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The Study of the Relationship between the Levels Of Heavy Metals in the Environment with the Incidens Of Autism in Bantul, Yogyakarta Province

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Abstract
Currently, the environment quality in Indonesia is declining do to naturally process (anthropogenic) such as disaster, volcanic eruption, earthquake and tsunami, or manmade (floods, landslides) associated with deforestation including industrialization, mining, agriculture and plantation. The pollutant, mainly the heavy metals (As,Hg,Cd,Pb) can affect the health of human, particularly if the level exceed the maximum contamination limit. The disease manifestation due to those metal in the human body depend on the chemical reaction in the organ. One of the disease allegedly related to environmental factors is autism.
This study was conducted to look the environmental cause of autism especially from heavy metals Arsenic (As), Mercury (Hg), Cadmium (Cd) and Lead (Pb) by measuring heavy metals levels in fish, vegetables, water, soil and heavy metals levels in hair of autism sufferers and control in Bantul Regency. A case-control study design was conducted by sampling fish, vegetables, water, soil and hair on September 18, 2017. Fish and vegetables were taken from 3 location, water and soil were taken from 4 location. While hair samples were taken from 30 autisim children and 10 non autism control. All of these sample were measured for heavy metals at The Laboratory (As and Hg by Neutron Activation analysis, while Cd and Pb by Atomic Absorbtion Spectrometry method).
Laboratory analysis was found the heavy metals with high levels and even axceeding the limit of contamination were mercury in fish, Mercury in vegetables and Lead in waters. While the hair was found to have heavy metals levels in the four types of heavy metals : Arsenic, Mercury, Cadmium and Lead.By statistical analysis, it was found that heavy metals (As, Hg, Pb) in autistic sufferers hair were higher than controls's, while Cd hair levels were equal between two groups. Is there any direct correlation between heavy metals in environment and heavy metal levels in autism hair, and how the heavy metals entered the children's bodies so could be detected in the hair, further research still needs?

Keywords: Autism, Environment, Heavy Metals

Introduction
Current environmental quality in Indonesia is declining due to disasters, naturally (volcanic eruptions, earthquakes, and tsunami) or man-made (floods and landslides associated with deforestation) including industrialization and mining. Likewise, the application of various chemicals in agriculture and plantations could further pollute the environment. The pollutant would lead to health problems, particularly if the levels exceed the maximum limit tolerable by the human body. The heavy metal contaminants enter the human body through air, food, and water, primarily within the food chain. The disease manifestations due to those metal in the human body depend on the chemical reactions in the organ. The United States Department of Health Centre concern on three types of heavy metals: arsenic (As), lead (Pb) and mercury (Hg) regarding their nature on inducing neurological disorders in the form of Attention Deficit Hyperactivity Disorders (ADHD), low IQ, and Autism Spectrum Disorders (ASD) even in low level (Mousavinejad 2017).[1] In this study, we measure the levels of four types of heavy metals in the environment (As, Hg, Cd, and Pb) to look for correlation to the

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incidence of autism. One can use blood, plasma, urine, or head hair samples to measure the level (Evrenoglou 2017).[2] after some considerations, this study used hair due to easiness in taking, collecting, storing, and observation of the samples.

Autism is a neurologic disorder (Pervasive development disorders) in children characterized by qualitative impairments in social interaction, verbal and nonverbal communication,and restricted repetitive and stereotyped pattern of behavior, interest and activities (Papandrew 2014 ; Irianto 2015).[3,4] The number of people with autism is increasing. Based on data from the Autism Research Institute, in 1987 in the United State only 1 patient with autism was found in every 5,000 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 (Geier *et al*. 2013).[5] Similarly Nationally, the number of people with autism has increased rapidly. In 1990 there were only 2-5 cases/10,000 births, increasing to 20/10,000 births (Anies 2015).[6] This increase was caused not only by the increasing number of cases, but also by improved diagnostic mothods, 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 disadvantages families (Kim *et al*. 2011).[7]

Untill now the cause of this disease has not been confirmed. Farida *et al.* (2015)[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 post natal). But of all these factors, the environmental and genetic factors are considered the most influential. Thus, the threat to the occurence of autism in the future increasingly because the increasing severity of the environmental damage due to the pollution including by heavy metals that comes from nature (anthropogenic), industry and agriculture (Akyuzlu *et al***.** 2014).[9] In this study, the determination of heavy metals in the hair of autism sufferers and controls were carried out in Bantul. In addition, heavy metal content in some environmental samples such as 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 occurance of autism and wether there were differences in levels of heavy metals from both autism and control group.

AIMS

The main objective of this study is to prove the relationship between heavy metal exposure in the environment and the occurence of autisme in Bantul regency. While the specific purpose is to prove the presence of heavy metal content (arsenic, mercury, cadmium and lead) in the environment (fish, vegetables, water and soil) whose levels exceed the maximum limit contamination, and prove the existence of heavy metal content in hair of people with autism in Bantul whose level exceed the maximum limit of contamination and exceed the levels of heavy metals in control hair.

Methods

This study was carried out with a case control study design by comparing the levels of heavy metals in environmental samples (fish, vegetables, water and soil) and levels of heavy metals in autism and control non autism hair. This research was conducted in Bantul Regency. Bantul is the Regency in Yogyakarta Province. The width of area 508, 1 km. The population are 947.568 2014. The north of this regency bordered by Yogyakarta City and Sleman regency. East bordered by Gunung Kidul regency and South are bordered with Hindian Ocean and west bordered by Kulonprogo regency. Geographical location between 07^0 $44^{'}$ $04^{''}$ South latitude and 110^0 $12^{'}$ $34^{''}$ West longitude. This regency has 17 subdistricts and 75 villages.

This reseach was carried out through several stages as follows:

First step; Submission for the issuance of Ethical Approval to Human Research Ethics Commitee 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 spesial education school (SLB) regarding the palanned implementation of this study. To the SLB principals, we asked for help to be fasilitated so that we could meet with parents of autism 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 September 18th 2018 in Bantul.

Third; Meeting with parents of people with autism who attended SLB. Through the SLB Bina Anggita Principals, we invited parents of autism childrens whose children will be included in this study. During the meeting we explain 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 contributed to humanitarian field. Hopefully, by knowing the cause of autism, the number of autism sufferer can be reduce in the future. After the meeting, all parents agreed that their children would be included as respondents in the study. Some of them even offered to have their normal children included as controls. After the meeting, all parents fill out the list of question that have been prepared, followed by signing the permit (informed consent).

Fourth; Environment samples (fish, vegetables, water and soil) was carried out on September 18, 2018. Samples taken from several places in Bantul so that they could represent environmental condition in all parts of the city. Fish samples were taken from Banguntapan region, Kampung Sawo Jetis (Sawo Jetis village) and region of Singosaren Imogiri. Vegetables samples were taken from Banguntapan area, Sawo Jetis village, Singosaren-Imogiri area and Bantul-Yogya area. Water samples are taken from well water in Banguntapan, well water on Jalan Parangtritis Km 12,5 and well water from Rejowinangun Dusun Pilahan. Soil samples taken from the paddy fields in Banguntapan and paddy fields on Jalan Parangtritis Km. 12,3.

Samples of fish and vegetables were taken each 1 kg. Samples inserted into plastic ziplock and then put into the cooler box which has been equipped with ice gel. The water samples were taken as much as 1 liter, then put into the plastic bottles. The samples were preserved by cooling. Soil samples were taken by the composite method in which samples were taken at three different points, and then samples from these three points were homogenously combined to reach 1 kg in weight. Samples were packed in ziplock plastic. All samples on the outside were labeled about the type of sample and the coordinates of sampling point.

Hair samples were taken from 30 children with autism consisted of 20 boys and 10 girls with the age range between 5,2 – 15,8 years, and 10 controls consisted of 7 boys and 3 girls with age range between 9 – 14,2 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). Before sampling, a selection of children will be included in the research, especially by looking at the domicile address of both the sufferers and controls, so that it could be represent the condition of the entire research area. Sampling hair done in Special School (SLB) Bina Anggita, SLB Qothrunada, SLB Dharma Bhakti and SLB Marsudi Putra III. Hair picking was done by a professional barber so that after the hair is taken, the child's hair remains tidy. Hair samples were taken in the occipital region up to 5 grams. Hair samples were then put into an envelope and labeled on the outside, which contains the name, adress, gender, and age.

Fifth; delivering all samples to Bandung, to the Laboratory of Applied Science and Technology Center on Jalan Taman Sari No. 71. Here the measurments heavy metals (Arsenic, Mercury, Cadmium and Lead) levels were done, both hair samples and environmental samples with Absorpsion Spectrometry methods for cadmium and lead, and Neutron Activation Analysis for arsenic and mercury.

Result and Discussion

Result.

All samples measured levels of heavy metals with the two methods. First, Activation Neutron Analysis methods for arsen (As) and merkuri (Hg). Second, Atomic Absorbsion Spektrometri Method for cadmium (Cd) and lead (Pb). The result of measurements of heavy metal levels in environmental samples (fish, vegetables, water and soil) can be seen in tables 1- 4, While the result of measurements of heavy metal levels in hair of autism sufferer and controls non autism can be seen in tables 5 – 6.

Tabel 1: Concentration of Heavy Metal (As,Hg,Cd,Pb) in Fish in Bantul.

NO.	Sampling Location	Coordinate	ARSEN (As) mg/kg	MERKURI (Hg) mg/kg	KADMIUM (Cd) mg/kg	TIMBAL (Pb) mg/kg
1.	Banguntapan	S 07° 51' 06,6" E 110° 23' 41,1"	0,013	0,295	≤ 0,0008	≤ 0,0128
2.	Jetis	S 07° 54' 39,1" E 110° 22' 07,3"	0,026	0,272	≤ 0,0008	≤ 0,0128
3.	Singosaren	S 07° 54' 22,1" E 110° 23' 09,6"	0,029	0,415	≤ 0,0008	0,045
Maximum Limit			0,25	0,06	0,10	0,20

Table 2: Concentration of Heavy Metal (As, Hg, Cd, Pb) in Vegetables in Bantul

NO.	SAMPLING LOCATION	COORDINATE	ARSEN (As) mg/kg	MERKURI (Hg) mg/kg	KADMIUM (Cd) mg/kg	TIMBAL (Pb) mg/kg
1.	Border Bantul-Jogja	S 07° 48' 44,9" E 110° 23' 56,7"	0,031	0,1628	0,0026	0,29
2.	Banguntapan	S 07° 54' 09,6" E 110° 23' 46,1"	0,002	0,248	0,0011	0,40
3.	Jetis	S 07° 54' 45,0" E 110° 22' 09,8"	<0,003	0,378	≤ 0,0008	0,07
4.	Singosaren	S 07° 54' 35,2" E 110° 23' 14,7"	0,005	0,17	≤ 0,0008	0,065
Maximum Limit			0,15	0,03	0,05	0,20

Table 3: Concentration of Heavy Metal (As,Hg,Cd,Pb) in Water in Bantul

NO.	SAMPLING LOCATION AND COORDINATE	ARSEN (As) mg/kg	MERKURI (Hg) mg/kg	KADMIUM (Cd) mg/kg	TIMBAL (Pb) mg/kg
1.	Banguntapan S 07° 47' 47,5" E 110° 24' 27,1"	≤ 0,007	0,0007	≤ 0,00004	0,02
2.	Parangtritis S 07° 54' 25" E 110° 21' 09,7"	≤ 0,007	0,0008	0,00005	0,0009
3.	Pilahan S 07° 48' 50,0" E 110° 24' 01,5"	≤ 0,007	0,0007	0,00009	0,0007
Maximum Limit		0,01	0,001	0,003	0,01

Table 4: Concentration of Heavy Metal (As,Hg,Cd,Pb) in Soil in Bantul

NO.	SAMPLING LOCATION	ARSEN (As) mg/kg	MERKURI (Hg) mg/kg	KADMIUM (Cd) mg/kg	TIMBAL (Pb) mg/kg
1.	Banguntapan	2,16	1,90	0,1192	33,2
2.	Parang Tritis	2,34	0,94	0,0612	27,8
Maximum Limit		75	840	85	420

Table 5: Consentration of Heavy Metals (As, Hg, Cd, Pb) in Hair of Children with Autism In Bantul.

No	Sex	Age (Year and Month)	Arsen (As) (mg/kg)	Merkuri (Hg) (mg/kg)	Kadmium (Cd) (mg/kg)	Timbal (Pb) (mg/kg)
1	Male	13,5	0,136	0,217	0,007	0,128
2	Male	7,5	0,058	0,359	0,040	0,382
3	Male	14,0	0,150	0,110	0,069	0,763
4	Female	9,2	0,032	0,207	0,020	0,440
5	Male	8,1	0,060	1,177	0,090	0,584
6	Female	8,7	0,034	0,204	0,026	1,713
7	Male	12,2	0,049	0,175	0,007	0,085
8	Male	9,0	0,029	0,221	0,007	0,224
9	Male	15,6	0,041	0,157	0,012	0,156
10	Female	14	0,078	0,397	0,042	1,290
11	Female	13,6	0,013	0,227	0,105	0,714
12	Male	16,0	0,052	1,167	0,007	0.808
13	Female	15,0	0,051	0,267	0,015	1,408
14	Male	15,8	0,061	0,152	0,014	1,083
15	Female	9,0	0,052	0,228	0,007	0,071
16	Male	9,5	0,050	0,374	0,048	0,255
17	Male	7,2	0,073	0,397	0,030	0,144
18	Male	11,0	0,103	0,500	0,037	0,320
19	Male	6,5	0,063	1,067	0,015	0,995
20	Female	6,0	0,066	0,600	0,031	0,195
21	Male	15,2	0,097	0,157	0,032	0,663
22	Male	14,0	0,043	0,138	0,141	0,969
23	Male	16,0	0,039	0,187	0,090	2,879
24	Male	9,2	0,138	0,731	0,017	0,189
25	Female	15,0	0,125	0,125	0,047	0,404
26	Female	14,0	0,048	0,107	0,013	0,475
27	Female	9,2	0,063	0,138	0,011	0,206
28	Male	5,2	0,417	0,015	0,050	0,563
29	Male	14,2	0,106	0,203	0,034	0,765
30	Male	10,0	2,191	0,185	0,050	0,135

Table 6: Consentration of Heavy Metal (As, Hg, Cd, Pb) in hair of control non autism in Bantul

No.	Sex	Age (Year and Month)	Arsen (As) mg/kg	Merkuri (Hg) mg/kg	Kadmium (Cd) mg/kg	Timbal (Pb) mg/kg
1.	Male	14,2	0,088	0,303	0,026	0,191
2.	Female	12	0,083	0,136	0,092	0,174
3.	Male	9	0,043	0,227	0,007	0,194
4.	Male	11	0,067	0,189	0,007	0,124
5.	Male	11,5	0,053	0,127	0,015	0,169
6.	Female	10	0,069	0,198	0,029	0,129
7.	Male	12,6	0,065	0,280	0,023	0,195
8.	Female	9,2	0,047	0,260	0,015	0,190
9.	Male	13	0,079	0,201	0,026	0,173
10.	Male	13,5	0,075	0,227	0,007	0,169

Discussion

Heavy metals such as arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) are a heavy metal that is not required by the body at all, and are toxic even small amounts (Hassanien 2011).[10] The children can be chronically exposure through the food chain for example through the fish, vegetables and drinking water. Those heavy metals have ability to bioaccumulate in the body and causing various diseases because they act as systemic toxin with specific effect such as influence behavior and impair mental and neurologic function via influencing neurotransmitter production and utilization (Schwartz

2010).[11]
Table 1 shows that the levels of heavy metals in fish that exceed the maximum limit of contamination based on the Head of BPOM regulation of The Indonesian Ministry of Health Number 23 year 2017[14] are mercury (Hg), where three fish samples with level (0,295 mg/kg, 0,275 mg/kg dan 0,415 mg/kg) all exceed the maximum limit of contamination (0, 06 mg/kg). It is dangerous if humans consume this fish, because they can experience accumulation in the body and will edanger health. The longer the fish lives in waters, its mercury levels will be higher because it will undergo a process of bioaccumulation and biomagnification in its body. So that the levels can reach a higher level and more dangerous for the life of aquatic animals and the health of humans who eat it (Mallongi 2017).[12] In waters, the activity of micro-organism will covert mercury into components of methyl mercury (CH3-Hg) which has toxic properties and strong binding capacity, especially in the bodies of aquatic animals. The speed of uptake of mercury by aquatic organism takes place faster than the excretion process, so that the mercury will accumulate if the up take place continously (Mallongi 2017)**.**[12] From where the mercury in fish come from ?. Because in Bantul there are no activities of the coal mining or gold mininng, allegedly derived from the air due to volcanic eruptions or burning coal for electricity generation.

Suyanto *et al*. (2010)[13] conducted their study in three centers in Central Java: Semarang, Tegal, and Pati. They observed that in two regions (Pati and Semarang), the heavy metal levels were similar to this study finding where the Hg level in fish exceeded the maximum limit of contamination. Nevertheless, in comparison, we should take note that this study used freshwater fish while Suyanto *et al.* used samples from fish-farming and Estuaries. The level was analyzed using the Atomic Absorption Spectroscopy method. The results showed that fish taken from Pati and Semarang had high Hg levels (0.08-0.12 mg/kg) exceeding the maximum limit based on the Decree of the Directorate General of Drug and Food Control (Ditjen POM) Republic of Indonesia (RI) Number 03725/B/SK/VII/89. Other heavy metals such as Pb, Cu, Zn, Cd, and as had contamination levels below the maximum limit. This data implicated that Hg contamination had occurred over a wide area.

The second environmental samples are vegetables. In table 2 it can be seen that in the vegetables samples, there were two heavy metal level exceeded than the maximum contamination limit is mercury (Hg) and lead (pb). Mercury levels are high in all samples and exceeded than the maximum contamination (0,03 mg/kg), while lead levels, of the four samples, only two of the levels exceed the maximum limit of contamination. This indicates that there are sources of Hg pollution around the area. The source of mercury are may be the same as the one that contaminating fishes. While Lead (Pb) from four samples, there are two sample that exceeds maximum limit of contamination (0.5 mg / kg), ie vegetables taken from Banguntapan (0.40 mg / kg) and from the border region of Bantul-Jogya (0, 3 mg / kg). Where does this Lead come from? Maybe from home industry activity, such as battery fusion? This require more research in the future.

The third environmental samples that measured heavy metal levels is water. The result can be seen in table 3. From this data it is seen that only one sample with lead (Pb) level exceeded the maximum contamination (0, 02 mg/kg), while the other heavy metals levels (mercury, cadmium dan lead) lower than the maximum limit of contamination. This is sample taken from Banguntapan area. If matched up with the other samples, it turn out that samples of vagetables taken from the region's, level of lead also high. It is very possible that this is related, because the water sources that contain lead are used to water the vegetables so the vegetables get high levels of lead.

The next samples is soil. In table 4 it can be seen that from two soil samples taken from Banguntapan area and Parangtritis area, the levels of heavy metals were lower than the maximum limit of contamination based on US Standards EPA 1992.[15]

Arsenic (As) Levels

In table 5, we can see the result of measurement of heavy metal in the hair of autism children in Bantul, while in table 6 are the heavy metal content in the hair of controls non autisme children. The highest arsenic (As) level in autism patients' hair was found in subject number 30 (2,191 mg/kg) followed by number 28 (0,417 mg/kg). When viewed from the domicile factor, both have a place to live close together. But why are arsenic level different? This may be influenced by the length of heavy metal exposure, because subject number 30 is 10 years old, while number 28 still 5 years 2 month. To prove whether there are differences in average arsenic (As) levels in the hair of autism children with controls non autism, statistical analysis were done.[16]

Table 7 showed that the mean Arsenic level of people with autism in Bantul was 0.122 mg/kg. The lowest level in people with autism is 0.066 mg/kg while the highest of 0.218 mg/kg. Furthermore, the mean Arsenic level in the hairs of the control group was 0.067 mg/kg. The lowest level in the control group was 0.038 mg/kg while the highest was 0.093 mg/kg.

Table 7. The hair arsenic levels on autism children and non-autism controls in Bontang.

Statistics	People on autism spectrum disorder	Control group
Total samples	30	10
Mean (mg/kg)	0.122	0.067
Median (mg/kg)	0.109	0.068
Standard deviation (mg/kg)	0.044	0.019
Minimum (mg/kg)	0.066	0.038
Maximum (mg/kg)	0.218	0.093

Furthermore, we analyzed the data to look for statistical significance in the difference of mean hair Arsenic level between autism children and control group using the parametric test (t-test) or non-parametric test (Mann-Whitney test). The t-test requires if normal-distributed data, while Mann-Whitney Test can be used in the non-normally

distributed data.
To find out if the data spread normally, the Kolmogorov Smirnov test was performed. The result it turn out data from children with autism sufferer and control hairs are normally distributed**.** As presented in Figure 2, the data were normally distributed ($p > 0.05$).

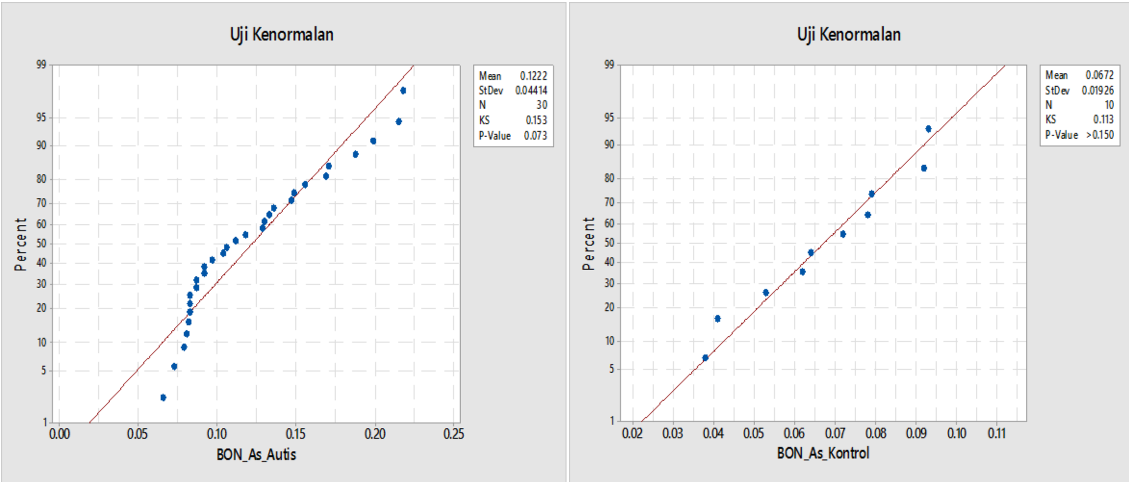

Fig. 2: Probability plot to the normality test of arsenic levels in the hair of (a) autism children and (b) non-autism control.

Regarding normally distributed data, we used the t-test to evaluate the significance of the difference between the two groups. The Ho hypothesis was that the mean hair Arsenic (As) level in autism children was not greater than non-autistic control (Ho: $\mu A < \mu B$). The H1 hypothesis was that the mean hair Arsenic (As) level in autism children was greater than non-autistic control (H1: $\mu A > \mu B$). We rejected Ho if p-value < α.
The t-test resulted in $\alpha = 0.05$ indicated that this study rejected Ho (and accepted the H1 hypothesis). The hair Arsenic level of people on autism spectrum disorder in Bantul was significantly higher than non-autism control. In summary, the arsenic levels affected the occurrence of autism in Bantul Regency.

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Two-sample T for BON_As_Autis vs BON_As_Kontrol

                  N    Mean   StDev  SE Mean
BON_As_Autis     30  0.1222  0.0441   0.0081
BON_As_Kontrol   10  0.0672  0.0193   0.0061

Difference = μ (BON_As_Autis) - μ (BON_As_Kontrol)
Estimate for difference:  0.0550
95% lower bound for difference:  0.0379
T-Test of difference = 0 (vs >): T-Value = 5.44  P-Value = 0.000  DF = 34
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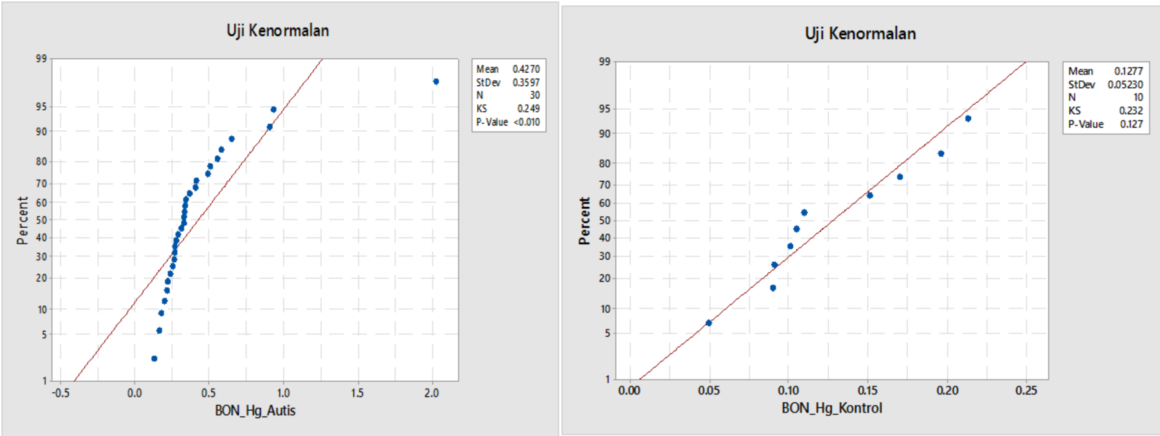
Fig. 3: The t-test result of hair arsenic levels of people on autism spectrum disorder and non-autism control in Bantul.

Mercury (Hg) Level.
Table 5 presented the levels of mercury in the hair of children with autism, whereas in Table 6 are the levels of mercury in children's hair control non autism. In this study included 30 (thirty) children on autism sufferer and 10 (ten) non-autism control to assess whether there was a significant difference in the level of Mercury between the two groups.Table 8 showed the statistical of mercury level on autism and control non autism children in Bantul.

Table 8. The hair mercury levels on autism children and non-autism control in Bantul.

Statistic	People on autism spectrum disorder	Control group
Total samples	30	10
Mean (mg/kg)	0.427	0.128
Median (mg/kg)	0.330	0.108
Standard deviation (mg/kg)	0.360	0.052
Minimum (mg/kg)	0.130	0.050
Maximum (mg/kg)	2.029	0.213

This study also evaluated the significance of the difference of the mean hair mercury level between two groups, preceeded by the normality test using the Kolmogorov-Smirnov test. The result showed non-normally distributed data regarding the p-value (< 0.05) on the autism group, while the data of hair mercury level in non-autism control distributed normally ($p > 0.05$), as presented in Figure 5.



a b

Figure 5. The normality test for the hair mercury level in (a) autism children and (b) non-autism control.

Because one of the data groups was not normally distributed, this study used the Mann-Whitney test to assess the significance of the difference in hair mercury levels between the two groups. It resulted in α = 0.05 implicating that the mean hair Mercury level in people on autism spectrum disorder in Bantul was higher than non-autism control. In conclusion, the mercury levels affected the occurrence of autism in Bantul. When associated with measuring mercury levels in environmental samples, mercury levels are quite high in fish samples (0,295 mg/kg, 0,272 mg/kg, and 0,415 mg/kg). Similarly with the vegetables samples, mercury levels were also quite high (0,378 mg/kg, 0,248 mg/kgand 0,17 mg/kg). Is there an effect of mercury levels in fish and vegetables on high levels of mercury in the hair? Maybe that is because the domicile of people with autism is not far from the sampling location. But how do heavy metals enters the body of a child with autism, still need further research, does this child often eat contaminated fish and vegetables? Or is the mother when pregnant often eating fish and vegetables with high mercury (Hg) levels?.

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Mann-Whitney Test and CI: BAN_Hg_Autis; BAN_Hg_Kontrol

                  N  Median
BON_Hg_Autis     30  0.3300
BON_Hg_Kontrol   10  0.1075


Point estimate for η1 - η2 is 0.1990
95.3 Percent CI for η1 - η2 is (0.1270;0.3050)
W = 755.0
Test of η1 = η2 vs η1 > η2 is significant at 0.0000
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Fig. 6: The Mann-Whitney test result of hair mercury levels of people on autism children and non-autism control in Bantul.

From previous research, Mohamed *et al.* (2014)[17] have studied children with autism in Egypt and also found an elevation of three types of heavy metals in autism sufferer's hair, namely mercury (Hg), lead (Pb) and aluminium (Al), when compared to level in control hair. The three types of heavy metals are positively related to fish consumption factor in the mother, also associated with the location of recidence or recidence near the fueling station and the use of aluminium pans. Whereas Filon (2017)[18] found a link between autism and epigenetic changes due to environmental factors, including toxic metal Hg. Moreover, a study conducted by Aizar (2014)[19] by taking the hair sample of 17 children with autism and 17 normal children in North Sumatra found the same thing with this research namely Hg levels in autistic subjects (1,82 ppm ± 0,52) significantly higher than normal children (0,91 ppm ± 0,68)) (p<0,05).

Cadmium (Cd) Levels

This study included 30 (thirty) people on autism children and 10 (ten) non-autism control to assess whether there were any differences in the levels of cadmium between the two groups. Table 9 showed the statistical of cadmium level in autism children.

Table 9. The hair cadmium levels of people on autism children and non-autism control in Bantul.

Statistic	People on autism spectrum disorder	Control group
Total samples	30	10
Mean (mg/kg)	0.020	0.020
Median (mg/kg)	0.012	0.018
Standard deviation (mg/kg)	0.019	0.013
Minimum (mg/kg)	0.007	0.002
Maximum (mg/kg)	0.072	0.040

Furthermore, this study used the Kolmogorov-Smirnov test to assess the data normality. The normality test results revealed that the hair cadmium levels of autism hair children were not normally-distributed shown as p-value < 0.05, while the hair cadmium level data in non-autism control distributed normally shown as p-value > 0.05, as presented in Figure 8.

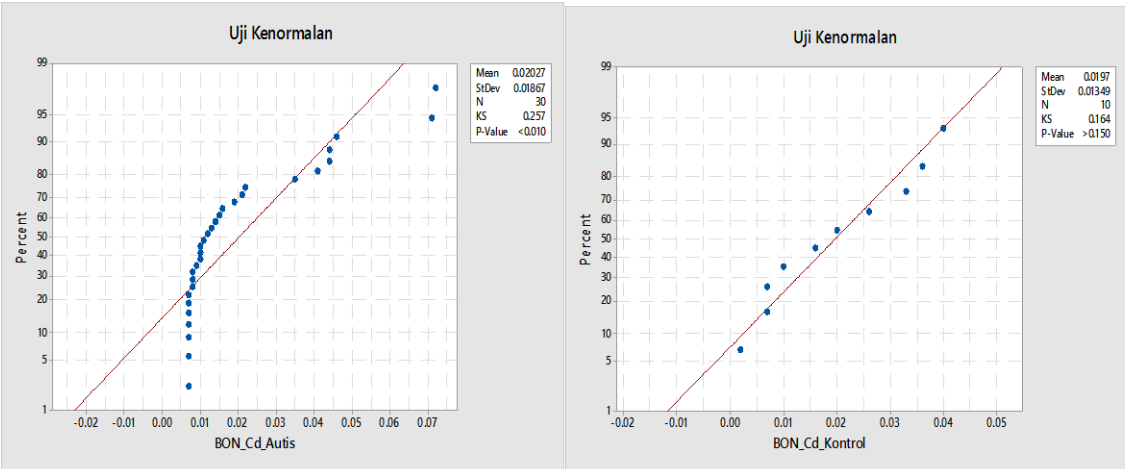

Fig. 8: The normality test for the hair cadmium level in (a) autism children and (b) non-autism control

As one of the data groups was not normally-distributed, this study used the Mann-Whitney test to assess the significance of the difference in hair cadmium levels between the two groups. It resulted in α ≥ 0.05 implicating that the mean hair cadmium level in people on autism spectrum disorder in Bantul was similar to non-autism control. In conclusion, cadmium levels did not affect the occurrence of autism in Bantul.

Mann-Whitney Test and CI: BON_Cd_Autis; BON_Cd_Kontrol

```
                 N   Median
BON_Cd_Autis    30  0.01150
BON_Cd_Kontrol  10  0.01800


Point estimate for η1 - η2 is -0.00000
95.3 Percent CI for η1 - η2 is (-0.01301;0.00800)
W = 611.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.9129
The test is significant at 0.9124 (adjusted for ties)
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Fig. 9: The Mann-Whitney test result of hair cadmium levels on autism children and non-autism control in Bantul.

There is no difference between the levels of cadmium in autism and control hair in statistical analysis. The inclusion of cadmium (Cd) as one of the heavy metals whose levels are measured because in other studies, for example, reasearch from Lee *et al.* (2018)[20] in Taiwan shows that cadmium (Cd) affect the abnormalities in nerve growth, including autism and attention deficit hyperactivity disorder (ADHD).

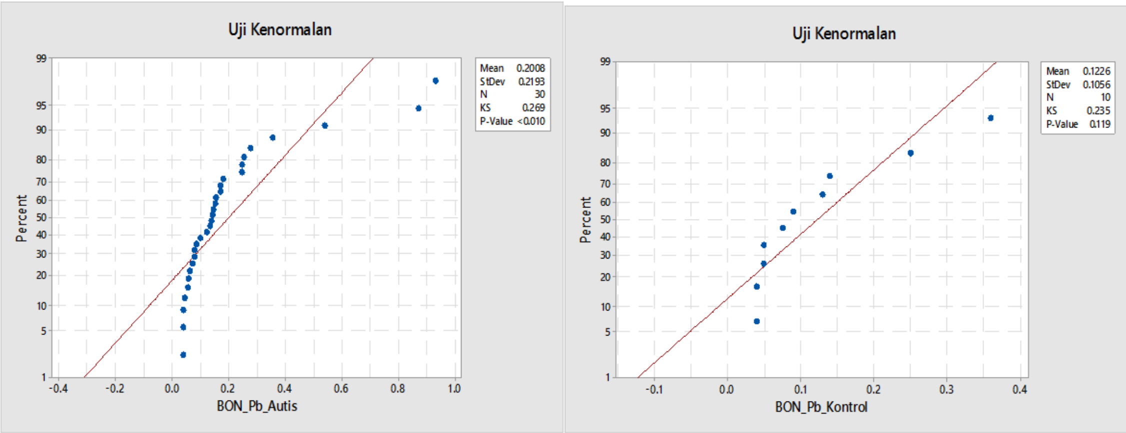
Lead (Pb) Levels

This study included 30 (thirty) people on autism spectrum disorder and 10 (ten) non-autism control to assess whether there were any differences in the levels of lead between the two groups. Table 10 shows the statistical data of lead (Pb) levels in hair of both groups.

Table 10: The hair lead levels of autism children and non-autism control in Bantul.

Statistic	People on autism spectrum disorder	Control group
Total samples	30	10
Mean (mg/kg)	0.201	0.123
Median (mg/kg)	0.142	0.083
Standard deviation (mg/kg)	0.219	0.106
Minimum (mg/kg)	0.040	0.040
Maximum (mg/kg)	0.932	0.360

Furthermore, this study used the Kolmogorov Smirnov test to **assess** the data normality**.** The result showed non-normally distributed data regarding the p-value (< 0.05) on the autism group, while the data of hair lead level in non-autism control distributed normally (p > 0.05), as presented in Figure 11.



(a) (b)

Fig. 11: The normality test for the hair lead level in (a) autism children and (b) non-autism control.

Because one of the data groups was not normally distributed, this study used the Mann-Whitney test to assess the significance of the difference in hair lead levels between the two groups. It resulted in α = 0.05 implicating that the mean hair lead level in people on autism spectrum disorder in Bantul was higher than non-autism control.

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Mann-Whitney Test and CI: BON_Pb_Autis; BON_Pb_Kontrol

                  N  Median
BON_Pb_Autis     30  0.1420
BON_Pb_Kontrol   10  0.0830


Point estimate for η1 - η2 is 0.0330
95.3 Percent CI for η1 - η2 is (-0.0160;0.1071)
W = 656.0
Test of η1 = η2 vs η1 > η2 is significant at 0.1029
The test is significant at 0.1027 (adjusted for ties)
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Fig. 12: The Mann-Whitney test result of hair lead levels on autism children and non-autism control in Bantul.

When we compared the lead levels in autism and control hairs and carried out statistical analysis with the Mann Whitney test, the result is that average lead (Pb) levels in autism children's hair is higher than non-autism controls, which means that Pb influences autism in Bantul. Likewise, from another study conducted by Akyuzlu *et al*. (2014)[9] in Ankara found the same thing with this study that the lead levels in autism patients' hair are higher (0,80 µg/ g) than levels in control hair (0,01 µg/g).

The entry of lead (Pb) into the human body is closely related to the precence of heavy metal lead pollution in the environment. The source of Pb exposure in children can come from paint, gasoline, batteries, contaminated drinking water, food (Illionis Department of Public Health 2015).[21]

Exposure to Pb in pregnant women, causing Pb into the body and through the placenta, Pb enters the body of the fetus so that it can be detected on examination of fetal blood, and one of the consequences of high levels of Pb in fetal blood is decreasin the IQ of children (Rahbar 2016).[22]

If compared with lead level in the environment samples, accordance with Table 1, it can be seen that environmental samples that have high lead levels are vegetable samples taken from the Banguntapan area (0,40 mg/kg) and Bantul-Yogya border area (0,29 mg/kg). This lead source is very likely to come from the air because the lead content in the soil is only 33 mg/kg from the maximum limit of 85 mg/kg. Or, there are source of lead that come from home industry such as battery fusion?

Conclusion

In Bantul, there has been environmental pollution of heavy metals, which can be seen in the high levels of mercury in fish and vegetables. Likewise, people living with autism have higher levels of mercury than control.

Lead (Pb) is also a heavy metal that has polluted the environment in Bantul. This can be seen in the high levels of Pb in vegetables and also the high levels of Pb in hair. But whether the Pb in the hair of autism and control patients comes from vegetables or the same source from the air, of course, it still needs verification.

While arsenic levels, although the levels in the environmental sample are low, some autism children have quite high arsenic levels in the hair. This is thought to be related to the home industry, which is likely to be in the form of battery amalgamation as a home industry activity around the area.

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Hopefully all the help given to us, could improve the management of autism in the future.

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