2 or $\text{Hg}(\text{OH})_2$, which may be rapidly converted into more dangerous forms like methylmercury or Hg^0 (Skyllberg et al., 2006). It is known that in soils with variable redox conditions and particularly in polluted soils, sulfide can compete with thiol groups in organic matter and precipitate nanoparticulate HgS as its metacinnabar form ($\beta\text{-HgS}$) (Gerbig et al., 2011). Such nanoparticles formed in situ in soils are able to be stabilized by organic matter (OM), remain structurally disordered in low-sulfidic environments, and could become more bioavailable for Hg methylation (Gilli et al., 2018). The formation of MeHg is mostly a biotic process formed by sulfate-reducing and iron-reducing bacteria, under reducing soil conditions (Gilli et al., 2018).

This study will be focusing on how the chemical soil properties can directly influence the speciation of Hg. It will highlight different Hg species and their interactions with some specific soil properties, especially that of organic matter (OM) and its species (total C, organic C, water soluble C). OM is considered to be one of the most important factors for Hg biogeochemistry, bioavailability, and risks due to its interaction with Hg (He et al., 2019). Besides the total amount of Hg, potentially mobilizable Hg species and methylmercury (the most toxic Hg species) will be described in these soils. All of these soil properties will be compared and contrasted against the behaviour and speciation observed with mercury in soils from mining sites in the Czech Republic.

2 Scientific hypothesis and aims of the thesis

The main goal of this research is to determine the drivers of speciation of mercury in some forest soils, whether naturally or anthropogenically derived, and to discover what affects the speciation of Hg species based on the origin of Hg. With this information, it could be evaluated which soil properties affect Hg species in which way. Ultimately, providing the information on how to remediate and mitigate the risk of the Hg pollution in these two sites.

Because both research sites are in the forest, both sites should be rich in organic matter coming from similar sources. Due to this fact, it is hypothesized that organic matter will interact differently with mercury based solely on its origin (anthropogenically and geogenically sourced in Horní Luby site, versus all anthropogenically sourced in Libčice), and not based on the environmental conditions. Further, it is expected that in Libčice, mercury will interact more with organic matter, and in Horní Luby mercury will interact more with the mineral fraction in soil due to its origin.

The overall hypothesis for this thesis is that Hg speciation in soils will depend on the origin of the mercury. This study aims to prove this hypothesis by showing differences in the behavior and speciation of mercury in comparable soils between the two mining sites with different mercury origins, examined in the Czech Republic. This will be done by evaluating various specific mercury species and soil parameters examined from soil sampling, mercury extraction, and statistical testing. With this information, it is expected there will be a better understanding on how to remediate such mercury contamination in soils, having further implications for whole biogeochemical cycles and ecosystems moving forward.

3 Literature research

3.1 Environmental impacts of Hg contamination

Hg is toxic and highly destructive to both plants and animals and has no good impacts on organisms; it also pollutes the air, water, and soil (Gworek et al., 2020). Even at low quantities, mercury is poisonous to plants, causing growth inhibition and a host of other negative effects (Ahammad et al., 2018). The growth and metabolism of plants are negatively impacted by an increase in mercury species in the soil in a variety of ways, including decreased photosynthesis, transpiration, water intake, and chlorophyll synthesis (Asati et al., 2016). As a consequence of mercury contamination for wildlife, more piscivorous (fish-eating) birds and mammals (than any other known aquatic ecosystem component) are exposed to mercury (Keating et al., 1997). Mercury has harmful consequences on fish, birds, and mammals, such as death, decreased reproductive success, stunted growth and development, and aberrant behaviour (Keating et al., 1997). Mercury is also well known for its biomagnification in food chains as well, having the potential to move up the food chain and have detrimental effects on keystone predators.

As established, diverse environmental sources, including mine tailings, industrial effluent, agricultural drainwater, and air deposition from the production of electricity, can expose biota to mercury (Száková et al., 2004). High trophic level feeders develop mercury toxicoses as a result of the bioaccumulation of both inorganic and methylated mercury compounds in the food chain (Száková et al., 2004). The main source of mercury in soil is litter deposition (Sommar et al., 2020). The link between increased total Hg mass and large increases in mass-dependent fractionation (MDF) in forest litter strata is most pronounced at high-elevation sites (Sommar et al., 2020). Since the majority of the mercury in litterfall is thought to have come from stomatal uptake of Hg^0 , it can be used to estimate the dry deposition of mercury in the atmosphere in a general and conservative manner (the portion that is retained in leaves) (Gworek et al., 2020).

3.2 The effects of Hg contamination on human health

Eating contaminated fish can expose people and animals to hazardous quantities of methylmercury (Schierow, 2006). In human adults via this consumption, methylmercury can be absorbed, where it then circulates in the blood and penetrates the brain, potentially causing structural damage (Schierow, 2006). According to certain research, the cardiovascular system may possibly be more vulnerable. (Schierow, 2006). Certain levels of mercuric chloride orally ingested can cause kidney damage, or exposure to elemental mercury can target the central nervous system and cause tremors (Davis et al., 1997). There has been ongoing and even increasing concern about mercury in the environment due to information showing that exposure to low amounts of mercury may also impair developing fetal neurological systems (Schierow, 2006). In the example of the U.S., women of childbearing age are advised by the Environmental Protection Agency and the Food and Drug Administration to avoid some big fish and to consume other fish in moderation (Schierow, 2006).

The physical damages from mercury intoxication in the body may cause tremors, loss of coordination, trouble walking, widespread weakness, impaired hearing and vision, tingling and numbness in the fingers and toes, and ultimately loss of consciousness and death (Schierow, 2006). Effects on the brain can be seen and are permanent at high exposure levels (National Research Council (US), 2000). For infants, methylmercury easily passes a pregnant woman's placenta (Wolfe et al., 1998). Concentrations in the developing fetal brain are around five to seven times higher than those in the mother's blood (Cernichiari et al., 1995). Methylmercury exposure in the womb can influence brain development, as seen in childhood by a child's capacity for learning and regular function after birth (Keating et al., 1997).

3.3 Biogeochemical cycling of Hg

Mercury is the only metal that exists in liquid form at typical earth surface temperatures and is a silvery liquid metal in its elemental condition at the earth's surface (Wershaw, 1970). It behaves similarly to other liquids in that it vaporizes and condenses in a manner that is influenced by both its own vapor pressure and the temperature and barometric pressure of its surroundings (Wershaw, 1970). Like other metallic elements, it reacts with a wide range of inorganic and organic compounds to form simple and complex molecules (Wershaw, 1970).

Like many other chemical compounds, the compounds of mercury are transported across rocks, soil, air, water, and living things through a complicated network of pathways (Wershaw, 1970). The most significant naturally occurring mercuric compound is cinnabar, a sulfide that typically forms under low temperatures (less than 300°C) and contains 86 percent mercury by weight (Wershaw, 1970). The primary sources of mercury are igneous rocks, which are those created by melting and cooling (Wershaw, 1970). The average mercury concentration in soils is 100 ppb, and variations occur within relatively small ranges (Wershaw, 1970).

The atmosphere measured at ground level close to mercury ore resources may contain as much as 20,000 ng/m³ (nanograms per cubic meter) of mercury in air due to mercury's propensity to evaporate (Wershaw, 1970). Regardless of the origin of atmospheric moisture, Hg patterns reacts to weather patterns and other natural laws. As a result, the greatest mercury concentration in the air is observed about noon, with substantially lower concentrations in the morning and the evening (Wershaw, 1970). As well as other other atmospheric components, Hg can be removed from the atmosphere by rain, meaning atmospheric Hg concentrations can drop to essentially 0 immediately following a large rain event (Wershaw, 1970).

A natural distribution of mercury in water is produced by the interaction of water with soil and rock during storm runoff, percolation into the earth, and movements under the surface where various geochemical stresses are present (Wershaw, 1970). Because of the prolonged and close contact with mineral grains, as well as other environmental conditions, subterranean waters are more likely to have higher Hg concentrations (Wershaw, 1970).

Although Hg is not known to be an important component of the food chain, animals living in situations where mercury is present naturally can absorb and assimilate it (Wershaw, 1970). The conversion of inorganic Hg by certain anaerobes to methylmercury (the more soluble form) is suggested to increase Hg concentrations in the environment (Wershaw, 1970). Apparently, certain plants have the extraordinary ability to concentrate mercury and even to separate it into metallic form (Wershaw, 1970).

3.4 *The effects of organic matter on mercury*

Organic matter (OM) is known to have some direct influences on the biogeochemical behaviour of Hg in the soil, particularly that decreases the bioavailability to plants and bioaccumulation of Hg and substantially reduces the risk correlated with Hg in soils or sediments (He et al., 2019). Mercury has a propensity to bind with soil organic matter or soil matrix surfaces, creating covalent bonds with this OM, unlike any other methyl (O'Connor et al., 2019). Because of its very stable complex structure, mercury can be considered a highly immobile metal in most soils (Liao et al., 2009). The majority of Hg in terrestrial systems (>90%) resides in soil and is associated with organic matter; where it can bond strongly to reduced sulfide groups (Selin, 2009). Sulfate-reducing bacteria convert Hg into MeHg, and this process is affected by factors such as the sulfur cycle, ecosystem pH, and especially the presence of OM (Selin, 2009). There are many different ways OM can influence the behaviour of soil Hg; for example, Hg can be released into the atmosphere when OM it is bound to is burned (Selin, 2009; Gilli et al., 2018).

Generally speaking, Hg in soils is controlled by inorganic and organic interactions, since it has an affinity to Cl^- , OH^- , S^{2-} , and S-containing functional thiol groups in organic ligands (Gilli et al., 2018). In terms of finding solutions for how to mitigate mercury contamination in the soil and its ecological and health hazards, this characteristic of OM has some implications, but we will discuss this further in later sections of the thesis, namely in the discussion and conclusion. Some earlier studies have claimed that there are beneficial attributes of OM and Hg interactions that would help alleviate remediate Hg contamination in soil, which can be broken down into three main factors of influence (Nugundu et al., 2016). First, OM has a strong affinity to Hg, therefore there is a strong effect on the partitioning and bioavailability of Hg in soils; this is now believed to be common knowledge on the subject after much review (He et al., 2019). Second, OM has been known to enhance the photodemethylation rates of MeHg, and consequently reduces Hg bioavailability (He et al., 2019). Third, that OM is capable of reducing MeHg production in soils (He et al., 2019). Other researchers (He et al., 2019) have shown the opposite, that OM readily facilitates the methylation of Hg, and therefore increasing its bioavailability and risk factors in the environment. These significantly increased Hg methylation rates can largely be attributed to an increase in microbial activity (He et al., 2019). Mercury in its inorganic form (IHg), when present in soils and sediments, can be subject to this methylation process in the presence of increased organic matter (He et al., 2019). Based on these points, the clear influence of OM on Hg methylation has not yet been clearly explained.

There are certain ecological conditions which also affect this production of MeHg, such as certain forestry operations where organic residues from logging and other OM can create environments similar to a wetland in such forest soils, which in turn facilitate the abundance of microbial communities, who then facilitate more MeHg production (He et al., 2019). The risk factors of Hg are significantly based on this balance between the conversion of IHg to MeHg, which is primarily controlled by microbial methylators under anaerobic conditions in the soil (He et al., 2019). Additionally, there are two primary factors for the effects of OM on IHg methylation; 1) the activity of microbial Hg methylators and 2) the microbial availability of Hg (Liem-Nguyen et al. 2016).

3.5 Mercury speciation

The speciation of mercury in contaminated soils can be determined by various factors, such as the geological origin of Hg, ore-processing methods, and the physico-chemical properties of the targeted soils (Hojdová et al., 2009). Additionally, this soil Hg speciation will determine the species solubility, mobility, as well as bioavailability in terrestrial ecosystems (Hojdová et al., 2009). Speciation will also play an important role in the general methylation process, as the different forms of Hg can prove to vary in their bioavailability to microbes (He et al., 2019). So, not only does the OM content in soil, as well as the environmental conditions play important roles in observing the risk factors of Hg contamination, however the species forms in which Hg is introduced in the soil also play an important role. HgS has a clear effect on interactions between OM and Hg due to high solubility in liquids as well as the presence of sulfur (He et al., 2019).

For these methylating microbial communities, the OM present in the soil can provide some resources which facilitates the MeHg production. OM provides available nutrition and an electron donor, and also provides a substrate which helps mediate microbial activity in the methylation process (He et al., 2019). Of course, there are various other environmental conditions which have an effect on OM and microbial activity, such as temperature, redox conditions, water saturation, pH, etc (He et al., 2019). Generally speaking, the most favourable conditions for anaerobic Hg methylators has shown to be under wet, anaerobic, and high temperatures; showing these conditions should theoretically yield the highest conversion of IHg to MeHg (He et al., 2019). As previously stated, Hg is mostly methylated by sulfur-reducing bacteria. The direct conversion of insoluble HgS (cinnabar) species can into the methylated Hg species is low or insignificant on its own, due to low bioavailability (O'Connor et al., 2019). However, under the right environmental circumstances (e.g., anaerobic, redox conditions) and with the presence of sulfur-reducing bacteria, we know this methylation process can be facilitated (O'Connor et al., 2019).

In this paper the following Hg species have been covered:

3.5.1 Total Hg

Total mercury refers to the total amount of mercury in a given sample or environment, consisting of all its various species (or chemical forms) which are present. Such groups include elemental, inorganic, and organic compounds of Hg. These Hg proportions are not a typical speciation but are given by the “operationally defined fractionation.” Organic soil components have a strong attraction for mercury. Therefore, organically rich soils like those found in forests, peat bogs, or rice paddies are frequently linked to higher total-Hg levels (Beckers and Rinklebe, 2017).

3.5.2 Potentially mobilizable Hg

As the term suggests, potentially mobilizable Hg (PM-Hg) is referred to the forms of Hg in a system which can be mobilized, i.e. the portion of total Hg which can be released or become bioavailable under the right conditions. The factors that affect the mobility and

bioavailability of Hg include pH, temperature, redox conditions etc. all have an effect on the potential mobility of mercury.

3.5.3 Methylated Hg

As previously stated, methylmercury (referred to commonly as MeHg, with compound symbol CH_3Hg^+) is the most toxic form of mercury known to occur in the environment. Being such a toxic, mobilizable, and bioavailable form of mercury, much of the focus regarding mercury contamination risk management is concerned with MeHg.

3.5.4 Water-soluble Hg

Water-soluble mercury is the portion of Hg in a system which can be dissolved or suspended in water. This soluble fraction of Hg, as well as nano-sized Hg particles, can play crucial roles in the level of Hg mobility, availability, and methylation in soils (O'Connor et al., 2019). Generally speaking, Hg is highly mobilizable with soluble Hg species, such as dissolved Hg, or soluble inorganic or organic complexes (O'Connor et al., 2019). Conversely, other species can be much less mobilizable under most conditions, such as mercuric sulfide (HgS) (O'Connor et al., 2019). Additionally, methylation is much less likely to occur with insoluble HgS forms of mercury (O'Connor et al., 2019). Although it is usually assumed that there is little direct conversion of insoluble HgS (cinnabar) species to Methyl-Hg in anaerobic soils, this is subject to alter when environmental factors promote HgS complexation (O'Connor et al., 2019). Different soil solutions and soil properties can directly influence the solubility of Hg compounds (O'Connor et al., 2019).

3.6 *Carbon species*

With between 48 and 58% of the total weight of soil organic matter made up of carbon (C), it is the predominant element present (Nelson & Sommers, 1996). For a variety of soils, the amount of C in soil organic matter varies greatly, and no one factor is suitable for all soils; this factor varies from soil to soil (Nelson & Sommers, 1996). Both organic and inorganic carbon can be found in soils; as a result, both forms of carbon are recovered during total carbon analysis procedures (Nelson & Sommers, 1996).

3.6.1 Total carbon

The sum of organic and inorganic carbon in soils is referred to as total C (Nelson & Sommers, 1996). Due to the breakdown of carbonate minerals that were once present in parent material during soil formation, not all soils contain inorganic C, meaning organic C can frequently be the dominant fraction of soil carbon (Nelson & Sommers, 1996).

3.6.2 Organic carbon

The soil's organic component, which includes microbial cells, plant and animal remains in various stages of decomposition, stable "humus" made from remains, and highly carbonized materials like charcoal, graphite, and coal, contains organic carbon (elemental forms of C) (Nelson & Sommers, 1996). Organic C determinations are commonly utilized as the basis for organic matter calculations through multiplying the organic C value by a factor (Nelson & Sommers, 1996). There are several methods for determining organic C, including: 1) analyzing a soil sample for total carbon and inorganic carbon and deducing the amount of inorganic carbon for the amount of total carbon; 2) determining the sample's total C after inorganic C has been destroyed; and 3) oxidizing organic C compounds with $\text{Cr}_2\text{O}_7^{2-}$ and then determining the amount of unreduced C (Nelson & Sommers, 1996).

3.6.3 Water-soluble carbon

The term "water soluble organic carbon" has been used to refer to the entire pool of organic carbon that is water soluble in soils, which can be applied generally to water-soluble C in soils (Tao & Lin, 2000). The soil water-soluble carbon (or WSC) can be used to measure the amount of C substrates released throughout the soil drying and rewetting processes and made accessible for C mineralization, and it may be seen of as an indication of the microbially available C from soil organic matter (Guo et al., 2014).

3.7 pH

pH is considered the typical indicator of how acidic or alkaline a solution is, and is one of the most often used analyses in soil and water testing (Addy et al., 2004). pH can be defined as the measurement for concentration of the ions H^+ and OH^- that make up water (Addy et al., 2004). Since the pH scale is logarithmic, a unit drop in pH corresponds to a tenfold rise in acidity (Addy et al., 2004). The pH of the soil has a significant impact on soil biogeochemical activities in the natural environment. The "master soil variable" that affects a wide range of soil biological, chemical, and physical qualities and processes that affect plant development and biomass output is hence soil pH (Neina, 2019). Both biological processes and soil biology are regulated by pH; soil pH and biogeochemical processes, especially in the soil, have a bidirectional link with terrestrial ecosystems (Neina, 2019). In this way, many biogeochemical processes are influenced by soil pH, whereas certain biogeochemical processes are also somewhat influenced by soil pH (Neina, 2019). The solubility, mobility, and bioavailability of trace elements (e.g., mercury) are governed by soil pH (Neina, 2019).

3.7.1 Potential pH

Soil consists of 3 phases (solid, liquid and gas) and 4 components (organic, mineral, liquid, gas). Potential pH is defined as the pH of solid phase (both mineral and organic components) of the soil. As established by Grove (1982), potential (reserve) acidity, which is a much greater component of total soil acidity, is found on the surface of soil clay and organic

matter particles. The soil's capacity to withstand changes in solution pH by either releasing or adsorbing H⁺ depends on that amount of clay or organic matter present (Grove, 1982).

3.7.2 Active pH

Active pH is defined as a pH of the soil solution. A particle's surface acidity is in balance with the solution active acidity (Grove, 1982). This resistance is the soil's ability to act as a buffer (Grove, 1982). The amount of active acidity and pH in soil plus water/simple salt suspensions can be the same in soils with varied textures (sandy, silty, or clayey), yet these soils will have quite different amounts (Grove, 1982).

3.8 *Other soil properties*

Aside from carbon species and pH as significant influences on the behavior and speciation of mercury in soils, the following soil properties were examined:

3.8.1 Cation exchange capacity

Cation exchange capacity (CEC) is well known as the potential for positively charged ions, or cations, which can be retained on the surface of a soil particle. This can then determine how available certain elements or compounds are to plants or soil organisms, namely the nutrients in the soil which often are in the form of cations. The level of soil organic matter can directly influence the level of CEC in the soil environment. Soil organic matter (SOM) has been shown to considerably affect certain soil characteristics such as the CEC and point of zero charge (PZC) (Ramos et al., 2018). Specifically, it seems to be the total organic carbon in a soil which is positively correlated with the level of CEC, and the removal of SOM is likely to decrease CEC; these results were made clear from a study on tropical soils in Brazil, where there was a clear linear relationship between SOM, total organic carbon, and CEC (Ramos et al., 2018).

3.8.2 Total sulfur

Around 95% of the total sulfur in soil settings is bonded to organic molecules and is also not immediately accessible to plants (Kertesz & Mirleau, 2004). There are many different forms of organic sulfur present in soils, some of which are found in the soil's organic matter and others in microbial biomass, and very little is known about the chemical makeup of the individual sulfur-containing molecules (Kertesz & Mirleau, 2004). The reactivity of various sulfur species to reducing agents has served to distinguish them (Kertesz & Mirleau, 2004). Immobilization of inorganic sulfur and mobilization of organically bound sulfur are thought to be the two key processes in sulfide cycling in soils, and they are both mediated by microbes (Ghani et al., 1992). However, it is not yet clear whether any particular members of the microbial community play a dominant role in catalyzing these processes (Kertesz & Mirleau, 2004).

Sulfur is generally known to immobilize mercury due to its affinity to bond with certain Hg compounds. Because stable Hg compounds like HgS or insoluble organic Hg-S compounds

can form when Hg reacts with S, the immobilization of mercury is likely when reacting with sulfur in the soil (Biester & Zimmer, 1998).

3.8.3 Carbon-nitrogen ratio

The carbon-nitrogen ratio (C/N) in soils can play an important part in the decomposition of organic matter, as it is directly linked to microbial activity. The microbial respiration of soil organic matter (SOM) can be significantly altered by the addition of C and N (Jílková et al., 2020). The decomposition of SOM that has been added to the soil will rely on the rate at which each of the C-containing components in the sample degrades, as organic materials added to soils may include a broad variety of C compounds (Chen et al., 2003). Generally speaking, a low C/N ratio means that organic matter decomposes more quickly (NRCS, USDA, 2011).

Jílková et al. (2020) showed that the addition of N to OM in soils resulted in an increase in litter respiration but a decrease in bulk soil respiration, indicating that the proportionate increase in microbial respiration is inversely correlated with the C:N ratio. Furthermore, interactions with the soil matrix may contribute to various reactions to the addition of N (Jílková et al., 2020). Depending on the SOM composition of the soil, different soils may experience different impacts from increases in C and N inputs (Jílková et al., 2020).

3.9 *The extraction and bioremediation of Hg contamination*

Determining the necessity and types of remediation activities requires accurate evaluation of the species and mobility of Hg in soils (Bollen et al., 2008). The extraction and bioremediation of mercury contamination, primarily by phytoremediation or microbes seems to be the most logical conclusion to the question posed, but this will take further research to follow up on the mechanics of this toxicological remediation. Hg contamination in soils has implications for further environmental damage, such as Hg leaching into groundwater beneath the contaminated soil. By treating contaminated soil with polysulfide solutions, Biester and Zimmer (1998) showed that matrix-bound Hg or HgCl₂ in soils may easily be converted into stable Hg compounds like metacinnabar (HgS) or insoluble organic Hg-S compounds. In this case, the immobilization of Hg into more stable or insoluble compounds in the soil, would be one way to prevent such groundwater leaching, and a first step towards the remediation of Hg contamination in soils. Findings of soluble Hg speciation indicate that filtering materials, such as particular resins or amalgamating metal alloys, should be tailored to ionic Hg species, in the case of groundwater leaching (Bollen et al., 2008). The chemistry of mercury necessitates the creation of unique extraction methods that are specifically tailored to Hg speciation and fractionation (based on the mobility of each species). (Bacon and Davidson, 2008).

When considering phytoremediation for the extraction and bioremediation of Hg, plants generally absorb pollutants from the soil through their root systems, which also contain the main defenses against contaminant toxicity (Ahhammad et al., 2018). The root system provides a huge surface area that collects and accumulates the water and nutrients that are necessary for growth, but it also absorbs other non-essential contaminants, because there is a propensity to create a heavy metal complex with inorganic compounds prevalent in organisms' bodies (Ahhammad et al., 2018). Most plants sort Hg mostly in the root, functioning as a barrier to

prevent that heavy metal from reaching the aerial sections of the plant; this process is considered as an excluder Hg (Patra & Sharma, 2000). Generally, low levels of organic and inorganic Hg are absorbed by plants from soil (Gworek et al., 2020). Thus, via direct absorption from soil, high soil Hg levels only result in small increases in plant Hg levels (Patra & Sharma, 2000). Hg in terrestrial vegetation is largely found from the atmosphere in the aboveground biomass, whereas Hg found in plant roots primarily comes from the soil (Selin et al., 2007).

Transgenic plants have been shown to cleave mercury ions from methylmercury complexes, reducing mercury ions to the metallic form, taking up metallic mercury through their roots (Patra & Sharma, 2000). The end result is a system that is economically viable, long-lasting, aesthetically pleasing, and in-situ, while lowering mercury toxicity by a factor of 10,000 and meeting or exceeding regulatory standards (Patra & Sharma, 2000). However, in some studies, even negative effects of Hg uptake by plants have been shown, such as for the case by Patra and Sharma (2000) when some transgenic plants were shown to be converting toxic HgCl₂ compounds in a nutrient base into a vapor form in the air, and these transgenic plants were converting volatily up to 7 times more than non-transgenic control plants. Otherwise, modified bacterial genes that decrease mercury ions and break down methylmercury are found in some genetically modified plants (Patra & Sharma, 2000).

Combining traditional phytoremediation techniques—which remove mercury from contaminated regions using scavenger plants—with the cultivation of such plants using both regular mutagenesis and genetic engineering might significantly lessen the problem of mercury pollution (Patra & Sharma, 2000). Future strategies could entail modifying soil conditions to make Hg behave in a way that is more conducive to removal or immobilization (O'Connor et al., 2019). In this regard, some researchers have recently started to examine the use of heat treatments assisted by citric acid for the cleanup of Hg polluted soils (Ma et al., 2015). Other strategies for the bioremediation of Hg from soils include the application of microorganisms for increased Hg flux (with capture) (Mahbub et al., 2017), and Hg biosorption techniques (Dash & Das, 2015). These sorts of methods would be preferred to develop as a more ecological remediation alternative to energy and resource-intensive typical Hg cleanup techniques like soil washing or conventional thermal treatment (Otto & Bajpai, 2007).

4 Materials & Methodology

4.1 Research area

Two locations have been looked into for this study. The areas were chosen based on where the Hg originated. The first place, Libčice, is completely contaminated with Hg from anthropogenic activity. The second, Horní Luby, has Hg from both anthropogenic and geological sources. Hg contamination has existed at both sites for a long period of time. The locations of the examined sites can be shown on the Czech Republic map below (Figure 1).

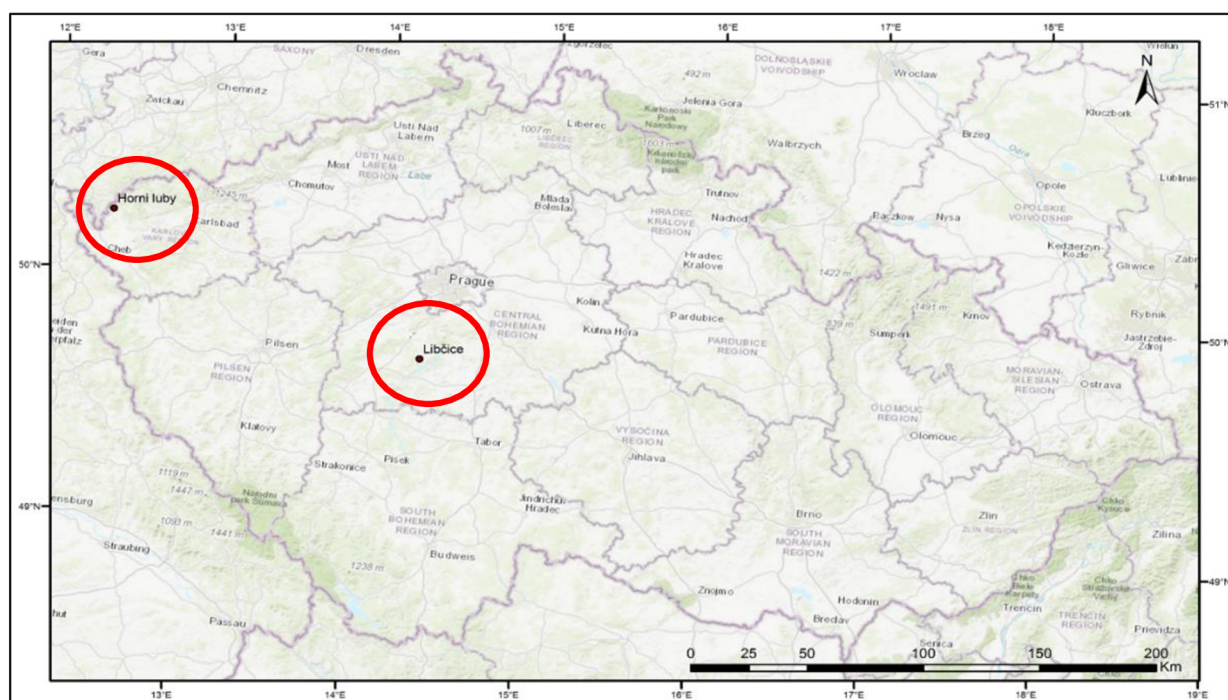


Figure 1: Locations of the examined sites on the map of Czech Republic

Mixed (mainly deciduous) forest and a thick layer of herbaceous vegetation cover both areas. According to some writers, the final destination of mercury in the landscape is determined by the kind of forest and the resulting variations in the delivery and assimilation of mercury into the soil (Demers et al., 2007).

For the purposes of this thesis project, we will be examining many factors about Hg content in soils; namely its distribution, speciation, and bioavailability. The specific Hg in discussion is from two separate mining sites in the Czech Republic; Libčice and Horní Luby. We will be comparing the two sites specifically because of their differences in the source of mercury content in soils. For Libčice, the Hg content is completely anthropogenically sourced from a local gold mining site, while the Hg content in Horní Luby is sourced from both geogenic inputs, as well as anthropogenic inputs from an abandoned cinnabar mining site.

4.1.1 Hg contamination from mining sites

One of the main producers of anthropogenic mercury come from the mining activities; especially from the waste which is produced in this process. It is well known that increased Hg concentration coming from mine wastes can lead to widespread contamination of the local environment (Hojdová et al., 2009). The specific cases which our studies are based on involve the mining of gold and cinnabar. In the example of one of our study areas in the Czech Republic, Libčice has an abandoned gold mine situated in the Central Bohemian region, where the most intensive gold mining took place from the seventeenth to twentieth century (Umlaufová et al., 2018). Gold mining can lead to the contamination and degradation of the local environment, is capable of affecting the soil structure, as well as physicochemical, biological properties, and even microbial ecology (Umlaufová et al., 2018).

Such contaminants left behind as by-products of the gold mining process include, of course, mercury, as well as other elements like cyanide, lead, and cadmium (Umlaufová et al., 2018). Mercury is capable of being dispersed from a gold mine through the atmosphere (since it is such a volatile element) and can later be deposited in the local surface forest soil (Tomiyasu et al., 2017). Once deposited in such soils, Hg can be methylated due to various factors in the soil organic matter, such as the presence of total organic carbon (TOC), where studies have found a linear relationship between the presence of TOC and MeHg (Tomiyasu et al., 2017). However, other factors may influence the formation of toxic MeHg in other types of soil environments, such factors as water content, redox conditions, and microbial activity (Tomiyasu et al., 2017).

4.1.2 Libčice

In the first half of the 20th century, Libčice was a gold mine; it has since been closed down for more than 70 years. This former gold mine's surroundings are distinguished by the presence of Hg-bearing mine tailings (from the amalgamation process) (Percival et al., 2014). Due to the Hg's anthropogenic origin as opposed to its natural occurrence at cinnabarite sites, the abandoned gold mine in Libčice (located in Central Bohemia, Czech Republic), where the amalgamation process was used for the separation of gold in the first half of the 20th century, was selected as an area of interest. In Libčice, it was shown by Umlaufová et al. (2018) that the soil texture showed a low percentage of clay minerals at this location. Therefore, soil physicochemical characteristics indicate favourable soil conditions for plant development, good sorption capacity of the soils,

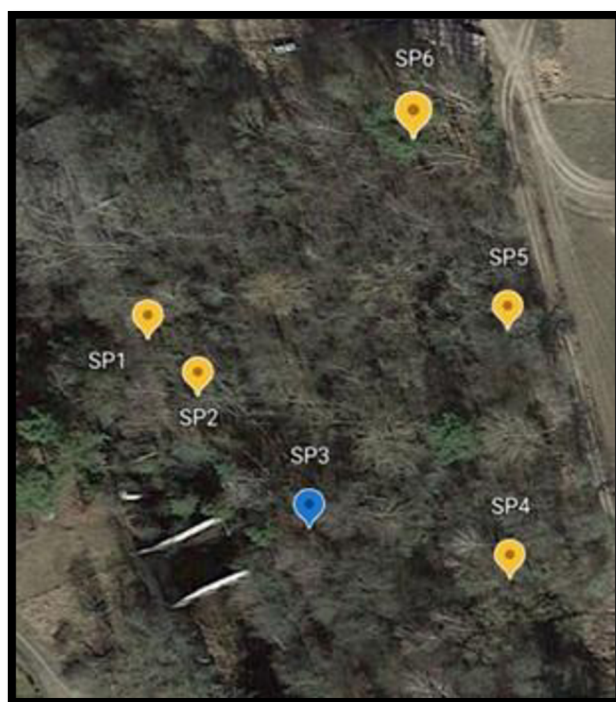


Figure 2: Sampling locations in Libčice

good retention capacity for nutrients, and potential for prediction of immobilization of the risk factors resulting from mining in these soils (Umlaufová et al., 2018).

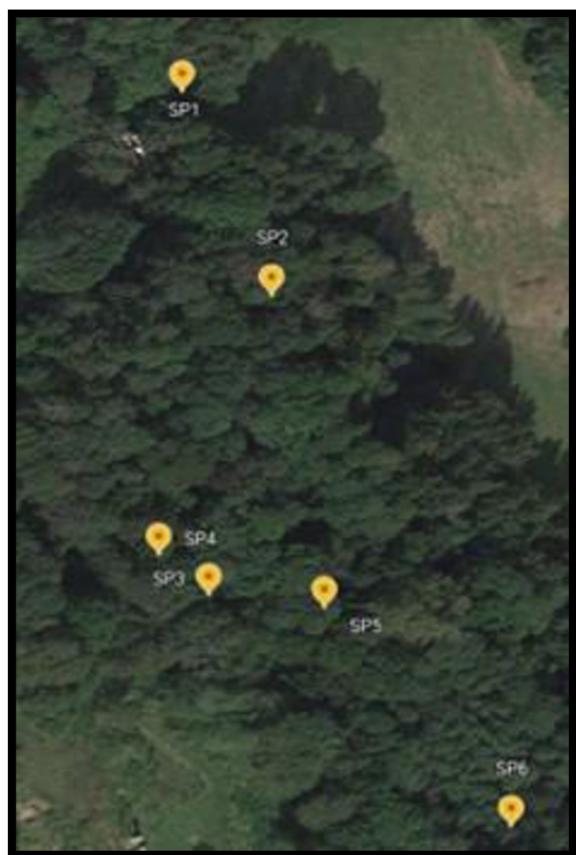


Figure 3: Sampling locations in Horní Luby

4.1.3 Horní Luby

For the location of Horní Luby (situated in North Bohemia, Czech Republic), cinnabar (HgS) was mined between 1520 and 1540, with a smaller portion between 1560 and 1570. At the time, mercury production ranged between 6 and 15 tons per year. In 1597, it was abandoned. The few, unsuccessful attempts to restart mining in the 17th, 18th, and 19th centuries were not especially substantial. According to Velebil and Zachariáš (2013), the ore that is mined at the Horní Luby mine is entirely made up of cinnabar.

4.2 Sampling

The sampling was carried at the pollution point sources at both sites. For Libčice, samples were taken at sampling points 1-3 (which near the buildings where the amalgamation process occurred) and 4-6 (at some distance from the buildings). It is expected that the Hg will accumulate near the buildings where the amalgamation process occurred. All sampling locations for Libčice are shown on the previous page in Figure 2.

For Horní Luby, sampling points (SP) 2, 5, were set up at the dumping grounds for the material excavated from the mine. SP 1 is close to the entrance to the mine, SP 3 is a lake, and SP 4 is right next to the lake (shown above in Figure 3). Soil samples were taken from the top layer of the soil (0-20 cm). Two profiles were opened at each sampling point: 15x30 cm profiles, 1.5 meters apart. Each soil horizon was taken separately, making four field repetitions of each horizon.

The organic layer was noticeably variable between the two sites, above the underlying mineral layers of the soil which were both considered in the analyses. In Libčice the O horizons (organic layers) ranged between 1-3cm deep (usually 2cm), A horizons (mineral and humic layers) went as low as 7-13cm deep (usually 10cm), and the remaining B horizons (subsoil mineral layers) were sampled up to 20cm (excluding samples taken at maximum 10cm depths due to large rock layers). Soils were evaluated as dark grey, hard, high clay content, and some or little organic matter.

In Horní Luby, the O horizon was noticeably thin and sporadically appearing in multiple occurrences, making it very difficult to sample separately from the A horizon, and in multiple cases only resulting in A horizon samples from a sampling point. The O horizon was

consistently <1cm, while the A horizon ranged from 0-20cm and the horizon Bg (gley soil due to water saturation and redox reactions) was also observed at one sampling point (SP3) between 12-20 cm. Also, F horizons (undecomposed organic layer) were observed from 0-4cm, as well as a Cg horizon (also gley soil due to water saturation and redox reactions) at another sampling point (SP6).

4.3 Sample preparation

Two sets of samples were brought into the lab. The refrigerator was used to transport one pair, and transportation with regular conditions for the other. For examination of soil chemical properties and total Hg, samples have been transported under regular conditions, air dried and stored at the room temperature. The samples that were transported in the freezer were have been freeze dried and stored at -40 degrees until the testing. All the samples have been crushed and sieved through 2 mm sieve.

4.4 Analytical methods

Total amounts of C, N, and S were measured by a CNS macro analyser machine. This machine is a macro elemental analyser (known as the vario MACRO cube), used for the purposes here of simultaneous C, N, and S determination from the samples. The machine is used for such macro elemental analyses for solids (in this case soil), as well as viscous and liquid samples. This CNS analyser has a precision of < 0.1 % absolute (100 mg glutamic acid) and an analysis time of ~ 3-4 min per element, self-optimizing according to element content and sample weight. The precision and analysis times are dependent also on sample type, analysis mode, and configuration. The detectors on the device are a Thermal Conductivity Detector (TCD) and an Infrared Detector for sulfur analysis.

CEC capacity was determined by using Gillman's method (Gillman, 1979). Potential pH of the solid phase was measured in KCl (Ash et al., 2013), and the active pH in water (Rayment and GE, 1992). Both measurements were made using an ISFET electrode and a Sentron SI400. The Tyurin method was used to oxidimetrically measure organic C (Borůvka, 2012). For the determination of total Hg in the extracts, inductively coupled plasma-atomic emission spectrometer (ICP-OES, Agilent 720, Agilent Technologies Inc., USA) equipped with a two-channel peristaltic pump, a Struman-Masters spray chamber, and a V-groove pneumatic nebulizer made of inert material was applied (the experimental conditions were as follows: power of 1.2 kW, plasma flow of 15.0 l/min, auxiliary flow of 0.75 l/min, nebulizer flow of 0.9 l/min).

4.4.1 Extraction

One gram of soil was weighed to the nearest 0.001 g into polypropylene (PP) 15-mL centrifuge tubes. For determination of mobile Hg fraction, extraction procedure validated by

Han et al. (2003) was used with some minor modifications (extractable with 2% HCl + 10% ethanol) – Han et al. 2003. This can be referred to as potentially mobilizable Hg in soil extract.

Soil samples were extracted in three consecutive steps with a solution containing 10% (v/v) ethanol (p.a., Penta Ltd., Czech Republic) in 2% (m/v) HCl (Analpure®, Analytika Ltd., Czech Republic). First extraction (7 mL of the extractant) was initiated by vigorous handshaking (10 s) and vortexing (10 s) resulting in soil suspension, then the samples were immersed in a water bath (55 ± 3 °C) and sonicated for 7 minutes. Soil slurries were centrifuged ($2700 \times g$) for 5 min, passed through a filter paper (particle capture >3 μm). The extraction was repeated two additional times with 4 mL of the extractant per extraction step. The filtrates were combined, and 10-fold diluted with Milli-Q water (≥ 18.2 $\text{M}\Omega$ cm^{-1} ; MilliQ system, Millipore SAS, France) prior to analysis.

4.4.2 Potentially mobilizable Hg in soil extract

Four mL of L-cysteine ($\geq 98\%$, Carl Roth Ltd., Germany) solution was added to 9 mL of soil extract to give a final concentration of 0.05% (m/v). The concentration of Hg was measured by an inductively coupled plasma mass spectrometry (ICPMS; Agilent 7700x, Agilent Technologies Inc., USA) operated in NoGas mode. The instrument was calibrated in the range of 0.03 to 30 $\mu\text{g L}^{-1}$ Hg in 1% (m/v) HNO_3 (Analpure®, Analytika Ltd., Czech Republic) containing 0.10% (m/v) cysteine, in which case an aqueous standard (1.000 g L^{-1} Hg, Agilent Technologies Inc., USA) acted as a source of Hg. Memory effects of Hg were avoided following the recommendation by Li et al. (2006). The signal of ^{202}Hg was compensated for by the use of an internal standard (Lu and Bi, each at 50 $\mu\text{g L}^{-1}$). For quality assurance of the analytical results, a standard reference material Mercury in water (SRM 1641d, NIST) and procedural blanks were analysed in parallel, including a spiking procedure based on that SRM.

4.4.3 Speciation analysis of Hg

An aliquot of diluted soil extract was additionally filtered (0.45 μm , Nylon), diluted with a mobile phase (see below) at a ratio of 1:1, and subjected to Hg speciation analysis employing a high-performance liquid chromatography (HPLC; Agilent 1260, Agilent Technologies Inc., USA) system hyphenated to ICPMS as a mercury detector. The HPLC system was equipped with an Eclipse Plus C18 column (100×4.6 mm, 3.5 μm ; Agilent Technologies Inc., USA), which was isocratically eluted at a flow rate of 1.2 mL min^{-1} . The mobile phase composition was adopted from Jagtap et al. (2011), which we modified as follows: 0.1% (v/v) 2-mercaptoethanol, 0.04 mol L^{-1} ammonium acetate ($\geq 97\%$, Carl Roth, Germany) in 5% (v/v) methanol (ROTISOLV®, Carl Roth, Germany), pH = 5.25 (adjusted with acetic acid). The sample injection volume was 20 μL and the column was heated to 30 °C. Standard solutions at 0.05, 0.25, 1.0, 5.0 and 10 $\mu\text{g L}^{-1}$ Hg of individual Hg species and MeHg, were prepared by dissolution and dilution of HgCl_2 ($\geq 99.5\%$, Honeywell-Fluka, Switzerland) and CH_3HgCl ($\geq 98\%$, Sigma-Aldrich, Germany), respectively.

4.4.4 Determination of water-soluble elements in soil (water-soluble Hg, and C content in soil)

Soil sample was weighed (to 0.001 g) and extracted by shaking with Milli-Q water at a soil:water ratio of 1:25 in 15-mL PP tubes placed on a rotator (30 rpm) for 24 h, following the recommendation by Reis et al. (2014). After shaking, the samples were centrifuged ($2700\times g$) for 10 min and passed through a filter paper (particle capture $>3\ \mu\text{m}$). For Hg determination, an aliquot of the filtrate was 8-fold diluted with a solution containing 0.05% (m/v) cysteine and 1% (m/v) HNO_3 in 0.25% HCl. For determination of the other elements, the addition of cysteine in the diluent was skipped. Elemental quantification was performed by ICPMS operated in NoGas mode (Hg and C). The instrument was calibrated externally using aqueous standards containing Hg (Agilent Technologies Inc., USA) and gravimetrically prepared carbon-containing solutions (sucrose; MANUFACTURER), which were diluted in the diluents specified above. The usefulness of a single quadrupole ICPMS in the determination of C has been demonstrated by Amaral et al. (2015). Similar values of limit of detection (LOD) and background equivalent concentration (BEC) for ^{13}C , $240\ \mu\text{g L}^{-1}$ and $6200\ \mu\text{g L}^{-1}$, respectively, to those reported by Amaral's group.

4.4.5 Total Hg determination in the soil

One of the most used methods for detecting trace amounts of total mercury in biological and environmental materials is the cold vapour AAS method (CVAAS) (Clevenger et al., 1997 & Yan et al., 1994). In quartz vessels with the $\text{HNO}_3 + \text{HClO}_4$ mixture, contamination-free digestion for the following mercury detection is possible at the ultratrace level (Száková et al., 2004). Specially purified acids are required for very low mercury contents (May & Stoeppler., 1984). The majority of Czech and Slovak analytical laboratories have been using the specialized, single-purpose Trace Mercury Analyzer TMA-254 atomic absorption spectrometer since 1986, and later on the primary technique for determining mercury in soil became the Advanced Mercury Analyzer AMA-254. (Száková et al., 2004). According to the findings of Száková et al. (2004), the mechanical, optical, and chemical advancements made during the AMA device's creation contributed to the analytical system's increased stability in Hg determination when compared to the device's predecessor, the TMA. Evidently from Száková et al. (2004), this approach is best suited for quickly and accurately determining the presence of Hg in a variety of agricultural and environmental materials, leading to an accurate assessment of Hg pollution of the environment.

4.5 *Statistical methods*

4.5.1 Shapiro-Wilk test for data distribution

One of the most effective omnibus tests for evaluating the univariate normality hypothesis is the Shapiro-Wilk's test (SWT), according to Thode (2002). In this study, the Shapiro-Wilk test was done to determine the normality of the distribution for each variable. This can be seen

below in Table 1 for Libčice and Table 2 for Horní Luby (shown in results and discussion). JASP version 0.17.1 was used to run the Shapiro-Wilk test.

4.5.2 Median +/- median absolute deviation (MAD)

To further examine values between the Hg characteristics and specific soil properties, the medians for all measured values of these factors were calculated, plus or minus the median absolute deviations (MAD) of these values. This analysis can be found for reference in the Appendices section of this thesis. There, the overall medians of the original factors are also shown for reference, but will not be included in the main paper (see Tables 5 and 6 in Appendices). Tables 5 and 6 were created using Microsoft Excel version 16.16.27.

Some studies have determined the median absolute deviation to be a stronger statistical method compared to the typical use of mean plus or minus standard deviation, particularly for the presence of outliers (Leys et al., 2013). The benefit of the median over the mean in a given sample size is that it is far less susceptible to the presence of outliers than the mean is and has even been considered as the strongest dispersion scale/measure in their presence (Leys et al., 2013).

4.5.3 Spearman Correlation

A widely used method for evaluating the rank correlation of two data sets is the Spearman Correlation (Liu, 2017). One benefit of using Spearman over other correlation coefficients, such as Pearson, is that the relative rank of the value, rather than the original value series, is what matters most in this coefficient (Liu, 2017). The Spearman correlation, often known as Spearman's rank correlation coefficient, is regarded as a non-parametric version of the Pearson correlation, and a suitable indicator of the strength and direction of link between ranked data sets (Liu, 2017). The monotonic relationship of a dataset's strength and direction are described by the Spearman correlation, although frequently, there is no substantial monotonic link between the relevant value series in the data sets under comparison (Liu, 2017).

In Tables 3 and 4 (shown below in results and discussion, section 5.2), the Spearman correlation was used to determine the correlations between the factors of Hg and the specific observed soil properties. The correlations in these tables were calculated using Statistica version 13.0. The sample number (N) for Libčice is 32, while N for Horní Luby is 25. The higher the number of samples per site, the smaller a correlation coefficient was used. The p-value for the correlation datasets at each location was <0.05. The relationships observed were considered as positive or negative based on this p-value.

5 Results & Discussion

All of the data except pH show characteristics of a non-normal dataset. Therefore, the non-parametric method of correlation, the Spearman Correlation, was chosen to examine the influences between these variables. Additionally, Graphs 1-24 shown below in section 5.1 display statistics for these properties as well in boxplots; created using Microsoft Excel version 16.16.27). Shown below in Tables 1 and 2 are the results of the Shapiro-Wilk tests, as well as descriptive statistics between the two study locations.

Table 1: Shapiro-Wilk test results for Libčice

Descriptive Statistics												
	Potential pH	Active pH	CEC	Total S [mg/kg]	C/N	Total C [mg/kg]	C org [mg/kg]	C_ws [mg/kg]	Total Hg	potentially mobilizable [mg/kg]	MeHg [mg/kg]	Hg_ws
Valid	32	32	32	32	32	32	32	32	32	32	32	32
Missing	1	1	1	1	1	1	1	1	1	1	1	1
Mean	5.660	6.782	246.394		15.678	95.735		39.925	5.182	1.059	0.018	0.145
Std. Deviation	0.312	0.371	204.529		2.484	85.544		36.674	6.778	1.344	0.015	0.145
Shapiro-Wilk	0.906	0.962	0.775		0.837	0.858		0.731	0.584	0.701	0.553	0.818
P-value of Shapiro-Wilk	0.009	0.311	<.001		<.001	<.001		<.001	<.001	<.001	<.001	<.001
Minimum	4.960	5.935	56.510		12.600	10.700		7.962	0.210	0.050	0.010	0.000
Maximum	6.050	7.515	950.400		23.940	288.433		156.951	35.000	6.700	0.060	0.620

Note. Not all values are available for Nominal Text variables

Table 2: Shapiro-Wilk test results for Horni Luby

Descriptive Statistics												
	Potential pH	Active pH	CEC	Total S [mg/kg]	C/N	Total C [mg/kg]	C org [mg/kg]	C_ws [mg/kg]	Total Hg	potentially mobilizable [mg/kg]	MeHg [mg/kg]	Hg_ws
Valid	25	25	25	25	25	25	25	25	25	25	25	25
Missing	85	85	85	85	85	85	85	85	85	85	85	85
Mean	4.861	4.955	109.018	4.219	12.972	92.339	65.367		18.795	2.538	0.017	0.125
Std. Deviation	0.992	0.771	94.416	6.794	3.652	112.069	75.789		16.903	2.663	0.015	0.126
Shapiro-Wilk	0.897	0.906	0.735	0.627	0.949	0.721	0.705		0.876	0.835	0.567	0.741
P-value of Shapiro-Wilk	0.016	0.024	<.001	<.001	0.239	<.001	<.001		0.006	<.001	<.001	<.001
Minimum	3.225	3.690	23.561	0.000	6.840	3.500	3.798		1.760	0.080	0.010	0.020
Maximum	6.120	6.030	339.649	25.168	21.465	441.550	295.560		66.140	10.050	0.060	0.500

Note. Not all values are available for Nominal Text variables

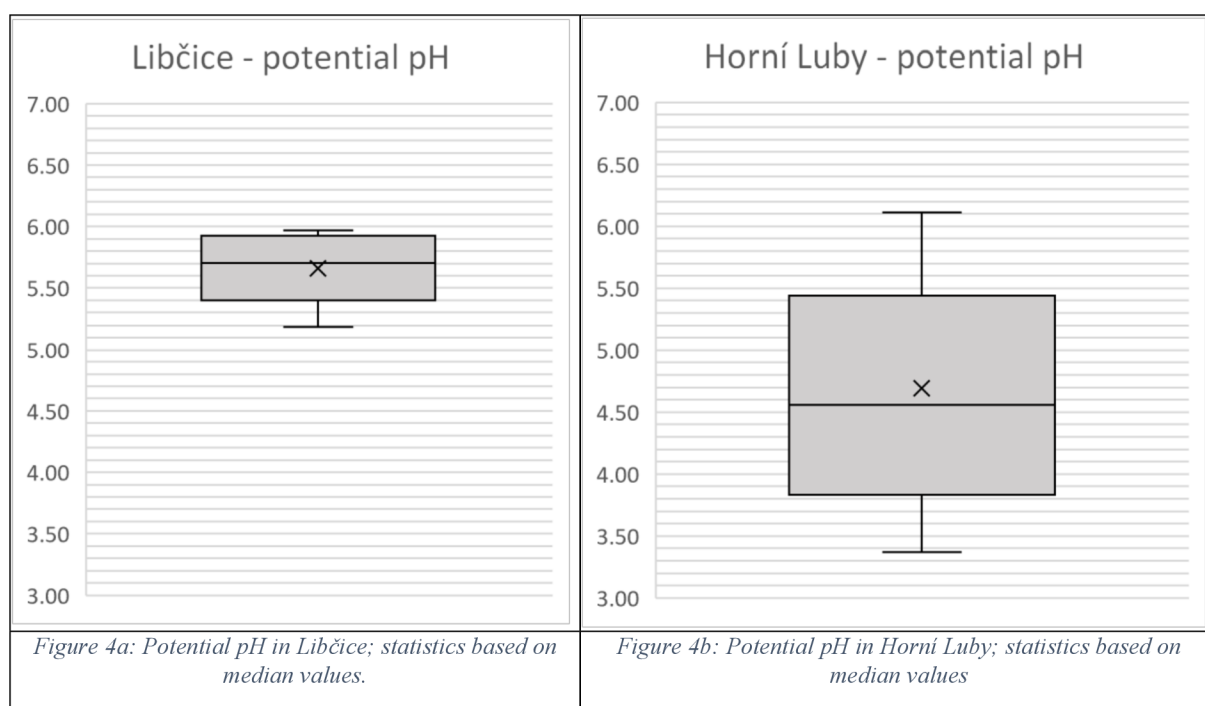
Various correlations between the Hg and some soil properties have been observed. Described below are the correlations with the various species of Hg observed, compared with specific soil properties. For mercury, specifically observed were the species of total Hg, PM-Hg, methylated Hg (MeHg), and water-soluble Hg (Hg_ws). The specific soil properties observed were potential pH, active pH, cation exchange capacity (CEC), total sulfur (S), the carbon-nitrogen ratio (C/N), total carbon (C), organic carbon (C org), and water-soluble carbon

(C_ws). The comparison of these various factors should help to further understand how this Hg speciation can occur, influenced by the many soil properties observed. In Tables 3 and 4, the Spearman correlation between observed Hg species and soil properties may be seen.

5.1 Compared values of mercury and soil properties between sites

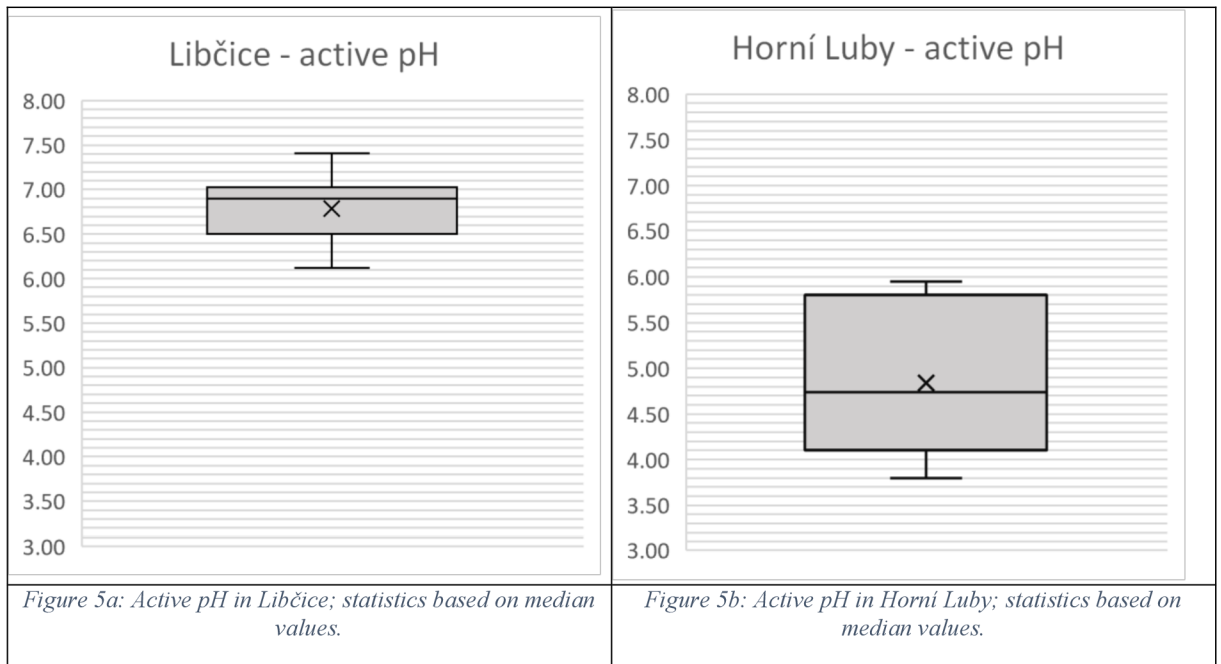
In the following section, the mercury and soil properties discussed thus far will be shown and compared statistically using initially median values, then applying mean, maximum and minimum values of the datasets, as well as outliers - displayed in the boxplots below (Figures 4-28).

5.1.1 Potential pH



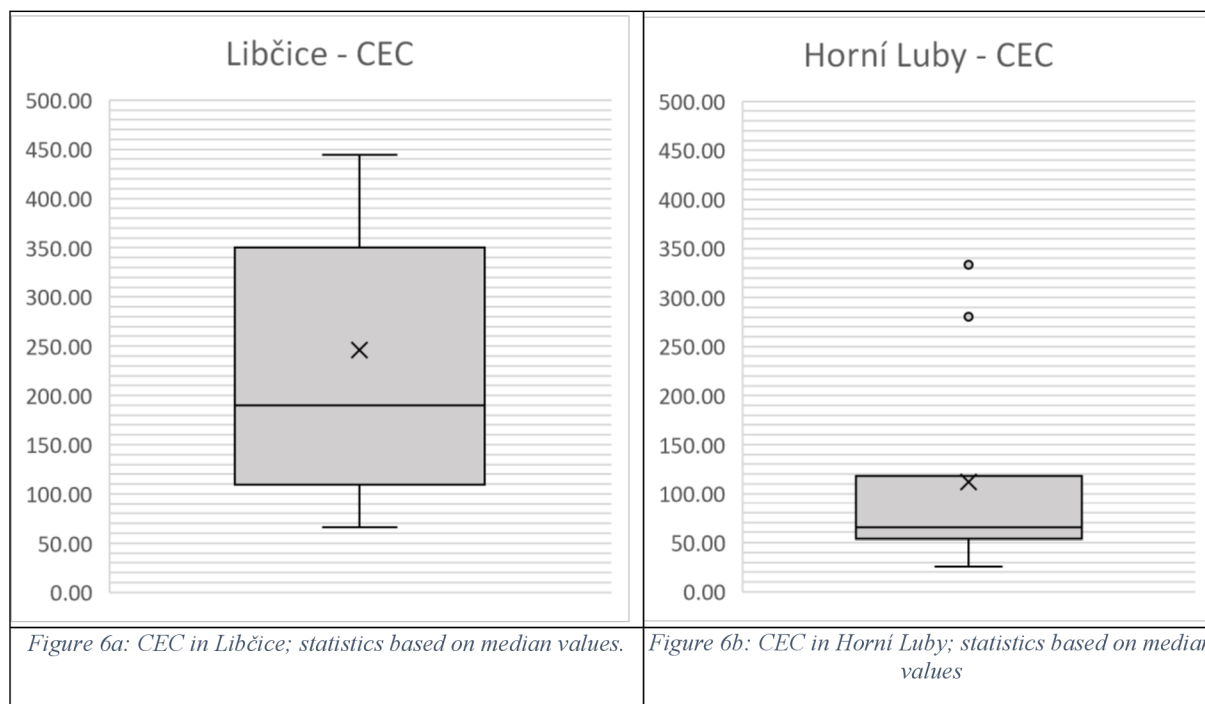
As is shown in Figures 4a and 4b above, the potential pH was generally higher in Libčice soils compared to Horní Luby soils. That is to say to say that generally, the pH of the solids in the soils are more acidic at the Horní Luby location. It is typically expected that Hg mobility is directly influenced by pH; lower pH and acidic conditions will increase Hg solubility and mobility, and higher pH will immobilize Hg (Gambrell et al. 1980). Because of the generally higher pH in Libčice compared to Horní Luby, it is expected that Hg is more mobile in Horní Luby.

5.1.2 Active pH



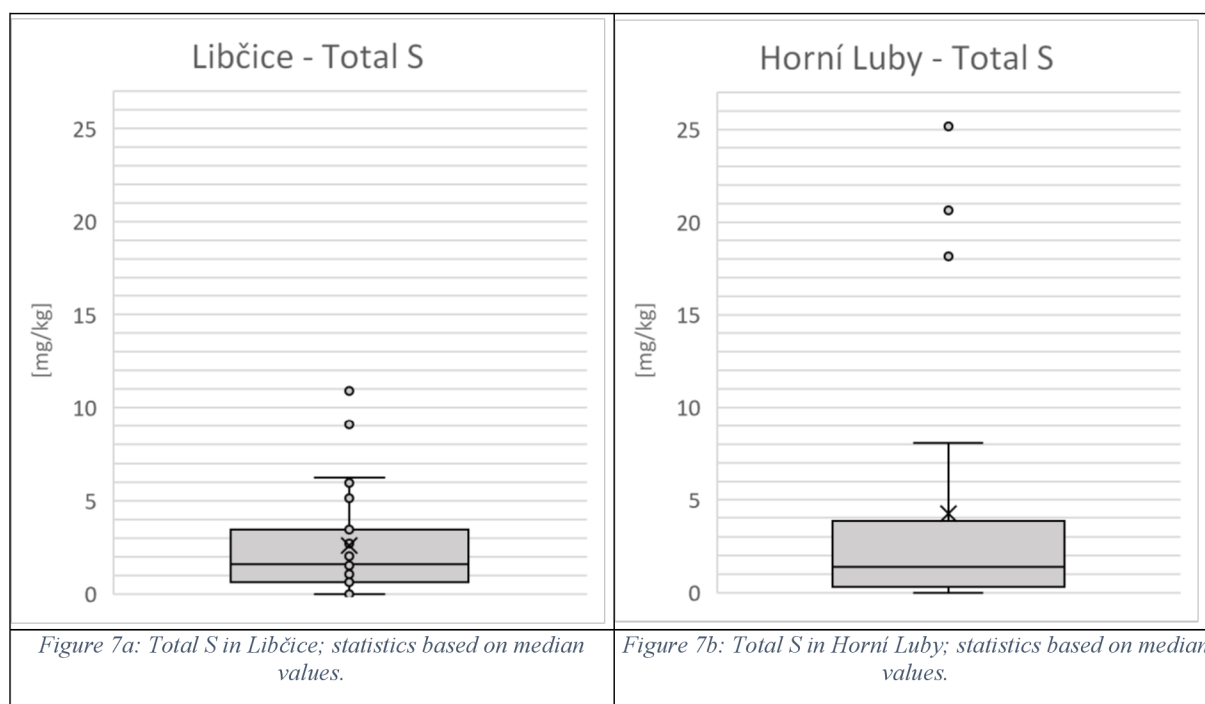
As is shown in Figures 4a and 4b above, the potential pH was generally higher in Libčice soils compared to Horní Luby soils. That is to say to say that generally, the pH of the solids in the soils are more acidic at the Horní Luby location. It is typically expected that Hg mobility is directly influenced by pH; lower pH and acidic conditions will increase Hg solubility and mobility, and higher pH will immobilize Hg (Gambrell et al. 1980). Because of the generally higher pH in Libčice compared to Horní Luby, it is expected that Hg is more mobile in Horní Luby.

5.1.3 Cation-exchange capacity



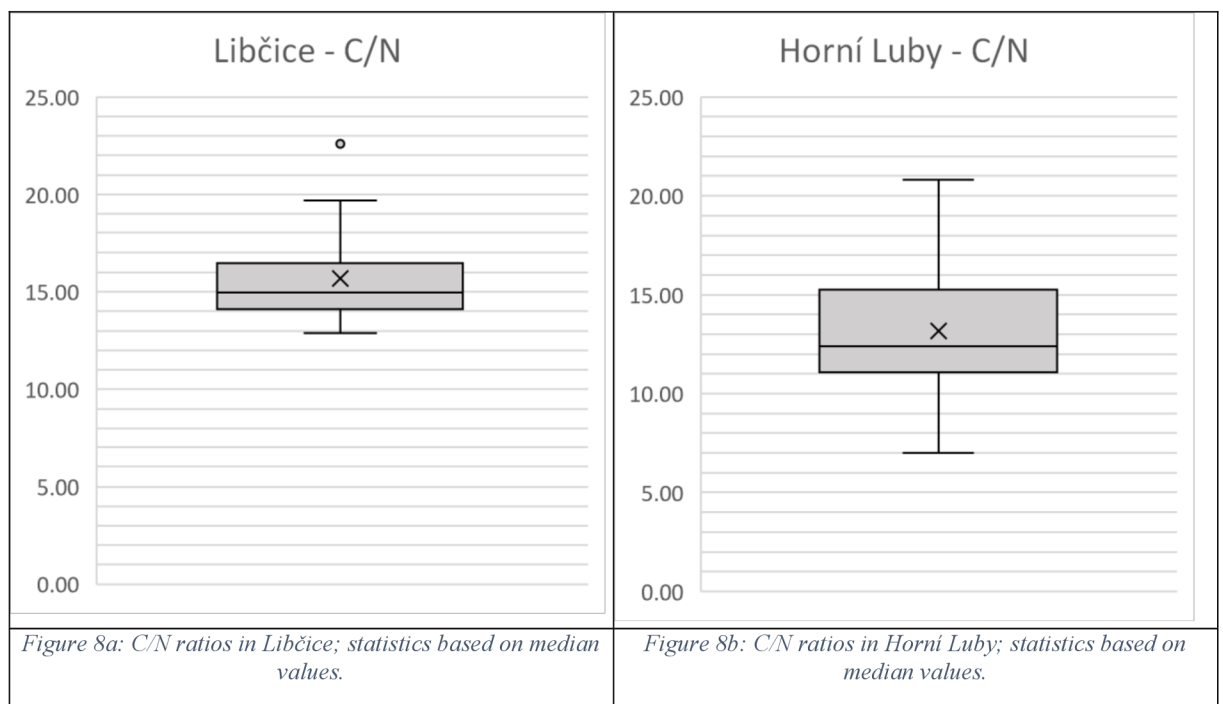
In Figures 6a and 6b above, the average CEC was considerably lower in Horní Luby than in Libčice. It is expected that there are two main factors leading to these differences in CEC for both sites, 1) that organic matter was higher in Libčice or 2) that clay content was higher in Libčice, as it was established from Ramos et al. (2018) that CEC is positively correlated with OM and C content, as well as clay content in soils.

5.1.4 Total sulfur



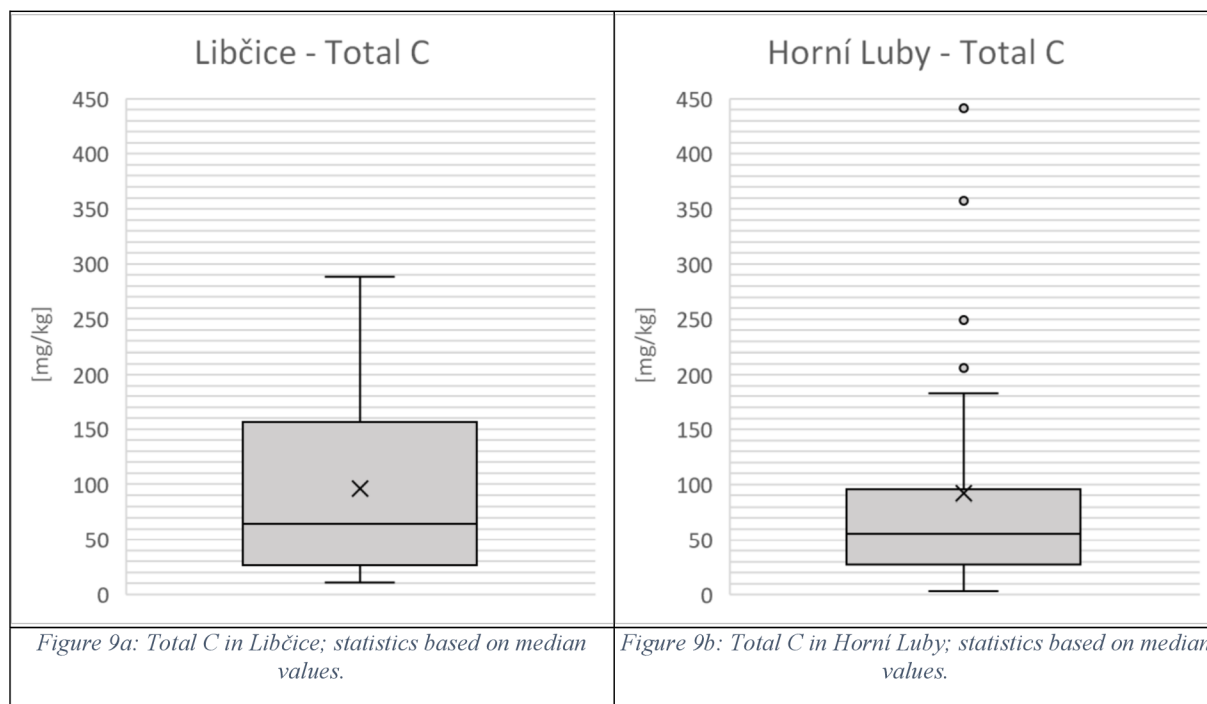
As can be seen in Figures 7a and 7b above, sulfur levels were quite comparable between the two sites. It is expected that although S levels are comparable between sites, that they from different sources. Because of the cinnabar mining in Horní Luby, the sulfur is mostly derived from high cinnabar content at that location in the form of HgS. Alternatively, in Libčice there was no cinnabar content and only gold, which was present in sulfidic minerals. In principle, sulfidic minerals can be found in both locations. To reiterate regarding Hg, the source of it at Horní Luby is geogenic, whereas Hg was added anthropogenically in Libčice for the gold mining process. However, as proposed by Šípková et al. (2014), it might be concluded that mercury species bound to sulfur are more prevalent in areas with higher anthropogenic contamination than they are in samples with lower contamination. This would imply that more Hg could be bound to sulfur Libčice overall due to the more anthropogenic origin of Hg, and could therefore be less detected and available.

5.1.5 Carbon-nitrogen ratio



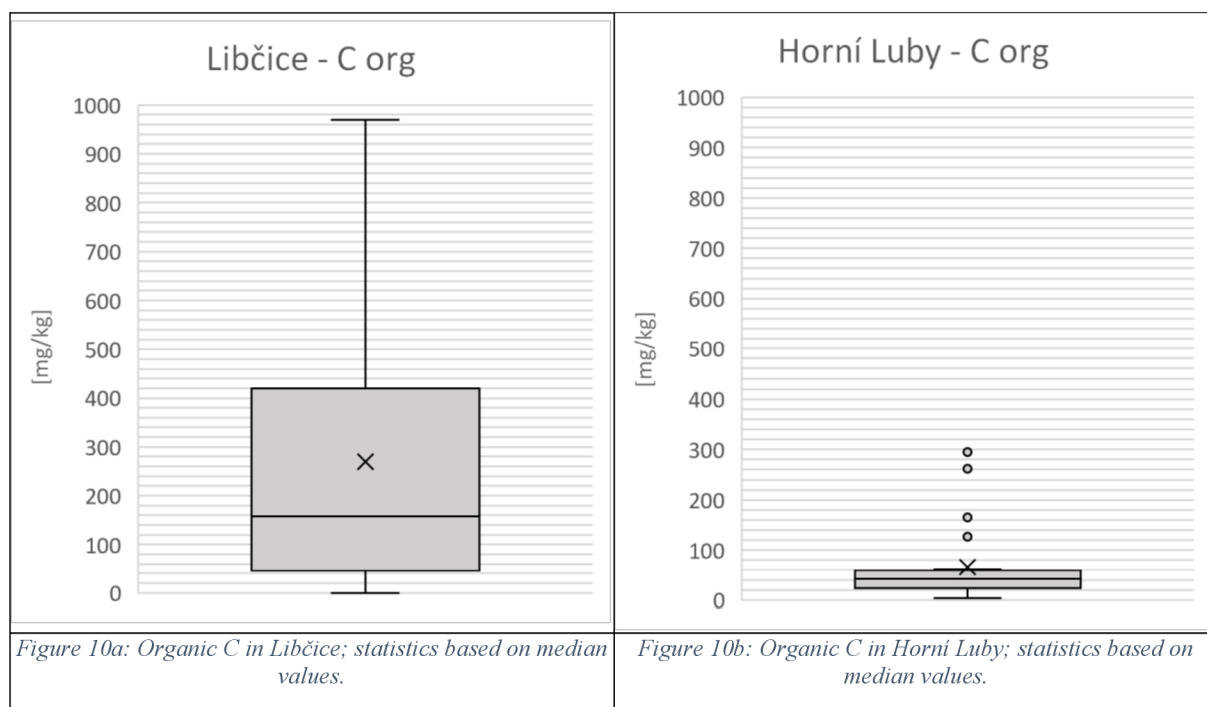
C/N ratios were comparable between sites, although slightly higher in Libčice, shown in Figures 8a and 8b above. As is shown in the following graphs for carbon species, generally there seems to be higher organic matter in Libčice, making it logical to see overall higher C/N ratios at the location as well.

5.1.6 Total carbon



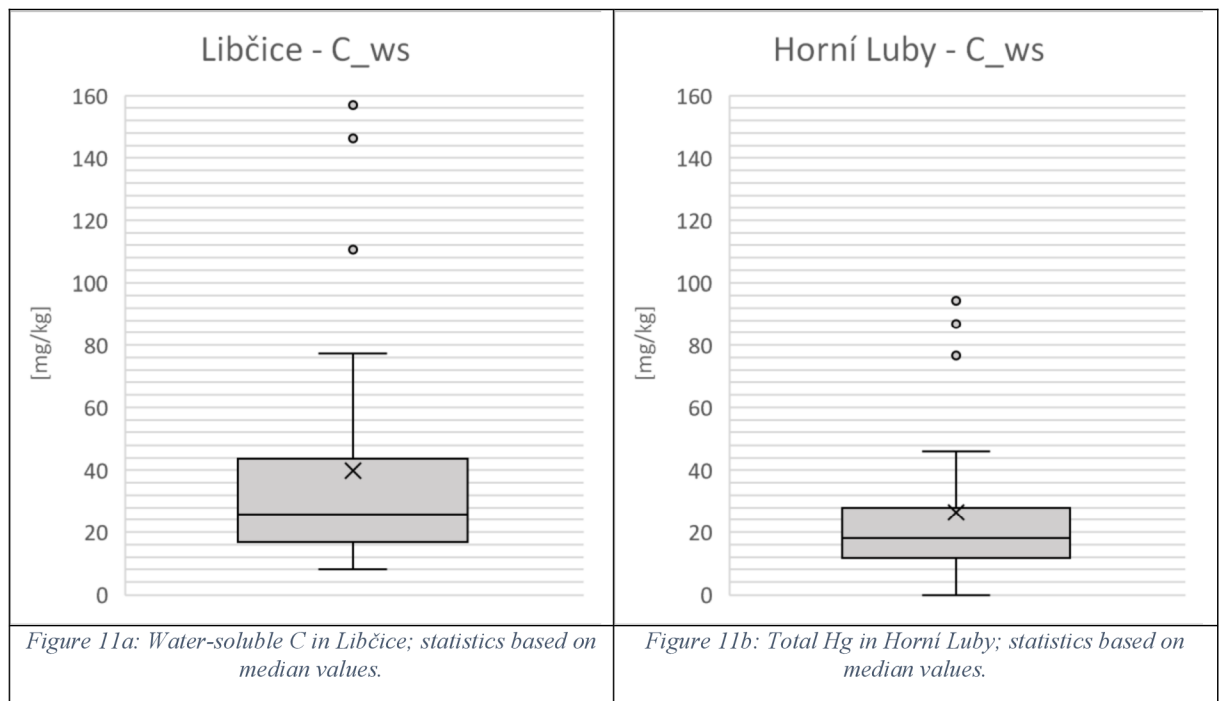
Total C levels showed some comparable values as well between sites, as can be seen in Figure 9a and 9b above. While the total C levels are quite comparable between the two, what is shown in the next section for organic C should have deeper implications for how Hg show be differing between the sites.

5.1.7 Organic carbon



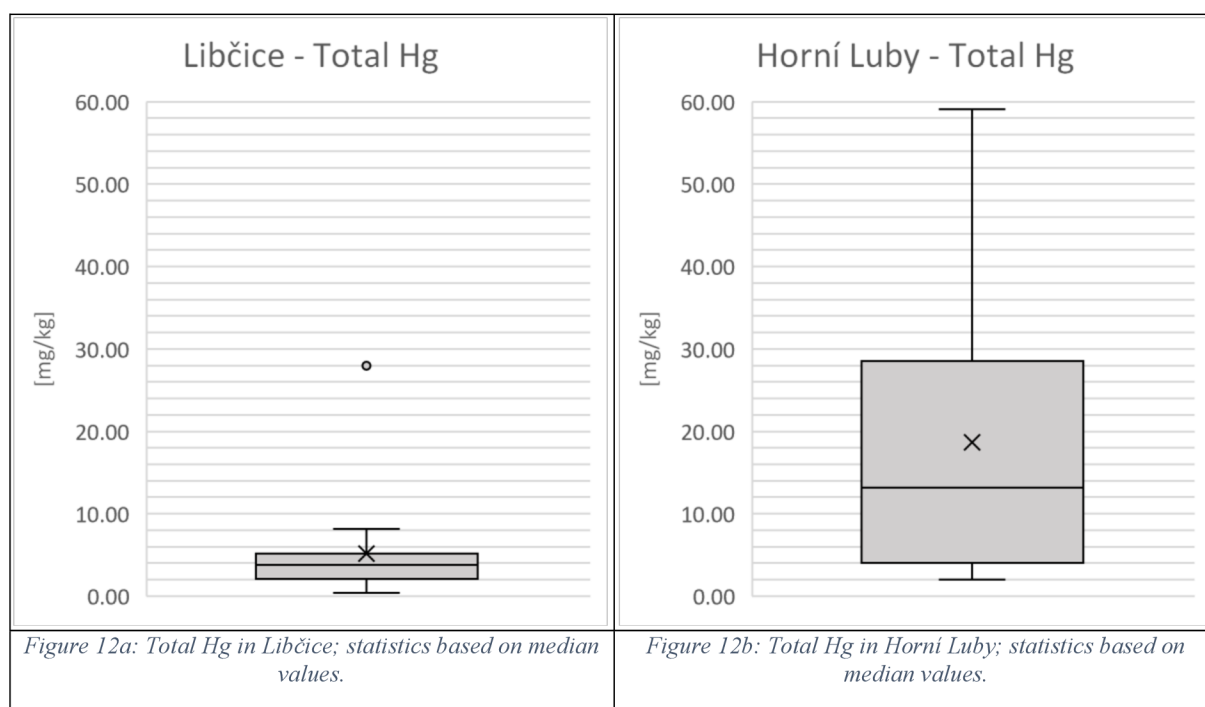
Organic C levels were considerably lower in Horní Luby compared to Libčice, as shown in Figures 10a and 10b above. As previously reviewed, it is known that organic matter has an affinity to bond to Hg compounds. Therefore, when higher organic C (correlating to higher OM) is observed, it is expected that Hg is less mobile in the case of Libčice. Because of the implied higher OM levels in Libčice and therefore the lowered mobility of Hg, it is expected that overall Hg levels as well will be considerably higher. As was shown with Šípková et al. (2014), levels of highest OM content can directly correlate to highest Hg values in soils. As established earlier on, Tomiyasu et al. (2017) described a linear relationship between the presence of total organic carbon and MeHg; so, the considerably higher organic C observed in Libčice should be hypothetically be facilitating more Hg methylation in Libčice.

5.1.8 Water-soluble carbon



In Figures 11a and 11b shown above, water-soluble C levels were generally higher in Libčice, which does have some implications for Hg mobility and speciation. As seen in the research of Barkay et al. (1997), water-soluble carbon species have been shown to reduce the bioavailability of Hg species, and that this is especially more pronounced under neutral conditions compared to acidic. This would also mean that there should be less Hg methylated under the conditions of higher water-soluble C content.

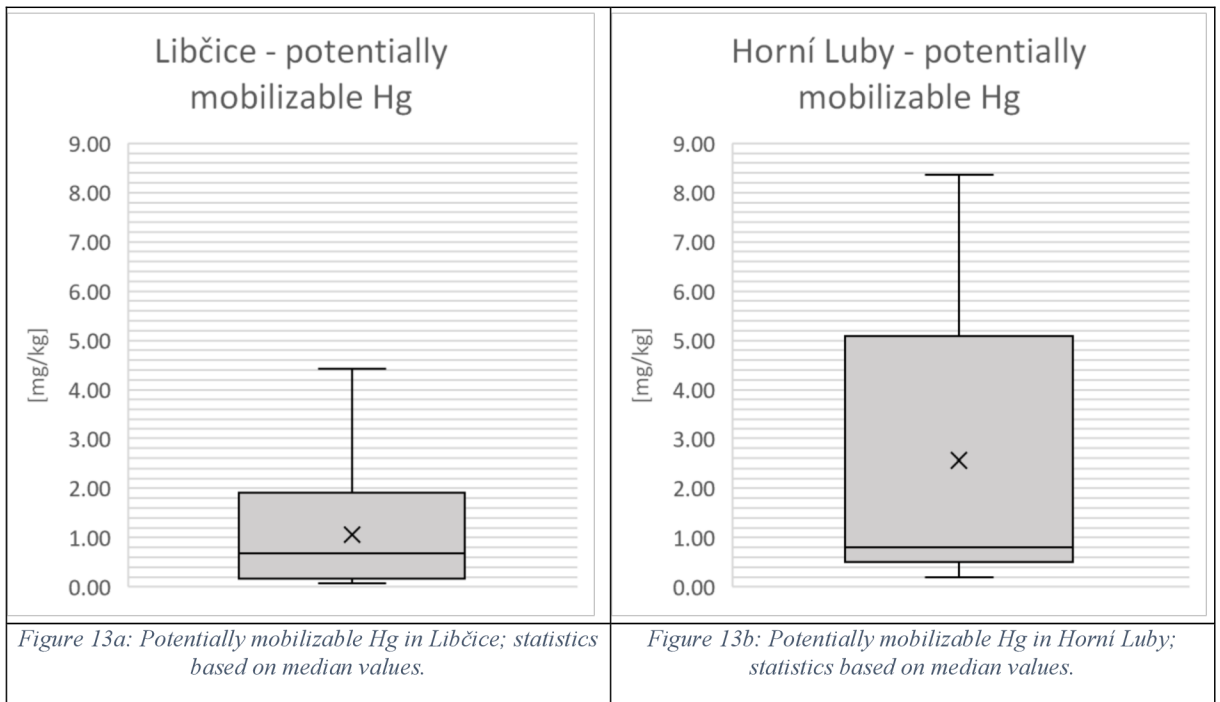
5.1.9 Total mercury



As is clear from Figures 12a and 12b above, total Hg levels were generally quite higher in Horní Luby than in Libčice. It is expected that this significantly higher total Hg observed in Horní Luby is due to the messy nature of the cinnabar mining, compared to the gold mining in Libčice. Generally, this study showed lower total Hg in Libčice as well as the variety of Hg species detected compared to Horní Luby, which is interesting to find considering Umlaufová et al. (2018) reported generally high levels of Hg in Libčice soils. They claimed that the high levels of Hg and the high variability of soil Hg contents found there pointed to the element's anthropogenic origin in the soil due to amalgamation technology used at this location for the gold mining process (Umlaufová et al., 2018).

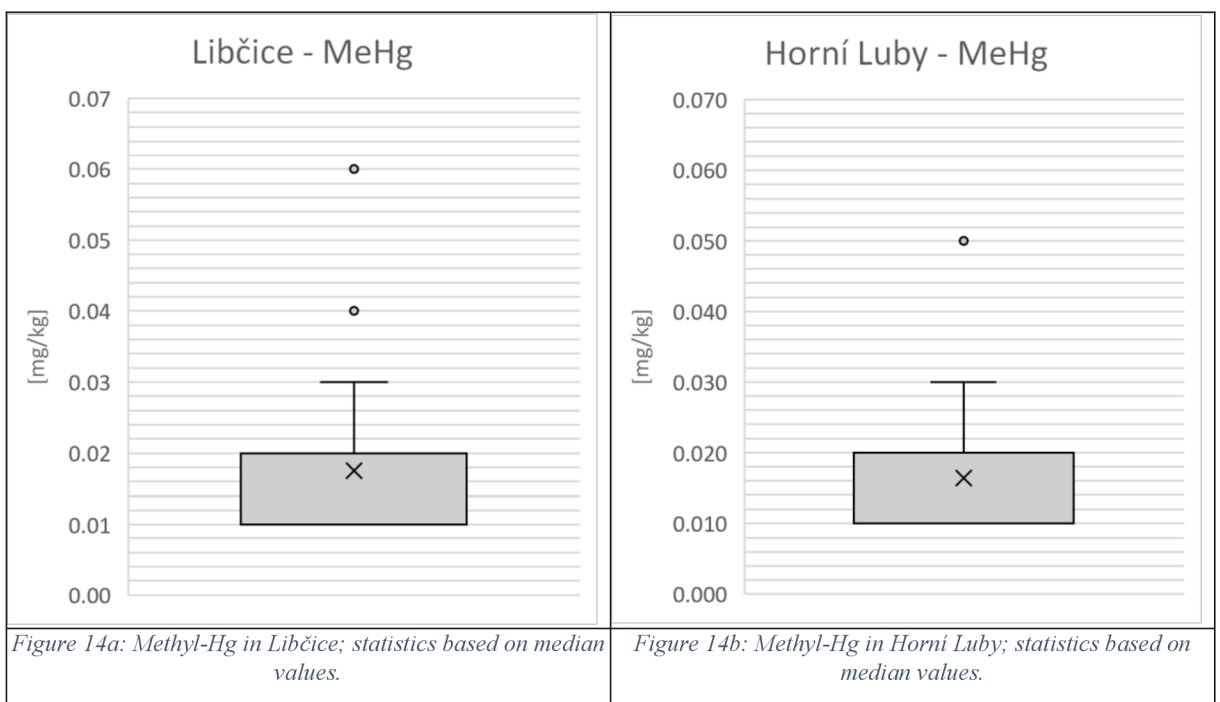
High Hg contamination levels remain an ecological and human health threat, surpassing tolerable limits of contamination. According to public notice No. 153/2016 about the conditions for the protection of the agricultural soil quality (legal code of the Czech Republic, pp. 2692–2699), the so-called preventive value of total Hg in soils is 0.3 mg/kg. Additionally, Debnárová et al. (2010) established pollution indexes based on tolerable limits (from state legislative document or criteria for soil pollution) of Hg contamination in the Czech Republic. In comparison with an example from an Hg contaminated site in the Czech Republic, highest total Hg values occurring in Horní Luby on average were found at 13.21 mg/kg.

5.1.10 Potentially mobilizable mercury



As might also be expected, PM-Hg levels showed some higher maximum and minimum values in Horní Luby, values are comparable on average between the sites (shown in Figures 13a and 13b above). It is believed that the proportion of Hg in Horní Luby is bound to OM and clay minerals, which could explain the reason for PM-Hg levels to show higher max and min values. These proportions of Hg can only be released using strong extraction agents, they are tightly bound, but not bioavailable.

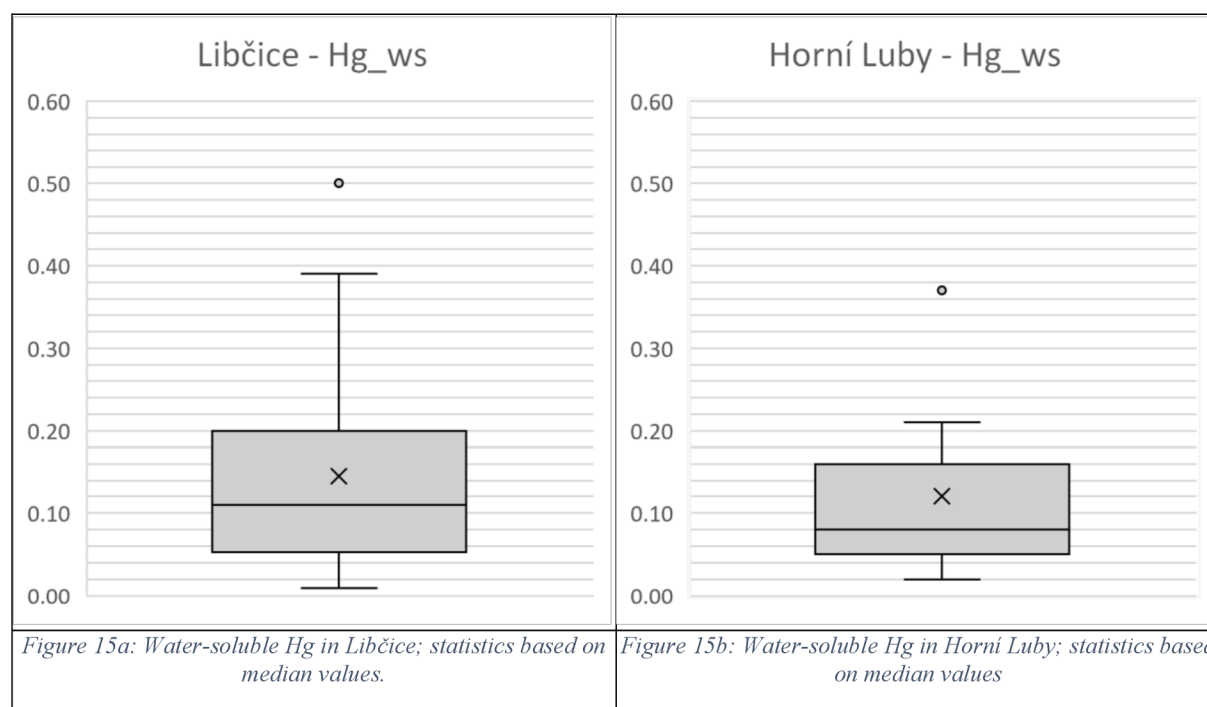
5.1.11 Methylmercury



MeHg levels appeared to be almost identical between the two sites, as shown in Figures 14a and 14b above. One important thing to note about Libčice is that because of lower total Hg, but elevated levels of pH, CEC and OM – this should result in less mobile forms of Hg. However, this was not shown in the results between the two sites, as the values of MeHg are almost identical between them. Another thing to note about Horní Luby is that it is usually assumed that there is little direct conversion of insoluble HgS (cinnabar) species to Methyl-Hg in anaerobic soils, but this is subject to alter when environmental factors promote HgS complexation (O'Connor et al., 2019). This would mean that the conditions at this site must be more favourable for this HgS complexation. The relationship between methylation potential and intermediate geochemical compounds resulting from rate-limited Hg-sulfide precipitation processes is currently unclear (O'Connor et al., 2019). Positive correlations with total sulfur could be indicative of the fact that, as previously stated, sulfate-reducing bacteria are capable of facilitating the Hg methylation process (Lefebvre et al., 2007). As established, in mildly sulfidic environments, microbial Hg methylation occurs most quickly (Benoit et al. 1999). As seen above in Graphs 7 and 8, both sites both showed mildly sulfidic environments.

In Libčice, it is known that Hg was added from anthropogenic origins directly from gold extraction, and not as tightly bound to minerals. Conversely in Horní Luby, Hg is more stable in soils and less bioavailable.

5.1.12 Water-soluble mercury



As shown in Figures 15a and 15b above, water-soluble Hg levels were relatively comparable between the two sites. This would imply that in this regard, Hg should be equally as mobilizable (O'Connor et al., 2019), however this was clearly not observed in the PM-Hg values above when comparing the two sites.

To review, generally pH values were higher in Libčice, and it was established that lower pH (and thus lower mobility of cations) and acidic conditions will increase Hg solubility and mobility, and higher pH will immobilize Hg (Gambrell et al. 1980). CEC was also higher in Libčice, and as it is known that CEC is correlated with OM and C contents from Ramos et al. (2018), it made sense that much higher overall carbon levels were also observed in Libčice. All of these factors imply that less methylation will occur in Libčice compared to Horní Luby. Additionally, it was observed that Libčice does have less total Hg, but approximately similar mobile forms of Hg (MeHg, Hg_ws). This is interesting and seems to imply that Hg could be methylating through a different pathway based on the contamination origin, considering the factors that should suppressing Hg methylation in Libčice. One consideration and possibility to explain the more mobile forms of Hg present in Libčice than expected is that although Hg has an affinity to bond to OM and, this is more likely to occur under low pH conditions, however the pH was still observed as high in Libčice.

5.2 Correlations of mercury and soil properties between sites

In the following section, correlations between mercury and the specific soil properties are discussed, from the perspective focusing on Hg and its relation to each factor.

Table 3: Correlations between Hg characteristics and soil properties for Libčice

	Potential pH	Active pH	CEC	Total S	C/N	Total C	C org	C_ws	Total Hg	PM-Hg	MeHg	Hg_ws
Potential pH	1.00											
Active pH	0.91	1.00										
CEC	0.71	0.61	1.00									
Total S	0.62	0.55	0.82	1.00								
C/N	0.38	0.22	0.64	0.66	1.00							
Total C	0.55	0.43	0.92	0.83	0.77	1.00						
C org	0.46	0.35	0.85	0.64	0.74	0.88	1.00					
C_ws	0.60	0.54	0.85	0.67	0.60	0.86	0.83	1.00				
Total Hg	0.18	0.33	0.00	0.31	-0.10	0.01	-0.16	-0.03	1.00			
PM-Hg	0.12	0.22	-0.26	-0.16	-0.49	-0.35	-0.51	-0.38	0.55	1.00		
MeHg	0.41	0.34	0.57	0.40	0.26	0.50	0.44	0.48	-0.12	-0.05	1.00	
Hg_ws	0.16	0.28	-0.15	-0.07	-0.41	-0.22	-0.40	-0.19	0.65	0.83	-0.17	1.00

*N = 32, **p < 0.05.

5.2.1 Total Hg

Total Hg levels shows positive correlations with potentially mobilizable Hg (0.55) and Hg_ws (0.65). This makes sense on a fundamental level that total Hg showed positive correlations with all most other forms of Hg, since total Hg can be considered more or less a sum of the parts of the various other Hg species. MeHg does not show a significant correlation with the total Hg, showing that this process is not dependent on the amount of Hg in the soil. Typically, the ratio of MeHg to total Hg is quite low when observing Hg methylation in soils and sediments, as described by Yang et al (2018) in a study of the two factors in a landfill environment where the correlation was already as low as 15-20%.

5.2.2 Potentially mobilizable Hg

PM-Hg showed positive correlations with Hg_{ws} (0.83). These positive correlations potentially mobilizable Hg could be attributed to the fact that Hg is categorically known to be more mobilizable in its ionic and water-soluble form, compared to most other Hg species (O'Connor et al., 2019). This would imply that large portions of this Hg species are immediately available.

PM-Hg also showed negative correlations with C/N (-0.49), C_{org} (-0.51), and C_{ws} (-0.38). As previously discussed, we know that OM can have either mobilizing or immobilizing effects on Hg, depending on other soil factors (Gilli et al., 2018). In the cases of C_{org} and C/N ratios, this might be related to the strong bonding and affinity of Hg to OM. Typically, OM should be high in organic carbon, as well in C/N ratios, and because of this, these Hg-OM bonds could have the potential to immobilize Hg under certain conditions (Hojdová et al., 2009). Because it has been established that low C/N ratios in soil can lead to faster decomposition of OM, and also that Hg has an affinity to bond to OM in soil, this leads to some implications. Hg could release into the soil solution during decomposition if it is bound to the organic matter. Therefore, there is a negative correlation between C/N and PM-Hg due to lower C/N and higher Hg release.

5.2.3 Methyl Hg

MeHg showed positive correlations with potential pH (0.41), CEC (0.57), and Total S (0.40). It is known already that both biotic and abiotic processes in the environment can produce methyl-Hg. The conditions that encourage sulfate-reducing bacteria, which are primarily responsible for methylation, include low oxygen levels, low pH, low Hg bioavailability, temperature, redox potential, and high quantities of dissolved organic molecules (Gworek et al., 2020). Given that the results for Libčice show different conditions for Hg methylation, it is possible that Hg is being methylated through a different pathway than described. Positive correlations with total sulfur could be indicative of the fact that, as previously stated, sulfate-reducing bacteria are capable of facilitating the Hg methylation process (Lefebvre et al., 2007). It is known that in mildly sulfidic environments, microbial Hg methylation occurs most quickly (Benoit et al. 1999). Additionally, according to recent research (Zhang et al., 2012), nano HgS may be accessible for methylation by microorganisms.

For carbon species, positive correlations were observed with total C (0.50), C_{org} (0.44), and C_{ws} (0.48). Correlations with total carbon could also be due to the binding of OM and its high carbon levels bonding with MeHg and the methylation of Hg from the OM-bound fraction (He et al., 2019). To repeat, once deposited in soils Hg can be methylated due to various factors in the soil organic matter, such as the presence of TOC, where studies have found a linear relationship between the presence of TOC and MeHg (Tomiyasu et al., 2017).

5.2.4 Water-soluble Hg

Hg_{ws} showed negative correlations with C/N (-0.41) and C_{org} (-0.40). This makes sense as it has been seen before that high levels of water-soluble Hg can be due to low organic

matter (and by proxy C/N and C org), therefore an inverse relationship is possible between the parameters (Tazisong et al., 2012). This result showed the same case as for PM-Hg, shown in section 5.2.2 above. This shared relationship is logical for water-soluble and potentially mobilizable fractions to share with C/N and C org.

As previously elaborated, Hg_ws also showed positive correlations with total Hg and PM-Hg. As stated, this correlation is quite expected due to the mobility of Hg in its water-soluble forms.

Table 4: Correlations between Hg characteristics and soil properties for Horní Luby

	Potential pH	Active pH	CEC	Total S	C/N	Total C	C org	C_ws	Total Hg	PM-Hg	MeHg	Hg_ws
Potential pH	1.00											
Active pH	0.96	1.00										
CEC	-0.13	-0.28	1.00									
Total S	-0.42	-0.54	0.87	1.00								
C/N	-0.53	-0.62	0.73	0.85	1.00							
Total C	-0.44	-0.55	0.89	0.96	0.89	1.00						
C org	-0.28	-0.43	0.94	0.90	0.84	0.94	1.00					
C_ws	-0.31	-0.40	0.84	0.83	0.60	0.82	0.79	1.00				
Total Hg	0.10	0.14	-0.15	-0.26	-0.05	-0.13	-0.09	-0.29	1.00			
PM-Hg	0.00	0.08	-0.39	-0.47	-0.26	-0.35	-0.33	-0.42	0.76	1.00		
MeHg	-0.10	-0.04	-0.11	-0.12	0.01	-0.09	-0.09	-0.22	0.54	0.60	1.00	
Hg_ws	0.38	0.37	0.13	0.00	0.07	0.02	0.02	-0.10	0.36	0.06	0.37	1.00

*N = 25, **p < 0.05.

5.2.5 Total Hg

Total Hg showed positive correlation with potentially mobilizable Hg (0.76), MeHg (0.54). As previously stated regarding the Libčice site, it is logical that overall, total Hg is correlated with almost all Hg species, however this does contradict earlier information discussed regarding the ratio of MeHg to total Hg in soils and sediments typically, so this correlation is not necessarily expected in this case. The next important question is to ask why MeHg was more correlated with total Hg at the Horní Luby site, whereas it was not as correlated with the Libčice site (which was more in line with expectations).

5.2.6 Potentially Mobilizable Hg

PM-Hg showed positive correlation with MeHg (0.60) (and total Hg, as previously explained). Negative correlations were observed with C_ws (-0.42). The inverse relationship of water-soluble C and potentially mobilizable Hg implies that the more mercury which is added to soil yields less C (Barkay et al., 1997). Using the inverse logic, it could be possible that as an Hg immobilizing technique, carbon is mobilized in the soil. At least as was shown by Rózański et al. (2016) in the case of organic C, readily soluble mercury compounds have been shown to be strongly associated with organic carbon.

Negative correlations were also observed with CEC (-0.39) and total S (-0.47). The inverse relationship of potentially mobilizable Hg and total S is logical, as it is expected that due to the high affinity of Hg compounds to soil, Hg will be immobilized (Biester & Zimmer, 1998). In this regard, the use of sulfur-containing reagents could be used to decrease mobility of soluble or volatile Hg compounds in soil. That is because the formation of stable Hg compounds such as HgS or insoluble organic Hg-S compounds are likely to be formed when Hg reacts with S (Biester & Zimmer, 1998).

5.2.7 Methyl Hg

As previously stated, MeHg only showed positive correlations with total Hg and PM-Hg. This was a key difference between the two sites that MeHg was correlated with total Hg in Horní Luby, but expectedly this was not the case in Libčice.

5.2.8 Water-soluble Hg

Water-soluble Hg did not show any significant correlation with any other soil parameters. This is a peculiar result in itself, as generally Horní Luby showed comparable, if not more overall content of Hg in soils despite conditions that should have shown more Hg overall in Libčice, yet water-soluble shows no significant correlation in this case. It is possible also that because of the lower number of samples in Horní Luby, that there was not enough data to further develop these correlations.

6 Conclusion

- Mercury speciation in soils is highly dependent on various soil parameters such as pH, sulfur content, and most importantly for the focus of this study – organic matter. As discussed, certain elements bind to mercury in soils, such as the insoluble and geogenically sourced cinnabar in the case of Horní Luby – in these cases, Hg is not readily methylated due to low bioavailability for methylating bacteria (O'Connor et al., 2019). Organic matter tends to bind to soils especially in low pH and high organic matter conditions, becoming less available for methylation (Gambrell et al. 1980).
- Given that the results for Libčice show different conditions for Hg methylation, it is possible that Hg is being methylated through a different pathway than described. Due to these results, more importance should be devoted in describing methylation in these sites. There seemed to be some limiting factor for Hg methylation in Libčice. Despite the conditions favouring more methylation in Libčice, such as lower total Hg, but elevated levels of pH, CEC and OM – this should result in less mobile forms of Hg. However, this was not shown in the results between the two sites, as the values of MeHg were very similar between them.
- Regarding the potentially mobilizable forms of mercury (and more available), this was found to depend on the rate of decomposition of organic matter, specifically in Libčice. The results showed that a lot of Hg is bound to the OM in Libčice, supported by the fact that organic C was negatively correlated. This would mean that organic C immobilizes the Hg in Libčice. In contrast with Horní Luby, PM-Hg was positively correlated with MeHg, meaning that more methylation was occurring from the PM-Hg there.
- The inverse relationships of carbon content in the soils and the bioavailability potential methylation of Hg suggest that Hg contamination could be remediated with carbon additives of some form in the soil, however that this might not be as effective for Hg origins from historical gold mining locations, such as Libčice. More research would be needed to support this hypothesis.
- High Hg contamination levels remain an ecological and human health threat, surpassing tolerable limits of contamination. According to public notice No. 153/2016 about the conditions for the protection of the agricultural soil quality (legal code of the Czech Republic, pp. 2692–2699), the so-called preventive value of total Hg in soils is 0.3 mg/kg. Debnárová et al. (2010) established pollution indexes based on tolerable limits (from state legislative document or criteria for soil pollution) of Hg contamination in the Czech Republic. Meanwhile, highest total Hg values occurring in Horní Luby on average were found at 13.21mg/kg. This serves as a reminder that Hg contamination is still plentiful in the environment and deserves much more research on how to best remediate this pollution.

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8 Appendices

Median	L7B	L7A	L7O	L5B	L5A	L5O	L4B	L4A	L4O	L3A	L3O	L2B	L2A	L2O	L1A	L1O	Sample names
5.7	5.67 ±0.38	5.74 ±0.09	5.95 ±0.04	5.23 ±0.21	5.64 ±0.18	5.89 ±0.04	5.38 ±0.29	5.84 ±0.03	5.94 ±0.08	5.53 ±0.01	5.94 ±0.03	5.19 ±0.23	5.34 ±0.06	5.46 ±0.06	5.97 ±0.03	5.88 ±0.08	Potential pH
6.9	7.10 ±0.28	6.93 ±0.05	6.96 ±0.08	6.27 ±0.10	6.76 ±0.18	6.95 ±0.04	6.70 ±0	6.87 ±0.03	6.99 ±0.04	6.61 ±0.02	7.04 ±0.04	6.30 ±0.36	6.12 ±0.06	6.47 ±0.05	7.41 ±0.11	7.06 ±0.05	Active pH
189.8	109.66 ±0.07	192.76 ±21.20	860.08 ±90.33	73.47 ±0.30	186.84 ±32.60	336.45 ±66.96	93.91 ±15.66	202.84 ±14.54	355.11 ±74.55	216.55 ±0.04	444.34 ±71.72	65.85 ±9.34	109.52 ±28.93	161.49 ±4.46	152.24 ±21.61	381.19 ±139.01	CEC
1.38	0.91 ±0.40	2.48 ±0.30	5.96 ±0.31	0.15 ±0.11	0.65 ±0.12	2.96 ±0.15	1.15 ±0.13	1.62 ±0.42	3.02 ±1.09	8.58 ±1.36	5.78 ±0.06	0.17 ±0	0.01 ±0.01	1.01 ±0.03	0.83 ±0.19	4.70 ±2.09	Total S [g/kg]
14.98	13.25 ±0.65	14.1 ±0.42	16.54 ±0	12.90 ±0.12	14.47 ±0.11	15.80 ±0.07	14.66 ±0.60	14.17 ±0.17	16.25 ±0.31	22.60 ±1.35	19.69 ±0.87	13.97 ±0.07	0.28 ±0.28	16.96 ±0.10	14.96 ±0.32	15.53 ±0.56	C/N
67.43	12.76 ±0.66	69.04 ±11.74	276.23 ±12.52	23.34 ±0.71	65.81 ±6.76	145.66 ±3.48	19.48 ±3.70	60.29 ±6.71	161.43 ±46.88	144.53 ±2.86	205.92 ±11.86	10.15 ±2.39	2.88 ±2.88	91.11 ±6.28	33.52 ±2.35	196.95 ±89.59	Total C [g/Kg]
61.98	16.49 ±7.21	45.14 ±3.29	185.96 ±9.81	18.69 ±0.28	68.55 ±19.41	97.64 ±0.41	8.87 ±2.31	44.24 ±6.17	71.39 ±9.48	76.85 ±3.64	104.88 ±8.56	20.59 ±4.84	3.20 ±3.20	87.99 ±4.10	37.4 ±4.92	182.02 ±27.67	C org [g/kg]
2632.91	1440.76 ±255.41	3301.4 ±752.26	10605.98 ±4295.45	1421.84 ±199.22	2341.95 ±106.32	3880.00 ±460.62	1230.85 ±273.64	2582.40 ±468.64	10599.14 ±4050.50	2283.94 ±153.21	4040.65 ±906.7	977.19 ±265.86	135.84 ±135.84	4327.90 ±266.73	2683.42 ±307.82	9305.82 ±1708.66	C_ws
3.79	6.70 ±2.78	4.21 ±0.98	1.72 ±0.19	5.03 ±0.27	3.73 ±0.13	3.07 ±0.06	4.96 ±0.41	3.70 ±0.23	3.23 ±0.63	5.25 ±0.15	3.85 ±0.14	0.40 ±0.19	0.02 ±0.02	0.51 ±0.11	8.19 ±5.18	27.99 ±7.01	Total Hg
0.68	4.43 ±2.27	0.86 ±0.28	0.13 ±0.06	2.64 ±0.41	0.78 ±0.01	0.70 ±0.21	2.09 ±0.01	1.44 ±0.67	0.21 ±0.01	0.31 ±0.05	0.66 ±0.37	0.07 ±0.01	0.02 ±0.02	0.10 ±0	2.07 ±0.10	0.29 ±0.17	potentially mobilizable [mg/kg]
0.006	0.01 ±0	0.03 ±0.02	0.01 ±0.01	0.0 ±0	0.02 ±0.01	0.06 ±0	0.01 ±0	0.01 ±0	0.04 ±0.02	0.01 ±0	0.02 ±0	0.01 ±0	0±0	0.01±0	0.01±0	0.01±0	MeHg [mg/kg]
0.11	0.39 ±0.10	0.09 ±0.01	0.06 ±0.01	0.50 ±0.12	0.11 ±0.03	0.11 ±0	0.21 ±0.02	0.15 ±0.07	0.11 ±0.02	0.09 ±0.03	0.05 ±0.01	0.01 ±0	0±0	0.02 ±0	0.24 ±0.08	0.17 ±0.08	Hg_ws

Table 3: Median (+/-) MAD for Hg characteristics and soil properties in Libice

Table 4: Median +/- MAD for Hg characteristics and soil properties in Horní Luby

Sample names	H1A	H2F	H2H	H2A	H3A	H3B	H4A	H5A	H6F	H6A	H6C	Median
Potential pH	5.36 ±0	3.83 ±0.08	3.37 ±0.14	3.75 ±0.01	6.11 ±0.02	6.10 ±0.02	4.56 ±0.14	4.86 ±0.11	5.44 ±0.19	3.98 ±0.13	4.27 ±0.06	4.56
Active pH	5.8 ±0.08	4.07 ±0.02	3.80 ±0.11	4.10 ±0.07	5.82 ±0.05	5.95 ±0.08	4.74 ±0	4.97 ±0.07	5.10 ±0.08	4.32 ±0.09	4.50 ±0	4.74
CEC	53.7 ±6.3	333.44 ±6.20	118.21 ±26.39	54.63 ±0.57	117.29 ±21.49	49.6 ±2.55	56.62 ±11.66	64.82 ±0.58	280.06 ±28.71	74.02 ±2.29	25.75 ±2.19	64.82
Total S [g/kg]	0.25 ±0.14	7.42 ±0.16	3.27 ±0.08	1.67 ±0.52	2.47 ±63.34	0 ±11.07	0.17 ±0.03	0.37 ±0.09	4.41 ±0.58	3.35 ±0.25	0.83 ±0.46	1.67
C/N	11.08 ±0.67	20.80 ±0.67	18.27 ±0.41	13.88 ±0.41	12.76 ±0.27	7.00 ±0.72	11.30 ±0.49	10.79 ±0.48	15.27 ±0.36	11.45 ±1.88	12.39 ±0.80	12.39
Total C [g/Kg]	26 ±1.75	390.90 ±7.80	147.37 ±14.32	48.70 ±8.98	52.83 ±5.52	5.69 ±1.28	30.26 ±0.94	34.95 ±0.65	210.29 ±27.74	59.76 ±8.89	28.11 ±5.19	48.70
C org [g/kg]	22.13 ±0.18	231.27 ±25.77	115.96 ±3.33	34.41 ±5.07	91.07 ±9.52	9.80 ±2.21	32.14 ±0.46	31.73 ±0.75	150.36 ±25.34	47.50 ±0.92	17.83 ±4.40	34.41
C_ws	1252.72 ±104.81	7058.12 ±2047.53	2589.34 ±159.20	1289.53 ±14.43	1768.68 ±214.31	1275.46 ±438.71	1533.43 ±139.64	1990.82 ±8.23	9892.07 ±1362.80	3679.08 ±809.01	1072.46 ±186.200	1768.68
Total Hg	29.19 ±4.28	4.02 ±1.71	13.21 ±1.19	28.49 ±8.20	26.65 ±1.31	3.63 ±1.75	59.11 ±7.03	23.04 ±12.46	11.71 ±9.67	4.21 ±0.69	2.00 ±0.24	13.21
potentially mobilizable [mg/kg]	4.56 ±0.2	0.50 ±0.41	0.80 ±0.03	5.09 ±0.26	1.67 ±0.62	0.77 ±1.10	8.36 ±1.69	5.45 ±0.64	0.25 ±0.09	0.55 ±0.04	0.2 ±0.02	0.80
MeHg [mg/kg]	0.03 ±0.01	0.01 ±0	0.01 ±0	0.01 ±0	0.02 ±0.01	0.01 ±0	0.01 ±0	0.05 ±0	0.01 ±0	0.01 ±0	0.01 ±0	0.01
Hg_ws	0.16 ±0.04	0.1 ±0.07	0.07 ±0	0.08 ±0	0.08 ±0.03	0.21 ±0.14	0.37 ±0.13	0.15 ±0.05	0.05 ±0	0.03 ±0	0.02 ±0	0.08