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Adult Chronic Exposure to Neurotoxic Metals Associated With Atmospheric Aerosols: A Case Study in The Urban Area of Turin (NW Italy)

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Abstract

Particulate Matter (PM) pollution still represents a great concern for its adverse effects on human health. Among the emerging risks, the neurotoxicity of PM-associated metals has been evidenced by the scientific literature. Since PM inhalation plays an important role with respect to its toxicity, there is a recognized need for improving the studies with respect to its deposition sites. This work is focused on the assessment of PM_{10}-associated metals in the urban environment of Turin (NW Italy), one of the most polluted European areas. Data of PM_{10}-associated metals mass size distribution, percentage repartition associated to the respiratory apparatus, relative concentration peaks, together with preferential deposition sites and the daily inhaled dose have been calculated. The analyzed metals were: As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, V and Zn. The data are limited, considering a precautionary approach, to the winter season, when the intensity and number of sources is greater and only in the case of stable good weather conditions, when the atmospheric removal processes have a more limited impact on the aerosol dynamics. The result show that the element concentrations in Torino atmospheric PM are similar to those of other considered European towns. The measures evidence a preferential association of each metal with different areas of the respiratory apparatus Furthermore, ordering the analyzed metals with respect to the daily inhaled dose, from higher to lower, we have: Fe, Zn, Sn, Cd, Mn, Cu, Pb, Ni, Cr, Mo, V, As and Co. Even if the risk doesn't seem to be significant up to now (apart from Cr), the evolving knowledge on the effects of chronic sub-threshold exposure might give a different evidence in the future. PM_{10} size repartition measurement, together with the analysis of PM_{10}-associated compounds, could well intermittently complement the routine air quality measures with the purpose of increasing the present body of knowledge with respect to PM neurotoxicity, allowing to improve both the existing risk assessment procedures and the public health management.

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1 Introduction

Air pollution represents one of the greatest concerns of urban environments. A great attention has been paid to Particulate Matter (PM), due to the correlation between fine PM exposure and adverse health effects. Considering the PM penetration into the respiratory apparatus, airborne particulate matter (PM) can be classified either as PM_{10} (particles with an aerodynamic diameter less than 10 μm) or $PM_{2.5}$ (with an aerodynamic diameter less than 2.5 μm, which can reach the lungs) or even PM_1 (with an aerodynamic diameter less than 1 μm). The coarse fraction (with aerodynamic diameter between 2.5 and 10 μm) has predominantly natural sources (geological material, such as fugitive and resuspended dust, and biological material, such as pollen and endotoxins), and its composition changes depending on the geology of the site considered. The fine fractions ($PM_{2.5}$ and its sub-ensemble PM_1) are dominated by combustion-derived particles, consisting mainly of organic and inorganic elements adsorbed onto the surface of a carbonaceous core (Bruggemann et al., 2009) and secondary particles produced by photochemical reactions in the atmosphere (sulphates and nitrates). The carbonaceous fraction consists of aggregates of organic and inorganic carbon, on which are adsorbed transition metals (Pb, Cd, V, Ni, Cu, Zn, Mn, Fe), organic compounds and biological constituents (EPA, 1996). Coarse particles are mainly deposited in the extrathoracic region, while some inhaled fine particles reach the alveolar region of the lung (Kawanaka et al., 2011). The PM coarse fraction has been associated with pro-inflammatory and cytotoxic effects (Gualtieri et al., 2010; Hetland et al., 2004; Schilirò et al., 2010); the PM fine fractions have been associated mainly to a higher genotoxic potential (Billet et al., 2008; de Kok et al., 2005; Traversi et al., 2009). The mechanisms responsible for these biological effects have been continuously undergone review, and some relevant aspects are still open, since the sources characteristics have been changing along the time and due to the fact that the toxicological effects of the different chemical compounds associated to PM are still under study. For example, the metals and their compounds are found distributed among various dimensional fractions of the PM in the atmosphere. The transport and distribution of aerosol particles strictly depends on their size, besides on the weather conditions (Poschl, 2005; Stone et al., 2009). For this reason, concentration, composition and size distribution of atmospheric metals particles are temporally and spatially highly variable. On the other hand, the particles size depends mainly from emission sources: typically, those emitted from anthropogenic sources are smaller than those emitted from natural sources (Harrison et al., 2012). Metals are associated both to the coarse and to the fine fractions, in which they occur generally as different chemical compounds and in different oxidation state. On the basis of existing scientific evidence, many metals (often depending on their oxidation state) may have a direct or indirect active role in the mechanisms of biological action (Ziemacki et al., 2003). In particular, some chemical-physical factors such as hydrosolubility, dimensional distribution and the incorporation into aerosol particles, could influence the bioavailability of metals. Since toxic and carcinogenic properties are well known for many metals, research on deposition of particle-bound mutagens in the atmosphere - first of all at respiratory level - is demanded for assessment of the influence of PM on human health. In fact, deeper investigations on atmospheric concentrations, chemical characteristics and physical-chemical properties of the various metals present in the atmosphere are generally more limited respect to the past. This is also true for the potential risks to which humans are undergone in relation to their chronic presence in atmosphere.

More generally, the health burden associated to PM air pollution is one of the main environmental health concerns, which has been raised by World Health Organization (WHO, 2006). In the year 2004, outdoor air pollution in urban areas was responsible for almost 1.2 million deaths (2% of all deaths) and 0.6% of the global burden of disease (WHO, 2009). Transportation-related air pollution, which is a significant contributor to total urban air pollution, increases the risks of cardiopulmonary-related deaths and non-allergic respiratory disease. Some evidence supports an association of transportation-related air pollution with increased risks of lung cancer, myocardial infarction, increased inflammatory response and adverse pregnancy outcomes (e.g. premature birth and low birth weight) (Krzyzanowski. 2005). Exposure to particulate matter, including metals, has been linked to a range of adverse health outcomes, including modest transient changes in the respiratory tract and impaired pulmonary function, increased risk of symptoms requiring emergency room or hospital treatment, and increased risk of death from cardiovascular and respiratory diseases or lung cancer. Particulate matter is estimated to cause about 8% of deaths from lung cancer, 5% of deaths from cardiopulmonary dis-

ease and about 3% of deaths from respiratory infections (WHO, 2004; WHO, 2007).

Nonetheless, the adverse effects are not limited to the cardiopulmonary ones. Several works investigate the neurodegenerative effect of metals associated to aerosol particles. Increasing evidence links diverse forms of air pollution to neuroinflammation and neuropathology. In particular, air pollution-induced neuroinflammation may precede preclinical markers of neurodegenerative disease in the midbrain, even if the effects of long-term exposures are still poorly understood (Levesque et al., 2011). Exposure to black carbon (surrogate for traffic-related particles) was associated with cognitive impairment in children (Franco Suglia et al., 2008), while prenatal exposure to air pollution has been associated with autism (Becerra et al., 2013) as well as higher incidence of attention deficit/ hyperactivity disorder (Newman et al., 2013). In the elderly, prolonged exposure to air pollution has been associated with decreased cognitive function, a hallmark of the age-dependent neurodegenerative disorder, Alzheimer's disease (Power et al., 2011; Weuve et al., 2012). Accumulating evidence from both human and animal studies show that brain is a target of air pollution. Multiple epidemiological studies have now linked components of air pollution to diagnosis of autism spectrum disorder (ASD), a linkage with plausibility based on the shared mechanisms of inflammation (Allen et al., 2015).

From a toxicological perspective, PM adverse effects appear to be associated to microglia, which generate the nitrogen species and mediate oxidative stress in neurodegeneration, play a critical but as yet unexplored role in PM-neurotoxicity (Gillespie et al., 2013). A recent study was performed on healthy children and young adults who died suddenly, resident in cities with high air pollution. Authors found that exposure to air pollution causes neuroinflammation, an altered brain innate immune response, and accumulation of A#42 and alpha-synuclein starting in childhood; carriers of the APOE ε4 allele are considered at a higher risk of developing Alzheimer's disease if they reside in a polluted environment (Calderón-Garcidueñas et al., 2008). The induction of inflammation in human airway epithelial cells by exposure to diesel soot and other PM_{10} particles throughout a process mediated by free radical/oxidative stress mechanisms has been shown by different in vitro studies (Edwards and Myers 2007). There have been increasing reports that nano-sized components of particulate matter can reach the brain and may be associated with neurodegenerative diseases (Block et al., 2004; Oberdörster et al., 2004; Tin-Tin-Win-Shwe et al., 2008). Nanoparticles can gain access to the brain by two different mechanisms: trans-synaptic transport after inhalation through the olfactory epithelium, and uptake through the blood–brain barrier. Their effects are mediated by oxidative stress, lung and systemic inflammation and different mechanisms of internalization and translocation. It is indeed emerging that one of the main unifying mechanisms of action of several metals is the interference with cellular redox regulation and induction of oxidative stress (Wang and Fowler, 2008; Beyersmann and Hartwig, 2008). Inhaled PM produces the oxidative modification of biomolecules, which can reach the systemic circulation, which and, considered to be biomarkers of systemic oxidative stress and inflammation, when found in higher concentrations than normal. In particular, metabolic stressors produced in the lung have a number of effects in tissues other than the lung, such as the brain. In this sense there is evidence, which suggests that inflammation in the lung is an important connection between air pollution and chronic inflammatory diseases such as autoimmunity and neurodegeneration (Gomez-Mejiba et al 2009). Finally, it has been shown that even sub-chronic exposure of concentrated ambient particulates produces indeed neuropathological damage in the neurons from the substantia nigra nucleus compacta of genetically-modified mice (ApoE-deficient) characterized by elevated levels of oxidative stress in the brain (Veronesi et al., 2005). Since the dopaminergic neurons of the nucleus compact are specifically targeted in Parkinson's disease, authors consider these data a confirmation of the environmental role for the development of neurodegeneration in oxidative stress-susceptible individuals.

Metals play an important role in such toxic mechanisms. An old work showed that concentrations of copper (Cu), iron (Fe) and zinc (Zn) have been measured in the rims and cores of senile plaques (SP) and in the neutrophil of the amygdala of several Alzheimer's Disease (AD) patients, finding an elevate concentration of these elements in SP in AD. This is of interest in light of the observation that Cu, Fe and particularly Zn, can accelerate aggregation of amyloid beta peptide (Lovell et al. 1998). More recent studies reveal the important role of metal ions in several neurodegenerative (ND) diseases (Charlet et al., 2012). More recent data suggest that an inverse relationship between size and toxicity occurs not only with respect to cardiopulmonary systems but also in terms of significant neuronal loss (Gillespie et al., 2013). Many metals have the property to catalyze the formation of ROS which damage key proteins causing protein denaturation, aggregation, and a

failure of the ubiquitin/proteasome system to eliminate these defective proteins (Crichton et al., 2008). If it happens, in specific brain regions it can have critical consequences and may be involved in the aetiology and pathogenesis of neurodegenerative processes (e.g., in substantia nigra, cortex, locus ceruleus for PD, cortex, hippocampus, basal forebrain, brain stem for AD, striatum, basal ganglia, cortex for HD, spinal motor neurons for ALS) (Migliore and Coppedè, 2009). Finally, there is the experimental evidence suggesting that PM–metal neurotoxicity causes brain damage in young urbanites, since the olfactory bulb is a target of air pollution and participates in the neuroinflammatory response (Calderón-Garcidueñas et al., 2013).

The interest about brain disorders has emerged recently since it represents an enormous burden of disease, accounting, in the European case, for 35% of the total (Olesen and Leonardi, 2003). The economic costs of brain disorders are correspondingly large. In Europe the estimated total cost of brain disorders was €386 billion per year in 2004 (including direct costs of treatment and care plus indirect cost of lost workdays and lost productivity) - twice the estimated cost of cancer (Andlin-Sobocki et al., 2005). A more recent survey from the same group (including a wider range of disorders and costs, plus new EU member countries) estimated the total cost in 2010 to be €798 billion, of which 60% was attributable to direct costs and 40% to lost productivity (Gustavsson et al., 2011). The most-known neurodegenerative disorders partly involve complex interactions of genetic and environmental influences, that can affect the brain in many ways. This is the case for Alzheimer's disease (AD) and Parkinson's Disease (PD). Such an interplay between environmental stressors related to air quality and neurodegenerative diseases might require more appropriate actions, considering the increasing societal impact, which is worsening also due to the ageing of society. In order to give a quick look to the present situation, worldwide Alzheimer's Disease International (ADI) estimates that there are 35.6 million people with AD as of 2010, and that this will grow to 115.4 million people by 2050 (ADI, 2010). Only in the US, the Alzheimer's Association (AA) estimates that 5.4 million people have AD (Alzheimer's Association, 2012), which might affect a number between 11 million and 16 million Americans by 2050, with one new case appearing every 33 seconds. ADI estimates that for 2010 the global cost of dementia (of which Alzheimer's is the major cause), including medical costs and cost of formal and informal care, is $604 billion – about 1% of world gross domestic product. If present trends continue, this cost is projected to grow to $1.1 trillion per year (in 2012 dollars) by 2050 – an overwhelming economic burden. Worldwide numbers related to PD are difficult to obtain, but in industrialized countries the prevalence of PD is about 1% for people over 60, with estimates of up to 4% for people in the highest age groups (de Lau and Breteler, 2006). The risk of developing PD rises sharply with age after the age of 60, so the number of cases is likely to grow significantly as populations become older throughout the world. In the US, a prevalence doubling is estimated by 2030 (Dorsey et al., 2007). The economic cost of PD in the US has been estimated of $23 billion per year (in 2002 dollars). Other estimations indicate a total cost of $10.78 billion per year or $21,626 per patient per year (in 2007 dollars) (O'Brien et al., 2009). In Europe, the 2010 estimated cost of PD was €13.9 billion (Gustavsson et al., 2011).

Despite the evidence given by the toxicological literature, a little has been done to assess the characteristics of metals associated to atmospheric particles, focusing the attention on their role as as environmental cofactors in the insurgence of AD and PD and other neurological or neurodegenerative disorders. Data are needed from the areas of inhalation, neurology, and metal toxicology in experimental and human studies after inhalation exposure. An increased understanding of the neurotoxicity associated with air pollution exposure is critical to protect susceptible individuals in the workplace and the general population (Lucchini et al., 2012). Furthermore, since the effect is size-dependent, both the inhaled dose and the size distribution should be defined, considering that these factors are known to be non-universal, being affected by their sources (in terms of intensity and distribution), the local conditions (e.g.: complex terrain, orography, and so on), and meteorological factors, which affect the transport and scavenging of particles. Consequently, no precautionary counter-measures are generally taken to reduce the atmospheric concentrations of such metals. Due to these facts, this paper is focused on the area of inhalation, considering the size-distribution of inhaled metals associated to PM.

In order to manage the risk derived from the potential neurotoxicity derived by the chronic exposure to PM-associated metals in the urban environment, where the exposed population is higher, the attention of this paper is focused on the specific case-study of the metropolitan area of Torino (area of 130.2 km^2; 908.551 in-

habitants), which is the most populated urban center of NW Italy. Within this context, the data related to neurodegenerative pathologies are partial and defective. The numbers, derived from the data collected by the Regional Epidemiological Service in year 2012, show that 32.000 are affected (about 1% of the regional population) by such pathologies. Nonetheless a complete picture of the current situation is missing. The choice of this specific case study is also due to further reasons. The first is that the metropolitan area of Torino is the most populated of NW Italy, as written before. Furthermore, the study of size-segregated PM_{10} mass concentration in the Po Valley started in the seventies and, from 1979, we have also started a Long Observation Period (LOP) of size-fractioned PM_{10} mass concentration in the city of Torino, that is still continuing at present, in the same place and using the same instrumental and analytical techniques (Casazza and Piano, 2003; Casazza et al., 2013; Malandrino et al., 2016). This gives the possibility of obtaining the metals mean size mass distribution over an incredibly long period. Finally, the western area of the Po Valley, where Torino is located, is known to be still one of the most polluted over Europe with respect to PM indicators (European Environmental Agency, 2014 – data referred to the year 2012), with prevalence of particles of finer fraction (the sub-micrometer particles have increased from about 40% to 65% of the total PM_{10} mass concentrations over the last 30 years), as observed in a previous study (Casazza et al., 2013). The results that will be discussed, after indicating the used instruments and methods, are:

1. the mass repartition of metals associated to PM_{10}, expressed in form of mass percentage with respect to the total collected mass. The purpose is to find the preferential deposition sites for each of the analyzed metal;
2. The preferential deposition sites along the respiratory apparatus with respect to each analyzed metal;
3. The calculation of the daily dose, limited to inhalation and for adults only.

The considered data are obtained selecting the worst available day (the days of highest levels of emission and lowest aerosol removal in association with the presence of typical seasonal atmospheric phenomena, such as thermal inversions, which trap the pollutants in the lower layers of the atmosphere - i.e. winter days with stable good weather conditions, limited to the atmospheric stability class A (Pasquill, 1974)), considering, thus, a precautionary approach to the study.

2 Instruments and methods

Aerosol samplings were carried out in autumn and winter 2011-2012 in one site localized near the historical centre (45 °3' 6.412" N, 7 °40' 51.919" E) at an elevation of 15 meter with respect to the road level (top roof with no other nearby buildings creating street canyon) using the 8-stages Andersen MkII nonviable cascade impactor with cut-off at: 11.0, 9.0, 5.85, 4.0, 2.7, 1.6, 0.88 and 0.54 μm of particle aerodynamic diameter. A back-up filter was mounted after the last stage. Sampling duration for each sample set was 7 days (168 hours), at a flow of 28.3 l/min (actual conditions) as prescribed by the impactor instructions, for a total volume of sampled air of 284 m^3. The air flow was maintained with a Tecora mod. Charlie (Milan, Italy) air sampling pump. On each stage a quartz tissue filter (83 mm diameter, PALL tissuquartz 2500QAT-UP) was placed as sampling media to collect the size-segregated particulate samples. The backup filters were of the same material but with a diameter of 43 mm. Before sampling the filters were treated with ultrapure concentrated HCl atunder reflux for two hours, washed several times with ultrapure water and treated at 700 °C for one hour, then stored in a desiccator over silica gel for 24 hours and weighed (see below). After each sampling, filters were stored in a desiccator over silica gel for 24 hours before weighing, then stored at -18 °C until before chemical analysis. Filters were weighed on a Sartorius BP 211D analytical balance with 0.01 mg resolution by using reference masses with the single pan double substitution method. PM mass was obtained as a difference between the filter masses after and before sampling. The procedure ensures uncertainties of 0.01 – 0.03 mg (95% confidence) on PM mass. Given the volume of sampling and the uncertainty on the flow of the sampling pump (5%), the uncertainties on the PM mass concentration are in the range 0.2 – 1.2 μg m^{-3}.

Weather conditions (wind speed and direction, temperature, barometric pressure, relative humidity and total solar radiation) were registered at the same site. In particular, the main meteorological parameters have been monitored in order to check the conditions under which the aerosol and pre-cursor gases measures were taken. The following sensors were used: a temperature sensor is a SIAP TM7722 (measure interval: -30 °C –

50 °C; instrument sensibility: ±0.3 °C); a solar radiation sensor is a EPPLEY PSP (measure interval: 0 W/m^2 – 2800 W/m^2; wavelength interval: 0.285 μm ÷ 2.8 μm; instrument sensibility: ±1 %); a RH sensor is a SIAP UM 5716 (measure interval: 0 % - 100 %; instrument sensibility: ±5 %); a pluviometer MTX model PPI030C (measure interval: 0 mm ÷ 51 mm; instrument sensibility: ±0.5 %). The measures have been collected with a sampling interval of 1 minute.

2.1 Determination of metals in size fractionated PM

The aerosol-loaded filters were cut in two parts by plastic scissors, which were separately weighed and digested by a microwave oven (Milestone, MLS-1200 Mega) in 100 ml tetrafluoromethoxyl vessels. The digestion mixture was composed of 2 ml of HNO_3 and 0.5 ml of H2O2: at a ratio of 4:1; nitric acid was purified by sub-boiling distillation and hydrogen peroxide was ultra-pure grade (Sigma-Aldrich). The following heating steps were applied: 1 min at 250W, 2 min at 0 W, 5 min at 200 W, 5 min at 350 W, 5 min at 550 W and 5 min at 250 W. The resulting solutions were filtered on cellulose filters (Whatman Grade 5) to eliminate the undissolved filter parts and diluted to 15 ml with Milli-Q (Millipore) ultrapure water (18.2 MΩ cm). By this method, the concentrations of trace elements not enclosed in silicate matrix were determined.

As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, V and Zn were determined by a magnetic sector inductively coupled plasma mass spectrometer (SF-ICPMS, Thermo Finnigan Element 2). Mass resolution and isotope selection were optimized for each element to ensure resolution of spectral interferences and maximize sensitivity. The following isotopes of the investigated elements were monitored: 60Ni, 207Pb and 208Pb at low resolution (R=400); ^{75}As, ^{114}Cd, ^{59}Co, ^{52}Cr, ^{65}Cu, ^{56}Fe, ^{55}Mn, ^{95}Mo, ^{120}Sn, ^{51}V, ^{64}Zn and ^{66}Zn at medium resolution (R=4,000). The instrument parameters are reported in Table 1.

Table 1. Instrumental and measurement parameters

Plasma and sample introduction		
Power	1.30 kW	
Plasma gas flow rate	16.0 l min^{-1}	
Auxiliary gas flow rate	1.25 l min^{-1}	
Carrier gas flow rate	0.95 l min^{-1}	
Sample uptake rate	1 ml min^{-1}	
Nebulizer	Concentric glass	
Spray Chamber	Scott double-pass	
a)		
b) Measurement		
Scan mode	Electric	
Resolution [a]	300 [b]	4000 [c]
Points per peak	10 [b]	20 [c]
Dwell time	10 ms [b]	20 ms [c]
Scans per replicate	10 [b]	6 [c]
Replicates	3 [b]	3 [c]

Notes:
[a] Defined as M/ΔM, where M is the mass of the lightest of two adjacent peaks of similar intensity and ΔM is the mass differences between them (if the valley between the peaks drops to 10% of the peak height) or as the mass divided by the peak width at 5% of the peak height.
[b] low resolution
[c] medium resolution

A minimum of triplicate 180 s analyses on each sample was conducted following a 60 s uptake and stabilization period. Between samples the nebulizer system was rinsed for 2 min with 2% sub-boiling HNO_3, which eliminated carry-over and reconditioned the sampler cone. Sets of instrumental blank and calibration verifica-

tion checks were run at frequent intervals during the batch sequence. The calibrations were performed with standard solutions prepared in aliquots of sample blanks. Process blanks were incorporated into the dissolution and analytical procedure to assess metal contribution from the filters, bombs, Milli-Q water and purified acids used in this procedure. The filter blank values together with limits of detection (LOD) and quantification (LOQ) for all the elements investigated are reported in Table 2. All signals for samples were obtained after subtraction of their appropriate process blank values. The relative standard deviation for all elements in each sample was always lower than 5 %.

Table 2. Filter blank values, LOD and LOQ for selected elements

Element	Filter blank ($\mu g\ l^{-1}$)	LOD ($ng\ l^{-1}$)[a]	LOQ ($ng\ l^{-1}$)[b]
As	0.26	1.34	13.4
Cd	$0.64\ 10^{-3}$	0.06	0.64
Co	0.21	0.25	2.47
Cr	2.60	3.02	30.2
Cu	2.08	0.11	1.15
Fe	25.7	49.3	493
Mn	1.07	2.94	29.4
Mo	0.38	1.69	16.9
Ni	0.62	0.52	5.24
Pb	0.005	0.53	5.30
Sn	2.35	0.12	1.23
V	0.12	3.34	33.4
Zn	28.5	11.7	117

Notes:
[a] The limit of detection (LOD) was estimated as three times the standard deviation of the blank signal.
[b] The limit of quantification (LOQ) was calculated multiplying LOD by 10.

NIST SRM 1648a (Urban Particulate Matter) was used to verify that analyte concentrations were within 15% of the expected values before proceeding with sample analysis. The metal recovery rates of certified elements (Table 3) were within 85% and 115% for all analytes, except for Cr, extractable completely only with HF. The mass concentration for each metal has been derived from the analyzed samples, calculating the difference between the analyzed samples and the blank filters concentration values. Each obtained value can be associated to a specific collection stage of the Andersen impactor, which has been used for the environmental sampling. This instrument was designed to provide a clear association among different tracts of the respiratory apparatus, corresponding to the different stages of the impactor, and the PM_{10} size repartition. Thus, different concentration values can be associated to different areas, from the mouth to the pulmonary alveoli. This allows the identification of different preferential PM_{10}-associated metals deposition sites. In order to allow a better visualization for the data, instead of reporting the mass concentration values, the values have been converted into percentage respect to the total mass concentration collected for each metal, using a consolidated approach in the literature (e.g.: Noll and Pilat, 1971; Casazza and Piano, 2003). The peaks of percentage mass concentration, which have been derived from such distributions, being put in relation within the deposition sites along the respiratory apparatus, are indicated as preferential airways deposition sites with respect to each metal, allowing to build a figure, which will be shown in the following section of this work.

Table 3. Recovery rates for selected elements in certified reference material NIST SRM 1648a

Element	Recovery Rate
As	96.2
Cd	113.1
Co	87.1
Cr	62.2
Cu	97.4
Fe	93.2
Mn	98.1

Ni	97.4
Pb	113.6
V	96.5
Zn	99.4

The inhaled daily dose for an adult has been calculated with respect to each metal. The data have been computed according to standard EPA methods (EPA, 2009), calculating the daily dose, expressed in units of mg, from the total inhaled Delivered Dose (DD), expressed in units of (mg/Kg), considering an adult of mean weight (i.e.: 70 kg) and a respiratory rate of 12 l/min. Calculation for other respiratory rates, referred to different activity levels have been omitted. The calculation for children exposure has also been omitted, considering that the size repartition of the stage impactor is also referred to the characteristics of an adult, with respect to the respiratory apparatus. A wider study of exposure, starting from the same database, has been already developed and published in a previous paper (Romanazzi et al., 2014), knowing that the inhalation RfC are applicable to chronic exposure situations, including also neurotoxicity data (EPA 1989, 1998, 2009, 2011).

3. Results and discussion

The average elemental concentrations, expressed in ng/m^3, in Turin are reported in Table 4, together with the annual mean elemental concentrations detected in other Italian and European towns (Vecchi et al., 2007; Amato et al., 2015; Dongarrà et al., 2007; Toscano et al., 2011; Johansson et al., 2009; Heal et al., 2005; Querol et al., 2001). We can compare these data, considering that winter samplings cannot be representative of the whole year.

Most of the elements present lower concentrations in Turin than in other considered urban areas; this is in part due to the decreasing trend associated to the recent European directives for the control of emissions into the atmosphere have been applied for longer times. Taking into account the great variability present in atmospheric conditions along a year, it is however possible to state that the element concentrations in Turin atmospheric particulate matter are similar to those of the other considered towns.

Table 4. Mean concentration in ng m^{-3} measured during winter time in Turin (NW Italy) and annual mean concentration observed in some Italian and European urban areas.

	As	Co	Cd	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sn	V	Zn
Turin	0.07	0.04	1.00	0.70	2.28	140	2.61	0.28	1.26	1.89	14.4	0.22	23.8
Milan[a]				13	72	1830	45		10	71			180
Milan[b]				4.1	72.8	1158	16.7		6.3	20.4	21.1	1.4	84.2
Palermo[c]	1.5	0.3		6.5	49	496	12	3.9	5.5	18		20	48
Venice[d]	3		1.9		8	247	14		6	16		6	47
Florence[b]	0.5		0.2	3.3	22.7	448	7.1	1.8	1.4	4.4		2.1	18.9
Stockholm[e]	0.88	0.15	0.11	2.3	7.7		5.5	1.6	2.3	3.4	14	1.6	17
Edinburgh[f]	0.37		0.34	1.60	4.93	183	2.94		3.43	14.1		1.14	13.3
Barcelona[g]				6.0	74		24		7.0	149		13	250
Barcelona[b]	0.4	0.1	0.2	3.0	19.6	481	10.2	9.4	1.8	7.6	4.6	4.4	68.1
Porto[b]	1.5	0.1	0.5	4.6	31.7	828	13.9	4.1	2.8	14.7	8.1	4.5	99.3
Athens[b]	0.6	0.1	0.1	2.5	6.3	413	7.0	1.8	2.6	4.2	1.5	4.0	18.0

Notes: [a] Vecchi et al. (2007); [b] Amato et al. (2015); [c] Dongarrà et al. (2007); [d] Toscano et al. (2011); [e] Johansson et al. (2009); [f] Heal et al. (2005); [g] Querol et al. (2001).

The mean mass concentration distribution of PM_{10}-associated metals, expressed in % of the total collected mass, with respect to their cut-off aerodynamic diameter (Noll and Pilat, 1971), measured in μm, is represented in Table 5.

Table 5. PM_{10}-associated mean mass concentration repartition, expressed in % of the total collected mass, for the analyzed metals, with respect to each impactor stage cut-off diameter, expressed in μm. The maxima within each metal distribution, are evidenced in italic

Cut-off diameter	As	Co	Cd	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sn	V	Zn
0.54	8	16	7	*19*	13	15	*19*	12	16	7	14	9	7
0.88	9	11	*27*	16	11	10	11	10	17	4	8	6	8
1.60	7	12	16	15	19	14	13	15	*20*	5	13	9	12
2.70	8	11	22	17	*25*	*23*	18	*23*	11	5	*25*	14	*22*
4.00	9	8	10	12	16	18	11	16	7	6	20	9	14
5.85	14	9	8	8	8	19	9	9	7	16	8	10	4
9.00	*23*	11	5	7	4	1	11	7	8	27	5	17	11
11.00	*23*	*21*	6	6	4	1	9	8	15	*30*	6	*26*	*22*

Each analyzed metal has a characteristic peak of mass concentration. In particular: As, Co, Pb, and V peaks are associated to bigger size particles; Cd, Cr, Mn and Ni peaks are associated with the lower fractions; Cu, Fe, Mo and Sn are associated to the middle stages of the impactor (between the aerodynamic diameters of 2.70 μm and 5.85 μm); Zn peaks are associated both with the middle stages of the impactor and with higher aerodynamic diameter particles. The reason of the highest Zn peak might be partially related to its crustal origin. Nonetheless, the reason for the presence of two peaks should be further investigated. With respect to previously published data (Casazza and Piano, 2003), the size distributions have changed. This fact suggests the change of source characteristics, which can be related both to the improved regulations with respect to air quality and to the technological improvements. Further measuring is ongoing to improve our knowledge about the association between sources and related pollutants, including also the analysis of precursor gases and organic compounds.

The PM_{10}-associated metals have been related to their maximum deposition sites along the respiratory apparatus, as shown in Figure 1.

As written in the previous section, a complete risk assessment has been already carried out, considering the available data, for both child and adult receptors living in Torino (Romanazzi et al., 2014). The study showed that the only metal, which comes closest to the risk values, was Cd. This result was clearly correlated to the high and remarkable Cd concentrations evaluated in this study probably due to the working activities related to the restoring of a building nearby the sampling site. However, since exposure to metals occurs also via ingestion and dermal contact and, if these routes are considered, the estimated risks might be higher (Slezakova et al., 2013).

Even if the data derived from the risk assessment do not give general risk indications, the specific neurotoxic risk is emerging and the body of literature has grown only in the last years. Considering the need of correlating, trough toxicological and epidemiological studies, the exposure to the potential adverse health impacts, the basic data for inhaled PM10-associated metals are reported. In particular, the values of inhaled DD and the derived value of the daily inhaled dose, are reported in Table 6. This fact could lead to long term effects, which could have not been previously considered or might be considered in a different way under the light of future studies. Ordering the metals with respect to the daily inhaled dose, from higher to lower, we have: Fe, Zn, Sn, Cd, Mn, Cu, Pb, Ni, Cr, Mo, V, As and Co.

Table 6. Delivered Dose (DD) trough inhalation, expressed in units of (mg/Kg), and daily dose, derived from DD, expressed in units of mg, for each analyzed metal. The data are only referred to an adult man exposure at respiratory rate of 12 l/min.

	As	Co	Cd	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sn	V	Zn
DD	0.01	0.00	0.74	0.07	0.18	10.84	0.19	0.02	0.08	0.09	1.10	0.01	1.74
Daily Dose	0.48	0.23	51.97	4.76	12.48	758.79	13.59	1.51	5.56	6.37	76.79	0.91	121.51

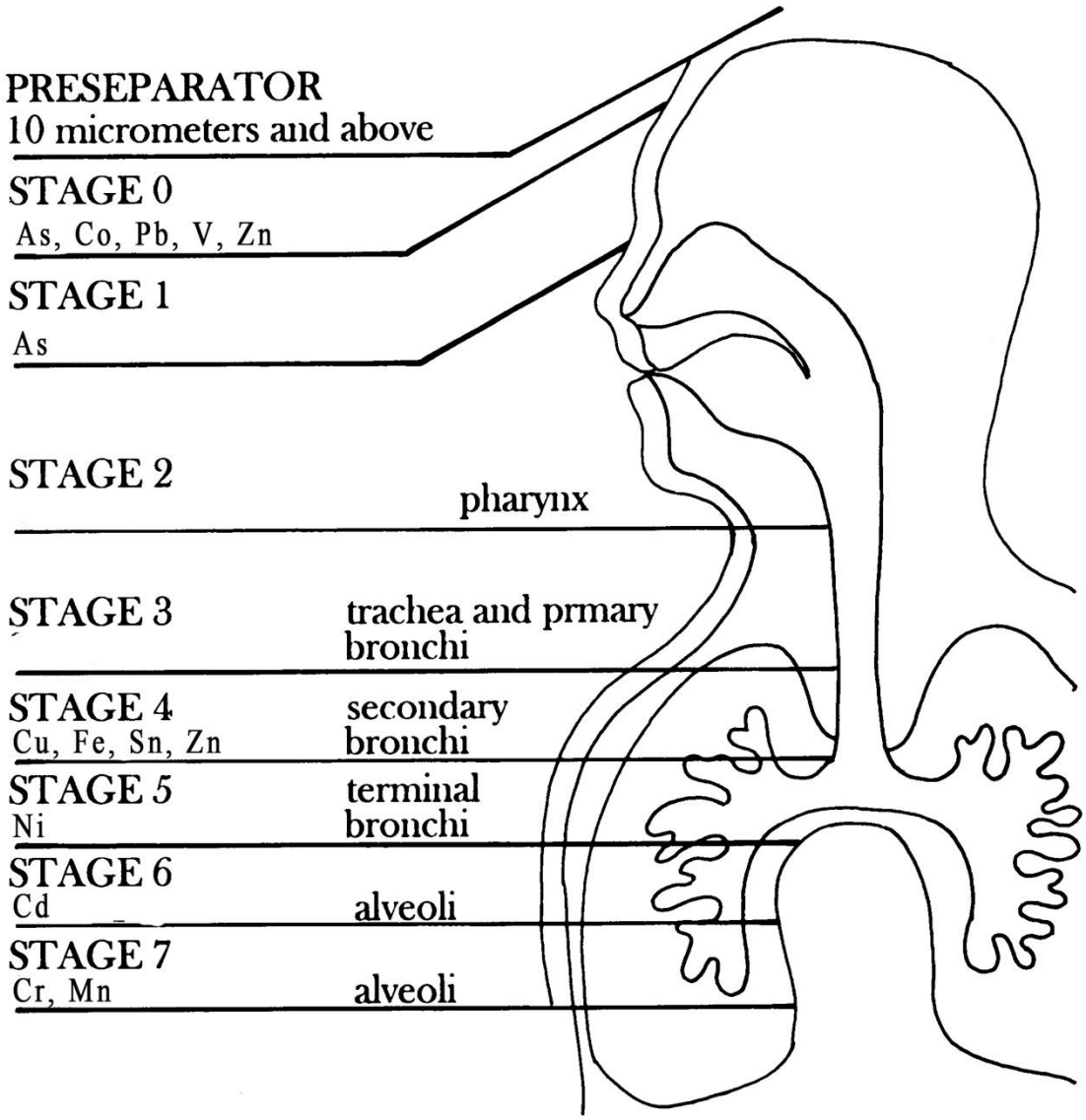

Fig.1. Preferential deposition sites for the inhaled PM_{10}-associated metals. The peak concentration for each metal, indicated with respect to the different stages of the Andersen cascade impactor, is associated with different tracts of an adult respiratory apparatus.

4. Conclusions

Among the emerging risks associated to PM_{10} exposure, there is the potential neurotoxic effect of some of its associated compounds, such as metals. This paper answers to the recognized need of increasing the body of knowledge with respect to inhaled atmospheric aerosols, considering also that the adverse effects are associated to their size, and, thus, to the consequent deposition sites along the respiratory apparatus followed by different translocation and interaction mechanisms, which are still under study.

This work is focused on a highly polluted area (Turin, NW Italy), using a cautionary approach, thus limiting the attention to the period of the year with highest emission levels and to the days, when the removal

mechanisms are limited, considering the weather conditions. Answering to the present needs of increasing the body of knowledge with respect to PM_{10}-associated metals and their potential neurotoxicity, the mean size distribution for As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, V and Zn has been computed. The metals concentration peaks have been associated to the different deposition sites along the respiratory apparatus. The delivered dose has been assessed for each element. Considering the fact that the exposure, even if at subthreshold levels, is chronic, and evidencing the fact that the exposure pathways are not only limited to the inhalation, the importance of the present findings might become more important under the light of the ongoing toxicological and epidemiological research.

In order to deepen the assessment of air pollution impacts on neurodegeneration and, potentially, on other environmental-related diseases, we stress the importance of improving the studies related to the influence of the size distribution, relative concentration peaks, preferential deposition sites of PM_{10}-associated metals on human health, which were more common in the past decades than now. The operational approach shown in this work could intermittently complement the routine measurements, performed for monitoring the air quality in the areas, where the population is more exposed to major pollutant sources, such as in the urban environments. This, in turn, could support a better environmental management trough the development of more effective preventive actions. In particular, a better identification of metal-associated neurotoxic effects and mechanisms might lead to the identification and consequent reduction of the identified emitting sources, thus achieving an important result with respect to public health. Finally, a new body of evidence with respect to the association between PM_{10} and neurodegeneration could lead to the improvement of the present risk assessment procedures for chronic exposure to PM.

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