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The Influence of Heavy Metal Levels on Autism Case in Bontang and Samarinda City, East Kalimantan – Indonesia

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Abstract

Autism is a disorder of pervasive development in children, which until now, the exact cause is unknown. The strongest allegations are genetic and environmental factors, especially heavy metals. To observe the influence of the environment (heavy metals) on the incidence of autism in two cities located in East Kalimantan (Bontang and Samarinda). measurements of heavy metal levels were carried out in each of the 30 autistic children and 10 control children respectively. Furthermore, with a case control study design, a comparison between the levels of heavy metals in autistic patients' hair and hair control was done.

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Also by measuring the levels of heavy metals in shells, fish, vegetables, water and soil and comparing the results with heavy metals in the hair. As the results, the levels of arsenic (As), mercury (Hg) and lead (Pb) were higher in autism children's compared to controls, but cadmium (Cd) levels were no difference between autistic and control hair, both in Samarinda and Bontang. Likewise, in environmental samples (shellfish, fish, vegetables, water and soil) there was an elevation of As, Hg, and Pb levels in some samples which corresponded to As, Hg and Pb levels in autistic sufferers' hair, but there were no environmental samples were found to have high levels of cadmium. This is also in accordance with the cadmium levels in sufferers'hair and control. High levels of heavy metals detected in children with ASDs at two cities in East Kalimantan indicate a correlation with environmental exposure. Further comprehensive investigations are recommended to assess environmental hazards at this location.

Keywords: Autism ; Environment ; Heavy metals.

1. Introduction

Autism is a neurologic disorder characterized by qualitative impairments in social interaction, communication, and restricted repetitive and stereotyped patterns of behavior, interests and activities [1]. The number of people with autism throughout the world is increasing. This increase was caused not only by the increasing number of cases, but also by improved diagnostic methods, as well as the increasing number of social institutions that cared for people with autism, so that autism sufferers were handled more, including patients from disadvantaged families [2] . Based on data from the Autism Research Institute, in 1987 in the United States only 1 patient with autism was found in every 5000 children, currently there is an increase. According to data from the Centers for Disease Control and Prevention in 2009, in the United States it was found at least 1 in every 110 children suffered from autism [3]. Similarly, nationally, the number of people with autism has also increased rapidly. In 1990 there were only 2-5 cases / 10,000 births, increasing to 20 / 10,000 births [4]. In Kalimantan, one of the provinces with high rates of autism is East Kalimantan. In East Kalimantan, the highest percentage of patients with autism is in Bontang City (181 people / 174,292 inhabitants**)** (Data from all special school in Bontang). The high number of autism sufferers is thought to be related to environmental factors because of the existence of several companies operating in this city. Such industries are not found in other regions. Even though it is lower than Bontang, the number of autism sufferers in Samarinda is also quite high (197 people / 812,597 residents) (Data from all Special School in Samarinda**)**. Allegedly related to coal mining activities, since there are several mining activities in the city, also the Mahakam River which divides the city has become main ships transportation line for the coal from upstream to downstream (sea) . The high number of autism sufferers is a matter of concern, because children with autism are mostly difficult to expect to be a qualified generation, and from a financial standpoint it will burden parents and families [5]. Although it was discovered by Leo Kanner in 1943, but until now the cause of this disease has not been confirmed [6]. Pathological abnormalities in the form of an inflammatory reaction accompanied by nerve damage are found in the brain of autism sufferers. Some xenobiotic species are thought to be the cause of this pathological disorder. But the most profound concern now is mercury [7]. In addition, Farida [8] suspect that autism is caused by multi factors such as genetics, environmental factors (heavy metals), certain foods, infectious diseases, vaccinations, psychosocial factors and factors associated with birth (prenatal, natal and postnatal). But of all these factors, the

environmental and genetic factors are considered the most influential [9]. In this study, the determination of heavy metals in the hair of autism sufferers and controls were carried out in two cities in East Kalimantan. In addition, heavy metal content in some environmental samples such as shellfish, fish, vegetables, water and soil will also be determined. The Purpose of this study was to assess the effect of heavy metal levels from environmental samples on the occurence of autism and whether there were differences in levels of heavy metals from both autisme and control groups.

2. Method

This study was carried out with a case control study design by comparing the levels of heavy metals in autism and hair control non-autism sufferers, as well as examining the levels of heavy metals in environmental samples (shellfish, fish, vegetables, water and soil) and their relation to autism. The location of this research is focused on two cities in East Kalimantan, namely Bontang and Samarinda as shown in Figure 1. The selection of the city of Bontang for this study was due to the high prevalence of children with autism in this city**.** Bontang is the smallest city in East Kalimantan Province, only 497,6 km^2. Geographical location between $0,137^0$ north latitude and $117,5^0$ east longitude. Bontang consist of three sub-districts and fifteen urban villages. The east of this city is bordered by the sea (Makassar strait) , the north and the west are bordered by East Kutai regency and south by Kutai Kartanegara district. The population of Bontang are 174.292 people .In the north of this city there are petrochemical industry (fertilizer industry) that serve the needs of farmers nationally, while in the south there are industries that produce natural gas liquid, whereas their product mainly for exports to several countries. While Samarinda is largest city and capital of East Kalimantan Province. The distance from Bontang to Samarinda is approximately 120 km. Samarinda has 10 sub-districts and 59 villages with population are 812,597 people. The city is divided by the Mahakam river . This river is the main traffic for ships carrying coal from upstream to downstream and also, this river for public transportation. In addition, this city has several coal mining areas which are feared to have a negative effect on public health.

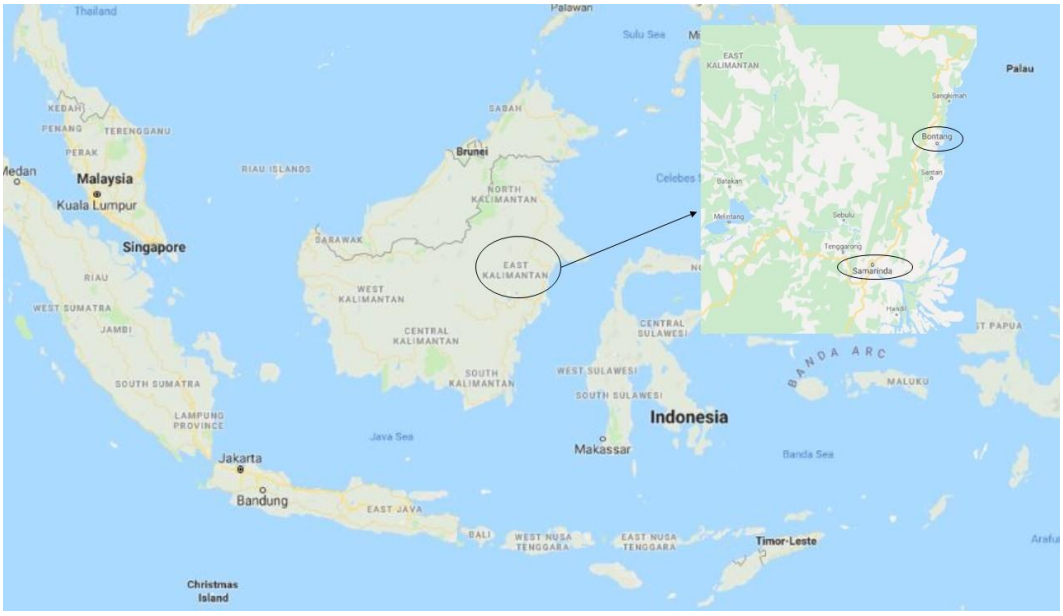

Figure 1: The sampling location in the cities of Bontang and Samarindah in East Kalimantan Indonesia

This research was carried out through several stages as follows:

First step ; after the completion of proposal, is submission for the issuance of Ethical Approval to Human Research Ethics Committee of The Bogor Agricultural University, with regards of the protection of human rights and welfare in research involving human subject. *Second* ; Requested permission from the Health Office, the Education Office and the Head of the local special education school (SLB) regarding the planned implementation of this study. To the SLB Principals, we asked for help to be facilitated so that we could meet with parents of autistic children who attended Special Education School (SLB). Similar to the Education Office, we asked permission to take junior or senior high school children into a control sample. This activity was held on 12 August 2017 in Bontang and on 27 August 2017 in Samarinda. *Third* ; Meeting with parents of people with autism who attended SLB. At the meeting, we conveyed the intent and purpose of this research, among others, to explain the causes of autism, especially heavy metal factors. The expectation was that parents willing to engage their children in the research, as well as giving informed consent. By including their children in this study, they have helped in terms of humanity so that people with autism will not increase in the future. After receiving this explanation, all parents agreed that their children would be included in the study. Some of them even offered to have their normal children included as controls. *Fourth* ; Hair sampling in Bontang was carried out on 4 and 5 October 2017. In Samarinda on 18 September 2017. The hair of children taken each of 30 children with autism in Bontang consisted of 20 boys and 10 girls, and 10 controls consisted of 7 boys and 3 girls. Likewise in Samarinda, children's hair taken consisted of 20 boys and 10 girls. Controls consisted of 7 boys and 3 girls. Age of children both sufferers and controls were between 6 and 16 years. We did not do the diagnosis of autism ourselves but have been diagnosed by a doctor before the child went to school at the SLB (Special school). We paid attention to the distribution of the domicile location of both the sufferers and controls, so that it could represent the condition of the entire city. The hair taken on the occipital part was 5 grams, then put into an envelope and labeled on the outside. Whereas environmental samples (shells, fish, vegetables, water and soil) were carried out in several places in Bontang and Samarinda so that they could represent environmental conditions in all parts of the city. Shellfish samples were only taken in the city of Bontang because there was no sea area in Samarinda. *Fifth* ; Delivering all samples to Bandung, to the Laboratory of Applied Science and Technology Center on Jalan Taman Sari No. 71. Here the measurements heavy metals (Arsenic, Mercury, Cadmium and Lead) levels were done, both hair samples and environmental samples with Neutron activation analysis methods for Arsenic and Mercury and absorption Spectrometry methods for cadmium and Lead.

3. Statistical Data Analysis

1. This research is to prove

a. Heavy metals in the hair of autism sufferers in Bontang large than Samarinda
b. Heavy metals in the hair of autism sufferers in Bontang large than normal.
c. Heavy metals in hair of autism in Samarinda large than normal.
d. Heavy metals in environment in Bontang large than Samarinda

Stages of statistical tests to answer or prove four points above:

1) To prove that heavy metals level in autistic children in Bontang is higher or lower than Samarinda.

a. Normality test was carried out, if the distribution was normal, then test on t 2 free samples was carried out. If the distribution was not normal then the Mann Whitney test was carried out according to city origin.
b. The result of the normality test with *one sample* KS turned out to be abnormally distributed ($p<0,05$), then followed by a Mann Whitney difference test .(attachmet 7).
c. The conclusion of Mann Whitney test, there were differences in the mean values of the heavy metal levels, where the heavy metal levels in hair autism children in Samarinda higher than Bontang. (attachment 1).

2) To prove that the heavy metals level in autism children in Bontang and in Samarinda is higher than healthy children (non autism).

a. Data normality test was carried out. If the distribution was normal, then test on t 2 free samples was carried out. If the distribution was not normal then the Mann Whitney test was carried out according to the diagnosis.
b. The result of normality test with *one sample* KS turned out to be abnormally distributed ($p<0,05$), then followed by a Mann Whitney difference test. (attachment 7).
c. The conclusion that can be drawn from the result of the different test , that there are differences in the average level of heavy metal (arsen, mercury, lead) between autism children in Bontang and Samarinda (mercury level were higher in autism children) axcept cadmium (no difference cadmium level between autism children and normal children in Bontang and Samarinda).

3) Heavy metals level in the environment (samples of fish, shellfish, vegetables, water and soil) in Bontang and Samarinda > normal → do not need to be tested, just compare with the quality standards or threshold values of each.
4) Heavy metals in the environment (samples of fish, vegetables, water) in Samarinda >Bontang ($p<0,001$). (Attachment 7).
5) The tiers analysis with Fisher exact test, had no difference between heavy metal level in soil in Bontang and Samarinda ($p>0,05$) (attachment 7).

4. Results And Discussion

Some heavy metals such as copper (Cu), zinc (Zn) and iron (Fe) serve as micronutrients at low concentrations, but they are toxic when in excess ,while other heavy metals as lead (Pb), cadmium (Cd) ,mercury (Hg) , arsenic, aluminium (Al) and nickel (Ni) that are toxic even in small amount. Through food chain those heavy metals have ability to bioaccumulate and children can be chronically exposed from several sources as air, water and food and causing various diseases because they act as systemic toxin with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effect and they can directly influence behavior and impair mental and neurological

functions via influencing neurotransmitter production and utilization [10]. In this research we measured hair level of arsen (As), mercury (Hg), cadmium (Cd) dan lead (Pb) and After the measurement of heavy metal content was carried out, our results are processed by tabulating the data. The concentration of heavy metals from hair samples collected in Bontang and Samarindah is presented in Tables 1 and Table 2 respectively, while for environmental samples can be seen in Figure 1 for arsenic, Figure 2 for Mercury, Figure 3 for Cadmium and Figure 4 for Lead. We discuss the soil samples specifically.

4.1. The Result of Measurement of Heavy Metals Level in Hair.

Table 1: Consentration of Heavy Metals (As, Hg, Cd, Pb) in Hair of Children With ASD and Control in Bontang

Number of Responden	Sex	Age (Year and Month)	Consentration of Heavy Metals in Hair (Mg/Kg)			
			Arsen (As)	Mercury (Hg)	Cadmium (Cd)	Lead (Pb)
1	Male	9,3	0,215	0,13	0,044	0,356
2	Male	7	0,149	0,271	0,044	0,54
3	Male	9,2	0,087	0,163	0,007	0,004
4	Female	8,1	0,171	0,369	0,015	0,173
5	Male	12	0,118	0,179	0,022	0,136
6	Male	6,3	0,097	0,27	0,009	0,173
7	Female	13,5	0,218	0,932	0,035	0,256
8	Male	12,5	0,083	0,343	0,012	0,124
9	Male	8,7	0,106	0,491	0,013	0,1
10	Female	8,5	0,066	0,279	0,007	0,145
11	Male	14,3	0,133	0,907	0,007	0,064
12	Male	14,1	0,092	0,331	0,007	0,139
13	Female	9,5	0,083	0,290	0,008	0,06
14	Female	9,7	0,104	0,199	0,016	0,249
15	Male	10,5	0,079	0,329	0,008	0,058
16	Male	14,2	0,199	0,216	0,071	0,278
17	Female	14,5	0,081	0,312	0,011	0,086
18	Male	11,6	0,073	0,339	0,021	0,156
19	Male	16	0,083	2,029	0,007	0,045
20	Male	15	0,112	0,556	0,007	0,181
21	Female	14,3	0,092	0,223	0,014	0,248
22	Male	16,0	0,147	0,406	0,01	0,073
23	Male	14,2	0,169	0,651	0,008	0,04
24	Female	13,5	0,082	0,265	0,019	0,079
25	Male	13,7	0,087	0,413	0,01	0,147
26	Female	15,4	0,136	0,332	0,008	0,04
27	Female	14,7	0,129	0,237	0,01	0,153
28	Male	16	0,13	0,256	0,046	0,08
29	Male	12	0,188	0,583	0,072	1,31
30	Male	15,5	0,156	0,508	0,041	1,26
Controls						
31	Male	7	0,079	0,213	0,033	0,04
32	Male	8,2	0,072	0,151	0,036	0,25
33	Female	9,1	0,093	0,196	0,04	0,36
34	Male	12,3	0,078	0,17	0,016	0,076
35	Female	7,8	0,092	0,05	0,026	0,14
36	Male	6,6	0,064	0,105	0,007	0,13
37	Male	8,8	0,041	0,09	0,007	0,04
38	Female	8,5	0,053	0,101	0,002	0,05
39	Male	16	0,062	0,091	0,01	0,09
40	Male	15,7	0,038	0,11	0,02	0,05

Table 2: Consentration of Heavy Metals (As, Hg, Cd, Pb) in Hair of Children With ASD and Control in Samarinda

Number of Responden	Sex	Age (Year and Month)	Consentration of Heavy Metals in Hair (Mg/Kg) Arsen (As)	Mercury (Hg)	Cadmium (Cd)	Lead (Pb)
1	Female	9	0,144	0,778	0,008	0,183
2	Male	7,1	0,067	0,362	0,007	0,171
3	Male	9,2	0,112	0,413	0,051	0,307
4	Female	8	0,152	0,408	0,044	0,367
5	Female	7,6	0,092	0,561	0,043	0,426
6	Male	14	0,103	0,575	0,03	0,276
7	Male	8,4	0,096	0,813	0,031	0,726
8	Male	11	0,097	0,653	0,007	0,214
9	Male	13	0,143	0,721	0,019	0,158
10	Female	9	0,091	0,834	0,015	0,246
11	Male	12	0,137	0,314	0,043	0,732
12	Female	15,1	0,072	0,092	0,007	0,359
13	Male	14,8	0,081	0,351	0,002	0,631
14	Male	6,5	0,121	0,279	0,007	0,532
15	Female	6,2	0,091	0,831	0,009	0,751
16	Male	16	0,085	1,321	0,035	1,151
17	Male	13	0,079	2,28	0,037	0,253
18	Female	12,5	0,185	0,417	0,034	0,873
19	Male	14,5	0,093	0,923	0,011	1,701
20	Male	13	0,189	1,431	0,081	0,952
21	Male	14	0,178	0,831	0,048	1,651
22	Female	12	0,093	0,753	0,05	1,345
23	Male	15	0,187	1,131	0,08	0,913
24	Male	15,4	0,053	0,451	0,048	0,852
25	Female	11	0,217	0,852	0,05	0,545
26	Male	12	0,194	1,473	0,08	1,753
27	Male	13	0,132	1,253	0,088	1,651
28	Female	14	0,115	0,371	0,055	0,851
29	Male	15	0,251	0,431	0,047	0,371
30	Male	13	0,213	0,583	0,082	0,521
Controls						
31	Male	6,5	0,033	0,213	0,021	0,21
32	Female	7,2	0,051	0,133	0,015	0,213
33	Female	8,5	0,023	0,151	0,01	0,131
34	Male	8,3	0,045	0,183	0,024	0,15
35	Male	9	0,031	0,315	0,015	0,09
36	Female	10,5	0,069	0,153	0,021	0,187
37	Male	10	0,131	0,183	0,027	0,137
38	Male	15,5	0,083	0,263	0,03	0,081
39	Male	16	0,091	0,514	0,017	0,193
40	Male	14	0,022	0,155	0,043	0,135

Arsen (As)

One of the heavy metals associated with autism is arsenic (As). The brain is an organ that is very sensitive to arsenic poisoning, because it can interfere the normal development of the brain. Busch and his colleagues [11] in his study of analyzing hair and urine of ASD children and comparing it with normal children, he found a significant difference between arsenic, cadmium, barium, cerium lead and also magnesium and zinc levels. Likewise, in this study, both in Bontang (Table 1) and in Samarinda (Table 2) higher arsenic levels were found in autism patients compared to controls. With further testing with Mann Whitney test it was found that arsenic

level was higher in autistic patients compared to hair control. In addition, age factors also determine the high arsenic levels in hair. The older age of autism, the arsenic level tends to be higher. For example, in Bontang (table 1), sample number 1 (age 9 years 3 months) had arsenic levels of 0.215 mg / kg higher than sample number 6 (6 years) with arsenic levels of 0.118 mg / kg even though the two sufferers were siblings. Likewise, sample number 16 (14 years, 2 months) with arsenic levels of 0.199 mg / kg siblings with sample number 18 (11 years 6 months) with lower arsenic levels of 0.073 mg / kg. Likewise, in the sample in Samarinda (figure 2), sample number 1 (9 years) with arsenic levels of 0.144 was higher than sample number 2 (7 years) with arsenic levels 0.067 mg / kg higher than sample number 2 even though the two sufferers were brothers. In terms of domicile, it can be seen that the closer it is to the source of pollution, the higher the arsenic level in the hair. From the results of measurements of the levels of heavy metals in environmental samples in Bontang, it was found that samples with high arsenic content were shellfish and fish taken from the sea near the fertilizer industry and natural gas industry it turned out that hair samples taken from the area (at a radius of up to 3 km) had high arsenic levels. The samples with domicile close to fertilizer industries waters include sample number 4 (0.171 mg / kg), number 20 (0.112), number 11 (0.133 mg / kg) and number 29 (0.188). And the one closer to to the sea near of the natural gas industries is sample number 26 with arsenic levels of 0.136 mg / kg. Similarly, in Samarinda, samples domiciled near coal mines (radius less than 2 km) had higher arsenic levels in their hair, including sample number 3 (0.112), number 6 (0.103), number 18 (0.185 mg / kg) and number 19 (0.093 mg / kg). Likewise, the sample lived near the Mahakam River which was the place where the coal was transported from the upstream, also had a fairly high arsenic level such as sample number 5 (0.092 mg / kg), sample number 7 (0.096 mg / kg), number 16 (0.085), number 25 (0.217 mg / kg) and sample number 30 (0.213 mg / kg). How the process of arsenic infiltration into a child's body until it can be detected in his hair, still requires deepening whether through his mother who later during pregnancy arsenic went through the placenta to the child, or the child who ate foods containing arsenic. The high arsenic levels in children's hair are not solely due to the inclusion of arsenic through food or drink. Arsenic from polluted environments, both water and air can also cause elevation of arsenic levels in hair [12].

Mercury (Hg) In tables 1 and 2, the mercury levels can be seen in the hair of autism children’s and controls in the cities of Bontang and Samarinda. According to the statistical analysis, in both Bontang and Samarinda, mercury levels in autistic childrens' hair are higher than hair control . Likewise, in terms of age, it seems that the older the respondents are, the mercury levels are getting higher. This can be seen in respondents who are brothers. Respondent number 6 (age 6 years) with a mercury level of 0.097 mg / kg has a lower mercury level than his brother, respondent number 1 (age 9 years 3 months) with levels of 0.215 mg / kg. Likewise, respondent number 16 (14 years 2 months) has arsenic levels of 0.199 mg / kg higher than his younger siblings, namely respondent number 18 (11 years 6 months) with mercury levels of 0.073 mg / kg. The same thing was found in Poland that mercury levels in patients were higher than controls and young age autism sufferers had lower mercury levels than the older ones [13]. In terms of domicile and its relation to mercury sources, in Bontang mercury levels were only found in shells from the territorial waters near of fertilizer industries (0.49 mg / kg). Although this is lower than the maximum contamination limit (1 mg / kg) but this is dangerous if consumed by humans because the Hg level will experience accumulation in the body which at one time will endanger health. There are four respondents whose domicile is close to the waters near of fertilizer industries (Less than 3 km)

are respondent number 4, 11, 20 and 29.Their mercury concentrations are 0.369, 0.907, 0.223, and 0.583 mg/kg respectively. Similarly, in Samarinda, mercury levels were obtained from fish taken from the Lempake area (around the coal mining area). The respondents who live in the area (less than 2 km) are samples number 6, 3, 18 and 19 . Their hair mercury concentration are 0.575 ,0.413 ,0.417 and 0.923 mg / kg respectively. Thus, both in Bontang and Samarinda it was found that the closer to the mercury source, the higher the Hg level in the hair. This can also be seen in controls where all mercury levels are low. Mercury is a toxic metal and has harmful effects on human health. Some studies show that if the mercury level is high in the blood, it becomes a risk factor for the occurrence of autism [14]. Another source of the infiltration of mercury into the blood is through vaccine namely Organic Hg exposure from Thimerosal which contains the hepatitis B vaccine given in the first six months of the child's age [14]. Autistic children had a significantly greater prevalens of adverse reactions after vaccinations and abnormal development than controls [15]. The source of mercury in the environment can come from fish, from old thermometers, vaccine preservatives, dental amalgams, burning coal on the power plants [16]. In this research, in order to observe the contamination by mercury, the samples which were measured for the levels of heavy metals were fish and shellfish, vegetables, water and soil due to suspected sources of pollution in the city of Bontang originated from industry and in Samarinda from the coal mines. Apart from hair and blood, mercury can also be monitored through urine. Wright and his colleagues [17] measured mercury in the urine of 56 autistic children (ASD), 42 siblings or normal siblings, 121 children who were normal and not related to autism sufferers and 34 children who attended special schools, found that excretion of mercury in urine is greater in children with autism than other groups. In relation to mother's breast milk, Yalcin and his colleagues [18] conducted an evaluation the relationship between the incidence of Hg levels in breast milk and maternal habits such as food habits, dental care, smoking, anemia, zinc (Zn) and Iron (Fe) levels in milk, as well as levels of selenium (Se), Zn and Copper (Cu) that affect the levels of mercury in mother’s breast milk. The pregnant women who smoked, contaminated food intakes, and anemia had an impact on increasing Hg levels in breast milk. Therefore, if there are risk factors, monitoring of the growth of children is carried out both at the time of exclusive breastfeeding and in the second year of life of the child. In this research, none of the mothers were either autistic mothers or controls who smoked.

Cadmium (Cd)

Of all respondents who were examined for cadmium levels in their hair, none of them had high cadmium levels (exceeding 0.1 mg / kg). This is in accordance with environmental samples (fish, vegetables, water and soil) which none of them exceeds the limit of contamination. Cadmium examination was carried out in this study because in previous studies got the excess of Cadmium level. For example, Akyuzlu and his colleagues [19] found a link between high cadmium levels in hair and urine with the onset of autism. In a study conducted in Ankara it was found that in patients with autism had the elevations of lead, cadmium and arsenic in hair, and in urine, it was also found high levels of lead and cadmium. This was compared to non-autism controls, which had low levels.

Lead (Pb)

Lead is heavy metal that can interfere with and cause neurological abnormalities, causing physiological

disorders and behavioral disorders in children. Interference due to Lead can result in preterm birth and small head circles of children [20]. Adams and his colleagues [1] conducted a study of 55 autistic children and 44 control children. Levels of heavy metals toxic in blood, red blood cells and urine from both groups were measured. It turned out that the autism group had high levels of Lead in red blood cells, Lead levels in urine and high levels of Thallium, Tin and Tungsten, and high levels of toxic metals associated with the severity of autism. Likewise, from the research conducted by Yalcin and his colleagues [18] by measuring 10 types of heavy metals and essential elements in the hair of children with autism and non-autism, it was found that in autistic sufferers' hair was found to be elevated from levels of Aluminum, Arsenic, Cadmium, Mercury, Antimony, Nickel, Lead and Ranadium. In the study, Yalcin also found a positive correlation between Lead levels and verbal communication disorders (P = 0.020). Its mean that verbal communication disorders will be more severe if level of lead exceeded. When looking at Pb levels in hair (table 1 and 2), there were only 3 hair samples in Bontang whose Pb levels exceeded 0.5 mg (Respondents No. 2, no. 29 and no. 30). Whereas in Samarinda (table 2) there were 17 autism respondents whose lead levels in their hair exceeded 0.5 mg. From statistical analysis **,** it is proven that lead level in autism children in Samarinda is higher than Bontang. When it was matched with environmental samples, in Bontang none of the environmental samples had high Pb levels. In Samarinda there was only one fish sample with high lead content (1.9 mg / kg), which was fish taken from Lempake (the distance of location less than 2 km from coal mining), or any other lead source in this area such as home industry for melting of used storage battery (need further research about it). The high Pb levels in hair in Samarinda could come from polluted air environments, for example from the results of burning fuel oil by motor vehicles. In Jamaica, although the ban on Pb to be used on gasoline has been carried out since 1998, Pb still exists until now in the environment in the form of air emissions and contaminates underground water [22] . It was like research conducted by Bandjar [23] in Sirimau District Ambon City . The study was conducted to observe Pb levels in the hair of traffic police (comparing Pb levels in police hair working in the field with police hair working in the office). The result showed that the police who worked in the field had Pb levels were 55.18 mg / kg and 53.21 mg / kg in their hair. While those who worked in the office, the level was only 23.77 mg / kg. The same study, to observe the existence of Pb pollution, Mahawati [24] conducted a study that the respondents were also traffic police. The research was conducted in Semarang (2011) using blood samples. It turned out that 39.4% of the Pb levels in the blood were still normal, 36.4% of the Pb level in the blood had increased but was still at a tolerable level, and 24.2% of the Pb level in the blood was excessive. In order to observe Pb levels in relation to exposure from the environment, Mayaserli [25] examined the Pb levels in the hair of gas station employees on Jalan Ir. Juanda. The result showed that the longer the employee has worked, the higher the Lead levels were found in his hair.

4.2. Environmental Sample

As environmental samples, shellfish, fish, vegetables, water and soil were taken. The shellfish samples were only taken in the city of Bontang because marine waters were not found in Samarinda. Of all these samples, the levels of four heavy metals were measured, namely Arsenic (As), Mercury (Hg), Cadmium (Cd) and Lead (Pb). For this reason, four type of heavy metal will be discussed as follows:

Arsenic Levels

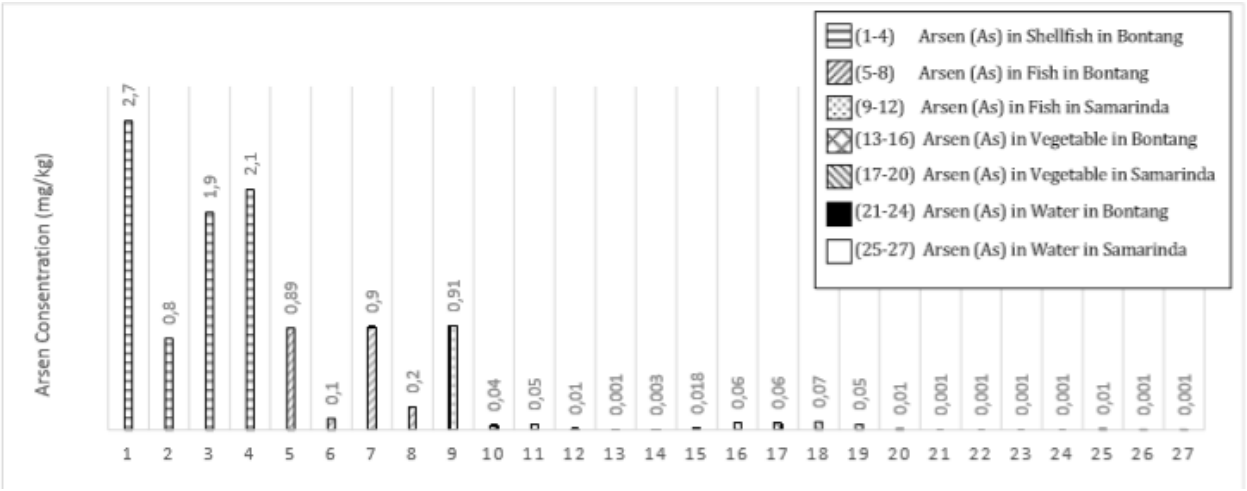

Figure 1: Concentration of Arsen (As) in shellfish ,fish, vegetable and water in Bontang and Samarinda

Arsenic levels in environmental samples can be seen in Figure 1. Four shellfish samples were taken from Bontang waters. Two were taken from the territorial waters close to fertilizer industry and two from the waters close to the gas industry. The maximum limit of arsenic contamination in shellfish based on the Head of BPOM Regulation of The Ministry of Health Number 23 Year 2017 [26] is 0,25 mg / kg. The arsenic levels in shellfish taken from waters close to the fertilizer industry are 2.7 and 0.8 mg / kg. While from the waters near the gas industry are 1.9 and 2.1 mg / kg. It means that all of four samples whose levels exceed the maximum limit of contamination. From the discussion on arsenic levels in hair in Bontang, it was seen that the domicile of 5 respondents with autism who had high arsenic levels, were close to fertilizer industries waters area (less than 3 km). The connection between arsenic in shellfish and high arsenic levels in hair certainly requires further exploration. From interviews with parents, it was found that the children rarely consume shellfish. Of the five parents whose domicile is close to this region, only two of them often ate shellfish while pregnant. The description of arsenic levels in shellfish shows that the marine area has been polluted with arsenic heavy metal. This is in accordance with the levels of Arsenic in fish which were also taken from the same water area: 0.89 mg / kg in fish taken from near fertilizer industry waters, 0.9 mg / kg in fish taken from the waters of gas industry. While the fish taken from Tanjung Limau waters and Tanjung Limau TPI had lower of Arsenic levels (0.1 and 0.2 mg / kg). Because arsenic levels from fish in this marine area are exceeded than the contamination limit (0,25 mg / kg), it is quite dangerous if these fish are eaten by humans because they will accumulate in the human body. And if the body's detoxification ability to heavy metals decreases, arsenic exposure from this environment can cause a clinical effect in the form of poisoning . In Samarinda, of the four fish samples taken, there was one sample whose arsenic levels were quite prominent, it was the fish taken from the pond in Lempake (0.91 mg / kg). This fish pond is located not far from the coal mining location (Less than 2 km). Possibly what happened was that water from the mine entered into this pond during rainy days. As for arsenic levels from vegetable samples (maximum contamination limit of 0,15 mg / kg, based on BPOM (Indonesia Health Ministry) regulation number 23 year 2017 it seems that all have low levels of Arsenic in both Bontang and Samarinda. Similarly, the water samples (maximum contamination limit of 0.05 mg / kg), there were not any samples in either Bontang or Samarinda that reached this limit. However, there was one sample which was 0.01 mg / kg, it was raw water taken from the Lempake Water Treatment Plant. Although the level is still lower than the

maximum limit of contamination, this requires caution because if this water is consumed by humans, the arsenic will accumulate in the body and at a certain level will cause health problems. Rahbar and his colleagues [22] in Jamaica found that the high levels of Arsenic in children's blood caused by the consumption of drinking water, avocado, *callaloo*, broccoli and *bok choy* which have been contaminated. This high levels of Arsenic caused behavioral disorders.

Mercury Levels.

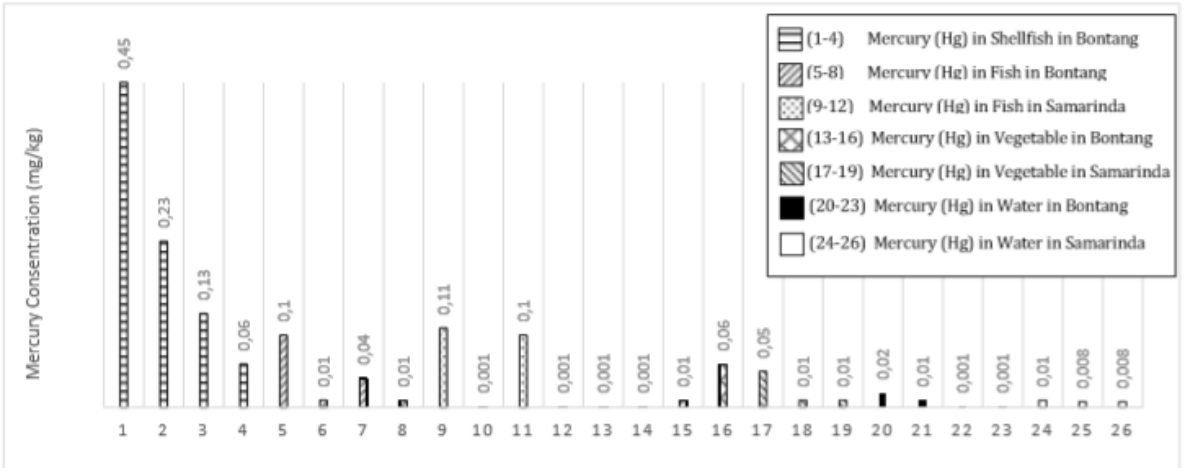

Figure 2: Consentratton of Mercury (Hg) In Shellfish. Fish. Vegeuble And Water In Ronung And Samannda

The Mercury levels in environmental samples can be seen in Figure 2. In the shellfish samples, the highest levels were found in shellfish taken from the waters of fertilizer industry (0.491 mg / kg). it's level exceeded than the maximum contamination limit (0,25 mg / kg), it is dangerous if consumed by humans because the human body will endanger health. The high level of mercury in the shells taken in Bontang waters can illustrate the contamination conditions in the area where the shells live, but it can also occur that the Mercury contamination at the place is low, but because of the long life the shells accumulate Hg levels in the body. In fish samples taken from this region the highest mercury level is 0.1 mg / kg. Although the level is still lower than the maximum contamination limit (0.25 mg / kg), but this is dangerous if consumed by humans, because it will experience accumulation in the human body so that one day it will endanger health conditions. Based on the mercury levels found in shellfish and fish, it can be concluded that this marine area has been contaminated with Hg heavy metals even though the contamination level is still in a low stage. In Samarinda, the Mercury levels were also found to be high in fish taken from the Lempake region (0.11 mg / kg). Although the levels are still lower than the maximum limit of contamination, this is dangerous if these fish are eaten by humans because they can experience accumulation in the body. When compared with mercury levels in the respondent's hair, it was found that in autism patients who live close to the mining area, Hg levels in their hair were higher, namely respondent number 3 (0.413 mg / kg), number 6 (0.575 mg / kg), number 18 (0.185) and number 19 (0.923 mg / kg).

Cadmium Levels

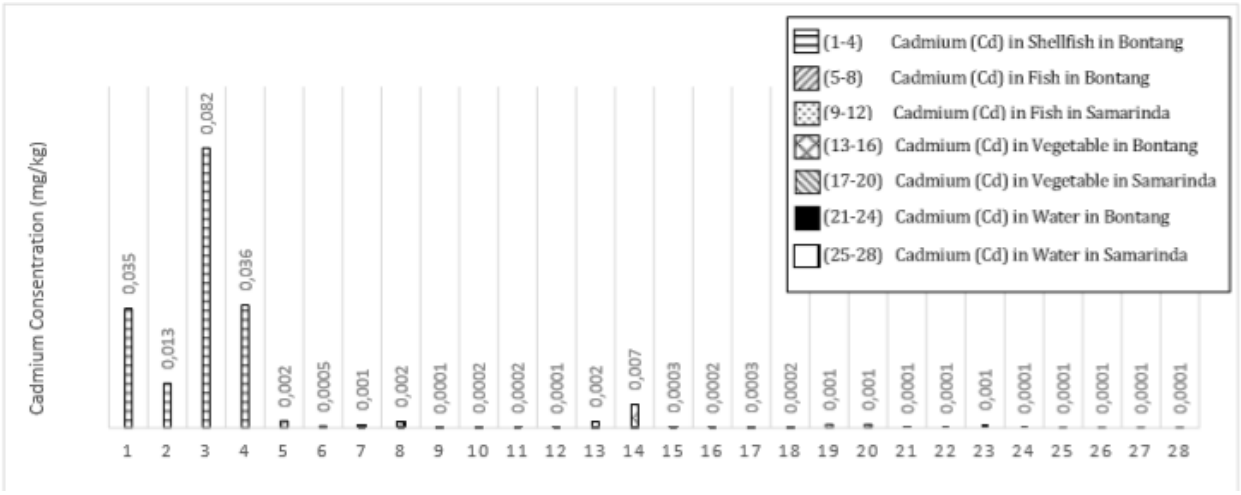

Figure 3: Consentratlon Of Cadminum (Cd) In Shellfish, Flsh, Vegetable And Water In Bontang And Samartnda

In figure number 3, Cadmium levels can be seen from all environmental samples. All samples showed low cadmium levels. Similarly, in measuring cadmium levels in hair samples, cadmium levels were also low. Measurements of cadmium levels were performed both on hair and on environmental samples because in previous studies, Akyuzlu and his colleagues [19] found a link between high levels of cadmium in hair and urine with the occurrence of autism. Research conducted in Ankara found that in patients with autism, the levels of lead found to be elevated, cadmium and arsenic in the hair, and high levels of lead and cadmium were also found in the urine. This is compared with non-autism control, which has low levels.

Lead Levels

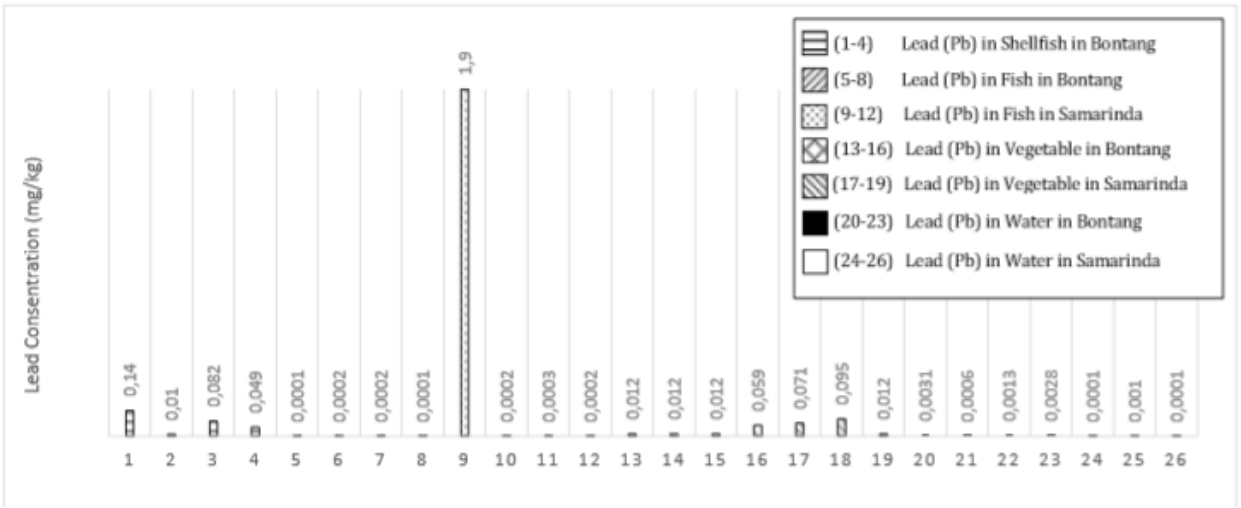

Figure 4: Consentration Of Timbal (Pb) In Shellfish. Fish, Vegetable And Water In tiontang And Sarttarinda

All environmental samples in Bontang have low lead levels . Whereas from three fish samples taken in Samarinda, the three low levels of samples were 0,0002; 0,0002; and 0,0002 mg / kg. While the sample taken

from Lempake was very high (1.9 mg / kg). The source of the high levels of Lead in fish in this region needs to be explored further, whether from coal mines or from other sources, but it is clear that four respondents who lived near this area (less than 2 km) had high lead levels namely respondent number 3 (0.307 mg / kg), number 6 (0.276 mg / kg), number 18 (0.873 mg / kg) and number 19 (1,701 mg / kg). How the effect of Lead levels on fish towards high levels of Lead in respondents' hair in Samarinda certainly requires further exploration. The high levels of Lead in autism sufferers in Bontang do not parallel to the levels of Lead in the samples (all samples of the environment are low). Where did the lead come from? Was it from the mothers? Or was it from food that contain Lead which were eaten by children or by their mothers during pregnancy? For example, from a study conducted by Ariansyah and his colleagues [27] by examining heavy metal contents in Kamplang crackers in Southern Indralaya District, Ogan Ilir Regency, it was found that Kamplang crackers originated from marine fish and were dried on the roadside had much higher levels of Pb compared with crackers originated from freshwater fish and dried in the village. Rahbar and his colleagues [16] also examined the connections between Lead levels and the occurrence of autism in children in Jamaica. It was found that the children who lived on highway sides had higher Lead levels in their blood and also those who ate Ackee fruit.

Soil Samples

Of all soil samples taken in the cities of Bontang and in Samarinda, the levels of heavy metals were lower than the maximum limit of contamination based on US Standards EPA number EPA/630/R-92/001.1992 (US EPA 1992) [28]. For example, the highest level of Arsenic found in Bontang was 10.3 mg/kg, while the contamination limit is 75 mg/kg. For mercury, the highest level found in Bontang was 5.18 mg/kg and the maximum limit contamination is 840 mg/kg. The highest grade cadmium found in Bontang was 0,107 mg/kg while the maximum contamination limit is 85 mg/kg, and the highest levels lead found in soil in Samarinda was 32,6 mg/kg and the maximum contamination limit is 420 mg/kg.

5. Conclusions

From the discussions above, it is concluded that from four types of heavy metals been discussed (Arsenic, Mercury, Cadmium and Lead), there are three of them which are high in hair, namely Arsenic, Mercury and Lead. While the Cadmium levels are low. When compared with Arsenic, Mercury and Lead levels in hair control, the levels are higher in autistic childrens'.

Bontang City's environment has been polluted by heavy metals. This can be seen from the levels of both Arsenic and Mercury in shells found in the waters near the fertilizer industry and near the natural gas industry. Similarly, the Arsenic and Hg levels in fish taken from the region are also high.

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Attachment 1

Statistical Analysis

1) To prove that heavy metals level in autistic children in Bontang is higher or than lower in Samarinda.

Table 1a: Average distribution and standard deviation of heavy metals level

Descriptive Statistics					
	N	*Mean*	*Std. Deviation*	*Minimum*	*Maximum*
Lead levels of respondents	80	.365488	.4135623	.0400	1.7530
Arsenic levels of respondents	80	.109775	.0513664	.0220	.2510
Cadmium levels of respondents	80	.027213	.0219474	.0020	.0880
Mercury levels of respondents	80	.491963	.5032819	.0500	3.1400
City of origin of respondents	80	1.50	.503	1	2

Table 1b: Distribution of the average rank of heavy metals according to the city of origin

Ranks				
	Respondents' city of origin	N	*Mean Rank*	*Sum of Ranks*
Lead levels of respondents	Bontang	40	27.68	1107.00
	Samarinda	40	53.33	2133.00
	Total	80		
Arsenic levels of respondents	Bontang	40	39.91	1596.50
	Samarinda	40	41.09	1643.50
	Total	80		
Cadmium levels of respondents	Bontang	40	32.84	1313.50
	Samarinda	40	48.16	1926.50
	Total	80		
Mercury levels of respondents	Bontang	40	32.06	1282.50
	Samarinda	40	48.94	1957.50
	Total	80		

The results of the normality test with *one sample* KS turned out to be abnormally distributed ($p<0.05$), then followed by a Mann Whitney difference test.

Table 1c: The results of the test of the degree of difference in concentration according to city

Test Statistics[a]				
	Lead levels of respondents	Arsenic levels of respondents	Cadmium levels of respondents	Mercury levels of respondents
Mann-Whitney U	287.000	776.500	493.500	462.500
Wilcoxon W	1107.000	1596.500	1313.500	1282.500
Z	-4.937	-.226	-2.957	-3.248
Asymp. Sig. (2-tailed)	.000	.821	.003	.001
a. Grouping Variable: Respondent's city of origin				

Conclusions there were differences in the mean values of the heavy metal levels (lead, cadmium and mercury with ($p < 0.05$), except arsenic ($p > 0.05$) in both cities (no difference arsenic level in Bontang and Samarinda).

Table 1 d: Distribution of average levels of heavy metals according to city of origin

Ranks				
	Respondents' city of origin	N	*Mean Rank*	*Sum of Ranks*
Lead levels of respondents	Bontang	40	27.68	1107.00
	Samarinda	40	53.33	2133.00
	Total	80		
Arsenic levels of respondents	Bontang	40	39.91	1596.50
	Samarinda	40	41.09	1643.50
	Total	80		
Cadmium levels of respondents	Bontang	40	32.84	1313.50
	Samarinda	40	48.16	1926.50
	Total	80		
Mercury levels of respondents	Bontang	40	32.06	1282.50
	Samarinda	40	48.94	1957.50
	Total	80		

Table 1 d above can prove that the heavy metals level of autism children in Samarinda is higher than in Bontang (Mean Rank is higher).

2) To prove that the heavy metals level in autism children in Bontang and in Samarinda is higher than healthy children (non autism).

Table 2 a: Distribution of the average value of the level of the heavy metals according to the diagnosis of autism.

Ranks				
	DIAGNOSIS	N	*Mean Rank*	*Sum of Ranks*
Lead levels of respondents	Normal Healthy	20	25.85	517.00
	Autism sufferers	60	45.38	2723.00
	Total	80		
Arsenic levels of respondents	Normal Healthy	20	16.85	337.00
	Autism sufferers	60	48.38	2903.00
	Total	80		
Cadmium levels of respondents	Normal Healthy	20	37.68	753.50
	Autism sufferers	60	41.44	2486.50
	Total	80		
Mercury levels of respondents	Normal Healthy	20	16.25	325.00
	Autism sufferers	60	48.58	2915.00
	Total	80		

Table 2 a above can prove that the average value of heavy metals level in autistic children is higher than in normal children.

Table 2 b: Test results for different heavy metals level according to the diagnosis

Test Statistics[a]				
	Lead levels of respondents	Arsenic levels of respondents	Cadmium levels of respondents	Mercury levels of respondents
Mann-Whitney U	307.000	127.000	543.500	115.000
Wilcoxon W	517.000	337.000	753.500	325.000
Z	-3.256	-5.257	-.629	-5.389
Asymp. Sig. (2-Tailed)	.001	.000	.529	.000
A. Grouping Variable: Diagnosis				

The conclusion that can be drawn from the results of the different tests in Table 2 b above is that there are differences in the average level of the heavy metals based on patient status for all heavy metals (lead, arsenic and mercury) except cadmium. (no difference cadmium level between autism children and normal children in Bontang and Samarinda).

3) Heavy metal in environment.

Table 3 a: Results of measurements of heavy metal levels in vegetables in Bontang and Samarinda

No	Sampling Locations	Sample Identity	Arsenic Level (mg/Kg)	Mercury Level (mg/Kg)	Cadmium Level (mg/Kg)	Lead Level (mg/Kg)
1	Bontang (Kel. Gunung Elai) (N 00^0 08' 00,0'', E 117^0 28' 30,1'')	mustard	0,006	0,0010	0,0028	≤ 0,0128
2	Bontang (Kel. Satimpo) (N 00^0 07' 36,1'' E 117^0 28' 20,7'')	mustard	0,003	0,0004	0,0074	≤ 0,0128
3	Bontang (Kel. Kanaan) (N 00^0 07' 51.7" E 117^0 27' 49,9")	water spinach	0,018	0,0124	≤ 0,0008	≤ 0,0128
4	Bontang (Kel. Guntung) (N 00^0 11' 22.1" E 117^0 28' 46,0")	water spinach	0,060	0,0012	≤ 0,0008	0,06
1	Samarinda (Lempake) (S 00^0 27' 00.8" E 117^0 11' 28,4")	mustard	0,006	0,0006	≤ 0,0008	0,072
2	Samarinda (Sempaja) (S 00^0 27' 26,7" E 117^0 10' 11,4")	water spinach	0,007	0,0007	0,0015	0,0955
3	Samarinda (Sempaja) (S 00^0 27' 26,7" E 117^0 10' 11,4")	mustard	0,006	0,0010	0,0013	≤ 0,0128
Maximum Contamination Limit			**1 ***	**0,03 ***	**0,2**	**0,5**

Table 3 b: Heavy metals level in the environment (samples of fish, vegetables, water and soil) in Samarinda > normal. There are differences in the levels of heavy metals between Bontang and Samarinda in environmental samples in the form of vegetables. (p <0.001)

Single Table Analysis

	A	M	C	T	TTL
Bon	193	13	30	400	636
Sam	63	8	12	600	683
	256	21	42	1000	1319

Chi Square for R by C Table

Chi Square=	113.4
Degrees of Freedom=	3
p-value=	<0.0000001

Cochran recommends accepting the chi square if:
1. No more than 20% of cells have expected < 5.
2. No cell has an expected value < 1.

In this table:
None of 8 cells have expected values < 5.
No cells have expected values < 1.

Using these criteria, this chi square can be accepted.

Expected value = row total*column total/grand total

Rosner, B. Fundamentals of Biostatistics. 5th ed. Duxbury Thompson Learning. 2000; p. 395

Table 3 c: Heavy metal levels in the environment in Bontang > Samarinda.

No	Sampling Location	Sample Identity	Arsenic Level (mg/Kg)	Mercury Level (mg/Kg)	Cadmium Level (mg/Kg)	Lead Level (mg/Kg)
1	Bontang (Kel. Guntung)	TB-1,2	8,45	4,20	0,0391	27,2
2	Bontang (Kel. Tanjung Laut)	TP-1,2,3	10,3	5,18	0,107	26,09
1	Samarinda (Sungai Mahakam)	TA.1	8,19	3,20	0,0194	30,7
2	Samarinda (Sempaja)	TA.2	9,08	3,45	0,0134	29,8
3	Samarinda (Lempake)	TA.3	2,67	2,003	≤ 0,0008	32,6
THRESHOLD LIMIT OF HEAVY METALS APPLIED ON SOIL			75	840	85	420

The tiered analysis results with Fisher's exact test had no difference ($p > 0.05$)