Addressing complexity of health impact assessment in industrially contaminated sites via the exposome paradigm

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ABSTRACT

BACKGROUND: this paper is based upon work from COST Action ICSHNet. Assessment of the health impacts related to industrially contaminated sites (ICSs) is a major scientific challenge with multiple societal implications. Most studies related to associations between ICSs and public health do not provide established mechanistic links between environmental exposure and disease burden, potentially resulting in suboptimal risk management measures.

OBJECTIVES: to assess the potential of the exposome paradigm to overhaul ICS risk assessment and management leading to precision prevention and targeted interventions.

METHODS: we selected the second largest waste landfill in Europe and the data collected in the frame of the HERACLES study on the exposome and health and analysed them together with clinical evidence of neurodevelopmental perturbations following the exposome-wide association study paradigm using the exposome analysis tools; briefly, these pertain to refined exposure assessment, internal dosimetry, and human biomonitoring, multi-omics/toxicity pathway analysis and advanced statistical tools for environment-wide association studies. Waste streams and the related contamination of environmental media are not viewed in isolation, but rather as components of the expotype, the vector of exposures an individual is exposed to over time. Thus, a multi-route and multi-pathway exposure estimation can be performed setting a realistic basis for integrated health risk and impact assessment.

The study was located in the area around the landfill of Fili, outside Athens (Greece). Since 2012, 325 children were recruited and have been followed using a combination of human biomonitoring, advanced-omics analysis on biosamples, environmental monitoring for metals and organic contaminants, and dietary pattern information.

The children were clinically tested for neurodevelopmental perturbations during different developmental stages and the results were analysed according to the exposome-wide association study methodology in conjunction with environmental exposure, but also socioeconomic, dietary, and metabolic determinants of internal exposure and health risk.

RESULTS: using the exposome analysis tools, we confirmed that proximity to a landfill and the consequent soil contamination with metals are critical for children neurodevelopment. However, it was found that additional parameters such as parental education level, socioeconomic status, and nutrition contribute either positively or negatively on child neurodevelopment.

CONCLUSIONS: the exposome concept comes to overhaul the nature vs. nurture paradigm and embraces a world of dynamic interactions between environmental exposures, endogenous exposures, and genetic expression in humans. In this context, the exposome paradigm provides a novel tool for holistic ICS health risk management. The effectiveness of the exposome approach is demonstrated in the case of Athens, the capital of Greece, where health effects associated to long term exposure to a major waste management facility (landfill) are presented.

Keywords: industrially contaminated sites, waste, exposome, omics, neurodevelopmental

KEYPOINTS

What is already known

- Industrially contaminated sites are commonly associated with health problems in the local population after long-term exposure.
- Proximity to industrially contaminated sites is a common proxy for population exposure.
- Complex exposures to toxicants characterize the population living close to industrially contaminated sites.

What this paper adds

- The exposome paradigm overhauls the health risk and impact assessment applied in landfills of municipal and industrial waste and in industrially contaminated sites in general.
- Exposome-wide associations allow joint consideration of all co-determinants of exposure and effects in industrially contaminated sites.
- Beyond environmental contamination and proximity to the site, dietary patterns, family socioeconomic status, and metabolic variability have been also considered as significant determinants of child neurodevelopment.
INTRODUCTION

Industrially contaminated sites (ICSs) have been recognised as a major public health issue the latest years, since they involve co-exposure to multiple environmental stressors and they are unevenly distributed within population of different sociodemographic characteristics. Several type of compounds might result in environmental deterioration in industrially contaminated sites, including heavy metals such mercury, arsenic, lead, nickel, and copper, as well as organic compounds such as polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, ethylbenzene, and xylene (BTEX) mixture, methyl tertiary-butyl ether (MTBE), and dicyclopentadienes (DCPD), among others. The recent years, exposure related to ICSs is also evidenced using biomonitoring. Exposure to heavy metals in school children in Poland in the vicinity of landfills containing lead, cadmium, and zinc has been verified using human biomonitoring (HBM) data, while the adverse effects in haematological parameters in children living near a non-ferrous smelter in Bulgaria have been associated using blood lead (Pb) and cadmium (Cd) levels. Given the well-established evidence of adverse health effects associated with exposure to the compounds identified in ICSs, recent studies aim at characterising the health impact of the populations living nearby, either based on epidemiology or on risk assessment methods. Key findings for assessing the health impacts of ICSs include the higher mortality, mesothelioma, and congenital anomalies of residents living nearby ICSs in Italy. However, health impact assessment in ICSs is a complex process that entails consideration of various technical, environmental, societal, and health-related aspects coupled to the associated uncertainties. These dimensions are often addressed separately in real context, and this is one of the reasons why a comprehensive approach to the problem is still lacking.

This paper contributes to the work carried out in an international network (COST Action on Industrially Contaminated Sites and Health Network; ICSHNet https://www.icshnet.eu/) aimed at: clarifying knowledge gaps and research priorities; supporting collection of relevant data and information; stimulate development of harmonised methodology; develop guidance and resources on risk and health impact assessment. The key questions in this regard can be summarised as follows:

- What type of compounds do we have to deal with? Typically, emissions vary significantly as a function of the industrially contaminated site activity type. Their intensity varies with the duration of activities, the environmental restoration measures applied and the amount of processed materials.

- How are these compounds distributed in the environment? Of particular interest in this regard are the environmental compartments in which chemicals are released and the physicochemical properties of the released compounds.

- What are the exposure levels to these compounds for on-site workers and the local population? Addressing this point requires assessing the contribution of multiple pathways and routes of exposure to the overall exposure burden, as well as reckoning the relative contribution of the industrially contaminated site sources to the exposure background in the vicinity.

- How does exposure to ICSs relate to adverse health outcomes? Answering the above questions requires integrated consideration of both epidemiological and toxicological evidence relating exposure to effects. This, however, would need to be sustained by the elucidation of the biological mechanisms underlying the exposure-to-effect continuum, i.e., the relevant adverse outcome pathway(s) linking the molecular biology of human disease onset and/or exacerbation and environmental co-exposure to the traditionally complex mixture of toxicants found in industrially contaminated sites.

In this work, we present a novel approach to health risk and impact assessment of ICSs that explores the connectivity among the different information layers pertinent to environmental characterisation on the one hand and the different scales of biological organisation in human beings (from gene regulatory networks to cell/tissue function allostatics to organism physiological perturbations).

A key sector of ICSs are the sites contaminated due to municipal and industrial waste management. Assessment of the health impacts related to hazardous waste is a major scientific challenge with multiple societal implications. Health impacts related to the operation of various waste management options have been investigated up to now only using associations of exposure proxies with specific health endpoints such as cancer prevalence and the presence of incinerators or the link between residence proximity to landfills and adverse birth outcomes. The complexity of assessing the health impact of hazardous waste is compounded by the variety of waste types, including both industrial compounds (e.g., plastisizers, dioxins, polychlorinated biphenyls – PCBs) and heavy metals. Exposure to these compounds is highly variable and the waste management option selection is critical. Overall, the importance of health effects of waste management and disposal activities has also been extensively recognized by the World Health Organization (WHO), where the need for multisite cohort studies and refined...
current risk estimates has been highlighted. It has also to be mentioned that the Declaration of the last Ministerial conference (Ostrava, June 2017) for the first time included waste and contaminates sites as one of the 7 priority environmental health areas.

The exposome represents the totality of exposures from conception onwards, including the exogenous and endogenous exposures and modifiable risk factors that predispose to and predict disease. Unravelling it will help us understand the intricate web of relationships between environmental exposures, lifestyle, genetics, and disease. Such a process implies that environmental exposures and genetic variance are reliably measured and linked through mechanistic analysis of toxicity pathways. To understand the interaction between environmental exposure and disease, we need to capture the biological perturbations initiated by chronic exposure to environmental stressors and identify the ones that overcome the homeostasis barrier inducing alterations of the cell/tissue environment and cascade down to disease phenotypes.

Based on all the above and accounting for the methodological difficulties for a comprehensive assessment of the impact that waste sites may have on public health, the present study describes how the exposome can improve our understanding in the complex relationships between exposure to an ICS and the related health effects. The ultimate objective is to enable cost-effective prevention strategies that curb hazardous exposures with precision at the individual (or susceptible subgroup) level and only limited uncertainty at the community level. This is demonstrated in the case of Athens (Greece), where the chronic effects of long-term exposure to heavy metals and organic chemicals leaching out from the second largest waste landfill in Europe is addressed.

**METHODOLOGY**

**OVERALL METHODOLOGICAL CONCEPT OF THE EXPOSOME**

Exposome science aims at capturing the mechanistic processes that describe the source-to-dose continuum. Towards this aim, several methodological tools are employed related to environmental and human biomonitoring. Starting from environmental exposure, various sensors including in situ systems for regulatory monitoring of environmental media, personal, wearable, and remote sensors are combined to determine the external exposome, feeding fusion algorithms for integrating functionally these datasets and filling adequately data gaps in space and time. External exposures are translated into internal dose both at systemic level and at target tissues using physiology-based biokinetic models that consider the different routes of exposure and the chemical and biochemical interactions among the pollutant mixture components. Biological perturbations at different levels of biological organization are captured with multiple-omics (including genomics, transcriptomics, proteomics, and metabolomics) and post-omics technologies including epigenomics.

Identification of the perturbations results in the identification of putative pathways of toxicity, that are verified by targeted multi-omics and functional assays. Advanced bioinformatics tools, such as support vector machines and clustering algorithms and systems biology models, allow us to identify the functional links among the data derived from high throughput testing platforms and disease phenotypes providing thus phenotypic anchoring of the mechanistic hypotheses made earlier. Finally, translation of individual outcomes into community-level effects is carried out using advanced statistical tools that allow the understanding of the connection among the investigated parameters, such as the environment-wide association studies (EWAS). Environmental factors that are correlated are not considered confounders; rather they are co-variates, which are in ’linkage disequilibrium’ with each other. Borrowing the linkage disequilibrium concept from genomics research in EWAS allows us to discern the causal co-determinants that define the relationship between exposures to contaminants in ICSs and adverse health outcomes.

Our approach to implementing EWAS is a population-based data analysis that correlates multiple environmental factors to disease. The EWAS approach has been introduced by Patel et al. and it is based on the Genome-Wide Association Study (GWAS) paradigm. In EWAS, the genome is replaced by the environment, i.e., the metrics of all the environmental exposures related to the population in the area. Specific metrics can be the amount of a chemical substance found in human matrices, self-reported historical exposure, behavioural traits (e.g., dietary patterns), and common well-being characteristics such as family and socioeconomic status (SES). To estimate the health impact, we use a statistical approach based on survey-weighted logistic multivariate regression adjusted for different covariates (age, sex, SES, etc.) linking internal doses with health effect considering the interdependence of the covariates (using as metric an analogy of the linkage disequilibrium metric used in genome-wide association studies). The general formulation of the approach is based on the following mathematical linkage of health endpoints (expressed in terms of odds ratio, $\rho$) with different covariates (age, sex, SES, etc.) and the internal dose in the target tissue ($X_{\text{factor}}$):

$$
\logit \left( \frac{\rho}{1 - \rho} \right) = \alpha + \beta_0 \cdot \text{cov}_1 + \beta_1 \cdot \text{cov}_1 + \beta_2 \cdot X_{\text{factor}} + \ldots + \beta_n \cdot \text{cov}_n
$$
where cov represents the different covariates used in the model and \( \alpha \) and \( \beta \) are the regression coefficients which take into account the interdependence between the covariates. The strength of the association is calculated by a 2-sided p-value for \( \beta_i \). The exponent of \( \beta_i \) serves as the odds ratio, or the change in the odds for the specific adverse health outcome disease versus a healthy status for a unit change of the factor under consideration (figure 1).

THE AREA UNDER STUDY
The methodology proposed above has been applied in a characteristic ICS, the Fili landfill outside Athens, the capital of Greece, servicing an area populated with around 4.5 million inhabitants. The average amount of solid waste is equal to 6 ktn/d.23 The average composition of the waste in Athens includes organics (42%), paper (29%), plastic (14%), metal (3%), and other material (6%). The average transportation distance of the municipal solid waste (MSW) is 19 km. It has to be noted that in the Fili landfill only non-hazardous urban and similar solid waste and no hazardous waste are deposited (figure 2).

However, it has to be noticed that in the close vicinity of this landfill the old Ano Liosia municipal solid waste landfill is located. That was the largest disposal site in Greece from 1973 to 2000, where hazardous (industrial) waste was dumped alongside municipal waste.24 Based on our best knowledge, the Ano Liosia landfill is expected to result in legacy contamination of the wider area.

ASSESSMENT OF LONG-TERM EXPOSURE EFFECTS ON CHILD NEURODEVELOPMENT
The HERACLES (Waste Management) Greek cohort is a study aiming at assessing the contribution of environmental contamination due to waste management practices in the urban and periurban environment including both municipal and industrial waste and associate it to perturbations in children neurodevelopment. The study was established in 2012. Around 350 children aged 3 to 8 years living in the proximity between 0.5 km to 12
km were enrolled. For the association, several exposure factors have been investigated, including:

- exposure to heavy metals, such as Cd, mercury (Hg), and arsenic (As) in urine, Pb in blood, manganese (Mn), and Hg in hair, which is due to landfills, is mainly associated with the PM that is actually formed in the landfill. This is the result of a combination of processes that include both the chemical composition of the waste and their compaction, as well as vehicle movement for handling the waste without excluding contributions from other sources related to urban PM, such as traffic, industrial activity, and biomass burning during wintertime.

- additional proxies of exposure, such as distance from the contaminated sites, concentration of heavy metals in the soil of the child address;

- additional factors considered as exposure and effects modifiers such as:
  - sociodemographic parameters (socioeconomic status, mother education, father education, stress events);
  - child anthropometric parameters and post-delivery factors (child body mass index – BMI, child gender, breastfeeding, presence of micronutrients, minerals and vitamins, selenium (Se) in maternal plasma during pregnancy, delivery, and in cord blood);
  - detailed dietary habits, such as consumption of meat products (pork meat, beef, lamb, sausages), fish, sea food, poultry (eggs, chicken), dairy products (milk, yogurt), nuts, fruits, vegetables, and snacks (biscuits, chocolates);

- analysis of metabolomics profile from urinary samples and pathway analysis, for identifying the pathways that are perturbed and associated with the respective neurodevelopmental progress;

- neurodevelopmental progress in children was estimated with the Wechsler Intelligence Scale for Children – Fourth Edition (WISC-IV), which is an individually administered measure of intelligence intended for children. WISC-IV yields measure of general intelligence as reflected in both verbal and nonverbal (performance) abilities and specific indices, including verbal comprehension, perceptual reasoning, working memory, and processing speed.

Methodologically speaking, we are not seeking simple statistical associations among these factors and indicators of adverse health outcomes using interaction terms in a classical epidemiological approach; instead, we identify associations of single factors with biological markers of allostasis (persistent perturbation of homeostasis). Then, we identify the exposure factors linked to the perturbed biological (in our case, metabolic) pathways associated with adverse health outcomes. We further check for linkage disequilibrium among these factors to isolate the ones with causal links with the identified adverse outcome pathways. Thus, when we identify factors that co-influence the induction/activation of specific adverse outcome pathways, this is not based on statistics alone. Rather our conclusions are based on biological plausibility of the adverse outcome pathways activated by the co-exposure to these factors, which leads to the observed clinical or subclinical health effect. The strength of the exposome paradigm is its ability to bring together the multitude of external exposure factors that determine the external exposome over one’s lifetime or during critical life stages and couple it with internal exposure profiles (e.g., whole metabolome profiling) and link these with biological pathways of toxicity. This allows us to seek exposome-wide associations with adverse health effects basing these associations on biological evidence instead of simple statistical associations. The introduction of the linkage disequilibrium among the determinants of adverse health outcomes permits us to identify causal linkages between exposure and health effects, very much the same way in which loci in the genome which are in linkage disequilibrium are causally associated with specific genetic traits.

RESULTS

POPULATION CHARACTERISTICS

The main population characteristics are illustrated in table 1. From the 350 children who participated in the study, 193 were females and 175 males. There was significant variability regarding parental educational level: almost 20% of the fathers held a bachelor degree or higher, while the same percentage was 16% for the mothers. Among the children who participated in the study, 194 live in families of lower SES, 109 of medium SES, and only 7 children in families of higher SES. Only 54 children were not breastfed at all, while most of the children were breastfed between 1 and 3 months. The median age of the mother at birth was 29 years. Finally, the median child BMI was found 18.7, while the maximum was 31.5. Assessing exposure fusing environmental and HBM data, heavy metals and organic chemicals were monitored in the soil and in biological matrices. In addition to environmental and biomonitoring data, dietary and sociodemographic information was collected by the population living near the Fili landfill. Levels of heavy metals were recently measured in the landfill in Fili and some indicative results are presented in figure 3. It has to be noted that metal concentrations in the landfill are 4 to 20 times higher.

The levels of the heavy metals found in human biological samples in the HERACLES study were used to estimate population chronic intake (figure 4). Ancillary information regarding the various contributors (diet, air pollution levels) was used to estimate the contribution of the various pathways and routes to the individuals.
Table 1. Population characteristics.

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>CATEGORIES</th>
<th>VALUE</th>
<th>CHARACTERISTICS</th>
<th>CATEGORIES</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (No.)</td>
<td>Female</td>
<td>193</td>
<td>Brest feeding index (No.)</td>
<td>No breast feeding</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>157</td>
<td></td>
<td>1 month</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 months</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 months</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12 months</td>
<td>6</td>
</tr>
<tr>
<td>Mother’s school title (No.)</td>
<td>Primary school</td>
<td>8</td>
<td>Age of the mother at birth (No.)</td>
<td>Min</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Secondary school</td>
<td>106</td>
<td></td>
<td>5h</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>High school</td>
<td>142</td>
<td></td>
<td>Median</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Bachelor degree</td>
<td>47</td>
<td></td>
<td>Mean</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Master degree</td>
<td>3</td>
<td></td>
<td>95h</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>PhD degree</td>
<td>0</td>
<td></td>
<td>Max</td>
<td>44</td>
</tr>
<tr>
<td>Father’s school title (No.)</td>
<td>Primary school</td>
<td>3</td>
<td>BMI (kg/m²)</td>
<td>Min</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>Secondary school</td>
<td>95</td>
<td></td>
<td>5h</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>High school</td>
<td>133</td>
<td></td>
<td>Median</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td>Bachelor degree</td>
<td>65</td>
<td></td>
<td>Mean</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>Master degree</td>
<td>7</td>
<td></td>
<td>95h</td>
<td>26.9</td>
</tr>
<tr>
<td></td>
<td>PhD degree</td>
<td>1</td>
<td></td>
<td>Max</td>
<td>31.5</td>
</tr>
<tr>
<td>Children’s SES index (No.)</td>
<td>Low</td>
<td>194</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Levels of heavy metals in soil (aggregated levels in living areas, spot measurements in landfill site).

Figure 4. Levels of heavy metals in the various human biospecimens.
EXPLORING CO-EXPOSURES AND THE ASSOCIATED HEALTH OUTCOMES

Associations observed among the various exposure parameters are given in the correlation globe in figure 5. Here, the parameters that are positively associated are connected with red arrows, while the parameters that are negatively associated are connected with blue arrows. The thickness of the arrows indicates the power (absolute value) of the association. EWAS analysis showed that distance of the child residence from the waste management site is a key factor associated with almost all the indices of the WISC-IV test. More specifically, this variable shows a robust statistical association (p-value <0.001) with the intelligence quotient (IQ), verbal comprehension index, perceptual reasoning index, working memory index. Analysis of the results shows a positive association with the WISC-IV scores indicating that living far from the waste management site has a positive impact on the cognitive function of children. Food consumption patterns influence neurodevelopmental indicators as well. Tomato consumption appears to be statistically (p-value: <0.05) associated with IQ (figure 6), verbal comprehension index and working memory index, while cereal consumption reveals a strong association (p-value: <0.01) with the perceptual reasoning index. Both food items are positively associated with cognition indices meaning that their consumption has potential positive effects on the cognitive functions of the children. Epidemiological evidence suggests that consumption of lycopene, a natural antioxidant present in tomatoes, is able to reduce the risk of chronic diseases such as cancer, cardiovascular diseases, as well as psychiatric syndromes. Li and Zhang reported that low serum levels of lycopene have been associated with increased risk of psychiatric disorders. A review of 22 studies examining the association of breakfast cereal consumption and academic performance in children and adolescents concluded that breakfast consumption of cereals may improve cognitive function related to memory, test grades, and school attendance.
Consumption of fish showed a hybrid behaviour depending on the type of fish and the neurodevelopmental indicator considered: higher consumption of white fish appears to have positive effects on the IQ and verbal comprehension index (p-value: <0.001), while revealed a negative effect (p-value: <0.01) on perceptual reasoning index and working memory index. Among sociodemographic parameters, parental educational level is positively associated with IQ and it is the second most significant parameter. The factors that have been identified as statistically significant (highlighted with red dots in the volcano plot in figure 6) are also presented in table 2.

MOLECULAR AND BIOINFORMATICS ANALYSIS

Based on the metabolomics analysis and the subsequent bioinformatics analysis, several metabolic pathways were identified. Overall, 89 different factors and 74 pathways were depicted in different colours in the correlation globe in figure 7. Association p-values from EWAS are shown as a separate track above each exposure; red points denote EWAS validated associations with positive effect size, indicating risk factors, while blue points indicate EWAS validated negative effect size, indicating protective effects. Line thickness is proportional to the size of the absolute

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>ASSOCIATION STRENGTH</th>
<th>STATISTICAL SIGNIFICANCE (P VALUE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>POSITIVE ASSOCIATION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance</td>
<td>0.332</td>
<td>6.1E-09</td>
</tr>
<tr>
<td>Mother educational level</td>
<td>0.301</td>
<td>2.9E-07</td>
</tr>
<tr>
<td>Father educational level</td>
<td>0.295</td>
<td>7.5E-07</td>
</tr>
<tr>
<td>White fish</td>
<td>0.173</td>
<td>0.008</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>0.147</td>
<td>0.013</td>
</tr>
<tr>
<td>Stess events</td>
<td>0.127</td>
<td>0.020</td>
</tr>
<tr>
<td>Mercury (Hg) in hair</td>
<td>0.121</td>
<td>0.032</td>
</tr>
<tr>
<td>Breast feeding</td>
<td>0.113</td>
<td>0.042</td>
</tr>
<tr>
<td><strong>NEGATIVE ASSOCIATION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese (Mn) in soil</td>
<td>-0.283</td>
<td>2.5E-06</td>
</tr>
<tr>
<td>Hg in soil</td>
<td>-0.267</td>
<td>6.3E-06</td>
</tr>
<tr>
<td>Lead (Pb) in soil</td>
<td>-0.184</td>
<td>0.004</td>
</tr>
<tr>
<td>Offal</td>
<td>-0.183</td>
<td>0.004</td>
</tr>
<tr>
<td>Chard</td>
<td>-0.175</td>
<td>0.018</td>
</tr>
<tr>
<td>Chocolate</td>
<td>-0.174</td>
<td>0.025</td>
</tr>
<tr>
<td>Crisps</td>
<td>-0.171</td>
<td>0.033</td>
</tr>
<tr>
<td>Cabbage</td>
<td>-0.163</td>
<td>0.038</td>
</tr>
<tr>
<td>socioeconomic status</td>
<td>-0.160</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Table 2. Statistically significant environmental, dietary, and exposure factors associated with IQ.
value of correlation coefficient. Among the various identified pathways, the following have been statistically associated with lower scores of neurological development:
- lactose degradation III;
- trehalose degradation II trehalase;
- sucrose degradation V for mammalians;
- UDP N acetyl D galactosamine biosynthesis II;
- glycogen degradation III;
- glycogen degradation II;
- GDP glucose biosynthesis;
- glycolysis gluconeogenesis;
- Hs AMP activated Protein Kinase .AMPK. Signaling WP1403 86073;
- Hs Cori Cycle WP1946 79691;
- mevalonate pathway I;
- cholesterol superpathway;
- geranylgeranyldiphosphate biosynthesis I via mevalonate.

Figure 7 captures in a correlation globe the most important significantly perturbed metabolic pathways, environmental contamination, dietary factors affecting the metabolome, and internal exposure as shown by human exposure biomarkers. Hg in child hair and maternal educational level are associated with perturbations in the mevalonate pathway. The mevalonate cascade is a key metabolic pathway that regulates a variety of cellular functions and is thereby involved in the pathophysiology of many brain diseases, including neurodevelopmental and neurodegenerative disorders. The mevalonate pathway was correlated with both the cholesterol and geranylgeranyldiphosphate biosynthesis pathways, resulting in central nervous system deregulation. In fact, geranylgeranyldiphosphate biosynthesis deregulation is associated with Hg in child hair, resulting in particular in deficiencies in IQ development. For example, the key biochemical finding for mevalonate kinase...
deficiency is the accumulation of urinary mevalonic acid/mevalonolactone. Moreover, 7-dehydrosterol, the last compound before cholesterol biosynthesis is involved in vitamin D₃ biosynthesis. Disactivation of the pathway blocks D₃ biosynthesis, a mechanism which has been clinically associated with neurodevelopmental disorders. According to Kocovsky et al., vitamin D deficiency may be an environmental trigger for autism spectrum disorder (ASD) in individuals genetically predisposed for the broad phenotype of autism and the recognition of this possibly important role of vitamin D in ASD.

**DISCUSSION**

In this study, the effect of contaminants on the neurodevelopmental progress of the children living in the proximity of the second largest landfill in Europe was investigated. Concurrent evaluation of environmental, societal, and dietary factors allowed the identification of the most critical parameters. Distance of the family residence from the landfill was identified as the most important parameter, highlighting the contribution of environmental contamination to child neurodevelopment, especially when considering the strong negative association between metals in the soil and distance of residence from the contaminated site. However, the beneficial effects of parental education and of children/family SES need to be underlined. Additional beneficial effects arise from specific dietary patterns, such as consumption of white fish and tomatoes, while the same occurs for breastfeeding. White fish is rich in omega 3 fatty acids. Direct actions of omega-3 polyunsaturated fatty acids on neuronal composition, neurochemical signalling, and cognitive function constitute a multidisciplinary rationale for classification of dietary lipids as ‘brain foods’. The validity of this conclusion rests upon accumulated mechanistic evidence that omega-3 fatty acids actually regulate neurotransmission in the normal nervous system, principally by modulating membrane biophysical properties and presynaptic vesicular release of classical amino acid and amine neurotransmitters. On the other hand, tomatoes are considered as strong antioxidants, providing protection against the reactive oxygen species generated by heavy metals that affect neuronal mitochondria and eventually children neurodevelopment.

With regard to significantly perturbed metabolic pathways, several pieces of indirect evidence exist that autophagy may degrade the glycogen in neurons, in a way similar to many other tissues. For example, defects in lysosomal enzyme acid alpha-glucosidase have been associated to glycogen accumulation in many tissues, including neurons; the concentration of glycogen in neurons provokes neurodegeneration. In practice, trehalose acts as a potent stabilizer of proteins able to preserve protein structural integrity and reduces aggregation of pathologically misfolded proteins. Trehalose is an mTOR-independent inducer of autophagy and, together with TSC, form a complex which is the key regulator of protein synthesis. Thus, they control cell growth. Recent data suggest that the TSC1/2 complex influences also neural polarity. Furthermore, our results have shown for the first time a mechanistic linkage between exposure to Hg (as measured by way of Hg concentration in child hair) and neurodevelopmental perturbations leading to IQ deficiency, through deregulation of the mevalonate pathway, which cascades down to deregulation of geranylgeranyldiphosphate and cholesterol biosynthesis. Deregulation of these two biochemical processes is linked with neurodevelopmental and neurodegenerative disorders including the induction of induction of ASD. Maternal educational level acts protectively in this respect.

The discussion above highlights the importance of addressing the complexity in the contamination-to-health continuum in ICSs encompassing the multifactorial interactions that predispose to human disease. The latter is of particular interest regarding ICSs in general and especially regarding waste management sites; landfills, in particular, are the places where all types of waste are accumulated, including a multitude of end-of-life products and organic waste. Thus, contaminants (industrial chemicals and heavy metals) with different physicochemical properties and environmental fate go through various exposure pathways and routes. Environmental exposure is one of the key triggers towards homeostasis perturbation. It is thus of particular scientific, regulatory, and societal interest to identify the contribution of the various genetic, epigenetic, environmental, dietary, and sociodemographic parameters on the health status of the population in areas hosting an ICS. Data paucity is one of the key obstacles to addressing the problem in its full complexity, as it further obscures the understanding of how socioeconomic disparities result in differences in exposure and adverse health effects. Spatial differentiation of exposure is another feature that introduces complexity, especially when accounting for the complex space-time trajectories characterizing individual lifespans. Space-time dynamics is also associated with the dispersion characteristics of the pollutants and the relevant pathways and routes of exposure involved. Finally, a fundamental problem for attributing the contribution of ICSs to adverse health outcomes is latency; although in principle it is easier to identify short-term health effects associated with acute exposure events, it is much more difficult to associate chronic exposure with health effects like neurodevelopmental/neurodegenerative disorders and cancer.
The connectivity-based exposome paradigm embraces these complexities and provides the methodological framework that helps structure the data and tools for the integration of all these parameters, treating confounding factors as co-determinants of disease. The fact that the exposome covers life-long exposure allows us to associate exposure at different periods of life (in particular during critical life stages) to later life health effects (e.g., how exposure to industrial contaminants in early life may affect not only neurodevelopment but also the onset of neurodegenerative disease). Use of multi-omics technologies for associating exposure to high dimension biological perturbations allows us to take stock of the mechanisms through which genetic and epigenetic factors influence the phenotype of disease even for the same level of exposure in the population. This complex analytical framework explains biologically the variance observed in adverse outcome phenotypes in the affected population. According to the conventional epidemiological paradigm, this variance is considered a source of uncertainty in the assessment and management of population health risk. In the exposome framework, this variance elucidates the overall susceptibility profile of the population in the area of an ICS, so paving the way towards more targeted and cost-effective interventions in order to minimize the overall population health risk in ICS areas. The possibility to decipher the adverse outcome pathways that connect complex environmental exposure to early effect biomarkers supports precision prevention in the population that is more at risk in an ICS, thus enhancing the efficiency of risk management measures.

Joint consideration of parameters that determine exposure (e.g., environmental contamination, dietary contribution to heavy metals, socioeconomic status) or effects (dietary elements such as omega 3 fatty acids, parental education level) could be elucidated only with the use of advanced statistical methods such as exposure-wide association studies (EWAS). Finally, advanced bioinformatics analysis of multi-omics results allows the identification of perturbations at various levels of biological organization and cellular/organism organization. In this sense, the development of early biomarkers of effect allows us to overcome the problem of traditional epidemiology associated to clinical observation. Briefly, it means that we do not need to wait to see clinical observations for associating specific contamination sources with clinical observations. Based on the above, we conclude that exposome science sets the premises for a paradigm shift in environmental health impact assessment applied in ICSs that goes far beyond proxies of exposure and clinical observations, elucidating an improved understanding of: * the compounds released from ICSs (including waste management sites); * the environmental fate of these compounds; * how population space-time lines cross with the space-time lines of these compounds; * the consequent effects on multiple biological organization levels and the corresponding regulatory networks (genetic, metabolic, etcetera).

As shown above, the highly dynamic interactions resulting from a variable in space and time complex mixture to which people are exposed through multiple pathways and routes during their lifespan could be captured only by taking stock of recent advances in both human exposure science, as well as molecular (multi-omics) and systems biology-based pathway analysis.

**CONCLUSIONS**

The question of human health in contaminated sites is multi-faceted and there is a need to identify appropriate and sound methodologies able to integrate knowledge and evidence coming from different disciplines dealing with the complex mix of environmental, health, and social issues at interplay in ICSs. Exposome science can overhaul the current environmental health impact assessment paradigm, allowing us to effectively address the complexity related to the interplay of genetic, epigenetic, environmental, dietary, and sociodemographic parameters. This requires coupling high dimensional biology and system science aiming at integration using big data analytics and bioinformatics. Towards this aim, the connectivity approach to the exposome elucidates toxicity pathways and assigns causal associations between environmental stressors and health. The application of the above methodology in a typical ICS (large-scale landfill) allowed us to conclude that proximity to landfills and exposure to heavy metals and organic compounds commonly found in waste streams, such as plasticisers, combined with sociodemographic and dietary parameters result in the perturbation of metabolic pathways that are mechanistically associated with lower neurodevelopmental scores (e.g., IQ values) in children. This contribution was encouraged by the ICSHNet COST Action and the approach is expected to support the achievement of the major action goal: the development of guidance documents on how to deal with the complex environmental health scenarios in ICS across Europe.

**Conflict of interest disclosure:** The authors declare they have no conflict of interest.

**Grant:** The work herein presented was done with the financial support of the COST Action IS1408 on Industrially Contaminated Sites and Health Network (ICSHNet).
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