

# An ecotoxicological approach for hazard identification of energy ash

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## Abstract

The Dept. of Applied Environmental Science (ITM), Stockholm University, and the Swedish Geotechnical Institute (SGI) have worked in projects with the main aim to improve the understanding of governing mechanisms for ecotoxicity of ash materials. With the ambition to provide recommendations on classification of ash according to H-14, we have characterised bottom and fly ash materials based on both total levels in solid materials and eluates. In addition, the eluates have been characterized with a battery of ecotoxicity tests. The most important findings from the projects are: (1) classification based on total content of substances over-estimates the ecotoxicological hazard potential as compared with ecotoxicity testing of eluates; (2) when leaching is performed at L/S <10 l/kg, components not classified as hazardous, in particular potassium, significantly influence the toxicity of the eluates, which is problematic from a hazard classification perspective, but likely negligible from a long-term environmental risk perspective.

*Keywords: Ash, H-14, classification, risk assessment, ecotoxicity tests*



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## BACKGROUND

According to the Waste Framework Directive (WFD; 2008/98/EC) waste and hazardous waste should be classified in accordance with the *List of waste* [1], in which so-called *mirror entries* concern waste types with the potential to be either hazardous or non-hazardous depending on their composition and concentrations of hazardous substances. Bottom ash from municipal and industrial waste incineration has such mirror entry, and for which hazard classification is thus mandatory.

The WFD further states that “*classification of waste as hazardous waste should be based, inter alia, on the Community legislation on chemicals, in particular concerning the classification of preparations as hazardous...*”. In this context, Regulation No 1272/2008 (CLP; European Regulation on Classification, Labeling and Packaging of chemical substances and mixtures) implements globally harmonized criteria for the classification of substances and mixtures according to their physical, health and environmental hazards (GHS; Globally Harmonized System of Classification and Labelling of Chemicals, Rev 4 United Nations 2011). Although no formal decision has been taken whether chemical and waste legislations should be harmonized in terms of hazardous properties and test procedures, the enforcement of the CLP for mixtures by 2015, in our view, makes it highly relevant that this will be the case.

Fifteen properties (i.e. H-criteria) of waste that may render it hazardous are listed in the WFD and, of these, criterion H-14 concerns the inherent ecotoxicological properties of waste. Currently, there are no harmonized quantitative criteria for H-14 classification, but several European countries have identified the need of biological test systems. In addition, an European ring test [2] based on the recommendations in CEN guideline 14735 [3] (*Characterization of waste – Preparation of waste samples for ecotoxicity tests*) was organised by the German Federal Environment Agency in order to define suitable test methods for the biological assessment of waste according to H-14. Based on a thorough evaluation of the waste and chemical legislations related to hazardous properties, our judgment is, however, that these recommendations only to a limited extent harmonize with the classification of substances and mixtures according to the CLP regulation. Considering harmonization of waste and chemical legislations, the classification of waste according to H-14 will involve drastic changes as opposed to the recommendations made from the European ring test, both related to choice of leaching conditions (pH, L/S ratio) and ecotoxicity tests (test species, endpoints).

Over the last 4-year period the Dept. of Applied Environmental Science (ITM), Stockholm University, and the Swedish Geotechnical Institute (SGI), financed by Värmeforsk (Thermal Engineering Research Association), Avfall Sverige (Swedish Waste Management) and the Swedish EPA, have worked in projects with the central aim to improve the understanding of governing mechanisms for ecotoxicity of ash eluates. The ambition has also been to provide recommendations on how these findings can be utilised within a formal classification framework of waste with respect to H-14. We have characterised 10 Swedish bottom and fly ash materials based on total levels in solid materials and levels in eluates generated using different leaching methods. Eluates have also been characterised using a range of ecotoxicity tests. The outcome of these projects is summarized below.

## PROJECTS

### **1. An ecotoxicological approach for hazard identification of energy ash [4].**

With the main aim to increase the general understanding on how to classify energy ash materials with regards to H-14 in the Directive on waste 2008/98/EC, the objectives of this study were to evaluate if a) clear concentration-response relationships could be achieved for the selected toxicity tests (bacteria, algae, crustacean and fish), b) some test(s) were more sensitive than others and c) the toxic responses were consistent with the chemical analyses. The test battery used was selected to represent a wide range of biological variation and routes of exposure to trace an array of toxicity mechanisms.

As model energy ash materials, bottom and fly ash from industrial, biofuel and municipal waste incineration were studied (see Table 1).

**Table 1.** The table shows abbreviations, type and origin of the tested ash materials.

Ash	Type	Origin	Age
A	Bottom ash	Municipal waste	ca 3 months
B	Stabilized fly ash	Municipal waste	ca 1 year
C	Fresh fly ash	Municipal waste	< 1 week
D	Fly ash	Industrial	> 3 years
E	Mixed ash (bottom and fly ash)	Industrial	> 4 years
F	Fly ash	Bio fuel	< 1 month
G	Aged bottom ash	Municipal waste	> 15 years

Extracted water eluates were prepared either with the batch method described in EN 14735:2005 [3] (L/S 10) or with a modified version of a recirculation column method, the ER-H method, developed by Gamst et al. (2007) [5], especially for leaching of nonvolatile organic compounds and validated for PAHs and chlorophenols. The ecotoxicological tests used on the eluates were; the Microtox® test [6]; a growth inhibition test with the micro algae *Pseudokirchneriella subcapitata* [7]; an acute test and a larval development test (LDR Larval Development Ratio, a (sub)chronic test) with the copepod *Nitocra spinipes* [8-9]; and an embryo toxicity test ((sub)chronic test) with sebra fish, *Danio reiro* [10-11]. Chemical analyses were made on both the solid matrices and on the eluates.

Overall, if considering both hazardous and non-hazardous substances in the eluates, the observed toxic responses were relatively consistent with the chemical analyses. However, classification based on total content of substances in solid matrices drastically over-estimates the toxic responses. Our results further show that, in general, the selected test organisms responded with distinct concentration-responses and that the (sub)chronic tests were much more sensitive than the acute tests (see Table 2).

**Table 2.** Effect concentrations (% eluate) for ash eluates A-G obtained from ecotoxicological testing on a bacterium, an alga, a copepod (both acute and (sub)chronic) and a fish embryo. Ninety-five percentage confidence intervals of the effect concentrations are presented in brackets for the algal and copepod acute test. For the (sub)chronic copepod test and the fish embryo test, effect concentrations are presented as NOEC-values (No Effect Concentration). Ash B was leached with both the batch and column method.

Ash eluate	Bacterium	Alga	Copepod (acute)	Copepod (LDR)	Fish embryo
	Effect value <i>EC</i> <sub>50</sub>	Effect value <i>EC</i> <sub>50</sub> (95%CI)	Effect value <i>LC</i> <sub>50</sub> (95%CI)	Effect value NOEC	Effect value NOEC
A (column)	>90.0	>80	121 (93-200)	8	3.2
B (column)	>90.0	>20	38 (24-43)	8	13
B (batch)	>90.0	>20	40 (---) <sup>a</sup>	<1.3	13
C (batch)	12.5-25.0	3.0 (2.6-3.4)	22 (19-25)	1.3	0.78
D (column)	>90.0	11(9.3-12)	16 (13-19)	0.5	>10
E (column)	>90.0	>80	78 (71-86)	20	>50
F (column)	>90.0	59 (49-72)	21 (19-24)	3.2	25
G (column)	>90.0	95 (87-108)	>100	>50	nm

<sup>a</sup> – It was not possible to calculate the 95% confidence interval

Consequently, our recommendation for classification of ash materials with regards to ecotoxic properties (H-14), is that focus should be on effects recorded in (sub)chronic/chronic tests. Since no single test or test organism proved most sensitive to all ash eluates, although the (sub)chronic test with *N. spinipes* was the most sensitive in five of the eluates, characterising ash eluates should be based on a battery of test organisms representing a wide range of biological variation and different routes of exposure. These findings are in agreement with guidelines in CLP. Finally, since preparation of eluates and eluate treatment prior to toxicity testing has a major influence on the concentration and speciation of released substances, further studies are needed in order to propose a relevant testing scheme that is harmonized with existing legislations on chemicals, in particular concerning the CLP regulation.

## 2. Improved understanding of key elements governing the toxicity of ash eluates [12]

Even though one of the main conclusions from the first study was that ecotoxicological test methods can improve the characterization of ash materials, challenges remain to be addressed before the mechanisms of ecotoxic effects can be fully understood. Parts of this challenge lie in further

investigating which elements caused the observed ecotoxic effects in the tested ash eluates to avoid the risk of misclassifications, i.e. due to toxic effects caused by substances that are not classified as hazardous according to the chemicals legislation.

In the first study, an attempt was made to identify which elements were potentially responsible for the ecotoxicity of the eight ash eluates. This was done by generating so-called Hazard Quotients (HQs) (see Table 4) for those elements that were present at high concentrations. The HQs were calculated based on the ratio of a measured concentration of a specific element in 100 % ash eluate and on the acute literature LC<sub>50</sub> value in the crustacean *Nitocra spinipes* [13] for the same element. This analysis pinpointed three elements normally not classified as ecotoxic according to the CLP regulation, (i.e. K, Ca and Al) that either alone or in combination could explain the observed ecotoxicity. Components normally considered as ecotoxic (i.e. Zn and Pb) may have contributed to the observed ecotoxic effects only in the eluates ranked the most hazardous. Although we regard these findings valuable for classification of ash according to H-14, our first study also revealed that the acute toxicity test with *N. spinipes* was overall much less sensitive than a (sub)chronic test with the same species when testing the actual ash eluates. The HQs based on acute toxicity data (further referred to as HQ<sub>acute1</sub>) should accordingly be considered only as preliminary estimations.

In order to confirm which elements were responsible for the observed (sub)chronic effects in *N. spinipes* exposed to the ash eluates in the first study, the primary aim of this follow-up study was to generate (sub)chronic data for the above-mentioned pinpointed elements. This was done using the LDR test with *N. spinipes*. The results made it possible to calculate (sub)chronic HQs (further referred to as HQ<sub>(sub)chronic</sub>). However, only four of the five pinpointed elements (i.e. K, Ca, Al and Pb) needed to be tested since Ytreberg et al. (2010) [14] recently published data for Zn using the same test method. Since for some of the eluates investigated in the first study the HQs for Cu were relatively high, we also included this element in the refinement activity (also results from Ytreberg et al. 2010). In the first study HQ<sub>acute1</sub> were calculated by dividing the measured analytical levels of the metals found in the undiluted ash eluates with the corresponding 96-hr LC<sub>50</sub> value for *N. spinipes* (literature data from Bengtsson 1978 [13]). The refined HQ<sub>(sub)chronic</sub> provided in this study are based on the dilution where we observed (sub)chronic effects on *N. spinipes* exposed to the eluates, taking background concentrations in dilution media in account. In order to be able to make a relevant comparison between acute and (sub)chronic data, a secondary aim of this study was to refine also HQ<sub>acute1</sub> according to the same principle, i.e. base the calculations on the dilution where acute effects was observed on *N. spinipes* exposed to the ash eluates (further referred to as HQ<sub>acute2</sub>).

The ecotoxicological data with NOEC/LOEC-values from the (sub)chronic test (either as significant differences in mortality or LDR) are presented in Table 3. In all these tests, the responses on mortality and LDR generally followed a concentration-related pattern and the measured concentrations were overall in line with the nominal concentrations added to the natural brackish sea water (i.e. within 63% to 97 %). The results show that Cu was the most toxic element to *N. spinipes* and the toxicity decreased in the following order Cu > Al > Zn > Pb > K > Ca.

**Table 3.** Compilation of nominal (NC) and measured (MC) concentrations for the obtained NOEC/LOEC-values together with percentage mortality and Larval Development Ratio (LDR) with corresponding approximate 95% confidence intervals of means ( $\pm 95\%$  CI) in *N. spinipes* exposed to Zn, Cu, K, Ca, Al and Pb. a - denotes significant differences from the control.

	Metal (mg/L)	NC (mg/L)	MC (mg/L)	Mortality	LDR	% measured to nominal
				mean ( $\% \pm 95\%CI$ )	mean ( $\% \pm 95\%CI$ )	concentrations ****
Zn*	Control	0	0.006	4.9 $\pm$ 4.7	48 $\pm$ 11	-
	NOEC	0.24	0.19	10 $\pm$ 6.6	31 $\pm$ 11	77
	LOEC	0.48	0.40	16 $\pm$ 8.1 <sup>a</sup>	9.0 $\pm$ 6.8 <sup>a</sup>	82
Cu*	Control	0	0.003	11 $\pm$ 6.9	61 $\pm$ 11	-
	NOEC	0.06	0.049	11 $\pm$ 6.9	51 $\pm$ 12	78
	LOEC	0.12	0.11	14 $\pm$ 7.5	7.2 $\pm$ 6.1 <sup>a</sup>	89
K	Control	0	82	2.8 $\pm$ 3.7	76 $\pm$ 9.9	-
	NOEC	35	117 <sup>**</sup>	8.1 $\pm$ 6.2	63 $\pm$ 12	97
	LOEC	105	190	10 $\pm$ 6.7	53 $\pm$ 12 <sup>a</sup>	96
Ca	Control	0	106	5.0 $\pm$ 4.8	68 $\pm$ 11	-
	NOEC	160	266 <sup>**</sup>	8.8 $\pm$ 6.2	55 $\pm$ 12	nm
	LOEC	320	nm	11 $\pm$ 6.9	37 $\pm$ 11 <sup>a</sup>	nm
Al	Control	0	0.0057	4.5 $\pm$ 5.0	61 $\pm$ 12	-
	NOEC	0.09	0.058	8.5 $\pm$ 7.1	76 $\pm$ 11	64
	LOEC	0.28	nm	20 $\pm$ 9.6 <sup>a</sup>	57 $\pm$ 13	nm
Pb	Control	0	0	8.8 $\pm$ 6.2	61 $\pm$ 11	-
	NOEC	0.27	0.17 <sup>***</sup>	19 $\pm$ 8.6	66 $\pm$ 12	nm
	LOEC	0.81	0.51	34 $\pm$ 10 <sup>a</sup>	67 $\pm$ 13	63

nm=Not measured.

NC=Nominal Concentration.

MC=Measured Concentration.

\* From Ytreberg et al., 2010 [14].

\*\* Background levels in the natural brackish sea water at 6‰ + NOEC NC.

\*\*\* Calculated value from the percent loss in LOEC MC<sub>0.51mg/L</sub> compared to LOEC NC<sub>0.81mg/L</sub>.

\*\*\*\* Differences in levels (in %) between NC and MC, background levels from natural brackish sea water added.

In Table 4, HQs with a black background indicate that a specific element contributed to the ecotoxicity of an eluate. Taken together, the calculated HQ<sub>acute1</sub> from the first study pinpointed five elements (K, Ca, Al and possibly Zn and Pb) responsible for the ecotoxicity in the ash eluates. However, when recalculating the HQ<sub>acute1</sub>, to HQ<sub>acute2</sub>, some of the elements in the fly ash eluates (i.e. Ash C and D) were over-estimated. Calculating the HQs using the newly obtained (sub)chronic data on individual elements resulted in HQ<sub>(sub)chronic</sub> > 1 for Al, Ca and K. Interestingly, in Ash B<sub>c</sub>, E and F, the toxicity of Al was under-estimated as compared to the two acute HQs. This indicates that Al, Ca and K can explain the observed (sub)chronic ecotoxicity for six of the eight ash eluates investigated.

**Table 4.** Hazard Quotients (HQs) based on acute or (sub)chronic data in *N. spinipes* exposed to ash eluates A-G (see tables 2 and 3). HQs with a black background indicate that a specific element have contributed to the ecotoxicity of an eluate.

	<b>Ash A</b> (LC <sub>50</sub> = □100%, NOEC = 8% eluate) HQ			<b>Ash B<sub>c</sub></b> (LC <sub>50</sub> = 38%, NOEC = 8% eluate) HQ			<b>Ash B<sub>b</sub></b> (LC <sub>50</sub> = 40%, NOEC = <1.3% eluate) HQ		
	<i>acute1</i>	<i>acute2</i>	<i>(sub)chronic</i>	<i>acute1</i>	<i>acute2</i>	<i>(sub)chronic</i>	<i>acute1</i>	<i>acute2</i>	<i>(sub)chronic</i>
	Zn	0.004	0.004	0.003	0.002	0.003	0.03	0.003	0.004
Cu	0.2	0.2	0.5	0.002	0.002	0.06	0.01	0.003	0.06
K	0.1	0.1	0.7	3.1	1.3	1.6	3.2	1.4	0.8
Ca	0.8	0.8	0.5	2.8	1.2	0.9	2.8	1.2	0.5
Al	1.0	1.0	14	0.1	0.03	1.2	0.1	0.03	0.2
Pb	0.002	0.002	0.003	0.001	0.0003	0.002	0.001	0.00009	0.001

	<b>Ash C</b> (LC <sub>50</sub> = 22%, NOEC = 1.3% eluate) HQ			<b>Ash D</b> (LC <sub>50</sub> = 16%, NOEC = 0.5% eluate) HQ			<b>Ash E</b> (LC <sub>50</sub> = 78%, NOEC = 20% eluate) HQ		
	<i>acute1</i>	<i>acute2</i>	<i>(sub)chronic</i>	<i>acute1</i>	<i>acute2</i>	<i>(sub)chronic</i>	<i>acute1</i>	<i>acute2</i>	<i>(sub)chronic</i>
	Zn	2.5	0.56	0.3	0.9	0.2	0.07	0.009	0.008
Cu	0.3	0.07	0.2	□0.001	□0.002	0.06	0.01	0.005	0.09
K	8.1	1.9	1.1	2.9	0.6	0.7	1.2	1.0	1.5
Ca	7.5	1.8	0.6	9.1	1.6	0.5	0.8	0.6	0.7
Al	0.001	0.0007	0.1	0.001	0.0006	0.1	0.1	0.06	2.8
Pb	3.7	0.8	0.6	7.5	1.2	0.4	0.08	0.06	0.2

	<b>Ash F</b> (LC <sub>50</sub> = 21%, NOEC = 8% eluate) HQ			<b>Ash G</b> (LC <sub>50</sub> = □100%, NOEC = >50% eluate) HQ		
	<i>acute1</i>	<i>acute2</i>	<i>(sub)chronic</i>	<i>acute1</i>	<i>acute2</i>	<i>(sub)chronic</i>
	Zn	□0.001	□0.003	0.03	0.03	0.03
Cu	□0.001	□0.001	0.06	0.01	0.01	0.2
K	6.0	1.4	2.5	0.03	0.03	0.4
Ca	0.2	0.2	0.4	1.1	1.1	1.3
Al	0.1	0.02	1.2	0.003	0.003	0.3
Pb	□0.0001	□0.003	0.001	0.0001	0.0001	0.001

To summarize, these findings clearly confirm our previous indications that the hazardous potential of eluates generated from ash materials from a range of Swedish incineration plants to a large extent is associated with elements not classified as hazardous. For six of the eight eluates (Ash A, B<sub>c</sub>, C, E, F and G, Table 1) the observed ecotoxicity can be explained by elements traditionally not classified as hazardous (i.e. Al, K and Ca). These elements will likely have significant implications for classification of ash according to H-14 and future strategies using ecotoxicological test methods for this purpose need to consider such artifacts, otherwise there is a risk for misclassification. At the same time, Cu, Pb and Zn were not identified as responsible for any of the observed effects of the eluates investigated in this study based on (sub)chronic data.

### 3. Influence of leaching conditions (pH, L/S ratio and particle size) for ecotoxicological classification (H14) of ash [16].

Given that chemical and waste legislation will be harmonized from 2015, leaching and ecotoxicity test procedures used for classification of waste according to H-14 must harmonize better with the classification schemes in the CLP regulation. In general, when testing and evaluating the aquatic ecotoxicity of ash and other waste types, the design of the leaching procedure is just as important as the choice and design of the ecotoxicity tests. The toxic response in ecotoxicity tests on waste eluates is dependent on the extent of leached toxic compounds that in turn is dependent on the characteristics of the material as well as the design of the leaching procedure. The leaching process is controlled by both the physical/chemical nature of the material being leached (e.g. pH, reducing properties, organic matter content) and the experimental conditions of the leaching test (e.g. pH of the leachant, duration time of the test, particle size of the leached material and the liquid to solid ratio (L/S) used) [17]. Standard leaching methods must be compatible with the subsequent bioassays; e.g. the eluate must have a pH that is tolerable to the organisms used and relevant from an environmental perspective.

The prevailing conditions will influence the ecotoxicological response of the tested eluates [18] and hence test methods should be chosen with great concern.

Harmonization of waste and chemicals legislations for classifications could involve rather drastic changes related to choice of both leaching and ecotoxicity test methods to be used for H-14 classification. For instance, the CLP regulation refers to the OECD Guidance on transformation/dissolution of metals and metal compounds in aqueous media [19]. Since ash consist mainly of a complex mixture of metal compounds and other inorganic substances [20], the recommendations of this OECD guidance document, in our view, should be highly relevant for ash materials. As opposed to the CEN guideline 14735 [3], which proposes an L/S ratio of 10 for aquatic ecotoxicity testing, the OECD document proposes three different L/S ratios should be used, i.e. L/S 10 000, 100 000 and 1 000 000. It further proposes that particle size should be the same as for the product on the market or, if unknown, < 1 mm, whereas CEN 14735 proposes a particle size of 4 mm. The OECD document also proposes that leaching should be done at a pH where a maximum leaching potential can be reached, yet within a range which is commonly found in the environment (i.e. 5.5-8.5), whereas CEN 14735 proposes that leaching should be done at the natural pH of the material.

The primary aim of the present study was therefore to evaluate the influence of these leaching parameters, i.e. pH (natural: ~10, and 7), L/S ratio (10 and 1000) and particle size (< 4 mm, < 1 mm, and < 0.125 mm), for subsequent chemical analysis and ecotoxicity testing in relation to classification of municipal waste incineration bottom ash (MSWIBA). A relevant ash material (age 3-6 months) from a Swedish incineration plant (Uppsala) was selected and extracted water eluates were prepared either with the batch method described in CEN 14735 or a modified pH static method (based on SS-CENTS 14997). The impact of these parameters were mainly evaluated based on calculations of the Toxic Index (TI) for Ca, K, Zn, Cu, Al and Pb, which previously have been identified as responsible for the observed toxicity of a range of ash materials from Swedish incineration plants (studies 1 and 2). For validation of the calculated TI values, two of the generated eluates were also tested with acute and (sub)chronic ecotoxicological test methods with the crustacean *N. spinipes*.

The findings from the present study clearly show that all three investigated parameters significantly influence the toxic potential of ash eluates and hence classification of ash. Calculated TIs were overall significantly higher at low particle size (<0.125 mm) as compared to particle fractions < 4 mm and < 1 mm, at natural pH of the ash material as compared to pH 7 and at L/S 10 as compared to L/S 1000.

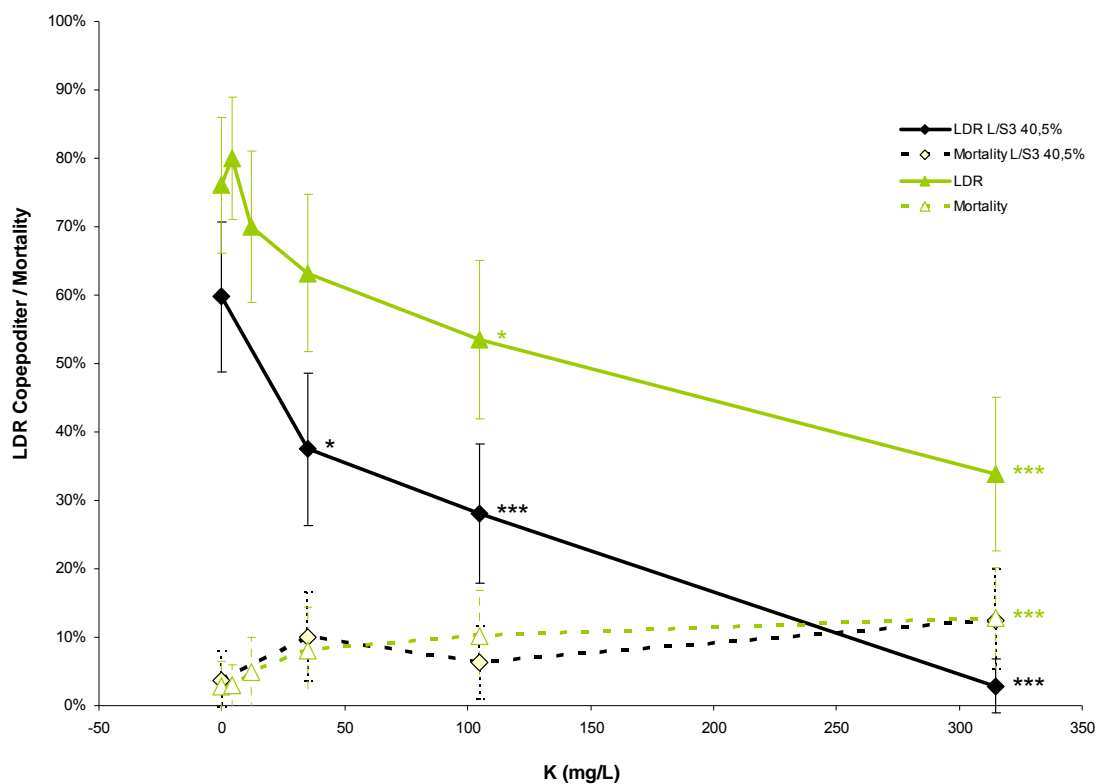
#### **4. Use of MSWIBA in constructions – Long-term ecotoxicological risks to the environment [21].**

In contrast to the other three studies presented above, this study focuses on the environmental long-term risks of ash utilized for filling or for construction purposes. Previous studies by the project group and also by others have shown that aging of MSWIBA may drastically reduce the toxic potential. We therefore selected a 5-year old MSWIBA from an incineration plant in Linköping, Sweden as a relevant study material. The ash material contains normal amounts of heavy metals and low amount of organic compounds, and is sufficiently aged for producing eluates with pH-values within an acceptable range for ecotoxicological testing (i.e. around 8). In the present study, we therefore chose to leach the selected MSWIBA at the lowest possible L/S value while still generating a leachant volume sufficient for toxicological testing using a sequential batch leaching approach from L/S 1 to L/S 3. With the main aim to identify toxic elements, which may cause long-term environmental risks in the aquatic environment, generated eluates were i) tested at 0.5%, 1.5%, 4.5%, 13.5% and 40.5% with the (sub)chronic ecotoxicity test with *N. spinipes*, which has been the most robust and sensitive species in our previous studies on ash (see Table 2), ii) analysed for dissolved metals (filtered 0,45 µm) , and iii) analysed with DGT- filters to obtain available fractions of selected elements. The rationale for using a range of L/S ratios was that we wanted to observe changes in leaching behaviour for specific components in the eluates and compare these changes to the toxic effects observed in the ecotoxicity test. To validate the outcome of these investigations, our final ambition was to spike the highest tested concentration of the eluate generated at L/S 3 (40.5%) with concentration series of the components identified as problematic (which in this study was K and Cu). These spiked eluates were tested with the (sub)chronic test with *N. spinipes* and the used test media were both analysed for both total content of elements and available fractions using DGT-filters.

The ecotoxicological testing of the three eluates generated at L/S 1-3 showed that only the eluate generated at L/S 1 caused significant effects from the control, manifested as a decreased LDR at the

highest tested concentration (40.5%). Calculated HQ values indicate that there were four elements that could be responsible for this significant effect, namely Ca, Al, Cu and K. Calculated HQ values for Ca and Al were close to 1 and stable for all studied eluates and thus indicate a risk of toxic effects. However, the used NOEC-value for Al is derived from a reactive form of Al, which exists in highly alkaline eluate (see above). The DGT-analysis showed that Al was present in the form of colloids, and it is therefore unlikely that Al can explain the observed changes in toxicity from L/S 1 to 3. Although the calculated HQ values for Ca were stable around 1, which hence indicate that this element is present at levels which could induce toxicity at all L/S ratios, these findings do not corroborate the findings of the toxicity of the eluates when exposed to *N. spinipes*. These findings indicate that the relatively high levels of Al and Ca in leachates from aged ash materials should not be problematic in an environmental perspective. Although the presence of Cu was slightly below levels where toxicity are expected we cannot exclude the possibility that this element may be problematic in aged bottom ash materials with higher leaching of Cu.

Instead, similar to the chemical and ecotoxicological investigations presented above, the findings from the present study show that K is dominating the toxicity also in eluates generated from aged bottom ash. However, the effects triggered by K in *N. spinipes* were not lethal but rather related to difficulties to acclimatize to rapidly changing conditions as shown in Figure 1, where K spiked to the highest concentration of the eluate generated at L/S 3 (i.e. 40.5%) is shown together with K dissolved in dilution media only. The figure shows that the shape of the two concentration-response curves for larval development are almost identical, but that the effect of K dissolved in the eluate is expressed at significantly lower levels as compared to when dissolved in dilution media. On the contrary, for mortality, no such shift in concentration-response was observed. This data provides indirect evidence that K was mainly responsible for the toxicity observed also at lower L/S ratios, but also reveals that the effect of the element was more pronounced when other elements were present at levels close to those where adverse effects are expected to occur. In conclusion, although K may be problematic from a classification perspective, long-term environmental risks of the element, at least for *N. spinipes*, are likely negligible.



**Figure 1.** Larval development ratio (LDR) and mortality in *Nitocra spinipes* exposed to K dissolved in dilution medium (triangle; brackish water) and 40.5% eluate at L/S 3 (rhomb). Error bars denote 95% confidence intervals of means. \* $p < 0.017$ ; \*\*\* $p < 0.001$ .

## OVERALL CONCLUSIONS

These studies have generated several important findings regarding classification of ash, of which the most important are that:

- classification based on total content of substances in ash drastically over-estimates the ecotoxicological hazard potential as compared to ecotoxicity testing of eluates.
- in order to harmonize with the classification according to the CLP, eluates for ecotoxicity testing should be prepared using much higher L/S ratios than prescribed in CEN 14735. This makes the classification less conservative but more in consistence with the chemical legislation and thus in coherence with the WFD.
- when testing ash eluates prepared at low L/S ratios components not classified as hazardous, in particular potassium (K), significantly influence the toxicity of the eluates, which is problematic from a hazard classification perspective (risk of misclassification), but likely negligible from a long-term environmental risk perspective.

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