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Original Article

Early Renal Effects of Chronic Co-Exposure to a Mixture of Toxic Metals in two Pediatric Age Groups

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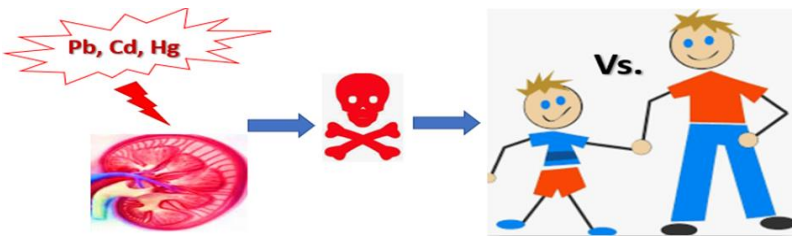
ABSTRACT

Sources of contamination by lead (Pb), cadmium (Cd), and mercury (Hg) are not regulated in Morocco, and have been reported to be responsible for renal effects in two separate cohorts of Moroccan children and teenagers. The aim of the present study was first to compare internal levels of Pb, Cd and Hg and their separate early renal effects between these two cohorts, living in the same urban, industrial and rural areas of Fez city, and second, to determine the early renal effects of chronic co-exposure to a mixture of these toxic metals. The Generalized Linear Model analysis was used to compare the vulnerability of the two groups to toxic metals' exposure, while the JMP 14 analysis was used to investigate high orders of metals interactions to evaluate the effects of the metal's mixture. The mean of blood lead levels (BLLs) was significantly and exclusively higher in urban young children (82.36 Vs. 57.39 µg/l) and may be responsible for the increase in their urinary Retinol-Bending Proteins (RBP) mean (128.15 Vs. 80.82 µg/g creatinine). These results testify to a high vulnerability to lead exposure in comparison to adolescents living in the same environment. Early alteration of the tubular renal function was evidenced in teenagers, due to potentializing interactions increasing levels of urinary RBP. This confirms the high vulnerability of teenagers, compared with young children, to the renal effects of the environmental mixture of lead and cadmium co-exposure.

GRAPHICAL ABSTRACT



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Introduction

Fez is a big city with more than a million inhabitants in the northwest of Morocco, characterized by high mining activities that are considered as a major source of trace elements, particularly Lead (Pb) and Cadmium (Cd), related to leaded-paint used in old houses and the continuous use of leaded-gasoline in the black market even after its ban in 2011. Therefore, official reports indicate that air levels of Pb, Cd and Hg trace elements remained constant during the last decade [1].

Main exposure sources to Pb are leaded-petrol and active or second-hand smoking [2], to Cd are active or passive smoking, contaminated vegetables and incinerators [3] and to Hg are fish intake and dental amalgam fillings [4].

Toxic metals, Pb, Cd, and Hg, are associated with an increase in microalbuminuria (urinary albumin>30 mg/g Creatinine) and in rates of retinol-binding protein (RBP > 300 µg/g Creatinine) as early alteration of kidneys glomerular and tubular functions respectively [5]. Blood levels are relevant biomarkers of exposure to Pb, Cd and Hg [4]. The threshold of toxicity in environmentally exposed children was defined as 50 µg/l, 2 µg/l, and 5 µg/l, respectively [3, 6]. Moreover, general populations are exposed to toxic metals as mixtures, leading to health deleterious effects, even at low levels of exposure [7]. Studies in animal models have shown that mixtures of toxic metals can have different combined effects compared with those noticed in models of separate effects of every toxic metal, especially on the renal and hepatic systems [8, 9].

In fact, it is well recognized that the renal function in children and adolescents is more vulnerable to toxic metals effects compared to adults [10, 11, 12]. However, to the best of our knowledge, comparisons of vulnerability levels between young children and teenagers have not yet been investigated. Furthermore, epidemiological data on health effects associated with the co-exposure to trace elements' mixtures, particularly in pediatric age groups, are scarce.

Accordingly, the aims of this study were: a) To compare the level of vulnerability to Pb, Cd and Hg toxic metals between two pediatric age groups (Young children Vs. Teenagers) living in the same areas and exposed to the same chronic environmental exposure to these toxicants, and reported in our two separate recent studies [13, 14] , b) to describe possible combined renal effects related to the co-exposure to a mixture of these trace elements in every cohort by investigating metals interactions, and c) to determine risk factors of exposure in the whole sample of young children and teenagers, aged from 6 to 18 years old.

Materials and Methods

Study Areas and Subjects

The study concerned two separate cohorts: 209 young children aged 6 to 12 years [13] and 149 teenagers aged 12 to 18 years [14]. In every cohort, we determined levels of environmental chronic exposure to Pb, Cd and Hg, related risk factors and individual early renal effects of exposure to each toxic metal.

In fact, young children and teenagers lived in the same areas (urban, industrial and rural) of Fez city, and according to official reports, are exposed to similar air levels of Pb, Cd and Hg and these levels did not change significantly in the period between 2010 to 2020 [1]. The three studied areas were: Urban (inner city, Bab fettouh area); 5 meters far from the main street but more than 5 kilometers far from industries, industrial (Sidi brahim area); 1 kilometer far from the main street but less than 100 meters far from the industrial areas' activities, and rural (Anousser area) considered as a control area; as it is located about 40 kilometers far from Fez city and more than 10 kilometers far from the main street and located in an area, which is free of any industrial activity.

Ethics

The study was approved by the Moroccan Public Health Ministry represented by the Ethics Committee of the Hassan II University Hospital (CHU Hassan II, Fez, Morocco). Contact was first established with the school management and then with parents, young children and teenagers using questionnaires containing a written description of the study. Written consent was signed by the manager of each school, and another by the child-parent or the person responsible for him/her.

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Before starting the study, oral consent of children had been obtained. Afterward, every child must be examined by the pediatrician before having the final permission to give biological samples. Participation in this study was voluntary and information obtained from volunteers was fully anonymous.

Questionnaires

Exclusion factors were history of drug use, chronic use of medication, diabetes, arterial hypertension, and liver, kidney, urinary, or endocrine system diseases. Parents' questionnaire revealed information about the risk factors for Pb, Cd and Hg exposure and included:

- Housing information: Infancy in urban or rural environment, number of years of living in this area, house built before 1970 (use of Pb in painting), leaded-based pipes, the closeness of house to an incinerator (Pb, Cd and Hg),
- Second-hand smoking (Cd and Pb in tobacco): Parental (or other people) smoking at home, and,
- Nutrition information: Adolescent's intake of offal (Cd), regular consumption of garden vegetables (Cd), fish consumption (Hg) (total, fresh, frozen and canned).

Anonymous interview/ examination of the young child or the adolescent presented data on alimentation out of home, use of amalgam fillings (Hg), a recent visit to a dentist (release of dental Hg and Pb), daily consumption of chewing-gum (release of dental Hg and Pb), and smoke attempt (Cd and Pb in tobacco).

Biological analysis

To determine blood trace elements, venous blood samples were obtained from the arm after cleaning with an ethanol swab. We used plastic 4 ml Lithium Heparin tubes for sampling (Greiner-Bio One GmbH, Frickenhausen, Germany). The levels of the three metals in these tubes were below 0.03 µg/l at leaching tests with 4 ml of 2% nitric acid. Then, every adolescent gave a urinary sample in a free-metal bottle that was used afterward to determine urinary proteins: Urinary creatinine (Creat-U), Albumin (Alb-U), and retinol-binding protein (RBP-U). Directly after sampling, all samples were stored in a 20 °C freezer.

Blood concentrations of Pb (Pb-B) and Cd (Cd-B) were determined by inductively coupled plasma mass spectrometry (ICP-MS) Thermo X7, Thermo Elemental, Winsford, UK [35, 36, 37]. Total blood Hg was determined in acid-digested samples by cold vapor atomic fluorescence spectrophotometry [38]. Urinary creatinine (Creat-U) was assessed by modified Jaffe reaction using a Beckman Synchron LX 20 analyzer (Beckman Coulter GmbH, Krefeld, Germany) [39]. Urinary albumin (Alb-U), and retinol-binding protein (RBP-U) rates were quantified by latex immunoassay [40].

Statistical analysis

Statistical analysis was carried out by using the SAS statistical package (SAS Institute Inc., Cary, NC, USA). The analysis of the variability of trace elements and renal markers for different parameters (continuous variables) was studied by the t-test of Student or by multivariate analysis of variance performed by the Generalized Linear Model (GLM) procedure that considered unbalanced numbers. Multiple comparison of means and their classification were provided by the Duncun test whenever the analysis of variance revealed significant differences. To elucidate several orders of interactions (up to fourth order) between Pb-B, Cd-B, Hg-B, age and BMI, multiple regression analysis was performed using the JMP 14_SAS. Geometric means were used for describing average levels. Results were expressed as Mean ± Standard Deviation and Regression coefficient ± Standard Deviation. Statistical significance was set at $p<0.05$.

Results and Discussion

The means of age and body mass index (BMI) in the cohort of teenagers were higher compared with those of the cohort of young children (age: 13.93, BMI:19.07 Vs. age: 9.07, BMI: 15.99 respectively, $p<0.0001$). These significant differences in age and BMI between the two cohorts were maintained even when analyzing the results according to the studied areas.

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Table 1: Comparison of trace elements concentrations between the two cohorts (Adolescents Vs. Young children) of Fez city

Parameters	Adolescents			Young Children			*p*
	N	GM ± SD	Range (Min-Max)	N	GM ± SD	Range (Min-Max)	*(Sign.)*
Pb-B (µg/l)	143	47.81 ± 29.98	11.39 - 195.24	196	51.71 ± 34.95	7.5 - 231.14	0.283 (NS)
Cd-B (µg/l)	143	0.29 [a] ± 0.14	0.09 - 0.89	196	0.21 [b] ± 0.09	0.07 - 0.68	< 0.0001 (***)
Hg-B (µg/l)	146	0.52 ± 0.59	0.03 - 3.91	192	0.45 ± 0.43	0.02 - 5.31	0.374 (NS)

Pb-B: Blood lead; Cd-B: blood cadmium; Hg-B: Total blood mercury; N: number of participants; GM: Geometric mean; SD: Standard deviation; Min: minimal value; Max: maximal value; Sign: Significance; (NS): Not significant; (***): p<0.001. Means without any symbols are not significantly different; but means with different letters ([a],[b]) are significantly different at p levels indicated in the table.

Table 1 indicates trace elements blood concentrations in the two cohorts of Fez city (Adolescents Vs. Young children).

The only significant difference was observed in blood cadmium levels (BCLs) mean that was higher in teenagers compared with children (0.29 µg/l Vs. 0.21 µg/l, respectively; p<0.0001).

Table 2: Comparison of trace elements concentrations between the two cohorts (Adolescents Vs. Young children) according to the area of residence.

Parameter	Cohort	Area 1: Industrial area					*p*	Area 2: Rural area					*p*	Area 3: Urban area					*p*
		N	GM	SD	Min	Max	(Sign.)	N	GM	SD	Min	Max	(Sign.)	N	GM	SD	Min	Max	(Sign.)
Pb-B (µg/l)	AD	50	43.59	25.31	16.14	165.93	0.281 (NS)	43	41.58	28.12	11.39	171.93	0.234 (NS)	50	57.39[b]	33.76	17.25	195.24	0.002 (**)
	Y CH	66	48.23	20.81	19.31	110.43		81	36.01	22.81	7.5	106.34		49	82.36[a]	45.99	35.74	231.14	
Cd-B (µg/l)	AD	50	0.33[a]	0.16	0.1	0.89	<0.0001 (***)	43	0.28[a]	0.13	0.09	0.62	0.0003 (***)	50	0.26[a]	0.11	0.1	0.62	0.027 (*)
	Y CH	66	0.22[b]	0.09	0.07	0.58		81	0.21[b]	0.08	0.09	0.68		49	0.21[b]	0.09	0.07	0.47	
Hg-B (µg/l)	AD	53	0.68	0.78	0.09	3.91	0.748 (NS)	43	0.34[a]	0.31	0.03	1.88	0.047 (*)	50	0.51	0.47	0.07	2.52	0.572 (NS)
	Y CH	64	0.64	0.69	0.07	4.25		80	0.23[b]	0.24	0.02	1.52		49	0.59	0.9	0.05	5.31	

Cohort AD: Adolescents; Cohort Y CH: Young children; Pb-B: Blood lead; Cd-B: blood cadmium; Hg-B: Total blood mercury; N: number of participants; GM: Geometric mean; SD: Standard deviation; Min: minimal value; Max: maximal value; Sign: Significance; (NS): Not significant; (*): p<0.05; (**): p<0.01; (***): p<0.001. Means without any symbols are not significantly different; but means with different letters ([a],[b]) are significantly different at p levels indicated in the table.

Table 2 shows the same result concerning BCLs means in each area, in addition to a higher blood lead levels (BLLs) mean in the urban young children compared with urban adolescents (82.36

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µg/l Vs. 57.39 µg/l; p=0.002), and higher total blood mercury levels (BMLs) mean in the rural adolescents in comparison to rural young children (0.34 µg/l Vs. 0.23 µg/l, respectively; p=0.046).

Table 3: Comparison of urinary rates of renal markers between the two cohorts (Adolescents Vs. Young children) of Fez city

Parameters	Adolescents			Young Children			*p*
	N	GM ± SD	Range (Min-Max)	N	GM ± SD	Range (Min-Max)	*(Sign.)*
Creat-U (g/l)	145	1.16 ± 0.51	0.22 - 3.23	179	1.07 ± 0.5	0.21 - 3.83	0.094 (NS)
RBP-U (µg/g Creat)	145	119.94 ± 74.43	8.32 - 499	177	131.47 ± 89.17	4.68 - 703.46	0.215 (NS)
Alb-U (mg/g Creat)	145	18.75 ± 34.56	0.39 - 221.07	178	19.64 ± 42.52	0.02 - 279.2	0.839 (NS)

Creat-U: urinary creatinine; RBP-U: urinary RBP (retinol bending protein); Alb-U: urinary albumin; N: number of participants; GM: Geometric mean; SD: Standard deviation; Min: minimal value; Max: maximal value; Sign: Significance; (NS): Not significant.

Table 4: Comparison of urinary rates of renal markers between the two cohorts (Adolescents Vs. Young Children) according to the area of residence.

Parameter	Cohort	Area 1: Industrial area					*p*	Area 2: Rural area					*p*	Area 3: Urban area					*p*
		N	GM	SD	Min	Max	(Sign.)	N	GM	SD	Min	Max	(Sign.)	N	GM	SD	Min	Max	(Sign.)
Creat-U (g/l)	AD	54	1.07	0.38	0.32	1.99	0.156 (NS)	42	1.19	0.62	0.28	3.23	0.143 (NS)	49	1.23	0.52	0.22	2.4	0.121 (NS)
	Y CH	62	1.21	0.58	0.41	3.83		74	0.99	0.44	0.21	2.63		43	0.99	0.42	0.29	2.1	
RBP-U (µg/g Creat)	AD	54	135.51	86.18	8.32	499	0.641 (NS)	42	145.59	66.43	33.23	281.13	0.564 (NS)	49	80.82[b]	47.32	12.43	193.92	0.0002 (***)
	Y CH	61	126.59	114.18	4.68	703.46		73	137.51	75.12	19.41	475.11		43	128.15[a]	70.11	37.45	381.03	
Alb-U (mg/g Creat)	AD	54	18.29	35.56	0.39	221.07	0.627 (NS)	42	29.86	43.11	1.21	157.69	0.431 (NS)	49	9.74	20.17	0.85	134.85	0.163 (NS)
	Y CH	62	15.26	31.66	0.39	173		73	22.91	46.72	0.02	240.83		43	20.42	48.71	1.81	279.2	

Cohort AD: Adolescents; Cohort Y CH: Young children; Creat-U: urinary creatinine; RBP-U: urinary RBP (retinol bending protein); Alb-U: urinary albumin; N: number of participants; GM: Geometric mean; SD: Standard deviation; Min: minimal value; Max: maximal value; Sign: Significance; (NS): Not significant; (***): p<0.001. Means without any symbols are not significantly different; but means with different letters ([a],[b]) are significantly different at p levels indicated in the table.

Tables 3 and 4 present urinary rates of renal markers in the two cohorts and according to the three areas, all rates were normal and the only significant difference concerned the urinary concentrations mean of RBP that was higher in the urban young children (128.15 µg/l) compared with urban adolescents (80.82 µg/l) with p=0.0002.

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Tables 5, 6, and 7 show the effects of Pb, Cd and Hg interactions, including age and/or BMI in the Multiple regression analysis model, in the studied renal markers in young children (n=209), in adolescents (n=149) and in the whole sample (young children and adolescents, n=339), respectively.

Table 5: Toxic metals interactions effects on renal markers in young children (N=209). R^2=0.252, adjusted R^2=0.079

Dependent variable	Independent variables	Regression coefficient	SD	*P*-Value (Sign.)
Creat-U	Age × BMI × Pb-B × Cd-B	- 0.0369	0.0136	0.0076 (**)
	Age × BMI × THg-B × Cd-B	- 1.4759	0.6341	0.0214 (*)
	Age × BMI × Pb-B × Cd-B × THg-B	- 0.1178	0.0576	0.0429 (*)

SD: Standard deviation; Sign: Significance; (*): p<0.05; (**): p<0.01; Pb-B: Blood lead; Cd-B: blood cadmium; THg-B: Total blood mercury; Creat-U: urinary creatinine.

Moreover, Table 5 demonstrates that the three toxic metals showed competitive interactions, which may lead to decreased levels of creatinine urinary rates (creat-U) in young children, particularly between Cd and the two other metals, as the interaction between Pb and Hg was not significant (p=0.536, result not shown in the Table 5).

Table 6: Toxic metals interactions effects on renal markers in adolescents (N=149). R^2=0.278 and adjusted R^2=0.063 for Creat-U and R^2=0.331 and adjusted R^2=0.134 for RBP-U.

Dependent variable	Independent variables	Regression coefficient	SD	P-Value (Sign.)
Creat-U	Age × BMI × Pb-B × Cd-B	- 2.13 × 10-8	8.98 × 10-9	0.018 (*)
RBP-U	Age × BMI × Pb-B	0.0003	0.0001	0.039 (*)
	BMI × Pb-B × Cd-B	2.96 × 10-6	1.29 × 10-6	0.023 (*)
	Age × Cd-B × Hg-B	- 0.35	0.14	0.012 (*)
	Age × BMI × Pb-B × Cd-B × Hg-B	1.2 × 10-8	4.82 × 10-9	0.014 (*)

SD: Standard deviation; Sign: Significance; (*): p<0.05; Pb-B: Blood lead; Cd-B: blood cadmium; Hg-B: Total blood mercury; Creat-U: urinary creatinine; RBP-U: urinary RBP (retinol bending protein).

In adolescents, Pb and Cd had also competitive interactions on creat-U. As for as RBP-U concentrations, its levels were increased by the three toxic metals interactions, which can be attributed to the direct effect of Pb in increasing RBP-U and to the interaction between Cd and Pb. On the other hand, Cd and Hg showed competitive interaction decreasing levels of RBP-U (Table 6).

Table 7: Toxic metals interactions on renal markers in the whole sample of children (Young children + Adolescents, N=339). R^2=0.138, adjusted R^2=0.039.

Dependent variable	Independent variables	Regression coefficient	SD	*P*-Value (Sign.)
Creat-U	Age × BMI × Pb-B × Cd-B	- 0.0048	0.0023	0.0351 (*)
	Age × BMI × Pb-B × Cd-B	- 0.3322	0.1683	0.0494 (*)

SD: Standard deviation; Sign: Significance; (*): p<0.05; Pb-B: Blood lead; Cd-B: blood cadmium; Creat-U: urinary creatinine; Alb-U: urinary Albumin.

In the whole sample of young children and adolescents, we found a competitive interaction between Cd and Pb on creat-U found in young children and adolescents separately. This interaction was observed even on the urinary rates of albumin (alb-U) (Table 7).

With regard to risk factors of environmental chronic exposure to the studied trace elements in the whole sample of young children and adolescents (aged 6 to 18 years, n=339), we noticed that children with daily consumption of chewing-gum (n=189) had higher BLLs mean than

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their respective control (n=150), (52.94 µg/l vs. 46.44 µg/l, respectively; p=0.008), while overweight children (high BMI according to the age of the child) (n=21) had increased BMLs mean (0.85 µg/l) compared with normal children (n=318) (0.85 µg/l Vs. 0.46 µg/l, respectively; p=0.026) (results not shown in the Tables).

Environmental exposure to lead

While the average blood lead levels (BLLs) was similar in the two cohorts of young children and adolescents, the mean obtained in urban young children was higher (82.36µg/l) than that of urban teenagers (57.39µg/l) (p<0.01). There were no differences between BLLs means in the other areas. This result may predict high lead intake in young children compared with teenagers living in the same environment. This intake became significantly higher in young children when the environmental exposure to lead was relatively high as the case in the urban area, characterized by intensified traffic, releasing excessive quantities of dust from the combustion of leaded gasoline. The increased intake of lead may partially be responsible for the elevated RBP-U mean observed in the urban young children compared with the mean in urban teenagers (128.15 vs. 80.82 µg/g creatinine, p<0.001), as RBP-U rates means are similar in the two other areas. These results indicate that the relatively high chronic environmental exposure to lead may be implicated in the lead early side effects on the tubular function in urban young children. Hence, this implies the excessive vulnerability of the renal function of young children even at a relatively low level of chronic environmental exposure to lead. Furthermore, BLLs mean observed in young children were higher in comparison to that recently reported in Moroccan adults (n=124, BLLs mean: 38.53 µg/l, mean age: 25.9 years) [15]. These findings are in agreement with those of previous studies in which young children have been reported to be more vulnerable to lead effects compared to adults [10, 11, 12], but a report for the first time in Morocco revealed, to the best of our knowledge, a higher vulnerability to lead exposure even in comparison with adolescents. Therefore, young children's kidneys may be at higher risk of having high lead concentration leading to nephrotoxicity.

In fact, the average oxygen consumption of a child is higher than that of an adult and an adolescent, and it gradually decreases with maturity [5, 10, 12]. Hence, it can be argued that due to their increased metabolic rate and as environmental exposure to lead appears to be mainly via pulmonary pathways in urban areas, young children have a higher respiratory intake of lead than teenagers. Other factors of vulnerability that can increase Pb internal dose in young children are as follows [5, 10, 11]:

- internal organs are developing; especially the organs responsible for purification and excretion of trace elements, particularly the kidney and the liver,
- immune and antioxidant systems are immature,
- behavioral characteristics that include playing very close to road traffic, being closer to the ground due to their small size and bringing objects to their mouths.

BLLs reported in our series of children and adolescents (especially in the urban area), are higher than the level of environmental chronic lead intoxication at pediatric age (BLLs ≥ 50 µg/l); cases with higher BLLs are at risk of suffering from neurological, intellectual and behavioral disorders [3], and hence need medical and psychological follow-up, as well as adequate chelation therapy.

The high BLLs observed in the urban area, characterized by heavy traffic, confirm that the use of leaded-petrol is the major source of chronic environmental exposure to lead in the two samples of young children and teenagers, while the effect of industrial emissions is low. In Morocco, the late ban of leaded-gasoline in 2011 could not resolve the problem of exposure to leaded dust resulting from vehicle exhausts in urban areas with heavy traffic [1]. This situation can mainly be related to the continuous use of leaded-gasoline in Moroccan black- market. This factor may be potentiated by the high consumption of chewing gum, as shown in our whole sample, by mobilizing the dental lead to blood circulation [4, 16].

Environmental exposure to cadmium

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Blood cadmium levels (BCLs) means were higher in the cohort of teenagers in comparison to the cohort of young children, and the same result was obtained in every studied area. This finding can be explained by the fact that pubertal girls represented about 2/3 of the adolescents' sample, and it is well recognized that pubertal girls have higher internal levels of cadmium than non-pubertal, as a result of higher gastrointestinal absorption of cadmium due to low iron stores and/or to genetic factors [17, 18]. In spite of the fact that BCLs observed in our work were relatively low and far from the level of concern (≥2µg/l) [6], and even from the mean reported in the Moroccan adults (0.58µg/l) [15], girls in our series were susceptible to suffer from cadmium toxicity in their adulthood if the chronic environmental exposure to cadmium remains at this level, considering the great accumulation of Cd owing to menstruations decreasing blood iron and calcium stores and increasing intestinal reabsorption and fixation of Cd^{2+} in their molecular targets, particularly in bones, kidneys and teeth [17, 18].

Environmental exposure to mercury:

All total blood mercury levels (BMLs) means reported in our study were low, far from the level of health concerns (5 µg/l) [6], and lower compared to the risky mean recently reported in Moroccan adults (18.64 µg/l) [15]. The BMLs mean was similar in the two cohorts, but was higher in rural teenagers in comparison to rural young children; this difference was related to the use of dental amalgams. The main determinants for Hg in non-occupationally exposed populations are fish intake and dental amalgam restorations [4]. In fact, fish consumption was low in children and adolescents from the rural area, probably due to the low socio-economic level. This finding may explain the fact that fish consumption was not a determinant in our sample. Moreover, BMLs mean was higher in overweight children compared to normal children in the whole sample of children and adolescents. This determinant of mercury chronic intoxication was not elucidated when the two cohorts of young children and teenagers were studied separately, and corroborates other studies reporting that blood mercury concentrations were associated with obesity and metabolic syndrome, and that the association was dose-dependent across metabolic and weight phenotypes [19, 20]. The proposed mechanisms from studies in animals are that being overweight can lead to methylmercury metabolism alteration and distribution [21]. In fact, Methylmercury (MeHg) enters cells forming water-soluble complexes, followed by higher accumulation of MeHg in blood and brain, which may reflect the lower distribution of MeHg in adipose tissue [21, 22].

Environmental exposure to a mixture of Pb, Cd and Hg

Renal biomarkers were low and far from levels of nephrotoxicity (30mg/g Creatinine for U-albumin and 300µg/g Creatinine for U-RBP) [5]. Moreover, BLLs have been found to increase urinary rates of RBP in adolescents. This effect is well recognized in children as an early stage of tubular alterations caused by lead even at a relatively low level of environmental exposure [5, 11, 23, 24]. Nevertheless, many studies have confirmed that the co-exposure to an environmental mixture of the three toxic metals Pb, Cd, and Hg may cause more additive and complicated renal toxic effects in children [25, 26]. In fact, an environmental mixture of trace elements exhibits different types of interaction that can alter the renal glomerular and tubular functions; the main ones are additive, synergistic and antagonistic interactions [27, 28]. In our study, several interactive effects of the studied toxic metals have been demonstrated, such as competitive interactions with lead and mercury inhibiting cadmium effects on the urinary creatinine that causes hyperfiltration in the glomerular function, implicating also the age and the BMI in young children. Interestingly, this competitive interaction between lead and cadmium persists even in adolescents and in the whole sample of Children (young children + adolescents). Other interactions, of competitive nature, have been reported between cadmium and mercury on the urinary rate of RBP in adolescents, and between cadmium and lead on the albuminuria concentrations in the whole sample of Children. This outcome has been reported previously in children and can be explained by metals competition for intracellular binding sites,

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which implies the cadmium remove from its renal store [11, 29].

Toxic metals interactions with potentiation actions, mainly between lead and cadmium, concerned all effects on the function of the proximal tubule, increase in urinary concentrations of RBP, and also concerned teenagers exclusively. Actually, adolescents and young children have high energy requests and then a very high metabolic rate compared with adults, which may increase their intake of essential elements and thereby increase the internal dose of trace elements. Hence, the higher vulnerability of teenagers compared with non-pubertal young children to the renal effects of a co-exposure to a mixture of the three toxic metals Pb, Cd and Hg can be explained by hormonal changes characterizing the adolescence [3, 5, 11], and fit with previous studies in animals chronically exposed to a mixture of lead and cadmium, that reported renal nephrons damages in rats due to an additive interaction as glomerulosclerosis, cell necrosis and reduced functional mass of the kidneys [30, 31]. Other renal side effects are nephrons enlargement, elevated rates of oxidative stress mediators as malondialdehyde (MDA) and delta-aminolaevulinic acid (ALA), due to the reactive oxygen species (ROS) overproduction and the decline reduced glutathione (GSH) rates and enzymatic activities of antioxidants, i.e. catalase, superoxide dismutase and glutathione peroxidase. This oxidative stress-dependent toxicity is linked to histopathological changes and nuclear and/or mitochondrial alterations, and can be explained by the Fenton reactions and their sequels on the alteration of the antioxidant status in rats [28, 32, 33]. Furthermore, Jadhave et al., reported chronic toxicity in hematopoietic and immune systems due to sub-chronic exposure to a mixture of eight metals including Pb, Cd and Hg, that may be related to anemia and immune responses suppression in albino rats [32], while the exposure to a mixture of Pb, Cd, and Cr was associated with immune dysfunction in freshwater fish [34].

Conclusion

Our work reports a high vulnerability of young children to renal effects of lead exposure, particularly in the urban area, and a great vulnerability of adolescents to the renal effects of a mixture of the three toxic metals. These findings indicate that co-exposure to different trace elements may increase the risk of severe renal, i.e. damage during adulthood, if the lead chronic environmental exposure remains constant at this level.

Overall, BCLs and BMLs were low, indicating a low-level environmental exposure to cadmium and mercury. Nevertheless, the different types of interactions elucidated in our study between these two trace elements and lead are likely to complicate and to result in additive renal damages, particularly the early alterations of the glomerular function in young children (by alteration of GFR) and of glomerular and tubular renal function in teenagers, due to the alteration of GFR concomitant with the potentializing interactions of these toxic metals (especially Pb and Cd) increasing levels of urinary RBP. These findings confirm the high vulnerability of teenagers to the renal effects of a co-exposure to a mixture of toxic metals (Pb, Cd and Hg), which may be a result and a factor of hormonal imbalances characterizing adolescence.

BMLs were associated in the whole sample of young children and adolescents with the use of amalgams fillings and overweight; while BCLs risk factors have to be more investigated.

In the light of our results, there is an urgent need for a strict application of environmental laws concerning the prohibition of leaded-gasoline in Morocco; the establishment of a rational environmental education in the health authorities' plans, raising young people's awareness of their vulnerability to these toxic metals environmental exposure and their risk factors (particularly the daily consumption of chewing-gum and overweight) and setting up a standardized blood lead screening program, in particular for populations living in urban areas with an intensified traffic. These preventive strategies must be accompanied by a medical follow-up and psychological support to the intoxicated children. Thus, nutritional chelators can be added to their diet to reduce the reabsorption and to increase the excretion of lead, such as the consumption of a high intake of calcium.

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Authors' contributions
All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest
We have no conflicts of interest to disclose.

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